Medicinal and Aromatic Plants of the World

Ákos Máthé *Editor*

Medicinal and Aromatic Plants of the World

Scientific, Production, Commercial and Utilization Aspects

Springer

Medicinal and Aromatic Plants of the World

Volume 1

Series Editor
Ákos Máthé
Faculty of Agriculture and Food Science
University of West Hungary
Budapest, Hungary

Medicinal and Aromatic Plants (MAPs) have been utilized in various forms since the earliest days of mankind. They have maintained their traditional basic curative role even in our modern societies. Apart from their traditional culinary and food industry uses, MAPs are intensively consumed as food supplements (food additives) and in animal husbandry, where feed additives are used to replace synthetic chemicals and production-increasing hormones. Importantly medicinal plants and their chemical ingredients can serve as starting and/or model materials for pharmaceutical research and medicine production. Current areas of utilization constitute powerful drivers for the exploitation of these natural resources. Today's demands, coupled with the already rather limited availability and potential exhaustion of these natural resources, make it necessary to take stock of them and our knowledge regarding research and development, production, trade and utilization, and especially from the viewpoint of sustainability.

The series Medicinal and Aromatic Plants of the World is aimed to look carefully at our present knowledge of this vast interdisciplinary domain on a global scale. In the era of global climatic change, the series is expected to make an important contribution to the better knowledge and understanding of MAPs.

Thanks are due to the editors and authors of the nine volumes and large number of chapters, who are internationally recognized professionals, specialists in the field of medicinal and aromatic plants and will be primarily invited from the members of the International Society for Horticultural Science and the International Council for Medicinal and Aromatic Plants.

The Editor of the series is indebted for all of the support and encouragement received in the course of international collaborations started with his ISHS involvement, in 1977. Special thanks are due to Professor D. Fritz, Germany for making it possible.

The encouragement and assistance of Springer Editor, Mrs. Melanie van Overbeek, has been essential in realizing this challenging book project. Thanks are due to the publisher - Springer Science+Business Media, The Netherlands - for supporting this global collaboration in the domain of medicinal and aromatic plants.

We sincerely hope this book series can contribute and give further impetus to the exploration and utilization of our mutual global, natural treasure of medicinal and aromatic plants.

More information about this series at http://www.springer.com/series/11192

Ákos Máthé
Editor

Medicinal and Aromatic Plants of the World

Scientific, Production, Commercial and Utilization Aspects

Springer

Editor
Ákos Máthé
Faculty of Agriculture and Food Science
University of West Hungary
Budapest, Hungary

ISSN 2352-6831 ISSN 2352-684X (electronic)
Medicinal and Aromatic Plants of the World
ISBN 978-94-017-9809-9 ISBN 978-94-017-9810-5 (eBook)
DOI 10.1007/978-94-017-9810-5

Library of Congress Control Number: 2015942625

Springer Dordrecht Heidelberg New York London

Printed on acid-free paper

Springer Science+Business Media B.V. Dordrecht is part of Springer Science+Business Media (www.springer.com)

Foreword

The history of medicinal and aromatic plant (MAP) utilization dates back to the beginnings of mankind. Our forefathers used natural substances they could find in nature to ease and cure their sufferings and illnesses and to heal their wounds. This type of approach has survived in the traditional medicinal (TM) uses until today, since nearly 80 % of the world population still relies on MAPs in their medications. The renaissance of MAP use in the high-income countries of the world has brought about a different type of use in the form of herbal medicines (Complementary/Alternative Medicine (CAM)). MAPs have become "industrial products" with new concepts like phytotherapy and veterinary medicinal uses, aromatherapy, nutraceuticals, cosmeceuticals, and animal welfare uses widening the scope of their utilization. New, innovative, value-added applications include their use in functional foods, animal husbandry, as well as plant protection in agriculture. In this regard, the versatile utilization of essential oils is promising. Modern approaches in production and uses have brought about an increased focus on the importance of quality, safety, and efficacy of both MAPs and their produce. MAPs will also maintain their importance in the search for new, valuable sources of drugs and lead compounds.

Current areas of utilization constitute powerful drivers for the exploitation of these natural resources: Today's demands, coupled with the already rather limited availability and potential exhaustion of these natural resources, make it necessary to take stock of our resources and our knowledge regarding research and development, production, trade, and utilization, especially from the viewpoint of sustainability.

One of the main goals of the series *Medicinal and Aromatic Plants of the World* is, therefore, to offer practical knowledge, in a monographic form, on cultivated and noncultivated medicinal and aromatic plants with both therapeutic and commercial values, and all this in a global perspective.

Volume No. 1 will summarize basic knowledge on MAPs ranging from botanical aspects to marketing and much more. It is meant to serve as a tool for facilitating the digestion (understanding/utilization) of the knowledge summarized about the MAPs in the subsequent eight volumes, from the various climatic zones of the world.

Due to the immense diversity of the plant and medicinal plant kingdom, individual volumes of the book series summarize recent scientific and traditional data on several unique medicinal plants with a wide range of bioactive and economic properties and introduces them as potential new crops and/or as new sources of various bioactive ingredients for the use of man.

It is a special honor that several leading scientists in the field of aromatic and medicinal plants from the world over have accepted our invitation to realize this daring vision of the editor and contribute their expertise and research experience in the assembling of knowledge for this book series.

The series will demonstrate the vast knowledge already available on MAPs on all four continents as well as the intensive and manifold activities going on in the R & D domain of medicinal plants with the attempt to utilize this precious resource of Mother Nature. Hopefully, the series *MAPW* will also contribute to further unfold our knowledge on the present and prospective potentials of this ancient, still ever-young group of economic crops.

The authors of the various volumes and chapters will be internationally recognized professionals, specialists in the field of medicinal and aromatic plants, and will be primarily invited from the members of the International Society for Horticultural Science and the International Council for Medicinal and Aromatic Plants, which also demonstrates the significance of MAPs that not only overarches the history of mankind but also global spatial dimensions.

So, when reading this extreme wealth of knowledge on MAPs compiled by our international authors collectively, we will realize how important it is to get to know and to preserve this precious natural resource of ours, as worthily worded by the Chiang Mai Declaration (1988): "Saving Lives by Saving Plants."

Budapest, Hungary
November 2014

Ákos Máthé

Contents

Chapter 1
Introduction: Utilization/Significance of Medicinal and Aromatic Plants

Ákos Máthé

Abstract The history of Medicinal and Aromatic Plant (MAP) utilization dates back to the beginnings of mankind. Our forefathers used natural substances, they could find in nature, to ease, cure their sufferings, illnesses, to heal their wounds. This type of approach has survived in the Traditional Medicinal (TM) uses, until today, since nearly 80 % of the world population still relies on MAPs in their medications. The renaissance of MAP-use in the high-income countries of the world has brought about a different type of use in the form of Herbal Medicines (CAM). MAPs have become "industrial products" with new concepts like phytotherapy and veterinary medicinal uses, aromatherapy, nutraceuticals, cosmeceuticals, and animal welfare uses widening the scope of the utilization. New, innovative, value added applications include their use in functional foods, animal husbandry, as well as plant protection in agriculture. In this regards the versatile utilization of essential oils is promising. Modern approaches in production and uses have brought about an increased focus on the importance of quality, safety and efficacy of both MAPs and their produce. MAPs will also maintain their importance in the search for new, valuable sources of drugs and lead compounds. In view of the steadily increasing demands on these important natural resources, attention should be paid to the sustainable forms of production and utilization.

Keywords Medicinal and aromatic plants • Utilization • Traditional medicine • Herbal medicines • Phytotherapy • Aromatherapy • Nutraceuticals • Food-/feed additives • Veterinary medicine • Legislation

Á. Máthé (✉)
Faculty of Agriculture and Food Science, University of West Hungary, Budapest, Hungary
e-mail: akos.mathe@upcmail.hu

Á. Máthé (ed.), *Medicinal and Aromatic Plants of the World*, Medicinal and Aromatic Plants of the World 1, DOI 10.1007/978-94-017-9810-5_1

1 Introduction

1.1 Notion of Medicinal and Aromatic Plants (MAPs)

Medicinal plants constitute an independent unit in the Plant systems of Economic botany (Simpson and Conner-Ogorzaly 1986). Nonetheless, in these systems aromatic plants (i.e. plants with a pleasant taste and odor) are generally not contained. These species are ranked among spices, condiments and flavoring plants.

The term medicinal plant is mostly applied to indicate that the plants contain biologically active substances with alleged medicinal properties, i.e. effects that relate to health, or have been proven to be useful as drugs by Western standards, or which contain constituents that are used as drugs (Farnsworth and Solejarto 1991).

In the simplest way, however, one can distinguish between medicinal or therapeutic (pharmacological) and miscellaneous (culinary, cosmetic, food, etc.) applications (Agri-Food Canada 2013).

1.2 A Historical Glimpse at MAP's Use

The history of MAP utilization dates back to our forefathers, who used natural substances they could find in nature to ease, cure their sufferings, illnesses, to heal their wounds, etc.

Even to date, medical plants (in *sensu stricto*) are extensively used in self-medication and in national health services, alike. MAPs constitute an important part of our natural wealth, and they serve as both therapeutic agents and raw materials for manufacturing several diverse products. Similarly to MAPs their products are also manifold. They include products ranging from traditional and modern medicines, products used in the medical (health care), through food industry (incl. the everyday culinary uses) to cosmetic uses (Hornok 1992).

The long history of MAPs, roughly, overarches the time span from the *Doctrine of signatures and similitudes* to the present day *Evidence based medicine*. According to Baser (2005), for the first time, MAPs have become industrial products for world-wide use with new concepts, e.g. nutraceuticals, cosmeceuticals, phytotherapy, aromatherapy, etc. widening their use and new applications in functional foods, animal husbandry. National and international legislative and regulatory authorities seem to have an ambivalent approach to the increased use of MAPs in conventional medicine.

2 Medicinal and Pharmacological Applications of MAPs

Therapeutic activity refers to the successful prevention, diagnosis and treatment of physical and mental illnesses; improvement of symptoms of illnesses; as well as beneficial alteration or regulation of the physical and mental status of the body (WHO 2003).

2.1 Traditional Medicine

Traditional Medicine (TM) is the sum total of the knowledge, skills, and practices based on the theories, beliefs, and experiences indigenous to different cultures. Historically the main centers of Traditional Medicines are: Traditional African Medicine, Bush medicine, Traditional Chinese Medicine, Traditional Medicine in India, Islamic Medicine, Mesoamerican Medicine, South American Traditional Medicine, South Asian Traditional Medicine (Magner 1992).

Traditional use of herbal medicines refers to the long historical use of these medicines. Their use is well established and widely acknowledged to be safe and effective, and may be accepted by national authorities.

In Africa, up to 80 % of the population uses traditional medicine for primary health care. Countries in Africa, Asia and Latin America also use TM to help meet some of their primary health care needs.

In high-income, industrialized countries, adaptations of traditional medicine are termed “Complementary“ or “Alternative” (CAM) and are used by up to 65 % of the population (Baser 2005). TM has maintained its popularity and its use is rapidly spreading in industrialized countries (Bagozzi 2003).

To promote the proper use of TM/CAM, WHO has elaborated a Traditional Medicine Strategy (WHO 1998, 2002, 2013) that focuses on Policy, Safety/Quality/Efficacy, Access and Rational use of TM/CAM.

Herbal medicines are also referred to as herbal remedies, herbal products, phytomedicines, phytotherapeutic agents and phytopharmaceuticals (Barnes et al. 2007). The use of herbal medicines in an evidence- or science-based approach for the treatment and prevention of disease is known as (rational) phytotherapy. This approach contrasts with the practice of traditional medical herbalism which uses herbal medicines in a holistic manner and mainly on the basis of their empirical and traditional uses (Barnes et al. 2007).

The most frequent forms of therapeutic uses of MAPs comprise traditional medicine, phytotherapy, aromatherapy, homeopathy and balneology.

2.2 Phytotherapy

The notion of phytotherapy is, in the simplest way, healing with various forms of plant-derived medicines (herbal medicines). Although differently interpreted in various countries, it comprises the major elements of both traditional (ethnomedicine) and modern medicine (Mills and Bone 2000). Phytotherapy (herbal medicine) involves the use of dried plant material or extracts of plant parts in therapeutic doses to treat the symptoms exhibited. In this latter respect, it is similar to conventional medicine (Barnes et al. 2007).

2.2.1 Herbal Medicinal Products (HMP)

According to the European legal definition, HMPs are medicinal products, exclusively containing as active ingredients one or more herbal substances or one or more herbal preparations, or one or more such herbal substances in combination with one or more such herbal preparations (DIRECTIVE 2001/83/EC of the European Parliament and of the Council (2001)).

Sales of herbal medicines are booming. This is particularly true in the United States, where the market for herbal supplements is now approaching $4bn a year. The fastest growth has been recorded for St John's wort, a herbal antidepressant whose sales increased in 1 year by 2,800 %.[1] Faced with such figures one might be inclined to ask where the evidence is. Are there rigorous trials to show that herbal treatments work (Ernst 2000)?

The answer is: yes, in an increasing number of cases there is sufficient evidence. Herbal pharmaceuticals are subjected to clinical trials before registration as HMPs by regulatory authorities. As Baser (2005) refers to a preliminary survey, globally *ca.* 748 clinical trials have been conducted on 96 plant species or their combination formulas and some 823 written papers have been published on the results of these clinical trials.

As a general rule, plant drugs and phyto-pharmaceuticals used by phytomedicine must be produced according to strict quality requirements (Máthá and Máthá 2008) and must have proven to be efficient; this can be based on earlier case records. Similarly, the safety of application (void of both toxicity and side effects, etc.) should be guaranteed along with their reliable good quality (Good Agricultural Practice = **GAP**, Good Manufacturing Practice = **GMP**, Good Laboratory Practice = **GLP**, etc.).

Irrespective of the regulatory pathway to access the market, the **quality** of the herbal medicinal product must be demonstrated.

In the European Community herbal monographs prepared by the European Medicines Agency (EMA) Committee on Herbal Medicinal Products (HMPC) are relevant for the traditional use registration as well as the well-established use marketing authorization. The European Scientific Cooperative on Phytotherapy (ESCOP) founded in 1989 by six EU national scientific organizations provides an umbrella organization for the assessment of HMPs. It supports scientific research and contributes to the acceptance of phytotherapy in Europe. Presently, ESCOP has published some 80 monographs on individual herbal drugs (Steinhoff 1999; Barnes et al. 2007).

Herbal medicines are one type of dietary supplements. People use herbal medicines to try to maintain or improve their health.

[1] http://www.ncbi.nlm.nih.gov/pmc/articles/PMC1127780/

2.3 Aromatherapy

As a therapeutic tool, aromatherapy has been known **since** ancient times. It implies a natural therapeutic method **based on** the effect of aromatic plants and plant extracts, essences to improve a person's mood, cognitive functions or health status. It is used by manufacturers of personal care, wellness and hygiene products, **as well as** practitioners, including massage therapists, chiropractors, nurses and doctors. Many essential oils are **potent** antimicrobials; therefore, they can be useful also in the treatment of infectious diseases. Aromatherapy **may be** applied in combination with other forms of alternative complementary treatments.

Scientific evidence on the pharmacological effects of essential oils and aromachemicals (e.g. antibacterial, anti-inflammatory, expectorant, sedative, etc.) has been increasing (Krings and Berger 1998; Rowe 2004).

2.3.1 Renaissance of Essential Oils

The effects of essential oils and aroma-chemicals on the human (and animal) organism (e.g. nervous system, gastrointestinal system, immune system, respiratory system), also in view of their antimicrobial and antifungal activities are intensively investigated. These studies, as recently exhaustively surveyed by Weiss (1997), Baser (2005), Bakkali et al. (2008), Baser and Buchbauer (2009), Máthé (2009), Franz et al. (2010) and others have already supplied an increasing number of proofs on the efficacy of essential oils and their components in conventional therapy of the following domains (Baser 2005):

- Anticancer Effects
- Antimicrobial Effects
- Plant Growth Promoting Activity
- Plant Growth Inhibiting Activity
- Pest Control
- Animal repellent Activity
- Enhancement of Soil Respiration
- Skin Penetration Enhancing Activity

These aspects of the essential oils will be discussed also in various relevant chapters of this volume.

2.4 Balneology and Mineral Therapy

Balneology is a method in alternative medicine, which refers to the use of baths of various types to relieve pain, muscle spasm, or stress, and hence promotes healing, an aspect of hydrotherapy (Routh et al. 1996). MAPs (such as the decoction of

Equisetum arvense in the case of rheumatic problems) have been used more and more frequently to add up to the beneficial effect of hot and cold water treatments (Ghersetic et al. 2000; Varga 2010).

2.5 Homeopathy

Homeopathy, or homeopathic medicine, is a holistic medical system that was developed in Germany more than 200 years ago and has been practiced in the United States since the early nineteenth century. Homeopathy is used for wellness and prevention and to treat many diseases as well as conditions (Dantas and Rampes 2000; Dantas et al. 2007).

According to its basic laws (by Christian Samuel Hahnemann), homeopathy seeks to stimulate the body's ability to heal itself by giving very small doses of highly diluted substances. The principle of similars (or "like cures like") states that a disease can be cured by a substance that produces similar symptoms in healthy people. To this end homeopathy makes use of the entire range of MAPs (Der Marderosian 1996; Jonas et al. 2003).

To date it is still a controversial area of Complementary and Alternative Medicine since a number of its key concepts are not consistent with the established laws of science, primarily chemical, biochemical and physical sciences.

2.6 Veterinary Medicine and Animal Welfare

The utilization of MAPs in treating/curing various animal diseases has a very long record, nearly as old as that of human medicine.

The worldwide interest in herbal products has grown significantly. Research in this field has intensified and there are numerous scientific publications justifying the importance of MAPs in veterinary medicinal applications, animal husbandry or animal welfare (Russo et al. 2009; Wallace et al. 2010; Laudato and Capasso 2013; Sabaldica 2011). According to Viegi et al. (2003) cattle, horses, sheep, goats and pigs represent about 70 % of the animals treated with herbal remedies, followed by poultry (9.1 %), dogs (5.3 %) and rabbits (4.3 %). This is not only due to a general trend towards the usage of natural products for curing illnesses but also due to the availability of considerable evidence regarding the efficacy of herbal remedies (Franz et al. 2010; Laudato and Capasso 2013).

3 Industrial Utilizations

Many useful drugs have been developed from plant sources. Numerous specialty materials such as essential oils, pharmaceuticals, colorants, dyes, cosmetics and biocides are obtained from plants. Several medicinal and aromatic plants (MAPs) species are cultivated for such industrial uses, however, most botanicals are still wild collected (Lubbe and Verpoorte 2011).

Following discovery of the penicillins, drug discovery from microbial sources occurred and diving techniques in the 1970s, opened also the seas. Combinatorial chemistry (in the late 1980s), shifted the focus of drug discovery efforts from Nature to the laboratory bench (Cragg and Newman 2013).

Natural product drug discovery has already yielded important drugs from natural sources that have revolutionized the treatment of serious diseases. Nature will continue to be a major source of new structural Effective drug development depends on multidisciplinary collaborations (Wermuth 2011).

3.1 Pharmaceutical Industry

The important role of MAPs for the pharmaceutical industry is explained by several factors. These plants are sources of numerous biologically active substances that, owing to their healing effects, can not be ignored by modern medical practice. Due to their frequently unknown features and complexity, they can however not yet be produced synthetically. Frequently, their synthetic production is irrationally expensive (e.g.: ergot alkaloids, cardiac glycosides, etc.).

The pharmaceutical industry also frequently uses the active principles of MAPs as starting materials for the semi-synthetic production of substances (as for example corticosteroids from *Solanum* alkaloids), or for isolating so called model compounds (e.g. secologanin), etc.

MAPs serve as raw materials for the production of numerous health care products, phyto-cosmetics and environment-friendly plant protection products (e.g.: pyrethroids).

3.2 Food and Feed Additives

Food additives are substances added to food to preserve flavor or enhance its taste and appearance. Some additives have been used for centuries; e.g. salting, as with bacon, preserving sweets or using sulfur dioxide as with wines. With the advent of processed foods in the second half of the twentieth century, MAPs are frequently used by the food-industry in the form of spices and condiments, as well as culinary herbs. Numerous new additives have been introduced, of both natural and artificial origin, their official list is contained in Codex Alimentarius (2014).

The significance of MAPs in **food additives** is raised also by their antimicrobial (bactericidal and fungicidal) properties, owing to which they make highly valued preservatives for both fresh vegetable and meat preparations as well as canned products (Davidson et al. 2005).

Based partly on their traditional uses in animal keeping, MAPs have recently been used to improve and to develop **healthy feed** for the safety of livestock and humans, and to find effective alternatives to address the removal from routine use of antimicrobials in animal feeds Codex Veterinary Drugs (CODEX Alimentarius 2014).

3.3 Dietary Supplements Versus Nutraceuticals

Food additives have been used for centuries to enhance the quality of food products. By the early 1960s about 2,500 different chemicals, among them medicinal and aromatic plants were used in the USA. Several plants are widely used in the daily diet that have medicinal and nutraceutical values, e.g. demonstrated high antioxidant activity or a potential as therapeutic agents for the management and prevention of chronic diseases, such as diabetes, stroke, etc. (Burdock 2010).

Dietary supplements include vitamins, minerals, and other less familiar substances — such as herbals, botanicals, amino acids, and enzymes. They are also marketed in forms such as tablets, hard- or soft-shelled capsules. While some dietary supplements are fairly well understood, others need further study.

Unlike drugs, supplements are not intended to treat, diagnose, prevent, or cure diseases (Enna and Norton 2012).

In the U.S.A. dietary supplements are *not* approved by the government for safety and effectiveness before they are marketed. The manufacturers and distributors of dietary supplements are responsible for making sure their products are safe before they go to market. Manufacturers are required to produce dietary supplements to minimum quality standards and ensure that they do not contain contaminants or impurities, and are accurately labeled (Paul et al. 2004).

FDA can take dietary supplements off the market if they are found to be unsafe, adulterated, or if the claims on the products are false and misleading (FDA 2014).

Nutraceuticals. The nutraceuticals industry has developed rapidly over the past decade. U.S. sales reached $32.5 billion in 2012, growing 7.5 %, according to Nutrition Business Journal (NBJ), Boulder, CO. Top supplement categories included vitamins ($10.6 billion), specialty/other ($6.1 billion), herbs and botanicals ($5.6 billion) and minerals ($2.4 billion). Meanwhile, the functional food and beverage market continues to gain significant momentum at a time when consumers are focused on disease prevention (Moloughney 2013).

A recently published volume edited by Bagchi (2014) offers an updated insight into the scope, importance, as well as continuing growth opportunities in the nutraceutical and functional food industries. The latest regulatory changes and their impacts are also explored. Public confidence in the quality of these products is based on sophisticated quality control, a broad spectrum of safety studies and GRAS (Generally Recognized as Safe), peer-reviewed publications and cutting-edge human clinical studies. An increasing number of additional populations around-the-world now seem to recognize the efficacy and functions of nutraceuticals and functional foods.

3.4 Cosmetic Industry

Herbal preparations are popular and are of significance in primary and individual home- and health-care. The popularity of health-care plant-derived products has been traced to their increasing acceptance and use in the cosmetic industry (Aburjai and Natsheh 2003), as well as to increasing public costs in the daily maintenance of personal health and well-being. About 1,400 herbal preparations are used widely, according to a recent survey in Member States of the European Union. Cosmetic products should be safe under normal or reasonably foreseeable conditions of use. In particular, risk benefit reasoning should not justify a risk to human health (Regulation E.U. 2009).

Herbal extracts are primarily added to cosmetic preparations due to their anti-oxidant constituents (e.g. carotenoids, flavonoids and polyphenols). Apart from these, herbal extracts have also been used for their topical anti-inflammatory properties (Kole et al. 2005).

Examples of such beauty-oriented therapeuticals are skin tissue regenerators, anti-wrinkling agents and anti-age creams. Skincare products such as skin creams, skin tonics, etc. derived from medicinal plants are grouped together as dermaceuticals (Hoarea and DaSilva 1999).

4 Drug Discovery

Nature has always been a valuable source of drugs and, despite the intensive research in medicinal chemistry, continues to deliver lead compounds. Originally, research on natural sources of chemicals was focused on terrestrial plants and microorganisms. More recently, however, organisms of marine origin are also being investigated (Grabley and Thiericke 1999; Tulp and Bohlin 2004).

5 Conclusion

The resurgence of interest in MAPs in the industrialized countries has greatly expanded the market demand on these natural resources that have been utilized since the early times of human civilizations. Similarly to the versatility of MAP species, their utilization is also manifold. Basically, however, therapeutic and industrial uses can be distinguished. Traditional Medicine has remained to be a priority in the health care systems of low income countries, whereas Herbal Medicines (CAM) have become popular in the developed parts of the world. Production, i.e. wild-crafting and cultivation of good quality MAPs to be used safely and with efficacy have become a precondition both for the various forms of uses and the preservation of our endangered natural resources. Sustainable utilization has become a necessity also as a prerequisite for the survival of these old natural resources.

References

Aburjai T, Natsheh FM (2003) Plants used in cosmetics. Phytother Res 17(9):987–1000

Agriculture and Agri-Food Canada (2013) Introduction to medicinal plants – Agriculture and Agri-Food Canada (AAFC). Retrieved 6 Oct 2014, from http://www.agr.gc.ca/eng/science-and-innovation/science-publications-and-resources/resources/canadian-medicinal-crops/introduction-to-medicinal-plants/?id=1300832855227

Bagchi D (ed) (2014) Nutraceutical and functional food regulations in the United States and around the world, 2nd edn. Academic, London/Waltham/San Diego, p 551

Bagozzi F (2003) Traditional medicine. What is traditional medicine? WHO Factsheet No 134. http://www.who.int/mediacentre/factsheets/2003/fs134/en/print.html

Bakkali FS, Averbeck S, Averbeck D, Idaomar M (2008) Biological effects of essential oils – a review. Food Chem Toxicol 46(2):446–475. doi: 10.1016/j.fct.2007.09.106

Barnes J, Anderson LA, Phillipson JD, Newall CA (2007) Herbal medicines, 3rd edn. Pharmaceutical Press, London, pp 346–351

Başer KHC (2005) New trends in the utilization of medicinal and aromatic plants. In III WOCMAP congress on medicinal and aromatic plants-volume 2: conservation, cultivation and sustainable use of medicinal and aromatic plants, 676:11–23

Baser KHC, Buchbauer G (eds) (2009) Handbook of essential oils: science, technology, and applications. CRC Press, Boca Raton, p 991

Burdock GA (2010) Fenaroli's handbook of flavor ingredients, 5th edn. CRC Press, Boca Raton, p 1864

Codex Alimentarius (2014) CODEX Alimentarius: Veterinary Drugs MRLs. Retrieved 6 Oct 2014, from http://www.codexalimentarius.org/standards/veterinary-drugs-mrls/en/

Cragg GM, Newman DJ (2013) Natural products: a continuing source of novel drug leads. Biochim Biophys Acta Gen Subj 1830:3670–3695

Dantas F, Rampes H (2000) Do homeopathic medicines provoke adverse effects? A systematic review. Br Homoeopath J 89(Suppl 1):35–38

Dantas F, Fisher P, Walach H, Wieland F, Rastogi DP, Teixeira H, Koster D, Jansen JP, Eizayaga J, Alvarez MEP, Marim M, Belon P, Weck LLM (2007) Systematic review of the quality of homeopathic pathogenetic trials published from 1945 to 1995. Homeopathy 96:4–16

Davidson MP, Sofos JN, Branen AL (2005) Antimicrobials in food, 3rd edn. Taylor & Francis, Boca Raton, p 720

Der Marderosian AH (1996) Understanding homeopathy. J Am Pharm Assoc NS36(5):317–321

DIRECTIVE 2001/83/EC OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL of 6 November 2001 on the Community code relating to medicinal products for human use. OJ L 311, 28.11.2001, p 67

Enna SJ, Norton S (2012) Herbal supplements and the brain: understanding their health benefits and hazards. FT Press, Upper Saddle River

Ernst E (2000) Herbal medicines: where is the evidence?: growing evidence of effectiveness is counterbalanced by inadequate regulation. BMJ 321(7258):395–396

Farnsworth NR, Solejarto DD (1991) Global importance of medicinal plants. In: Akerele O, Heywood V, Synge H (eds) Conservation of medicinal plants. Cambridge University Press, Cambridge, UK

FDA (2014) Food facts. Dietary supplements: what you need to know. US Food and Drug Administration Center for Food Safety and Applied Nutrition Information, May 2006, p 2

Franz C, Baser K, Windisch W (2010) Essential oils and aromatic plants in animal feeding – a European perspective. A review. Flavour Fragr J 25:327–340. doi:10.1002/ffj.1967

Ghersetic I, Freedman D, Lotti T (2000) Balneology today. J Eur Acad Dermatol Venereol 14:346–348. doi:10.1046/j.1468-3083.2000.00136.x

Grabley S, Thiericke R (1999) Drug discovery from nature. Springer, Berlin/Heidelberg, p 347

Hoarea, L. & E.J. Da Silva. 1999. Medicinal plants: A re-emerging health aid. Elect. J. Biotech. V: 2.

Hornok L (ed) (1992) Cultivation and processing of medicinal plants. Akadémiai Kiadó, Budapest

Jonas WB, Kaptchuk TJ, Linde K (2003) A critical overview of homeopathy. Ann Intern Med 138(5):393–399

Kole PL, Jadhav HR, Thakurdesai P, Nagappa AN (2005) Cosmetic potential of herbal extracts. Nat Prod Radiance 4:315–321

Krings U, Berger RG (1998) Biotechnological production of flavours and fragrances. Appl Microbiol Biotechnol 49(1):1–8

Laudato M, Capasso R (2013) Useful plants for animal therapy. OA Altern Med 1(1):1

Lubbe A, Verpoorte R (2011) Cultivation of medicinal and aromatic plants for specialty industrial materials. Ind Crop Prod 34:785–801

Magner LN (ed) (1992) A history of medicine. CRC Press, p 400

Máthé A (2009) Essential oils: biochemistry, production and utilization. In: Steiner T (ed) Phytogenics in animal nutrition. Natural concepts to optimize gut health and performance. Nottingham University Press, Nottingham, pp 1–18

Máthé Á, Máthé I (2008) Quality assurance of cultivated and gathered medicinal plants. Acta Horticult 765:67–76

Mills S, Bone K (2000) Principles and practice of phytotherapy. Modern herbal medicine. CAB International, p 643

Moloughney S (2013) Is the nutraceuticals industry passing its most critical assessments and how can companies improve? Nutraceuticals World, 1. Oct 2013. http://www.nutraceuticalsworld.com/issues/2013-10/view_features/testing-trends-takeaways/

Paul M, Coates M, Coates P, Blackman M, Blackman MR, Gordon M, Cragg ML, White JD, Moss J, Levine MA (2004) Encyclopedia of dietary supplements. CRC Press, p 840

Regulation EU (2009) 1223/2009. REGULATION (EC) No, 1223

Routh HB, Bhowmik R, Parish LC, Witkowski JA (1996) Balneology, mineral water, and spas in historical perspective. Clin Dermatol 14(6):551–554

Rowe DJ (ed) (2004) Chemistry and technology of flavors and fragrances. Blackwell, Oxford, UK. doi:10.1002/9781444305517.fmatter

Russo R, Autore G, Severino L (2009) Pharmaco-toxicological aspects of herbal drugs used in domestic animals. Nat Prod Commun 4(12):1777–1784

Sabaldica AC (2011) Medicinal plants for livestock healthcare. A primer on medicinal plants for livestock healthcare. Northern Marianas College Cooperative Research Extension and Education Service (NMC-CREES)

Simpson BB, Conner-Ogorzaly M (1986) Economic botany. McGraw-Hill, New York

Steinhoff B (1999) The contribution of the European Scientific Cooperative on phytotherapy and World Health Organisation monographs. Drug Inf J 33:17–22

Tulp M, Bohlin L (2004) Unconventional natural sources for future drug discovery. Drug Discov Today 9:450–458

Varga C (2010) The Balneology paradox. Int J Biometeorol 55(2):105–106. doi:10.1007/s00484-010-0378-1

Viegi L, Pieroni A, Guarrera PM, Vangelisti R (2003) A review of plants used in folk veterinary medicine in Italy as basis for a databank. J Ethnopharmacol 89(2–3):221–244

Wallace RJ, Oleszek W, Franz C, Hahn I, Baser KHC, Máthé Á, Teichmann K (2010) Dietary plant bioactives for poultry health and productivity. Br Poultry Sci 51(4):461–487

Weiss EA (1997) Essential oil crops. CAB International, Wallingford, p 600

Wermuth CG (ed) (2011) The practice of medicinal chemistry, 3rd edn. Academic, London/Burlington/San Diego, 923

WHO (1998) General guidelines for methodologies on research and evaluation of traditional medicine. WHO/EDM/TRM/2000.1, Geneva, p 128

WHO (2002) Traditional medicine strategy 2014–2023. World Health Organization, Geneva, http://apps.who.int/iris/bitstream/10665/92455/1/9789241506090_eng.pdf?ua=1

WHO (2003) WHO guidelines on good agricultural and collection practices (GACP) for medicinal plants. WHO, Geneva

WHO (2013) The WHO Traditional Medicine Strategy 2014–2023. WHO, Geneva. ISBN 978 92 4 150609 0

Chapter 2
Botanical Aspects of Medicinal and Aromatic Plants

Ákos Máthé

Abstract Botany, the science of plants, is linked with medicinal and aromatic plants in many different ways. The ca. 40,000 plant species used for ethnomedicinal purposes, since the beginning of recorded history, have traditionally been collected and gathered from the wild. Botanical sciences (like plant systematics, morphology and physiology) have been assisting the study and utilization of MAPs in a multiple of ways.

The study and utilization of MAPs should begin with the correct identification of plants. Formerly this was done mainly on the basis of morphological characters. With the progress of scientific – technical development, the chemical traits were also involved. Recent research trends have opened up new opportunities for revealing the DNA and biosynthetic causes of chemo-differentiation, and ultimately the information supplied by the plant metabolome. As thus, botany assisted by other scientific achievements, seems to open up promising perspectives for the breeding of new, highly powerful chemo-cultivars of medicinal and aromatic taxa.

In the case of medicinal and aromatic plants the inheritance patterns, as well as the interrelatedness of economically important traits is complex. Their variability as complemented by the ecological plasticity of plants make it difficult to arrive at reliable research conclusions, to sort out the inheritable characteristics making MAP-breeding still quite a challenge.

The efficiency of cultivation of these species is fundamentally dependent on the productivity of the plant biomass within which the active principles are synthesized and frequently accumulated. Their quantities and composition are important preconditions for utilization, therefore also intensively investigated botanical domains.

Floristics or Vegetation Science deals with plants in geographic dimensions. The knowledge and economic mapping of MAP resources is an important contribution to the sustainable management and utilization of these species.

Á. Máthé (✉)
Faculty of Agriculture and Food Science, University of West Hungary, Budapest, Hungary
e-mail: akos.mathe@upcmail.hu

Á. Máthé (ed.), *Medicinal and Aromatic Plants of the World*, Medicinal and Aromatic Plants of the World 1, DOI 10.1007/978-94-017-9810-5_2

In the wake of the Chiang Mai Declaration (1988), appropriate policies and legal frameworks, standards, etc. (GAP, GCP, GMP, Fair Trade, etc.) have been elaborated to safeguard the already frequently endangered natural resources of MAPs and to assist their the protection. These and also other guidelines (standards) are meant to contribute to the survival and sustainable utilization of medicinal and aromatic plant resources.

Keywords Medicinal and Aromatic plants • Botanical aspects • Systematics • Ecology • Active ingredients • Variability • Production ecology • Collection • Production • Utilization

1 Introduction: MAPs in the History of Botanical Sciences

The vast diversity of the plant kingdom, the approximately 40,000 plant species used for ethnomedicinal purposes, since the beginning of recorded history, have traditionally been collected and gathered from the wild (Trease and Evans 2002; Máthé 2011b). Similarly, the science of plants (scientia "amabile"), **Botany** takes its origin in prehistory, as herbalism with the efforts of early humans to identify – and later to cultivate – edible, medicinal and poisonous plants, has made Botany one of the oldest domains of science (Sumner 2000).

The link between humans and the different uses of plants constitutes the applied branches of botany (e.g. economic botany). Owing to their versatility, MAPs cannot be ranked into one category, but various classes of the economic-botanical system. In a most simple way, one can differentiate between **wild growing** (wild-crafted) and **cultivated** medicinal and aromatic plants. The latter category comprises species that have been domesticated or introduced into cultivation, from different climatic, geographic regions of the world.

Pharmacobotany or **Medical Botany** covers all pharmaceutical aspects of botany, including cytology, histology, morphology and taxonomy of plants species used in the pharmacological practice i.e. it deals with the botanical aspects of plants affecting man's health (Medicinal and Aromatic Plants).

2 Botanical Aspects of MAPs

The knowledge of MAPs begins with the description (Anatomy/Morphology) and classification (Systematics/Taxonomy) of plants, especially in view of their healing or simply other useful properties.

Botany, also called **plant science(s)** or **plant biology**, is the science of plant-life, a branch of biology. Even until the eighteenth century, botany was involved mainly with the description of plants and their classification. As a *quasi* contrast, the modern Science of Botany is dealing with plants in a broad, multidisciplinary way and is based on inputs from numerous other areas of science.

2.1 Plant Morphology

With regards to their botanical characteristics, medicinal plants are both rather specialized, and also diverse. In a broad sense this is a science that is concerned with the structure of plants. It involves external **morphology**, i.e. external form, arrangement and relationships of the various organs and **anatomy**, i.e. the internal structure, including the finer details of tissues (histology), cells (cytology). Before Charles Darwin's theories on the principles of inheritance, morphological traits of plants formed an important basis for plant systems. Sometimes even therapeutic significance was attributed to the plant form (which manifested itself in the Doctrine of Signatures) Plant organs with a similar shape to certain human organs, were claimed to have an effect and could be used for curing these organs (e.g. the liver shaped *Hepatica* sp. for liver problems, lungwort (*Pulmonaria sp.)* for pulmonary infections, etc.) The role of plant form was overestimated before the emergence of Darwin's theories. Darwin's influence facilitated the development of the first phylogenetic systems that based on the principles of inheritance, in addition to morphological features, took into consideration the results of also other sciences (e.g. geology, genetics, physiology, chemistry, etc.) in plant classification.

The morphological implications of morphological structures in MAPs have, however, maintained their importance, since the active principles are synthesized and frequently also accumulated in specific plant cells, tissues and organs. E.g.: essential oils, in the species of the Family Lamiaceae, accumulate in glandular hairs of the epidermis, whereas the tropane alkaloids of *Atropa, Datura, Hyosciamus* (Solanaceae) are synthesized in the root system of the plants from where they are transported to the aerial part of the plants via the xylem (Mothes et al. 1985). (For the sake of completeness, it should be mentioned that alkaloids can be synthesized and occur also in other plant organs, e.g.: leaves and stems and berries – *Solanum* sp., capsules – *Papaver somniferum*, etc.).

The discipline of **Pharmacognosy**, relies greatly on the morpho-anatomic characteristics, i.e. on the diverse presence or absence of morphological traits, since it needs well identifiable morphological/structural proofs both to determine the identity of crude drugs, and to eliminate adulterations. Histological structures e.g. crystolithic cells, starch grains, polygonal crystalloids and secretory structures (glandular hairs, schizolysigenous cavities and passage, lactiferous vessels, etc) aided by histochemical methods and electron microscopy facilitate drug identification, even in the dried crude drugs.

It has been long established that the basic physiological site for the synthesis of secondary metabolites can be found in the plant cells. It has been also long ago recognized that the occurrence/synthesis/accumulation of various secondary metabolites is organ specific, i.e. they are localized in the specific cells/tissues of specific plant organs. In practice, the utilization of plant organs and tissues is based on this feature. Mostly those plant organs and tissues are utilized (harvested) that contain the desired secondary metabolites in the highest optimal quality and quantity.

Regarding the active principle contents and morpho-anatomical features of MAPs, there is also a great deal of variability (inhomogeneity). Variability might exist in the course of the plant growth cycle. From the juvenile to the adult stage, the metabolic processes are changing which frequently can be related to plant organs. This, in turn, might influence the synthesis (quantity and quality) and accumulation of active principles (**time and organ specific variability**). The morphological features of plants (e.g. density of glandular cells) might also vary according to stage of growth and development. Similar morphological differences can be observed under the influence of ecological factors.

Knowledge of the optimal accumulation of active principles is important from the view-point of MAPs utilization. Herbal medicines are therefore also studied in detail by Pharmacognosy (the study of medicines derived from natural sources).

2.2 Plant Physiology

Plant physiology is a subdiscipline of botany concerned with the functioning or physiology of plants. It is an experimental science that observes the effects of variations in environment and in heredity on the life-processes and uses this information to explain and to control plant behaviour. The various intensity of the production of active principles is also an important link between MAPs and botany (Robbins et al. 1957).

2.2.1 Primary and Secondary Metabolism

In every plant cell there is a multitude of chemical reactions taking place at any single moment. The sum of chemical reactions and processes is called **metabolism. Primary metabolism** produces mainly carbohydrates, lipids, proteins and nucleic acids, as primary compounds out of which living organisms are made. **Secondary metabolism** produces a wide range of compounds that are not found in all species. They are used e.g.: for defense, specialized structures or reproduction (Graham et al. 2006). These processes generally use intermediaries from the primary metabolic pathway.

In MAPs it is mainly the secondary metabolites that are utilized. The synthesis and biological role of these chemical principles, as related to the products of the primary metabolism, are special. Frequently they are metabolic end products, with no relevant role in the metabolism (e.g.: essential oils excreted by glandular hairs). Some others are actively transported, even translocated throughout the plant (e.g. alkaloids) (Nowacki and Waller 1973).

Secondary metabolites are also frequently called **special principles** relating to their special role in the plant metabolism, as contrast to the universally occurring substances (Vágujfalvi 1992). Their occurrence within the plant is not ubiquitous: frequently these compounds are synthesized (accumulated) by special cells/tissues/organs and serve important ecological functions (defense, attractant, reproduction, etc.).

The utilization of MAPs is based on the fact that plants are capable of producing a multitude of biologically active principles with useful properties for man. When we ingest MAPs or herbal medicines, we are adapting the very same compounds which the plants use themselves (Daniel 2006). As an example, the plant antioxidants can be used by man for the same function of protecting from free radicals or oxidative damages related to aging.

Differences in the physiological activity of MAPs make them distinguishable from any other members of the plant kingdom (Tétényi 1987). In this respect, we distinguish the primary metabolism from the production of secondary metabolites. Whereas **primary metabolites** are chemical principles generally occurring in most plants, the **secondary metabolites** are the products of the special metabolism of valuable ingredients and should not be regarded as products of secondary importance, or final products of the metabolism.

The physiological basis of either synthesis or accumulation of active principles represents a speciality of the taxon. As the same constituents can be synthesized in different ways, the pathways of their synthesis are characteristic of any plant. A good example for this was published by Hegenauer (1986): different plant families can synthesize structurally similar naphtoquinones e.g.: plumbagin (Plantaginaceae, Droseraceae, Ebenaceae, Apocynaceae) and lawsone (Lythraceae, Balsaminaceae, or chimaphillin (Pyrolaceae).

It is a remarkable feature of medicinal plant physiology that under environmental pressure qualitative changes can take place in plants, i.e. plants have the ability to change their synthesis. This ontogenetically related phenomenon was first recorded by Nilov (1934) and Berry at al. (1937). Similar **ontogentic changes** in the metabolism of glycoside and terpenoid containing species were recorded also by Tétényi (1970).

Differences in morphological traits are frequently used by plant breeders, however, in MAP breeding their role is frequently insignificant or misleading. Examples for the failure in breeding include the case of large, ovate capsule opium poppy and high glycoside yielding *Digitalis lanata* (Tétényi 1978). In spice crops (like *Coriandrum sativum* used for direct consumption), however, morphological traits could be successfully used in breeding large fruited varieties.

2.3 Plant Systematics and Taxonomy

Medicinal and Aromatic Plants belong to numerous plant families which, frequently, produce characteristic similar active ingredients (as a result of similarities in the biosynthetic pathways). Thus, e.g. the plant-family Labiateae (Lamiaceae) comprises a large number of essential oil containing species (lavender, thyme, rosemary, sage, etc.) whereas other plant families, like the Solanaceae are characterized by the occurrence of several alkaloid containing species (belladonna, thorn apple, tobacco, etc.).

In view of the diversity of MAPs, Bennett and Balick (2008) emphasize the importance of correct and reproducible experiments in medicinal plant research. In this regards they state that MAP research should begin with a vouchered plant sample that has been accurately identified. Vouchers should be deposited in recognized herbaria. Also according to them a "voucher is more important than a correct identification", since an erroneous scientific name can be rectified or amended to reflect new taxonomic circumscriptions.

2.3.1 Taxonomic Value of Secondary Metabolites

The basic taxonomic unit of MAPs remains the *species* (sp.), with the related species constituting a *genus*. The categories *subspecies* (subsp.), *variety* (var.) and *form* (f.) are used to differentiate among dissimilar populations of wild-growing species. In an econo-botanical sense, both natural and cultivated species are divided into well distinguished *infraspecific varieties* (Terpó 1992). Cultivars are differentiated according to their features valued by human societies.

A special feature of MAPs is that sometimes a number of characteristic chemical, cytological, morphological, and occasionally even ecological properties may be used for their correct description. In these cases the species represents either a homogenous taxon of plants with little variation from one specimen to another, or it may include various varieties or races with distinctive features. Often, such varieties represent single gene mutations and are morphologically recognizable. In other instances the mutation gives rise to a variant having a different secondary metabolite profile, which is not necessarily noticeable in the morphological form. These are termed **chemical races** or **chemodemes** (Tétényi 1970, 1987).

In certain instances, there are also other genetic variations that affect the chemical constituents of the species, e.g. the appearance of polyploids, the addition of one or a few chromosomes above the normal complement (extra chromosomal types, gross structural changes to the chromosome, biotechnologically produced genetically modified plants).

Chemical races have been detected in numerous species including various chemical substances. E.g.: cyanogenetic glycosides in *Prunus communis*, alkaloids in *Duboisia* species, cardiac glycosides in *Digitalis purpurea*, essential oils in *Ocimum* spp, *Melissa* spp., *Thymus* spp., etc. (Trease and Evans 2002).

Phylogenetic systems classify medicinal and aromatic plants according to their purported evolutionary relationships or heredity. Remarkably, even to date, these systems are to a large extent based on the former **artificial system** of Linneaus (Linneaeus 1758). More refined taxonomic systems take into consideration the morphological traits of plants and evaluate them according to the principles of evolution and inheritance (Ehrendorfer and Heywood 1968; Cronquist 1981; Takhtajan 1997; Tétényi 1992).

In the second half of the last century, more and more attention was paid to the use of plant-derived chemical information: this was the advent of **Chemo-taxonomy** or **Phytochemical plant systematics** (Hegnauer 1962–1986; Swain 1976). The characters used in chemical taxonomy need to be ubiquitous, i.e. of intermediate distribution in the plant kingdom. Remarkably, many of these compounds (e.g. essential amino acids or common sugars) are of little taxonomic interest. Some secondary metabolites (e.g.: alkaloids, isoprenoids, flavonoids, characteristic glycosides, etc.), however have been studied frequently (Trease and Evans 2002).

Reason for this is that many secondary metabolites have been suggested to have important ecological functions in plants (Bennett and Wallsgrove 1994) e.g.:

- protect plants against being eaten by herbivores and/or being infected by microbial pathogens.
- serve as attractants (smell, color, taste) for pollinators and seed-dispersing animals,
- function as agents of plant-plant competition and plant-microbe symbioses.

Secondary metabolites have maintained their importance in chemical defense against predators, pathogens, allelopatic agents and help also in pollination, dispersal, etc. To date, however, their importance in plant systematics, however, seems to have somewhat eased (Gershenzon and Mary 1983; Singh 2004).

This can be attributed to the recent advances in the scientific and technical development of plant analytics, making it possible to study the molecular biological aspects of plants. This trend has led to the quantitative study of molecular structure and events underlying biological processes. The evolving new scientific domain, **Molecular Biology** deals with diverse issues like the study of the gene structure, function, and the mechanism by which genes are replicated and transcribed to control the metabolism (e.g.: synthesis of enzymes and proteins, and ultimately also secondary metabolites).

Recent trends, to use cytological and molecular biological traits in botanical classification (in **Molecular Systematics**), have established themselves as efficient tools and are expected to bring about also some changes in the already established phylogenetic systems (Minelli 1993; Stace 1991).

Modern botany has become a broad, multidisciplinary subject with inputs from other areas of science and technology. Its research domains include the study of plant structure, growth and differentiation, reproduction, biochemistry and primary/secondary metabolism, chemical products, development, diseases, evolutionary relationships, systematics, and plant taxonomy.

3 Vegetation Science Versus the Sourcing of Botanicals

Wild growing medicinal plants belong to natural ecosystems. **Floristics** (vegetation science) and **Biogeography** are subdomains of botany that study the distribution and relationships of plant species, including MAPs, over geographic areas.

The primary raw material resource for MAP production is gathering (wild-crafting). Over ninety percent of the traditionally used MAPs in economically poor countries are traditionally gathered. The natural resources of MAPs are, however, limited. If the biological equilibrium of natural ecosystems is impaired by irrational exploitation, this action could have disastrous repercussions also on the other components of the ecosystem (Bojor 1991).

It is not possible to establish a small- or medium scale industry based on the natural wild flora without assessing the quality and quantity of raw materials available for exploitation and without taking into account the protection of the environment (Bojor 1991). Mapping the spontaneous medicinal plant flora may generate important information that facilitates decision making on how to locate the required small industrial units used for the extraction of active substances, the drying equipment, the postharvest conditioning and primary processing of plants (Schippmann et al. 2002; Lange 2004; Maltry et al. 1975; Schippmann and Lange 2007).

To date it is a known fact that unthoughtful utilization has already lead to the overexploitation of natural resources endangering the survival of an increasing number of species and valuable incomes, especially for rural households in developing countries.

4 Sustainable Management of MAP Resources "Save Plants That Save Lives"

The belated growth in international awareness about the declining supply capacity of the world's medicinal plants, the over-harvesting of natural resources, the destructive harvesting practices accompanied by habitat loss, forest degradation of habitats, etc. have brought about alarming problems to biodiversity.

The need for the sustainable use of natural resources was recognized by the Chiang Mai Declaration (1988) that had expressed alarm over the consequences in the loss of plant diversity (WHO 1991). The Declaration highlighted "the urgent need for international cooperation and coordination to establish programs for the conservation of medicinal plants to ensure that adequate quantities are available for future generations". It has also called for a need to coordinate conservation actions based on both *in situ* and *ex situ* strategies.

The subsequent decades have been marked by several declarations and sets of recommendations calling for the **Conservation** and **Sustainable use** of biodiversity including also medicinal plants. Among these, The Convention on Biological Diversity (CBD), an international, legally binding treaty reached at the Earth Summit, in Rio de Janeiro (1992), established the following main goals: the conservation of biological diversity, the sustainable use of its components, and the fair and equitable sharing of the benefits from the use of genetic resources (Diversity 1992; Cunningham 1993).

The CBD that came into force in 1993, secured rights to control access to genetic resources for the countries in which those resources are located. In view of the objective to enable lesser-developed countries better benefit from their resources and traditional knowledge, the CBD rules that bio-prospectors are required to obtain informed consent to access such resources, and must share any benefits with the biodiversity-rich country (Shankar amd Majumdar 1995).

4.1 Methods and Standards for Sustainability

Several field-based methods have been developed for the sustainable harvest, assessment and monitoring of MAPs. Limiting the harvest to a sustainable level requires an effective management system including annual harvest quotas, seasonal or geographical restrictions and restriction of harvest to particular plant parts or size classes. In many cases harvesting techniques need to be improved, since collecting methods are often crude and wasteful, resulting in loss of quality and reduction in price (Iqbal 1993, 2002; Vantomme 2002). In addition, clarification of the access and user rights to the resources providing MAPs is part of the essential baseline information (FAO 1995; Leaman et al. 1997; Prescott-Allen and Prescott-Allen 1996; Schippmann 1997; WHO 2002; WHO/IUCN/WWF, 1993 revision in Kathe 2006).

In the followings we discuss only some of the major examples of international standards to be observed and followed:

4.1.1 International Standard on Sustainable Wild Collection of MAPs (ISSC-MAP)

ISSC-MAP's mission is to ensure the long term survival of medicinal and aromatic plant populations in their natural habitats, while respecting the traditions, cultures and livelihoods of all stakeholders if the process. Through its holistic approach, the ISSC-MAP is aimed at bridging the gap between the existing broad conservation guidelines and management plans for local conditions. It is meant to provide guidance for **sustainable wild collection** of MAPs and also a basis for audit and **certification** in wild collection (*incl. the* organic sector). Ultimately, the standard is expected to have several benefits: e.g. substantially contribute to the livelihood improvement of those involved in wildcrafting of MAPs, serve as **a** "communication tool" for the industry, serve as a guideline for MAP protection, harvest, and monitoring. For collectors, it can offer both insurance against resource and market failures, and for consumers, reliability of claims about ecological as well as social sustainability. Most importantly, at the level of species and habitats, it can contribute to maintaining biodiversity.

4.1.2 Guidelines for Good Agricultural (and Collection) Practice of MAPs (GA(C)P

Good Agricultural and/or Collection Practices (e.g.: Máthé and Franz 1999; EUROPAM 2006) for medicinal plants are the first step in quality assurance, on which the **safety and efficacy** of herbal medicinal products directly depend (Máthé and Máthé 2008). Harvesters of wild plants must apply collection practices that address not only their need to gain economic benefits from the sale of wild-harvested plants, but also make sure that each of the collected species survives. In addition to preserving plant populations, harvest practices must also minimize damage to local habitats (Akerele 1992).

These practices are also expected to play an important role in the **protection of natural resources of medicinal plants** for sustainable use.

4.1.3 FairWild/FairTrade

Initiated by the Swiss Import Promotion Organization (SIPPO), FairWild is a verification system that offers a comprehensive guidance framework and certification option for all sustainably collected wild plant, fungi and lichen species worldwide. FairWild Standard covers both ecological sustainability (based on the ISSC-MAP1) and aspects of fair trade as well as social sustainability.

The FairWild Standard is implemented either by the FairWild Foundation (2010) and/or its partners. Although specifically designed for wild collection situations, FairWild also includes the collection of plants, lichens or fungi or parts or products thereof on cultivated land, if the target species for collection are only a by-product and not the target of cultivation.

SIPPO supports small and medium-sized enterprises (SMEs) from emerging markets and markets in transition to access the Swiss and European markets.

Similarly Fair Trade (an organized social movement) aims at helping producers in mostly developing countries achieve better trading conditions and promote sustainability. With a special focus on commodities like medicinal and aromatic plants, or products which are typically exported from developing countries to developed countries. Fair Trade is an alternative approach to conventional trade based on a partnership between producers and traders, businesses and consumers. The international Fairtrade system – made up of Fairtrade International and its member organizations – represents the world's largest and most recognized fair trade system. Details on its structure, vision of activities, as well as the FairTrade Standards can be found at http://www.fairtrade.net/

5 Botanical Aspects of MAPs in Wild-Crafting

The production of high quality crude drugs with favorable contents (high amounts and favorable composition) of active substances has its scientific basis in the botanical sciences. Despite the huge diversity of both plant species and substances concerned, some general the aspects in the production process of botanicals are more or less similar. Among these, the main steps involved are the following: (expert) collection practices (see: Sects. 4 and 5) proper processing, drying, packaging and correct storage.

In the followings, our focus will be on wild crafting (wild harvesting), however, it is important to mention that several botanical aspects mentioned hereafter are valid for the harvesting of cultivated MAPs, too.

5.1 Knowledge of the Plant Habitats

This form of expertise can facilitate the identification of species which are difficult to distinguish, e.g. *Tussilago farfara* and *Petasites hybridus*. It can also provide useful information on the environmental load of plants, i.e. on areas that should be avoided due to heavy metal contamination, pesticide/herbicide residues, etc. Gatherers of MAPs should also avoid to operate in areas under environment protection, where collection would be subject to special permissions.

5.2 Determination of Plant Parts to Be Collected

Besides the knowledge of the plants *in se* – it is also essential to know which part of the plant and in which developmental state the drug can be collected. Typically, in the case of *Herba* drugs, where the flowering above-ground plant parts are collected, with the stems containing low amounts of active substances, the stem length and/or diameter is limited. Similarly the quality of flower drugs can be deteriorated by the higher rate of flower stalks, therefore these are also limited (e.g.: *Chamomillae flos*, *Sambuci flos*).

Likewise, in root drugs, the rate of stem parts should be taken into consideration (e.g.: *Valerianae radix*, *Primulae radix*). In the case of fruit drugs, the fruit color is often an indicator of ripeness and is therefore an important trait to be considered. E.g.: in two rose species *Rosa dumalis* and *R. rubiginosa*, color changes were characterized during ripening of rose fruits: a large increase in redness was observed for the first three harvest dates, whereas yellowness decreased during the whole study (rose hips became darker and less yellow). Changes in calculated hue (visible colour) were related to changes in total sugar content and other traits, therefore it was postulated that it is possible to use colour as an indicator of optimum harvesting time (Uggla et al. 2005). This type of knowledge and expertise is contained in the relative drug standards or pharmacopoeia, and gatherers of MAPs are to be educated and trained accordingly.

5.3 Determination of Harvesting Time

Medicinal plants are generally collected in the so called **technical stage of maturity**/ripeness. It has been found that in most cases this coincides with the maximum level of valuable active substances in the plant organs to be collected. Due to the large number of influencing factors the exact **determination of the harvesting time is a complex issue** that needs farther studies. This statement is especially valid for wild-growing MAPs.

In general, dry, sunny weather conditions are favorable for the wild crafting of MAPs. Due to seasonal changes in the metabolic processes of plants, and thus in the level of active substances, it has become common practice that underground organs (roots, rhizomes) are collected in the dormancy period, whereas barks (cortex) are gathered after the start of sap flow in spring. For similar reasons buds (gemmae) are collected after bud break, while for leaves this is done in a fully opened stage. The optimal time for the collection of flowers and inflorescences is usually in full anthesis (with the majority of flowers open) or in the case of *Herba* at the beginning of anthesis.

5.4 Determination of the Appropriate Method of Gathering

There seems to be a consensus that the wild-crafting of medicinal plants will remain the main source of raw materials for the industry (Leaman and Salvador 2005), Stakeholders of medicinal plant production and utilization should therefore find ways and means to limit incurring damages and losses. In this regard, the selection of appropriate collection methods/techniques has gained on importance. The **susceptibility** of species to over-collection can vary **according to their life forms and/or plant parts** used. Annual/biannual and even perennial species (e.g.: *Primula* sp., *Ginseng* sp., etc.) are highly susceptible to the wild-crafting of root-drugs, and their populations can be easily destroyed by the persistent indiscriminate practices. Ultimately, sustainable wild collection contributes both to the production of good quality drugs, and serves to maintain the ecological balance of plant communities.

As a rule of thumb it should be emphasized that valuable plant parts should always be dissected/collected at the biologically appropriate time, and in a circumspect way, so that any irrecoverable damage to plants can be avoided. In this respect sharp scissors or dissection knifes can be used. In the same way, overharvesting of plant species should by any means be avoided.

6 From Wild Plants to Cultivated Species Domestication and Introduction into Cultivation of MAPs

Medicinal plants have co-evolved with the ecosystems that form their natural habitats, therefore their transfer from natural habitats to a cultivated field is not merely a matter of transplanting. Changes in the growth environment of plants, such as those associated with a move from the natural ecosystem to the cultivated field, can produce substantial modifications in plant growth, development and active principle content, influencing the physical acceptance for cultivation and the chemical value of plant derived products (Bernáth and Hornok 1992; Bernáth and Németh 2008). Several researchers have attempted to develop definitive schemes for successful plant introduction.

Domestication and cultivation of a wild species requires manipulation of the eco-physiological factors to match the environmental requirements necessary for the plants to grow and reproduce; or the modification of the plant to meet the ecological conditions present in the field. The ecological goal of medicinal plant introduction is to minimize the differences between the wild and cultivated plant habitats that would detrimentally affect feasible production of economically important natural products.

6.1 Essential Tasks of Plant Domestication

According to Thompson (1990) the methods of new crop development (domestication) depend on the following factors: if it is developed by domesticating a wild species (1), adapting an existing crop from another area (2), making genetic changes in an established crop so that a new commodity is produced (3).

The system of new crop R + D is principally composed of five steps, i.e.: collection, evaluation, enhancement, development, utilization of germplasm in cultivar development, which is intimately involved with agronomic/horticultural evaluation, and the development of appropriate cultural and management systems, activities associated with full scale commercialization.

Planting systems, pest control procedures and soil preparation techniques must be tested to develop field environments suitable for vigorous plant growth with high chemical yield. Plant selection and breeding may be used to alter the plant, to increase homogeneity and improve characteristics that will enable the plant to withstand stresses caused by cultivation conditions (Máthé 2010). A variety of stresses has recently been shown to influence the resistance of plants to herbivores, while draught could make some plants susceptible to insect attack.

Remarkably, although one would assume that introduction of a plant into a new habitat would detrimentally affect its growth and chemical yield, this is not necessarily always valid. E.g.: *Chamomilla recutita* (Eliašová et al. 2004), *Catharanthus roseus* (Jaleel et al. 2008) and *Dioscorea bulbifera* (Narula et al. 2005) have been reported to accumulate higher levels of secondary metabolites under stressed conditions.

6.2 Input of Modern Botanical Sciences

In the process of domestication several botany related activities (tasks), like **bioprospecting, genetic resource/biodiversity assessment, conservation**, **management** have a basic, important role to play.

It seems that bringing medicinal plants into cultivation means both challenges and opportunities for plant biotechnology (Canter et al. 2005). The application of modern *in situ* and/or *ex-situ* technologies in the **germplasm preservation and improvemen**t of MAPs is already wide spread. Sophisticated *in vitro* propagation and breeding (selection) technologies (see: relevant chapter of this volume) aided by advanced phytochemical and molecular biology based analytical techniques can farther assist this progress.

These methods have a raison d'etre especially due to the high costs of the relevant chemical analyses making the breeding of MAPs rather costly. These and also further considerations have led to the elaboration of **new analytical methods** that are not only more cost effective but also have the advantage, that they can be used in the chains of quality assurance. The study of the metabolome can be regarded as such an important contribution, since one single **metabolome**, the sum of primary and secondary metabolites in a plant, contains about 4,000 compounds and it is also estimated that the total number of compounds present in the plant kingdom amounts to ca. 2,000,000 (Daniel 2006).

7 Scope of Heredity and Variability in MAPs

At different levels of evolution, the chemistry of living organisms, including medicinal and aromatic plants, is different. The rise of chemical taxa can be considered as the result of biochemical and metabolic processes mostly under genetic control. It is accepted that differentiation in cells is mostly manifested in **chemo-differentiation** at a molecular level and the fundamental differences in the course of ontogeny manifest themselves mainly in the differences of proteins present in the organism, i.e. ultimately in differences between the enzyme systems (Tétényi 1992). All other (morphological, anatomical) differences are only the consequences of these.

The secondary metabolites of MAPs, the end products of metabolic processes, if accumulated, can be directly observed by sensing (through color, taste, odor) and can be revealed by chemical analysis. Frequently, however, these chemical characters are hidden. Sometimes, they are accompanied by morphological divergence (e.g. cyanogenic glycosides in *Trifolium repens* and the presence or absence of a white spot on leaflets).

Differences in plant chemistry, i.e. in the special chemical features of MAPs, are attributed to **dissimilarities in the metabolism**. Since they are manifestations of the genetic background, they should be determined in a possibly most comprehensive way, when characterizing this special group of plants. This chemical fingerprint, i.e. the complexity of chemical traits, is called ***chemosyndrome***. It should be noted that the conclusions made on the basis of the chemosyndrome are not necessarily verifiable, since the accumulation of identical materials does not imply a relationship in chemism (e.g. the occurrence of the alkaloid bufotedine, in both the flowering plant *Piptadenia falcata* Benth. and toads) and it is also known that both rare (e.g. thebaine in certain *Papaver* species) and ubiquitous substances (e.g. chlorophyll) may also have homologous biosynthetic pathways. Therefore, their frequency of occurrence cannot be regarded as proof of relationship.

Recently, it is the metabolite fingerprint that is used with increasing popularity. **Metabolite profiling** may yield characteristic metabolic fingerprints that can be used to identify novel potential taxonomic markers, like it has been the case with *Glycyrrhiza glabra* (Farag et al. 2012).

It has also been found that chemical changes – e.g. infra-specific chemical modifications – can be caused by ecological and geographical conditions. These chemical differences are known as ***polychemism***. According to Tétényi polychemism is the materialized result of all chemical processes of the plant. It results from differences between the chemism and taxonomic units, i.e. chemotaxa can be established during chemical differentiation. Polychemism is known to be frequent in autogamous species (where a sudden chemical change can easily be inherited) and has been frequently described in cross-fertilized species, provided they are isolated (Baser et al. 1996).

In view of the abovementioned, investigators of MAPs are frequently faced with the question where are the limits of inherited or ecological factor triggered changes (This is frequently valid for the domestication process). Therefore, in the followings, we would like to briefly outline the major sources of ecology related variability.

8 Productivity of MAPs Under the Influence of Environment

Characteristically MAP productivity denotes the quantity of active principles synthesized or accumulated by the plant. It is composed of **biomass yield** and the ratio of **active principles accumulated within unit quantities of the biomass** (g, %).

There are several concepts to demonstrate these correlations (e.g. Máthé 1988; Bernáth and Hornok 1992). The scheme in Fig. 2.1 summarizes the interrelatedness among environmental factors and components of productivity according to Máthé (1994). In general, however, it should be noted that the variability of MAPs can be traced back to their genetic make-up, their geographic origin and the time of their study (as related to their phenophase and also in a diurnal way, during the day).

As a rule, plant growth and development of MAPs as well as the nature of secondary metabolites are affected by the **physical environment**, including light, temperature, rainfall, and soil properties. The impact of these has been studied by an increasing number of researchers growing various species under different climatic conditions, at different geographic locations and occasionally in a controlled environment (phytotron), which could form the basis of plant factories (Máthé 2014).

Variations have been also established according to the geographic origin of species (e.g. variations in the essential oil content of *Chamomilla recutita* on an

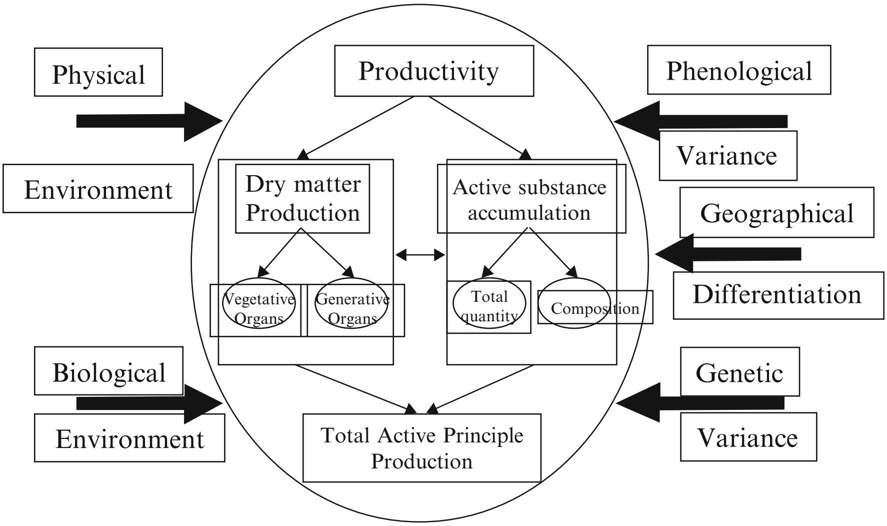


Fig. 2.1 Major factors influencing biomass, yield and active substance production in medicinal and aromatic plants (Máthé 1994)

Hungarian as well as European scale Máthé 1962; Franz and Novak 2009; Massoud and Franz 1990), as related to differences in ecological factors.

It should also be noted that the synthesis, accumulation or translocation of secondary metabolites can undergo changes in the course of the **plant life cycle**. Consequently, their presence and/or optimal concentrations must be determined specifically. These aspects are taken into consideration when determining the harvest and collection dates of the relevant species.

In certain cases of **diurnal variations,** the changes in the amount and quality of active principles occur in the course of even one single day. Due to their character and rate, they can be relatively substantial. They are generally caused by the translocation of metabolites between various organs, occasionally even between above- and under-ground organs. To avoid false conclusions, the effect of these factors must be investigated in the course of breeding.

Breeders of MAPs use well established traditional and biotechnological methods of plant breeding. Selection breeding is generally important to retain fitness while inbreeding is used mostly in increasing uniformity. The latter is frequently accompanied by the deterioration of certain traits, e.g. vitality in *Digitalis* sp.

Selection assisted by genetic markers seems to be an extension of traditional crop breeding, mostly used with food crops. This is a means to recognize genotypes at an early stage, so that the speeding up of selection process becomes possible (Canter et al. 2005). It is also foreseen that the so called "**omics**" revolution will take its due place in MAP breeding, similarly to the spread of genetic manipulations systems (e.g. to produce active principles, and improve basic agronomic characters, or/and crops resistance to stress factors). Pathway engineering, i.e. regulating key enzymatic processes, like in the case of *Ocimum basilicum,* where by way of metabolic engineering it has been possible to enhance the levels of aroma and flavor compounds (Lewinsohn et al. 2001) is also promising.

9 Botanical Sciences and Quality Control in MAPs

Quality control has always been a focal issue in the production and utilization of medicinal and aromatic plants. Plant materials are used as home remedies, over-the-counter drug products (OTC) and raw materials for the pharmaceutical industry, and thus represent a substantial proportion of the global drug market (WHO 1998). The World Health Assembly – in resolutions WHA31.33 (1978), WHA40.33 (1987) and WHA42.43 (1989) – has emphasized the need to ensure the quality of medicinal plant products by using modern control techniques and applying suitable standards.

Quality control requirements and methods of quality control assurance are determined by various international, national and regional pharmacopoeia, where botanical sciences have an established basic role.

The last decades have seen a new upsurge in the improvement of the **traceability and safety of natural products**. The increasing reliability in the production/collection practices of these species has greatly contributed to the increasing acceptance of these commodities. Automated, specialized quality-control systems that can spot erroneous data that might obscure important biological effects are needed urgently.

The introduction of modern control techniques and the use of modern standards have already yielded success. Based on the already available modern sample preparation techniques, like Solid-Phase Micro-extraction (SPME), Supercritical Fluid Extraction (SFE), Pressurized –Liquid Extraction (PLE), Microwave Assisted Extraction (MAE) and Solvent Micro-Extraction (SME) etc., the study of the plant metabolome is already is gaining on popularity (Dunn and Ellis 2005; Huie 2002). Advances in plant genomics and metabolite profiling (Oksman-Caldentey and Inzé. 2004) also seem to offer unprecedented possibilities in exploring the extraordinary complexity of plant biochemical capacity. State-of the art genomics tools can be used to enhance the production of known target metabolites and/or to synthesize entire novel compounds in cultivated plant cells by the so-called combinatorial biochemistry.

The application of **rapid analytical methods** has several relevant advantages, among them the most important being rapidity, the ability to select high-quality single plants from populations, progenies (e.g.: crossing experiments), as well as industrial uses for quality checks and supervision.

10 Conclusions

Botany, the science of plants, is linked with medicinal and aromatic plants in many different ways. The ca. 40,000 plant species used for ethnomedicinal purposes, since the beginning of recorded history, have traditionally been collected and gathered from the wild. Botanical sciences (like plant systematics, morphology and physiology) have been assisting the study and utilization of MAPs in various ways.

The exact scientific study of medicinal plants should begin with the collection and correct identification of a voucher plant specimen. Formerly identification was done mainly on the basis of morphological characters. Later on, with the progress in scientific and technical development, the chemical traits assisted this process. Research trends provide new opportunities for revealing the reality of DNA and biosynthetic causes of chemo-differentiation, and most recently, the information supplied by the plant metabolome. The latest scientific achievements are opening up promising perspectives for the breeding of new, highly powerful chemo-cultivars of medicinal and aromatic taxa.

The inheritance and variability of important traits in medicinal plants are governed by the same rules as in other crops, though in the case of medicinal and aromatic plants the interrelatedness of these traits is more complex. Reason for this is that the immense variability of traits complemented by the ecological plasticity of plants that are not easy to distinguish from the inheritable characteristics, thus making MAP-breeding quite a challenge.

The efficiency of cultivation of these species is fundamentally dependent on the productivity of the plant material (biomass) within which the active principles are synthesized and frequently accumulated.

Floristics or Vegetation Science is a domain of botany that deals with plants in geographic dimensions. The knowledge and economic mapping of MAP resources is an important contribution to the sustainable utilization of these species.

In the wake of the Chiang Mai Declaration (1988), appropriate policies and legal frameworks to guide the protection, Fair Trade, and applications of medicinal and aromatic plant materials have been elaborated (GAP, GCP, GMP, etc.). These and also other guidelines (standards) mean substantial contribution to the survival and sustainable utilization of our medicinal and aromatic plant resources.

References

Akerele O (1992) WHO guidelines for the assessment of herbal medicines. Fitoterapia LXIII (2):99–110

Baser KHC, Kirimer N, Ermin N, Kürkçüoglu M, Tümen G (1996) Essential oils from four chemotypes of Thymus zygioides Griseb. var. lycaonicus (Celak) Ronniger. J Essent Oil Res 8(6):615–618

Bennett BC, Balick MJ (2008) Phytomedicine 101: plant taxonomy for preclinical and clinical medicinal plant researchers. J Soc Integr Oncol 6(4):150

Bennett RN, Wallsgrove RM (1994) Secondary metabolites in plant defence mechanisms. New Phytol 127(4):617–633

Bernáth J, Hornok L (1992) Environmental factors. In: Hornok L (ed) Cultivation and processing of medicinal plants. Akadémiai Kiadó, Budapest, pp 56–68

Bernáth J, Németh É (eds) (2008) Gyógy- és fűszernövények gyűjtése, termesztése és felhasználása. (Collection, cultivation and use of medicinal and aromatic plants (in: Hungarian). Mezőgazda Kiadó, Budapest, p 256

Berry Ph et al (1937) J Chem Soc 1443

Bojor O (1991) Methodology of economic mapping of medicinal plants in the spontaneous flora. The medicinal plant industry. CRC Press, Boca Raton, pp 19–32

Canter PH, Thomas H, Ernst E (2005) Bringing medicinal plants into cultivation: opportunities and challenges for biotechnology. TRENDS Biotechnol 23(4):180–185

Cronquist A (1981) An integrated system of classification of flowering plants. Columbia Univ Press, Mew York

Cunningham AB (1993) African medicinal plants: setting priorities at the interface between conservation and primary health care. People and plants working paper 1. UNESCO, Paris, p 53

Daniel M (2006) Medicinal plants: chemistry and properties. IBH Publishing, Oxford

Dunn WB, Ellis DI (2005) Metabolomics: current analytical platforms and methodologies. TrAC Trends Anal Chem 24(4):285–294

Ehrendorfer F, Heywood VH (1968) Geographical and ecological aspects of infraspecific differentiation. In: Haywood VH (ed) Modern methods in plant taxonomy. Academic Press, New York, pp 261–296

Eliašová A, Repčák M, Pastirova A (2004) Quantitative changes of secondary metabolites of Matricaria chamomilla by abiotic stress. Z Naturforsch C 59:543–548

EUROPAM (The European Herb Growers Association): GAP/GWP – subcommittee Guidelines for Good Agricultural Practice (GAP) of medicinal and aromatic plants, Brussels, 3 Apr 2006. (EUROPAM GAP working copy no. 7.3)

Evans, WCh (ed) (2002) Trease and Evans Pharmacognosy, 15th edn. W.B. Saunders, Edinburgh. pp xiii + 585; ISBN 0-7020-2617-4; 59.95 GBP

FairTrade. http://www.fairtrade.net/

FAO (1995) Non-wood forest products for rural income and sustainable development. Rome (Non-wood forest products 7)

Farag MA, Porzel A, Wessjohann LA (2012) Comparative metabolite profiling and fingerprinting of medicinal licorice roots using a multiplex approach of GC–MS, LC–MS and 1D NMR techniques. Phytochemistry 76:60–72

Franz C, Novak J (2009) 3 Sources of essential oils. Handbook of essential oils: science, technology, and applications, 39

Gershenzon J, Mary TJ (1983) Secondary metabolites and the higher classification of angiosperms. Nord J Bot 3(1):5–34

Graham LE, Graham JM, Wilcox LW (2006) Plant biology. Pearson/Prentice Hall, Upper Saddle River, p 670

Hegenauer R (1986) Phytochemistry and plant taxonomy, an essay on the chemotaxonomy of higher plants. Phytochemistry 25(7):1519–1535

Hegnauer R (1962–1986) Chemotaxonomie der Pflanzen. Band 1-7. Birkhäuser Verlag, Basel/Stuttgart

Huie CW (2002) A review of modern sample-preparation techniques for the extraction and analysis of medicinal plants. Anal Bioanal Chem 373(1–2):23–30

Jaleel CA, Sankar B, Murali PV, Gomathinayagam M, Lakshmanan GMA, Panneerselvam R (2008) Water deficit stress effects on reactive oxygen metabolism in *Catharanthus roseus* impacts on ajmalicine accumulation. Colloids Surf B Biointerfaces 62(1):105–111

Kathe W (2006) Revision of the 'Guidelines on the conservation of medicinal plants' by WHO, IUCN, WWF and TRAFFIC: process and scope. Frontis 17:109–120

Lange D (2004) Medicinal and aromatic plants: Trade, production, and management of botanical resources. Acta Hort (ISHS) 629:177–197

Leaman DJ, Salvador S (2005) International standard for sustainable wild collection of medicinal and aromatic plants (ISSC-MAP), draft 2. Steering group for the development of practice standards and performance criteria for the sustainable wild collection. http://www.floraweb.de/proxy/floraweb/map-pro/draft_2.pdf

Leaman DJ, Schippmann U, Glowka L (1997) Environmental protection concerns of prospecting and producing plant-based drugs. In: International symposium on herbal medicine. A holistic approach. Documents, proceedings and recommendations, pp 1–4

Lewinsohn E, Schalechet F, Wilkinson J, Matsui K, Tadmor Y, Nam KH, Pichersky E (2001) Enhanced levels of the aroma and flavor compound S-linalool by metabolic engineering of the terpenoid pathway in tomato fruits. Plant Physiol 127(3):1256–1265

Maltry W, Pötke E, Schneider B (1975) Landwirtschaftliche Trocknungstechnik, 2nd edn. VEB Verlag Technik, Berlin

Massoud H, Franz C (1990) Quantitative genetical aspects of Chamomilla recutita (L.) Rauschert. J Essent Oil Res 2(1):15–20

Máthé I (1962) A kamilla évjáratonkénti és tájankénti fejlődési és hatóanyagváltozásai. (Annual and geographical variations in the development and active principle content of chamomile.) Bot. Közlem 49(3–4):280–288

Máthé Á (1994) Utilization of biodiversity in horticultural crops. Scientific Theses. Degree Doctor of the Hungarian Academy of Sciences, Budapest

Máthé Á (2010) A new look at medicinal and aromatic plants. In: XXVIII International Horticultural Congress on science and horticulture for people (IHC2010): a new look at medicinal and 925, pp 13–20

Máthé I Jr (1988) Növények biológiai szkrínelése (Biological screening of plants) Összehasonlító vizsgálatok a növényi szekunder anyagcsere-termékek hasznosítási célú értékelésére. Doctor's Theses, Hungarian Academy of Sciences, Budapest

Máthé Á, Franz C (1999) Good agricultural practice and the quality of phytomedicines. J Herbs Spices Med Plants 6(3):101–113

Máthé Á, Máthé I (2008) Quality assurance of cultivated and gathered medicinal plants. Acta Horticult 765:67–76

Minelli A (1993) Biological systematics: the state of the art. Chapman & Hall, New York

Mothes K, Schütte HR, Luckner M (1985) Biochemistry of alkaloids. VEB Berlin 18(37):107

Narula A, Kumar S, Srivastava PS (2005) Abiotic metal stress enhances diosgenin yield in Dioscorea bulbifera L. cultures. Plant Cell Rep 24(4):250–254

Nilov VI (1934) Soc Plant 11:21

Nowacki EK, Waller GR (1973) Alkaloid transport in plants. Zeitschrift für Pflanzenphysiologie 69(3):228–241

Oksman-Caldentey KM, Inzé D (2004) Plant cell factories in the post-genomic era: new ways to produce designer secondary metabolites. Trends Plant Sci 9(9):433–440. doi:10.1016/j.tplants.2004.07.006

Prescott-Allen R, Prescott-Allen C (eds) (1996) Assessing the sustainability of uses of wild species: case studies and initial assessment procedure (vol 12). IUCN

Robbins WW, Weier TE, Stocking CR (1957) Botany; an introduction to plant science. Botany; an introduction to plant science, 2nd edn. Wiley, New York, p 578

Schippmann U (1997) Plant uses and species risk. From horticultural to medicinal plant trade, pp 161–165. In: Newton J (ed) Planta Europea. Proceedings of the first European conference on the conservation of wild plants. Hyeres, 2–8 Sept 1995, London, Plantlife

Schippmann U, Lange D (2007) Annotated bibliography on plant resource assessment methods for non-timber forest products with a focus on medicinal and aromatic plants. Floraweb.de,. (2014). Retrieved 13 Oct 2014, from http://www.floraweb.de/map-pro/Bib_Vilm.pdf

Schippmann U, Leaman DJ, Cunningham AB (2002) Impact of cultivation and gathering of medicinal plants on biodiversity: global trends and issues. In: FAO. 2002. Biodiversity and the ecosystem approach in agriculture, forestry and fisheries. 9th regular session of the commission on genetic resources for food and agriculture. Rome, 12–13 Oct 2002. Inter-Departmental Working Group on Biological Diversity for Food and Agriculture. Rome

Shankar D, Majumdar B (1995) Beyond the biodiversity convention: the challenges facing the biocultural heritage of India's medicinal plants. In: Bodeker G, Bhat KKS, Burley J, Vantomme P (eds) Medicinal plants for conservation and health care, FAO, Rome. Non-Wood Forest Products, vol 11. pp 87–99.

Singh G (2004) Plant systematics. An integrated approach. Science Publishers, Enfield-Plymouth

Stace CA (1991) Plant taxonomy and biosystematics. Cambridge University Press, Cambridge, UK, p 561

Sumner J (2000) The natural history of medicinal plants. Timber Press, New York

Swain PM (1976) Chemical plant taxonomy. Academic, London/New York

Takhtajan A (1997) Diversity and classification of flowering plants. Columbia Univ Press, New York

Terpó A (1992) Taxonomy of medicinal plants. In: Hornok L (ed) Cultivation and processing of medicinal plants. Akadémiai Kiadó, Budapest, pp 42–55

Tétényi P (1970) Infraspecific chemical taxa of medicinal plants. Akadémiai Kiadó, Budapest

Tétényi P (1987) Botany of medicinal plants. Acta Horticult 208:15–28

Tétényi P (1992) Heredity and variability of medicinal plants. In: Hornok L (ed) Cultivation and processing of medicinal plants. Akadémiai Kiadó, Budapest, pp 10–18

Thompson AE (1990) Breeding new industrial crops. In: Janick J, Simon JE (eds) Advances in new crops. Timber Press, Portland, pp 100–103

Uggla M, Gustavsson K-E, Olsson ME, Nybom H (2005) Changes in colour and sugar content in rose hips (*Rosa dumalis* L. and *R. rubiginosa* L.) during ripening. J Hortic Sci Biotechnol 80 (2):204–208

Vágujfalvi D (1992) Active substances in medicinal plants. In: Hornok L (ed) Cultivation and processing of medicinal plants. Akadémiai Kiadó, Budapest, pp 19–41

WHO/IUCN/WWF (1988) Saving lives by saving plants. The Chiang Mai Declaration 1988. In

WHO (1991) Guidelines for the assessment of herbal medicines

WHO (1998) General guidelines for methodologies on research and evaluation of traditional medicine. WHO/EDM/TRM/2000.1, Geneva, pp 128

WHO (2002) Traditional medicine strategy 2014–2023. http://apps.who.int/iris/bitstream/10665/92455/1/9789241506090_eng.pdf?ua=1

Chapter 3
Chemical Diversity of Medicinal Plants

Imre Máthé

Abstract Exploring new biologically active ingredients of plants are in the focus of medicinal plant researches. The chemicals of plants determine the healing properties of plants. This chapter deals with the active ingredients that can explain the applicability of medicinal plants or can help in the evaluation of the plants from chemical point of view. Rather difficult thing to classify the medicinal plants on the bases of their active chemical ingredients as in the same plants several compounds belonging to various chemical groups can be found and, in addition, in many cases they contribute to the effect and applicability of the plants. In other cases the same type of compounds is present in plants bearing different utilization from medical viewpoint. Exceptions are the plants having compounds of strong biological effect and the ingredients from them as pure compounds are used after their isolation. In this chapter the plants are discussed according to the classical, broadly applied grouping: based upon the biosynthetic pathways of the main active ingredients. Consequently, the classes of primary and secondary metabolites are used. Primary metabolites are as follows: carbohydrates, fatty acids, fats, proteins. The main groups of secondary metabolites discussed are the phenolics (including polyketides), terpenoids and alkaloids. Examples, most frequently occurring in medicinal plant handbooks illustrate the chemical diversity of these compounds.

Keywords Medicinal plants • Primary and secondary metabolites • Drugs

1 Introduction

When speaking about medicinal and aromatic plants (MAPs) we refer to those plants that can be used either in a direct way, without the isolation of single compounds from them (e.g. the majority of herbal remedies). Similarly, they are medicinal plants that serve as starting materials for both isolates (e.g. sources of tropane, ergot, Vinca alkaloids) or those plants that as medical agents. In other cases chemicals from the plants are starting materials for medicines (e.g. steroid

I. Máthé (✉)
Faculty of Pharmacognosy, Department of Pharmacognosy, University of Szeged,
Eötvös Str. 6, Szeged 6720, Hungary
e-mail: mathe@pharm.u-szeged.hu

Á. Máthé (ed.), *Medicinal and Aromatic Plants of the World*, Medicinal and Aromatic Plants of the World 1, DOI 10.1007/978-94-017-9810-5_3

sapogenins are sources of chemical ingredients for chemical processing). To make differences among various groups of economic plants ranked among medicinal plants (e.g. spices, poisonous plants, dye staff producing plants, hallucinogenic species etc.) is rather difficult. It is also difficult to draw a border line between foods and medicinal plants, since more and more research evidence justifies that traditional foods may have healing properties. Probably that is why the categories of medical foods or food additives have been introduced into practice (Bishop and Jennings 1985; Cardelina 2002; Aggarwal et al. 2008). When surveying the scientific literature, one might have the feeling that at least potentially all plants can be regarded at least potentially as medicinal plants. In this chapter we discuss the traditionally applied plants as medicinal ones including the categories based on the EU phraseology, i.e. on the terms of herbal remedies, traditional medicines and food additives that are generally used in EU regulations. In healthcare, these terms also illustrate both the broad spectrum that medicinal plants cover and the fact how difficult it is to differentiate among medicinal plants. The difficulties lie in the fact that apart from the orthodox medicinal products, the herbal remedies are crude extracts containing a lot of various plant ingredients of varying composition. Due to this, the posology of the products is also less obvious.

So, why can the plants be used as medical agents? From biochemical viewpoint, the living world shows an amazing similarity. The biosynthetic processes, enzymes, biosynthetic cycles are similar in the majority of organisms that are governed by DNA via RNA. Genetic information is translated to proteins also in a rather similar mechanism. The greatest difference between plants and animals is that, with the exception of some primitive organisms, only plants can bind ambient CO_2 by photosynthesis and can convert light energy into chemical energy. At the same time, from CO_2 the plants can primarily provide the building elements of the bodies of other living entities.

Though the above-mentioned points refer to the similarities of the living organisms, including the plants, there are, also striking differences among the organisms, even within the plant kingdom. These include, beside the visible morphological characters, the differences in the chemistry of the plants. In most cases the morphological appearance refers to the chemistry of the plant, too. This is why we are able to recognize and name the MAPs. In several cases, however, the morphological characters do not reflect perfectly the chemical differences of the species.

Another important term to refer to in connection with MAPs, is “diversity” or “biological diversity”. The preservation of biodiversity has become a very important concern for human beings. Since the Rio de Janeiro world conference (1992), this expression has become an important and unavoidable question for not only scientists but also more and more for the public, at large. The preservation of the diversity of living world is one of the most important challenges for mankind (Baker et al. 1995). When speaking about biodiversity of plants also their chemical diversity is implied. In such cases we emphasize that the plants are regarded from their chemical composition. The presence of the so-called active ingredients of plants is the most characteristic feature of MAPs. These chemical principles are responsible for their biological effects. Therefore, in this chapter we focus our attention and discuss the chemistry of MPs. The scientific discipline, chemotaxonomy, deals with the distribution of the various chemicals in the plant kingdom at various levels. The information thus obtained is used in a taxonomic and systematic form. A more detailed discussion of MAPs and their active ingredients will be contained by the individual plant monographs of this series.

The two main groups of plant metabolites are as follows: primary and secondary metabolites. Some of farther important groups of metabolites and their occurrence in drugs are illustrated bellow according to the MAPs handbooks by Bruneton (1999), Hänsel et al. (1999), Evans (2000).

2 Primary Metabolites

Predominantly, the elements of nucleic acids. Generally, as direct active ingredients we rarely take them into account. However, regarding their role in the biosynthesis in all living materials and as they can be influenced by different ways (gene-expression, DNA transformation, GMO organisms, etc.) in the field of medicinal plants they have an increasing importance, among others, in the identification of plants. Primary metabolites, the products of photosynthesis, are synthesized with the help of chlorophylls. Chlorophylls can also be used in herbal products (e.g. from nettle).

It should, however, be noted that the Calvin cycle provides the carbohydrates (D-glucose, D-fructose, etc.) that serve as starting materials for farther biosynthetic (metabolic) cycles (e.g. Szent-Gyögyi - Krebs, Embden-Meyerhoff and other cycles) producing the small molecules that can serve as building blocks for all living materials, including secondary metabolites.

2.1 Carbohydrates

Carbohydrates are present in the plants as monomers, oligomers (up to ten monomer units), and polymers of the same or various other simple sugars. The number of C atoms in monomers is between 3 to 7, each but one carbon atom bears hydroxyl group. The remaining carbon atom is a part of carbonyl group. We are speaking of aldoses and ketoses depending on the feature of the carbonyl group. They are asymmetric compounds and provide two; D, and L series of isomers according to Fischer's projection which takes into account of the distal asymmetric center to the carbonyl group of the molecule. As the carbonyl groups react readily with alcohols, even if the hydroxyl group belongs to the same molecule and create five- or six-membered oxygen containing heterocycles: they are the furanose and pyranose structures of sugars. In these cases, the C atom of carbonyl group becomes asymmetric and the semi-acetal hydroxyl groups formed are the glycosidic hydroxyl, which chemically differ from the other alcoholic hydroxyl groups. The glycosidic hydroxyl may have two optical isomers α and β isomers. This difference may be of great significance in the formation of oligo- and polysaccharides and also when the sugar moieties are bound to other type of compounds (aglycones). Some common mono-saccharides in plants are as follows: D-glucose, D-xylose, D- and L-arabinose, D-mannose, D-galactose, etc.

We are speaking about the derivatives of sugars, in such molecules the ratio of hydrogen and oxygen is unlike to that in water as it is in the normal simple monosaccharides. They can also take part in the structures of oligo- or polysaccharides. Such derivatives are the uronic acids when the terminal C atom of sugars is oxidized to carboxylic group like in D-glucuronic, D-mannuronic, D-galacturonic, etc acids. Similar derivatives are the sugar alcohols, amino sugars, the sugar ethers and the dehydrosugars like L-rhamnose, etc. They can be found in the living organisms in free or bound forms, bound to other sugars providing oligosaccharides (up to 10 sugar monomers) or polymers when several hundreds of units may form the compounds. These polymers and also the oligomers can be built either from the same units like D-glucose (in starches and cellulose) or from different monomers including also the so-called sugar derivatives (mucilages, gums, alginates, etc.) These polymers may provide a strait chain or they can be ramified.

The sugar chain can be attached to non-sugars and other type of compounds, providing the glycosides of secondary metabolites. Sugar moiety is connected via glycoside binding to steroids, triterpenes in saponines. Similar glycosides are the iridoids, several flavonoids, phenylethanoids, etc. The presence of sugar in the molecules influences the polarity (e.g. solubility) of the compounds and also the biological effectiveness of the molecules.

The polysaccharides are emulsifier, mucilage forming viscous solutions. The polysaccharides are of pharmaceutical necessities, emulgents, mucilages, fixatives, etc. they may have immune modulating effect as relatively recently has been recognized (Bishop and Jennings 1982).

Some typical carbohydrates sources: Manna: *Fraxinus ornus* (Family Oleaceae), Tamarind: *Tamarindus indica* (Caesalpinaceae), Red Sorrel: *Hibiscus sabdariffa* (Malvaceae), Sugar beet: *Beta vulgaris* (Chenopodiaceae), Sugar cane: *Saccharum officinarum* (Poaceae); Alginic acid, alginates, carrageens, agar are in the following seaweeds: Kelp: *Fucus serratus, F. vesiculosa* (Families Fucaceae, Laminariaceae, Lessoniaceae), Irish moss: *Chondrus crisopus* (Gigartinaceae), Agar: *Gelidium, Gracilaria, Pterocladia* spp. (Rhodophyta algae), and fucans in *Furcellaria fastigiata* (Phaeophyceae).

Starch is ubiquitous in the plant kingdom. In exploitable quantities, it can be found in the most frequently used foods like in Wheat: *Triticum aestivum*, Barley: *Hordeum vulgare*, Rice: *Orysa sativa*, Maize: *Zea mays (*Poaceae), Potato: *Solanum tuberosum* (Solanaceae), Manihot: *Manihot utilissima* (Euphorbiaceae), Sweet potato: *Ipomoea batatas* (Convolvulaceae), Sago: *Metroxylon rumphii* (Palmae), etc., Cellulose can be gained in pure form from *Gossypium* ssp. (Malvaceae), fructans are in Chicory: *Cichorium intybus*, *Taraxacum officinale* (Asteraceae), The main sources of gums are Gum Arabica: *Acacia senegal* (Mimosaceae), Sterculia gum: *Sterculia urens* (Sterculiaceae), Tragacanth: *Astragalus gummifer*

(Fabaceae), Gum Ghati: *Anogeissus latifolia* (Combretaceae), Carob tree: *Ceratonia siliqua* (Caesalpinaceae), Guar: *Cyamopsis tetragonolobum* (Fabaceae), etc. Psyllium: *Psyllium afra, P. arenaria*, Ispaghula: *Plantago ovata*, Buckhorn Plantain: *P. lanceolata,* Great plantain: *P. major* (Plantaginaceae), Marshmallow: *Althaea officinalis*, High mallow: *Malva sylvestris* (Malvaceae), Lime Tree: *Tilia cordata, T. platyphyllos* (Tiliaceae), Flax: *Linum usitatissimum* (Linaceae), Quince: *Cydonia vulgaris* (Rosaceae) also sources of polysaccharides.

2.2 Lipids

Lipids are soluble in nonpolar organic solvents. Primary metabolites are the organic acids, fats (fixed oils). Organic acids have one or more carboxyl groups. Mainly those, forming the homologous series of fatty acids are interesting from the viewpoint of medicinal plants as esterifying agents of glycerol in fats (in fixed oils). Fats accumulate mainly in energy storing organs, in the seeds of the plans. They arise from acetyl-CoA and malonyl–CoA by bounding to a special protein which has more domains (ACP). The saturated long-chain fatty acids are synthesized step by step lengthening the chain with 2 carbon atoms. The unsaturated acids are derived from the saturated fatty acids. Some of the unsaturated acids have special importance for human being as they cannot be synthesized by our body but from biological viewpoint they are substantial so we should get them from our surroundings. These unsaturated acids are linoleic-, linolenic- and other more unsaturated acids. They are present in higher concentration in certain plants and especially in sea organisms (fish oils). So those plants having high concentration of these highly unsaturated fatty acids are of importance as medicinal agents (food additives). The high fixed oil containing plants are of transitional position between the medicinal plants and other ones predominantly used as industrial raw materials and foods.

The unsaturated fatty acids are in the plant kingdom almost exclusively in *cis* form. In recent years, the significance of the *trans* form that is obtained by isomerization of the oils on heating (cooking) have received special attention. The *trans* isomers can increase the risk of cardiac infarct. Consequently, marketing the fats of high *trans* fatty acid content has become limited by regulation in some countries.

Fixed oils are triacyl glycerols which are esters formed from glycerol (trihydric alcohol and three fatty acid molecules). The acids are long chain saturated and/or unsaturated acids. The most important saturated acids has the general formula

$$CH_3(CH_2)_nCOOH$$

If "n" equals 10, 12, 14, 16, 18, 20, the acids are lauric, myristic, palmitic, stearic, arachidic, lignoceric acids. The most important unsaturated acids are palmitoleic, oleic, ricinoleic acids with one double bound at C9 position, if the carbon atoms are numbered from the carboxylic carbon. Oleic and ricinoleic acids have 18 carbon atom long chain while palmitolic acid consist only of 16 C atoms. In addition to the double bound at C9, a hydroxyl group can be found at C12 position in the ricinoleic acid. In the linoleic acid 2 double bounds are (at the 9, and 11 position), Linolenic acids have three double bounds (each interspersed by one methylene group) and in the α- and γ-linolenic acids beside the double bound at C9 two additional ones are at the C12, C15 (α-linolenic acid) and at the C6 and C12 position (γ-linolenic acid) respectively. These unsaturated acids bearing 18 carbon atoms are essential for man. They can take part in the synthesis of the biologically important group of prostanoids in our bodies. The double bounds are with minor exception always in cis form in nature as mentioned above. When we are speaking on plant lipids it is worth mentioning that so-called unsaponifiable part of the oils which after acidic hydrolysis (saponification) as residue can be obtained. It may consist of valuable ingredients like vitamins, sterols, etc.

Some sources of fixed oils that can have relevance from the pharmaceutical viewpoint: Almond oil: *Prunus amygdalus* (Rosaceae), in its cake, obtained as the residue after expressing the oil, a toxic cyanogenic glycoside: amygdalin can be present, Arachis oil: *Arachis hypogea* (Fabaceae), Castor oil: *Ricinus communis* (Euphorbiaceae) contains ricinoleic acid (purgative action is due to this speciality of the oil). Ricin (a mixture of highly toxic proteins) and ricinine (an alkaloid) are present in the castor seed. Olive oil: *Olea europea* (Oleaceae) is of importance in the Mediterranean kitchen. Coconut oil: *Cocos nucifera* and Palm oil: *Eleis guinensis* (Palmae) have lauric acid as the dominant esterifying agent. Linseed oil: *Linum usitatissimum* (Linaceae) is highly unsaturated oil. In cake of the seed linamarine, lotaustralin and other cyanogenic compounds may be present. Soya oil: *Glycine max* (Fabaceae) produced in the largest quantity in the world. Theobroma oil: *Theobroma cacao* (Sterculuiaceae): Cocoa butter is the ester of mainly saturated fatty acids, Evening primrose: *Oenothera biennis* (Onagraceae) has highly unsaturated oil, Hydnocarpus oil: *Hydnocarpus wightiana, H. anthelmintica* (Flacourtiaceae) has cyclopentanyl fatty acids served in the past against leprosy, Carnauba wax: *Copernica cerifera* (Palmae), from the leaves the wax of myricyl cerotate can be obtained. Waxes contain mainly the esters of long chain monocarboxylic acids with long chain monohydric alcohols. The best known wax is the bee wax.

2.3 Some Other Primary Metabolites (Miscellaneous)

Amino acids, proteins of plants are predominantly as enzymes are interesting from pharmaceutical point of view. Hydrolyses, lipases and the proteases are of special importance. Beside them some toxic peptides (e.g. ricins) and other compounds may be interesting from medical viewpoint. Plant fatty acid amides for instance are interesting because of their immune-modulatory effects (Gertsch 2008).

3 Secondary Metabolites

The majority of the active ingredients of the known medicinal plants are secondary metabolites. They are relatively small compounds, show great variation and their distribution in the plant kingdom is not ubiquitous, their role in the life of plants in the most cases is not known. A complete definition for secondary metabolites is given by Luckner (1984).

In the synthesis of secondary metabolites the condensation, oxidation, reduction, substitution are involved as main chemical reactions. They take place in the formation of large groups of in their structure similar or different compounds. Related compounds often occur in the same species or in related ones. In other cases compounds of the same or similar structure are present in taxonomically non related plants. Studying the diversity of secondary metabolites is of great significance in chemotaxonomy (Dahlgren 1977; Hegnauer 1963–1992, 1984) and also in the discovery of new active ingredients for medicines.

The main groups of secondary metabolites are as follows: phenolic compounds, terpenoids and alkaloids.

3.1 Phenolic Compounds

They can be synthesized in different pathways in the plants but predominantly via the shikimic acid and partly by polyacetate-malonate pathways. There may be other ways of how the aromatic ring is formed under natural conditions. The main groups of phenolics that can be worth mentioning in the medicinal plants are Simple phenolics (Phenols, Phloroglucinols, etc.), Coumarines, Flavonoids, Tannines, Lignanes, Phenylpropanoides etc. and the subgroups of these larger groups.

3.1.1 Simple Phenolics

Some simple phenolics and their sources are: Male fern: *Dryopteris filix-mas* and other spp. (Polypodiaceae) have taenicide phloroglucinol derivatives (filicic acid, aspidinol, albaspidin, etc.) similar constituents are in Kamala: *Mallotus philippinensis* (Euphorbiaceae). Phloroglucinol derivatives are the antidepressant hyperforin in St John's wort: *Hypericum perforatum* (Fam. Hypericaceae); humulone and lupulone in the sedative Hope strobiole: *Humulus lupulus,* cannabinoids including the hallucinogenic compound THC (Δ^9-tetrahydrocannabinol) in Indian hemp: *Cannabis sativa* (Cannabinaceae). Diuretic, antiseptic arbutin (D-glucoside of methyl hydroquinone) is in the Bearbeberry leaves: *Arctostaphylos uva-ursi* (Ericaceae), the antiinflammatory salicin in Willow barks: *Salix fragilis, S. purpurea, S. alba,* etc. (Salicaceae), Meadowsweet: *Filipendula ulmaria* (Rosaceae).

Some relatively simple representatives of phenolics are the phenylpropanoids (anethol, oestragol, myristicine. asaron, etc.) are often the ingredients alone or mainly together with terpenoids of essential oils. Some of these compounds are toxic, like asarone, myricetine.

3.1.2 Naphtoquinones

Hanna: *Lawsonia inermis* (Lytharceae) has hepatoprotective activity. *Plumbago zeylanica* (Plumbaginaceae), *Juglans regia* (Juglandaceae) juglone, *Diospyros* spp. (Ebenacea), *Alkanna tinctoria*, *Lithospermum erythrorhizon*, *L. ruderale*, (Boraginaceae), *Galium*, *Rubia* spp. (Rubiaceae) have naphtoquinone derivatives. Fungicidal, antibacterial, insecticidal, cytostatic, anticarcinogenic effects are attributed to naphtoquinones.

3.1.3 Anthraquinones as Laxatives

The anthraquinones and anthrons are present in free and O- and C-glycosidic forms and they may be as monomers like emodin, aloe-emodin, rhein and dimers as dianthrones like Sennoside A-D, Rheidines, etc.. Senna: *Cassia senna, C. angustifolia, C. fistula* (Fabaceae), *Aloe barbadensis, A. ferox*, (Liliaceae), Rhubarb: *Rheum palmatum* (Polygonaceae), Cascara bark: *Rhamnus purshiana,* Frangula bark: *Frangula alnus* (Rhamnaceae) have laxative, purgative effects. In *Hypericum perforatum* hypericin and other dianthrones are thought to contribute to the plants antidepressant effect.

3.1.4 Flavonoids

Flavonoids are widely distributed in the plant kingdom. The "flavonoid" is a generic name which refers to various subgroups including flavons (apigenin, luteolin), flavonols (kaempferol, quercetin), flavanons (naringenin, eriodictyol), flavanonols (dihydroquercetin, dihydromyricetin), isoflavones (daidzein, genistein) in a broader sense the anthocyanidins (cyanidin, delphinidin, pelargonidin, petunidin, malvidin) which are aglycons of the glycosides anthocyanines etc. Antocyanines are in the form of salt bound by acids as coloring agents of plants, e.g. in flowers and on changing the pH values they change their color.

The basic skeleton of flavonoids provides several possibilities for substitution. Predominantly at the C 5, 7 and 4', 5' positions hydroxyl, alkoxy (mainly methoxy) groups are and often they are bound to sugars by glycosidic linkage. Mono- and oligo-saccharides, in rare cases, instead of O-glycosides C-glycosides (vitexin, isovitexin, orientin, iso-orientin) can also be found. The occurrence of the latter compounds and some others like isoflavanoids are less ubiquitous than the flavons, flavonols are. Dimeric flavonoids may also occur. C-C linkages can be seen between two flavonoid units like in the case of amentoflavone which is a biflavone. Isoflavonoids are of non-steroidal phyto-oestrogenes in Clover: *Trifolium pratense, T. repens,* Dyer's broom: *Genista tinctoria,* Soya*: Glycine max* (Fabaceae), many species of Lamiaceae, Asteraceae etc. families are significant also for their flavonoid content. They have antioxidant effect.

Birch leaf: *Betula pendula*, Calendula flower: *Calendula officinalis* (Asteraceae), Elder flower: *Sambucus nigra* (Caprifoliaceae), Lime flower: *Tilia cordata T. platyphyallos* (Tiliaceae) are rich in flavonoids. Field horsetail: *Equisetum arvense* (Equisetaceae) has flavon glycosides. They enhance renal excretion. Passion flower: *Passiflora incarnate* (Passifloraceae) is sedative, affects CNS (cardiac symptoms of nervousness). It has flavon C-glycosides. Maidenhair tree: *Ginkgo biloba* (Ginkgoaceae) is used in case of senile cerebral insufficiency against dementia, in case of symptoms of memory loss, etc. Its leaf extract contains flavonols, biflavonoids and ginkgolids (diterpenes) as active, antioxidant ingredients.

3.1.5 Tannins

They can combine with proteins and in the case of animal hides transform them into lather. Two large groups are distinguished: the hydrolysable and non-hydrolysable. Hydrolysable tannins (gallic and hexahydroxydiphenic, ellagic acids are esters with themselves and with sugars. Condensed tannins (proanthocyanidines) are polymers of flavanol unites bound by carbon-carbon bonds. Tannins are broadly occurring compounds in the plant kingdom. Medical purposes are used the galls of Oak: *Quercus infectoria* and other Quercus spp. . (Fagaceae), Hamamelis leaf: *Hamamelis virginiana* (Hamamelidaceae), Hawthorn: *Crataegus monogyna, C. laevigata* (Rosaceae) and other species from that family like Herb Bennett: *Geum urbanum*, Wild strawberry: *Fragaria vesca*, French rose: *Rosa gallica*, Blackberry: *Rubus* spp, Tormentil: *Potentilla erectam.* Lady's mantle: A*lchemilla glabra*. Rhatany: *Krameria triandra* (Krameriaceae), Gambir: *Uncaria tomentosa* (Rubiaceae), Bistort: *Polygonum bistorta* (Polygonaceae), Hazel: *Corylus avellana* (Corylaceae), Vine: *Vitis vinifera* (Vitaceae), Black catechu: *Acacia catechu* (Mimosaceae), *Rhus hirta* (Anacardiaceae), etc. are also the source of tannins. They are astringents, antioxidants, enzyme inhibitors.

3.1.6 Coumarines

The coumarins are derivatives of hydroxy-coumaric (phenyl propionic acid). At the C7 position of the aromatic ring hydroxyl group (except coumarine itself) generally occurs. Beside it compounds with additional substituents (aesculetin, umbelliferon, scopoletin, herniarin, umbelliferon, etc.) are common in plant kingdom. We are speaking furanocoumarines and pyranocoumarins when additional five- or six membered oxygen containing rings are attached in an angular or linear arrangement to the basic coumarine skeleton. These are characteristic ingredients of some plant families like Apiaceae, Rutaceae, Asteraceae, Fabaceae, etc. They have among others photosensitizer effect on skin. The related compounds, the γ-chromones are of medical importance because of their cardiovascular effect. The coumarine structure can be recognized in some fungi toxins (aflatoxines) and also in antibiotics.

Aesculin in Hors chestnut: *Aesculus hypocastanum* (Hypocastanaceae), melilotsid in Sweet clover: *Melilotus officinalis* (Fabaceae), khellin (furanochromone), visnadin in Khella: *Ammi visnaga, A. majus* (Apiaceae), Mouse Ear: *Hieracium pilosella* (Asteraceae), Angelica: *Angelica archangelica* (Apiaceae), Sweet woodruff: *Galium odoratum* (Rubiaceae), Tonka Bean: *Dipteryx odorata* (Fabaceae) are of significant coumarin content. Photoxicity may occur in the case of *Heracleum sphondylium, Pastinaca sativa* (Apiaceae), *Ruta graveolens*, *Citrus* sp. (Rutaceae), *Ficus carica* (Moraceae). It is due to the coumarines present in these species.

Chromones (and xanthones) are related compounds to coumarines. Sometimes they are present with coumarines in the same plants like in *Ammi visnaga*.

3.1.7 Lignans

They are dimeric compounds of two molecules of phenylpropane derivatives. May apple: *Podophyllum peltatum* (podopyllotoxin) (Berberidaceae) is of potent anti-cancer effect. Its derivatives are used in clinical practice. St'Mary thistle: *Sylibum marianum* (Asteraceae) has flavanolignans (among them silybin) bearing hepatoprotective effect and used in cases of liver damages. It holds true of Scizandra: *Schizandra chinensis* (Schizandraceae) which is used against hepatitis. Lignanes are present in many plants like in Mistletoe: *Vixcum album* (Loranthaceae), Siberian ginseng: *Eleutherococcus senticosus* (Araliaceae) which has adaptogenic effect.

3.2 Terpenoids

Terpenoids follows the mevalonic acid biosynthetic pathway. Mevalonic acid is synthesized from acetyl-CoA and acetoacetyl-CoA unites. From mevalonic acid isopentenyl and dimethylallyl pyrophosphates arise. These 5 membered compounds by coupling give rise to monoterpenes (geraniol), with an additional five membered unit sesquiterpenes (farnesol), with two geraniol molecules diterpenes (geranyl-geraniol), with two sesquiterpene molecules triterpenes (squalene) and from triterpenes with some steps of degradation and rearrangement the steroids (cholesterol) can be derived. Two diterpene units give rise to tetraterpenes (carotenoids) and by coupling of many isopentenyl pyrophosphates polyterpenes (rubber) can be obtained. At lower levels of polymerization cyclisation is a common phenomenon though non-cyclic compounds may also be found.

3.2.1 Monoterpenes

These compounds can be grouped in two: volatile and non-volatile (iridoids) monoterpenes. Volatile monoterpenes may be the component of essential oils. In an exploitable quantity they can be found in a great many plants belonging to various families (Lamiaceae, Apiaceae, Asteraceae, etc.). Essential oils are mixtures sometimes of a great number of compounds (more than 100 terpenoids and other type of ingredients could be identified in certain essential oils, like citrus oils). The main components of essential oils are the monoterpenes, sesquiterpenes, phenylpropanoids. Other ingredients which can be steam distilled may also be present in the essential or volatile oils. The monoterpenes may be linear type non-cyclic, cyclic and bicyclic compounds. They may have double bonds and hydroxyl, carbonyl, acetoxy and other type of substituents. They mainly occur in plants of the gymnosperm and angiosperm families. Predominantly some genera, belonging to the families Lamiaceae like Lavender: *Lavandula angustifolia* (linalyl acetate, linalool, geraniol, etc.), Rosemary: *Rosmarinus officinalis* (1,8-cineole), Sage: *Salvia officinalis* (α-, β-thujone, borneol, etc.), Spearmint: *Mentha spiata,* Peppermint: *M. piperita* (menthol, menthone), Sweet Basil: *Ocimum basilicum* has linalool, methylcavicol chemotypes and used for gastrointestinal disturbances. Thymol, carvacrol are present in Thyme: *Thymus vulgaris* and other *Thymus* spp., Horsemint: *Monarda punctata*, in Oregano: *Origanum vulgare*, in Winter savory: *Satureja montana*, etc. (Lamiaceae). The spices Caraway: *Carum carvi* (carvone), Coriander: *Coriandrum sativum* (linalool), Dill: *Anethum graveolens*, Anise: *Pimpinella anisum* (anethole), Fennel: *Foeniculum vulgare*, Lovage: *Levisticum officinale Cuminum cyminum*, (Apiaceae family), Juniper: *Juniperus communis* (Cupressaceae) are also rich in essential oil. Palmarosa (Indian geranium or Lemon grass): *Cymbopogon citratus* (Gramineae) (geraniol), Star anise: *Illicium verum* (Illicaceae), *Pinus* spp. (α-, β- pinene), (Pinaceae) etc. are of mainly monoterpene ingredients, while Cinnamon: *Cinnamomum verum, C. cassia, C. zeylanicum* (cinnamic aldehyde) phenylpropanoids are as chief components. *C. camphora* (camphor) (Lauraceae), Orange: *Citrus aurantium orange*. Lemon: *C. limon*, (limonene, citral-a and -b, citronellal, etc) (Rutaceae), Wormwood: *Artemisia absinthium*, Yarrow: *Achillea millefolium* (chamazulene), Matricaria: *Matricaria recutita* (azulenogen sesquiterpenes, chamazulene, α-bisabolol), (Asteraceae), Eucalyptus: *Eucalyptus globules* (cineol), Clove: *Syzygium aromaticum*, Cajeput: *Melaleuca cajuputi* and other species (cineol) (Myrtaceae), Nutmeg: *Myristica fragrans* (pinene, camphene, myristicin, eugenol), etc. Sweet-flag: *Acorus calamus* (Araceae) (acorone, asarone), Cardamom: *Elettaria cardamomum* (Zingiberaceae) with terpenyl acetate, cineol belong to the drugs as sources of essential oils.

3.2.2 Iridoids

Predominantly monoterpene glycosides the sugar part of which is in the most cases D-glucose. The main groups are the Nepeta lactons, normal iridoids having a cyclopentan-c-pyran ring (iridane skeleton), seco-iridoids which arises from loganin by the cleavage of the cyclopentan ring and forms a six membered lacton ring. In a special case only esters are bound to the iridan skeleton. They are the ingredients of the so-called valepotriates of *Valeriana* species. The word "valepotriates" is coined on the bases of the characteristics of these compounds. It refers to their plant origin and beside the tri esters character and the presence of an epoxide ring in the molecules. Seco-loganin is also a key precursor of the largest group of alkaloids occurring in Loganiaceae, Apocynaceae, Rubiaceae families (see later on). Iridoid containing plants belong to a rather well-defined group of plants. Dahlgren (1977), Hegnauer (1963–1992) and others use them as chemotaxonomic markers. They are also the ingredients of a lot of MAPs. Valerian: *Valeriana officinalis* (Valerianaceae) in which the valepotriates have sedative effect. Devil's claw: *Harpagophytum procumbens* (Pedaliaceae) has harpagoside content. This drug has choleretic, appetite-stimulating, anti-inflammatory, analgesic effects. Olive tree leaves: *Olea europea* (Oleaceae) has seco-iridoid content (oleuropein, oleacin) which is of among others, hypotensive affect and traditionally used to enhance the renal and digestive elimination. Yellow gentian: *Gentiana lutea* (Gentianaceae), European centaury: *Centaurium erythraea* (Gentianaceae), Buckbean: *Menyanthes trifoliate* (Menyanthaceae) contain bitter secoiridoids (sweroside, gentiopicroside, amarogentin, etc) and used to enhance the appetite. European vervain: *Verbena officinale* (Verbenaceae) has similar effect than Gentian has and also used in case of skin disorders. White dead nettle: *Lamium album* (Lamiaceae) like many other species of Lamiaceae family and Bedstraw: *Galium verum, G. aparine*, etc. (Rubiaceae) contain of significant amount of iridoids (aucubin, catalpl, asperuloside).

Pyrethrum: *Tanacetum cinerariifolium* (Asteraceae) has irregular monoterpenes the so-called pyrethrins. They are toxic for coldblooded animals and used as insecticides.

3.2.3 Sesquiterpenes

The 15 number long carbon chain of farnesyl pyrophosphate gives rise to several ring systems. They, together with the differences in substituents provide a large group of natural products. The substituents, like lactone rings and ketones render the compounds not steam distillable. Some of the lactones (proazulenes) during processes can lose its substituents and become steam distillable and sometimes due to the formation of polyunsaturated products the color of them may be blue or greenish blue (like chamazulene from matricine in chamomile and some other Asteraceae species like *Artemisia*, *Achillea* spp.). Matricine is a non volatile sesquiterpene lacton. To this type of lactones several biological properties can be rendered. Among them the allergic properties should be mentioned which is due to sesquiterpene lactons bearing methylene group on the lacton ring. The most typical non-volatile sesquiterpene lactons are the following structures: germacranolide, guaianolide, eudesmanolide, pseudoguaianolide, elemanolide. They can be found in Elfdock: *Inula helenium* (alantolacton, isoalantolacton are responsible for allergic dermatitis), Arnica: *Arnica montana*, Fewerfew: *Tanacetum parthenium* (Asteraceae) suggested against migraine headache, Sweet wormwood: *Artemisia annua* (artemisinin is an important antimalarial drug, which can also be used in the case of chloroquine resistant *Plasmodium falciparum*), *Ambrosia maritima* is used against mollusks in Africa. *Matricaria* and other related species contain sesquiterpene lactons together with monoterpenes. Anti-inflammatory, spasmolytic effects are ascribed to essential oil ingredients like α-bisabolol, chamazulene, Yarrow: *Achillea millefolium* (Asteraceae), is used as substitute of Matricaria, etc.

3.2.4 Diterpenes

They have also a lot of forms. Several predominantly cyclic compounds can be derived from the geranyl-geranyl pyrophosphate. Some more important skeletons are: abietane, clerodane, kaurane, cembrane, tiglane, taxane, etc. *Salvia milthioria* (tanshinones I and II) helps the excretion of urea, and used against angina pectoris, White Horehound: *Marrubium vulgare* (marrubiin) is expectorant, choleretic, Germander: *Teucrium chamaedris* is used to treat the symptoms of digestive diseases, neurotonic disorders, Black Horehound: *Ballota nigra*, (neurotonic disorders), Ground Ivy: *Glechoma hederacea,* has a similar effect to horehound, *Coleus forskohlii* works antihypertensive (forskolin), etc. (Lamiaceae).

Stevia rebaudiana contains the sweetener stevoiside, Grindelia: *Grindelia squarrosa*, *G. robusta, G. humilis,* etc. are gum producing plants with grindelic acid content (Asteraceae).

Taxus brevifolia (Taxaceae) contains the anti-carcinogenic taxol in the bark Croton, Euphorbia (Euphorbiaceae), Daphne, Thymelaea (Thymeleaceae) species contain highly toxic diterpenes.

3.2.5 Triterpenes

They arise from the 30 C atoms containing squalene that can be found in some fish oils in larger quantities, and also in certain plants. The more important skeletons that can be found in medicinal plants are the pentacyclic triterpenes ursane, oleanane, lupane and tetracyclic dammarane. To date, many more are known. These skeletons may have double bounds, carboxylic groups and hydroxyl groups (ursolic acid, oleanolic acid), oxo-groups. They are frequently the member of glycosides and esters (sugar moieties may be attached both to hydroxyl and carboxyl groups of the triterpenes). Saponins are present, in triterpene and steroid form, in many species.

Licorice root: *Glycyrrhiza glabra* (Fabaceae) has the sweet glycyrrhizin content which is a glycoside of glycyrrhetic acid, together with flavonoids and isoflavonoids. It has anti-inflammatory effect and a sweetener, used in food industry. Horse chestnut: *Aesculus hyppocastanum* (Hyppocastanaceae) with protoaescigenin, barringtogenol C has anti-inflammatory effect. Common Ivy: *Hedera helix* (Araliaceae) (hederacosid C) is used in cosmetic preparations, in the treatment of skin disorders, Primrose: *Pirimula veris* (Primulaceae) (protoprimulagenin A, echinocystic acid), promotes secretion, used in the treatment of cough and skin disorders, etc, *Centella asiatica* (Apiaceae) (asiatic acid is the main aglycon of the drug) is applied in venous insufficiency, skin disorders, is an emollient), Marigold: *Calendula officinalis* (faradiol is one of the main triterpenes in the drug which are together with flavonoids) is used topically for treating skin disorders, has anti-inflammatory effect. (Asteraceae), Ginseng: *Panax ginseng* (Araliaceae) (ginsenosides with dammarane skeleton like protopanaxadiol and protopanaxatriol), Siberian ginseng: *Eleutherococcus senticosus* (Araliaceae) eleutheroside I-M are described together with a lot of various other ingredients. Both ginsengs are adaptogenic.

Quillaja: *Quillaja saponaria*, (quillaja saponins are detergents, used in hygiene, topically in emollient, itch-relieving products.) (Caryophyllaceae), Soapwort: *Saponaria officinalis* is expectorant like Gypsophila: *Gypsophila paniculata*. The main aglycone of the saponines of both species is gypsogenine. Alfalfa: *Medicago sativa* (Fabaceae) lowers blood cholesterol, Senega root: *Polygala senega* (presenegenin glucoside with antitussive effect, against cough) (Polygalaceae).

Cucurbitacins are toxic, and purgative, emetic compounds. Limonoids, quasanolides have similar effects.

3.2.6 Steroids

Cardiac glycosides (cardenolides, bufadienolids), Pregnane derivatives, Sterols, Ecdysterols, Steroid saponins, Steroid alkaloids are the main groups of the plant steroids. Several subgroups exist.

Pregnane derivatives can be found in *Vitex agnus-castus* (Verbenaceae), *Asclepias* spp. (Asclepiadaceae) and together with cardiac glycosides in many plants (*Digitalis*, etc. species).

Phytosterols like Sitosterols (β-sitosterol, sigmasterol, etc.) are present ubiquitously in the plant kingdom. They are frequently accumulates in the fixed oils and can be isolated from their non-hydrolysable fractions. They are present in free and ester form, sometimes in glycoside. Nettle: *Urtica dioica* (Urticaceae) is the source of sitosterols, Pumpkin: *Cucurbta pepo* (Cucurbitaceae) has Δ^7 sterols. Both drugs are of beneficial effect on benign prostate hypertrophy.

Yams: *Dioscorea* species, like *D. floribunda, D. composita, D. deltoidea, D. mexicana* etc. (Dioscoreaceae) contain diosgenin, tigogenin, etc. *Agave sisalana*

(Agavaceae) hecogenin, Fenugreek: *Trigonella foenum-graecum* (Fabaceae) diosgenin in the form of glycosides. Certain types of the sapogenins (diosgenin, hecogenin) can be used as starting material for the hormone-steroid industry.

The length and composition of the sugar moiety in the cardiac glycosides, the substituents on the steroid skeleton can influence the cardiac activity of the glycosides.

Purple foxglove: *Digitalis purpurea* and *D. lanata* (Purpurea glycoside A-E and Lanatosid A-E are the glycosides of digitoxigenin, gitoxigenin, digoxigenin) etc. (Scrophulariaceae), *Strophanthus kombe*, *S. hispidus, S. gratus, S. sarmentosus,* etc. (Apocynaceae), *Adonis vernalis* (Ranunculaceae) and Lily of the valley : *Convallaria majalis* (Liliaceaea) are of strophantidin glycosides. Squill: *Drimia maritime (Scilla maritime, Urginea maritime)* (Liliaceae) is the main representative of bufadienolids like proscillaridin and in the Black hellebore: *Helleborus odorus* and other Helleborus spp. (Ranunculaceae). Hellebrigenin is the main component of the aglycones of its cardiac gylcosides.

3.2.7 Tetra- and Polyterpenes

Carotenoids are acyclic (lycopene) or have one or two cyclic rings (β-carotene, provitamin A). *Capsicum* spp. (Solanaceae), Annatto Tree: *Bixa orellana* (Bixaceae) bixin, Saffron: *Crocus sativus* (Iridaceae), crocetin, crocin are sources and the representatives of carotenes and their oxygenated derivatives. Lutein is an agent in prevention of age–related macula degeneration. In large quantity it can be obtained from *Tagates erecta* (Asteraceae).

Polyterpenoids are composed of a lot of isoprene unites. Rubber: *Hevea brasiliensis* (Euphorbiaceae), Gutta-percha: *Palaquium* and *Payerna* spp. (Sapotaceae) are less used recent years for medical purpose.

3.3 Alkaloids

Alkaloids like the other main groups of secondary metabolites cannot be simply classified. According to Hegnauer (1963) real, proto- and pseudo- alkaloids can be roughly distinguished. The true alkaloids are those that have a N-atom in a heterocyclic ring or ring system and the N originates from one of the essential amino acids. Proto-alkaloids also arise from amino acids, but their N atom is not in a ring system. Pseudo-alkaloids, predominantly terpene alkaloids have a N atom in their molecule but its origin is obscure: with other words it does not arise directly from amino acids. According to the classical definition alkaloids, they are basic compounds that have strong biological effects and are of plant origin. The majority of them are white solid compounds. All of the mentioned characteristics are not valid for all the alkaloids. There are several exceptions, consequently it is rather difficult to categorize the alkaloids.

The basic character of alkaloids can be used for isolating and separating them from the other plant materials. In most cases they are present in the plants in the form of salts of organic or inorganic acids, while their bases are generally not water but organic solvent soluble and provide a solid material, in basic conditions. These characteristics can be used in the isolation of the alkaloids.

Regarding their occurrence, alkaloids can be predominantly but not exclusively found in the Dicotyledonous species. Some of their representatives are of medical importance. They are listed according to the amino acids they arise from together with their main drug sources:

3.3.1 Ornithine-Derived Alkaloids

Tropane alkaloids are present predominantly in the Solanaceae and Erythroxylaceae families. Their main representatives are hyoscyamine (its raceme mixture is atropine), hyoscine (scopolamine). They occur in Deadly nightshade: *Atropa belladonna*, Scopolia: *S. carniolica, S. japonica*, Henbane: *Hyoscyamus niger, H. muticus*, Thorn Apple: *Datura stramonium, D. innoxia, D. metel, D. sanguinea,* Corkwood: *Duboisia myoporoides, D. leichardtii*, Mandrake: *Mandragora officinalis* (Solanaceae) and other tropane (ecgonine) alkaloids like cocaine, cinnamylcocaine, α-truxilline are also in the species Coca: *Erythroxylon coca, E. novogranatense,* (Erytroxylaceae). The variation of tropan alkaloids is mainly due to various esterifying organic acids. Hyoscyamine is a stimulant on the CNS, whereas hyoscine has a sedative effect. Cocaine is a local anaesthetic and an illicit drug.

Pyrrolizidine alkaloids are toxic, due to their hepatic tumor inducing action. They are present in the genera *Doronicum, Eupatorium, Petasites, Senecio, Tussilago,* etc. (Asteraceae) and in the species of the genera *Alkanna, Petasites, Echium, Lithospermum, Symphytum, etc.* (Boraginaceae).

3.3.2 Lysine-Derived Alkaloids

They are present as quinolizidine alkaloids: sparteine, lupanine, cytosine, anabasine in Broom: *Cytisus scoparius* (Fabaceae). Lobelia: *Lobelia inflata* (Lobeliaceae) contains lobeline, lobelanine, lobelanidine and their closely related compounds. Lobelin is used in the treatment of asthma, bronchitis, in the resuscitation of new-born infant. Piperine is in the spice Pepper: *Piper nigrum* (Piperaceae) it is mainly condiment. Nicotine in Tobacco: *Nicotiana tabacun, N. glauca*, *N. rustica* (Solanaceae) arises from nicotinic acid and lysine. Nicotine after stimulation followed by depression of ganglia. It is highly toxic. In Pomegranate: *Punica granatum* (Punicaceae) pelletierine is of anthelminthic effect. It is astringent and used in the treatment of diarrhoea.

Reduced pyridine derivatives are arecoline in the masticatory Asian Betel nut: *Areca catechu* (Palmae), and the highly toxic compounds coniine, γ-coniceine are in the common European weed, Hemlock: *Conium maculatum* (Apiaceae). The latter compounds may not be of lysine origin.

3.3.3 Phenylalanine and Tyrosine-Derived Alkaloids

Several alkaloids belonging to various subgroups arise from these amino acids. The proto-alkaloids are the simple phenethylamines like mescaline in Peyote: *Lophophora williamsii* (Cactaceae), ephedrine (against asthma, hay fever) in Ephedra: *Ephedra dystachia, E. sinica, E. gerardina*, etc. (Ephedraceae), cathinone (CNS stimulant, like amphetamine) in Khat: *Catha edulis* (Celastraceae). Autumn crocus: *Colchicum autumnale* contains the proto-alkaloid colchicine which is highly toxic (Fam. Liliaceae). It is used to relieve gout. For the multiplication of chromosomes in cell can be used.

Isoquinoline alkaloids: Hydrastine, berberine, etc, in Goldenseal: *Hydrastis canadensis* (Ranunculaceae) is used in uterine haemorrhage. Fumitory: *Fumaria officinalis* (Fumariaceae) is choleretic. In the family Papaveraceae phenantridine, protopine and other types of alkaloids can be found in various combinations, like in Celandine: *Chelidonium majus,* Bloodroot: *Sanguinaria canadensis*, California poppy: *Escholtzia californica*, Corn Poppy: *Papaver rhoeas, etc*. The most important alkaloid is morphine (analgesic, hypnotic), codeine (against coughing) and thebaine occur with several other alkaloids, like papaverine (spasmolitic, narcotine, narceine, etc. in Opium poppy: *Papaver somniferum*. The alkaloids can be obtained both from dried latex (opium) of the fruit and the dried poppy straw according to the Kabay's process. Morphine, and its derivatives (e.g. diacetyl derivative which is heroin) and also opium serve as illegal drugs in many parts of the world. *P. orientale, P. bracteatum* has no morphine content. Some of their chemovarieties may produce thebaine which may serve as industrial source of other morphinane alkaloids.

Various phenylalanine-derived alkaloids are present in the genera *Galanthus, Leucojum, Narcissus*, spp., etc. (Amaryllidaceae). They are used in the treatment of Alzheimer's disease.

Ipecacuanha: *Cephaelis ipecacuanha, C. acuminate* (Rubiaceae) are of emetine, psychotrine, cephaline etc. content. These alkaloids arose from the condensation of dopamine and secologanine. Ipecacuanha is an expectorant and emetic and used also in the treatment of amoebic dysentery.

One type of curare the so-called Menispermaceae curare as arrow poisons was used in South America. They are crude plant extracts containing alkaloids, among them the dimers bisbenzyltetrahydroisoquinolines. Curare of *Chondrodendron* spp. like *C. tomentosa, C. candicans*, etc. (Family Menispermaceae) containing the mixture of several compounds including several dimer isoquinoline alkaloids. The most important of them is tubocuraine which is a muscular relaxant and used in surgical operations.

Aporphinoids (e.g. apomorphine) are often co-occurring with other types of alkaloids. Boldo: *Peumus boldus* (Monimiaceae) from Chile has boldine content as the principal alkaloid in the bark of the plant. It has anti-hepatoxic and anti-inflammatory properties.

3.3.4 Indole Alkaloids

They arise from the amino acid tryptophane or tryptamine. They can be classed in two main groups: The smaller group is the 'simple' indole alkaloids, bearing some substituents on their indole ring, e.g. the hallucinogenic psilocybin in the mushrooms Psylocibe: *Psilocybe aztecorum* (Agariaceae). The strong toxic cholinesterase inhibitor physostigmine in Calabar bean: *Physostigma venenosum* (Fabaceae) also belongs to this group.

The larger group of indole alkaloids can be combined with monoterpene building units. Dimethyl-allyl-pyrophosphate is involved in the biosynthesis of ergot alkaloids. They are important medical agents used for different purposes and synthesized by Ergot of rye: *Claviceps puprpurea* (Clavicipitaceae) and other related fungi. Beside the simple amides (ergonoviene is a potent oxytocic), of lysergic acids the ergopeptines: ergotamine (vasoconstrictor), ergometrine, ergocristine, ergocryptine, ergocornine and other alkaloids and their synthetic derivatives can be used broadly in medical practice. The hallucinogenic LSD lysergic acid diethylamide is an artificial product.

The largest group of the alkaloids is the monoterpene indole alkaloids. Strictosidine is a key compound in the biosynthesis of this group. It arises from tryptamine and secologanin (see iridoids) by a condensation reaction. As this compound has a lot of reactive sites, it serves as an intermediate of a large number of compounds (estimated number of alkaloids is over 2000) creating several chemical structures, subgroups of indole alkaloids (Bruneton 1999). The main drugs having alkaloids arise from strictosidine are as follows:

Loganiaceae Family

Gelsemium, *Strychnos* (200 alkaloids from *Strychnos* species) genera are of importance. Nux vomic: *Strychnos nux-vomica* has strychnine, brucine while Yellow jessamine: *Gelssemium sempervirens* has oxindole alkaloids of them gelsemine is the principal ingredient. They are highly toxic, they can be used against certain types of neuralgic pain, spasm (facial, dental neuralgia).

Curare of the South American *Strychnos* species: *S. toxifera, S. jobertiana, S. guianensis* have dimer indole alkaloids, like toxiferines I-XII. (see above the Menispermcea curare).

Rubiaceae Family

The fourth largest family of flowering plants is Rubiaceae. 10 % of the 611 genera of the family (divided into subfamilies) is known to contain medicinal plants. In the tribe Psychotrieae: *Cephaelis, Cinchonoideae,* in Naucleae: *Adina*, in Cinchoneae: *Cinchona, Corynanthe, Pausinystalia, Remijia, Mitragyna, Uncaria. Pausinystalia* genera are of medicinal plant representatives.

Yohimbe: *Pausinystalia yohimbe* contains yohimbine, corynanthine as principal agents in the trunk bark. It is an aphrodisiac, used for the treatment of impotence.

The rubane alkaloids of *Cinchona* species are of tryptophane origin. Chincona: *Cinchona* spp. (*C. pubescens, C. ledgeriana*, etc.) have in their bark quinine, quinidine, cinchonidine, cinchonine as the main components of the alkaloid mixture. Quinine is an important antimalarial, bitter tonic. Quinidine has antiarrhythmic property.

Mitragyna speciosa has indole and oxindole alkaloids, like mitragynine. It is antitussive, analgesic.

Uncaria gambir, U. rhynchophylla have rhynchophylline (oxindole alkaloid) content with lasting hypotensive effect, *U. tomentosa* is an immunostimulant.

Apocynaceae Family

In the Plumerioideae subfamily; Tribus Carisseae: *Hunteria, Melodinus, Picralima*, Tribus Alstonieae: *Alstonia, Aspidosperma, Catharanthus, Vinca, Amsonia, Rhazya,* Tribus Tabernaemontaneae: *Tabernaemontana, Tabernanthe, Voacanga*, Tribus Rauwolfeae: *Kopsia, Ochrosia, Rauwolfia, Vallesia* are of indole alkaloid rich species.

Madagascan Periwinkle: *Catharanthus roseus* (*Vinca rosea*) contains different types of indole alkaloids, among the some 20 dimers. Vinblastine, vincristine are dimer (binary) alkaloids. They can be synthesized from vindoline and catharanthine. They and some of their derivatives possess anticancer effect and clinically used as anticancer drugs.

In Rauwolfia: *Rauwolfia serpentine, R. vomitoria, R. tetraphylla* reserpine and rescinnamine are antihypertensive, sedative, neuroleptic. Ajmalicine (raubasine) is α-blocking spasmolytic and used to treat the symptoms of senility. Ajmaline is toxic.

Common Periwinkle: *Vinca minor*. Vincamine and an artificial product from vincamine: vinpocetine are antihypertensive and predominantly the latter is used against cerebral problems, senility (e.g. in memory loss). Recently, *Camptotheca acuminate* (Nyssaceae) has received special attention. Camptothecine and some of its derivatives have anticancer effect. This compound has also quinoline structure arising from strictosidine, consequently from tryptamine.

3.3.5 Imidazole Alkaloids

Relatively few alkaloids of a possible histidine origin are known. Imidazole alkaloids: pilocarpine, pilosine and their related compounds belong to them. They can be found in the South American *Pylocarpus* species like in *P. microphyllus, P. jaborandi*, etc. (Family Rutaceae). Pilocarpine is used in ophthalmic practice, in the treatment of glaucoma.

3.3.6 Glycine Derived Alkaloids

Caffeine, theobromine and theophylline are closely related alkaloids and follow the biosynthesis of nucleotides. They can be found in many plant families and frequently are present in beverages like those from Caffee: *Coffeea arabica*, and *C. liberica, C. canephora*, Tea leaves:Thea sinensis (Theaceae), Cocoa seed: *Theobroma cacao,* Cola: *Cola nitida, C. acuminate, C. vera*, etc., (Sterculiaceae), Guarana:*, Paulinia cupana* (Sapindaceae), Mate leaf: *Ilex paraguarensis* (Aquifoliaceae) species. In beverages caffeine is a stimulant of the central nervous system (CNS), a weak diuretic.

3.3.7 Terpene Alkaloids

Monoterpene alkaloids, sometimes, supposedly cannot be found in the living plants, but are artificially produced in the course of the isolation procedure, e.g. from iridoids, in the presence of nitrogen sources. Such alkaloids may be present in iridoid containing plants, as co-occurring compounds.

In recent years the diterpene pseudo- (and proto-) alkaloid, the taxol has acquired great importance, together with its derivatives, as potent anticancer agents. Taxol can be found in a rather small quantity in the barks of *Taxus* species. Another important group of the pseudo alkaloids is of diterpene origin, like the *Aconitum* alkaloids (Liliaceae) that are used in the treatment of Alzheimer disease.

Among the triterpene alkaloids of the *Veratrum* species (cevine, protoveratine alkaloids) are worth mentioning. They can be found in Veratrum: *Veratrum album, V. viride*, and Cevadilla seeds: *Schoenocaulon officinale* (Liliaceae family) are used as insecticides. Protoveratrines are hypotensive. Steroid alkaloids, first of all, the spirosolane and solanidane skeletons containing ones (solasodine, tomatidine solanidine, etc.), are present in many *Solanum, Lycopersicum* species, among them in potatoes and tomatoes. *Solanum* (spirosolan alkaloids containing 27 carbon atoms) have received attention as possible raw materials of the semi-syntheses of hormonal steroids. In plants, they are present in the form of glycosides. Some *Solanum* species (*S. laciniatum, S. kashianum, S. aviculare*, and in certain parts of *S. nigrum, S. americanum*, etc.) may contain the glycosides of solasodine in considerably high concentrations. Δ^5-tomatidenol and solasodine glycosides are present in the thoroughly investigated Bittersweet (Woody nightshade): *Solanum dulcamara*, and the solasonine (with solanidane skeleton) in Potato: *S. tuberosum* and tomatidine in Tomato: *S. lycopersicum*.

In nature, together with other steroids, e.g. pregnane derivatives (C21 steroids) other pseudo and proto alkaloids can also be found, predominantly in the Apocynaceae, Asclepiedaceae families (e.g. conessine, holaphylline). Some of them, like conessine is the ingredient of *Holarrhena antidysenterica, H. pubescens, H. floribunda, etc.* (Apocynaceae) and are used against amoebic dysentery.

3.4 Other Nitrogen and Heteroatom Containing Compounds

Cystein and some other amino acids are associated with the glykosinolates. They are S glycosides. Mustard oils arise from amino acids, via glucosinolates. Other sulphur containing non alkaloid compounds are the pungent allicine and its derivatives in the Garlic: *Allium sativa*, Onion: *A. cepa* (Liliaceae family). They are important ingredients of the garlic oil.

4 Conclusions

In this chapter we have surveyed some 200 plant genera and species as potential MAPs. They are only examples though illustrate the order of magnitude of the MAPs, used mainly in Europe. As a comparison, a list of plants evaluated as MAPs, in four European countries (Belgium, France, Germany, United Kingdom) by De Smet (1993) contains round 500 species and genera that are officially regarded as safe and unsafe for pharmaceutical use. The most reliable European handbooks on medicinal plants may contain round one thousand Latin names (Bruneton 1999; Hänsel et al. 1999; Evans 2000). Only a small proportion of the plant kingdom, an estimated 422,000 plant species (Schippmann et al. 2003) is exploited as medicinal plant in Europe. This number can differ according to publications and can amount to 50,000 species. E.g.: the less evaluated plants in the Chinese and Indian Ayurvedic and other traditional medicines is certainly much higher than the MAPs in Europe.

The plant kingdom is moderately known from the chemical viewpoint. As regards the extent of studies, they vary from 10 to 25 % (Bates 1985; Schippmann et al. 2003; Máthé et al. 2009). It is remarkable however, that even the most thoroughly studied species are under continuous, steady investigations. The increasing number of scientific publications illustrates this phenomenon. For instance the number of publications on sage: *Salvia officinalis* has doubled between 2000 and 2008 – as illustrated by a survey, based upon the SciFinder database

(Máthé et al. 2009). This also implies the huge unexploited possibilities offered by the plant kingdom.

The plant kingdom can produce a great number of structurally similar natural substances. Theoretically, plants as living entities, produce biologically existing compounds that have a statistically higher probability of being compatible with the receptors of other living organisms than the synthetic chemicals, e.g. random combinatorial chemical products have. Among the natural products, at present, mainly the secondary metabolites are of medical importance (Dobson 2004). Primary metabolites serve very often as medical necessities. According to Lipinsky's rule of five secondary metabolites are more promising targets as medicines (druglikeness). The compounds, with a molecular mass of less than 500 daltons (like several secondary metabolites) are expected to affect human cells more than other type of compounds. (Lipinski and Hopkins 2004; Dobson, 2004).

The variability of chemical ingredients may differ according to plant species (Nicolaus and Maria 2008). The same ingredients can be synthetized by sometimes only distantly related species. This explains that the same substances may be present in plants belonging to different families (e.g. the steroid sapogenin diosgenine is in both the monocotyledonous *Dioscorea* and the dicotyledonous *Solanum spp*. (Solanaceae), *Trigonella* spp. (Leguminosae), *Digitalis* spp. (Scrophulariaceae family), etc. species, or the same essential oil components in the species of several plant families, etc.

Frequently also chemical races can be distinguished within the same species. From the practical viewpoint, these differences can determine the applicability of drugs imported from different regions, sometimes from different continents (e.g. *Acorus calamus* from America, Europe and India are of different toxic β-asarone content. Its presence in the essential oils depends upon the ploidy of the plants (Bruneton 1999). Similar differences were observed in case of the alkaloid containing *Solanum dulcamara*. The Eastern and Western European samples show great differences in the steroid alkaloid composition which determines the applicability of this plant as source of starting material of hormonal steroids (Máthé and Máthe Jr 1973). This kind, so-called infraspecific variations, due to genetic and/or ecological factors should be taken into account of the utilization of the MAPs.

Various types of secondary metabolites can be present in the same plant. In Lamiaceae species, the flavonoids, other phenolics including rosmarinic, caffeic acids, di-, triterpenes, betalains are present. They show variations in the plants independently from the principal essential oil content which is also vary in its chemical composition. (Máthé and Csedő 2007). Differences due to genetic and ecological factors could be observed when the occurrence of main chemical components was studied in relation to their geographic distribution (e.g. vincamine) of *Vinca minor* (Máthé and Précsényi 1966)), and alkaloids of *Solanum dulcamara* (Máthé and Máthé 1973), iridoids of *Galium verum* (Máthé et al. 1988).

The chemical agents in plants are so diverse that it is impossible to get a perfect knowledge about them. It is to be expected that DNA sequencing (bar-coding) and comparative studies may also contribute to the better description of plant species and as such can provide more information on plants as references for further chemical evaluations of MAPs (Xu et al. 2002).

Ethnobotanical approaches accompanied by intensive taxonomic research may help to speed up these processes (Lewis 2003; Grayer et al. 1999).

In recent years, besides the terrestrial species (Butler 2004; Newman et al. 2003; Newman and Cragg 2007; Mishra and Tiwari 2011), also marine organisms (Cragg et al. 2006) have received remarkable attention. The number of anticancer drugs (Cragg et al. 2006), or medicines against infections, especially against resistant bacteria, well illustrate that natural products are competitive with the synthetic drugs (Newman et al. 2003; Gibbons 2008; Csupor-Löffler et al. 2009).

Sometimes the biologically active substances occur in such minute quantities that it can be justified to look for similar compounds, also in farther species. The history of the anticancer compound taxol illustrates this well (Baloglu and Kingston 1999; Oberlies and Kroll 2004). Taxol was first isolated from the bark of *Taxus brevifolia* (Fam. Taxaceae) in very low concentration. Consequently other sources had to be found to meet the medical needs. By another compound, 10-deacetylbaccatin III found in the leaves of other *Taxus* species, in an order of magnitude larger quantity, serve as starting material for producing anticancer compounds. *Taxus* spp. and their ingredients of taxan-skeleton have been investigated intensively and now some hundred compounds of taxan-skeleton are known and tested (Baloglu and Kingston 1999; Oberlies and Kroll 2004).

The reinvestigation of already tested plants by using new methods may also be successful (Spjut 1985). It is also possible to change the character of certain "useless" compounds e.g. by chemical or biological means in order to get active agents for medical purposes (Ramallo et al. 2011).

With increasing knowledge on the biochemistry of the human body, we can expect to have more opportunities to influence these biochemical processes. (Grabley and Thiericke 1999). As, at present these targets are not or less known, new, yet unknown active ingredients will be necessary for affecting these targets.

There are new diseases that we did not meet in the past. Against these, new medicines (active ingredients) will be needed. E.g. the appearance of HIV, in the second half of the last century, initiated new researches for new active ingredients among them also from plant sources (Yang et al. 2001). It is generally believed that at present neglected plants could be utilized as sources of active ingredients against such new diseases.

According to Sucher and Carles (2008) DNA fingerprinting is important in the identification of drugs. In the genetic-manipulation of plants, in e.g. in tissue/cell cultures, genes are modified that can also influence the characteristics of plants. E. g.: the antimalarial compound, the artemisinin, production could be enhanced (Chen et al. 2011). DNA modification, DNA engineering of MAPs will, therefore, remain to be an important research target (Walker and Rapley 2000; Kreis et al. 2001). The spreading the GMO organisms can also provide new ingredients. Plant tissue cultures may also provide new substances different from those found in the intact plants. As such biotechnology can farther increase the chemical diversity of plants (Kreis et al. 2001; Walker and Rapley 2000).

The rapid progress in natural product biology, chemistry and technology (Garbley and Thiericke 1999), including metabolomic approaches (Wang et al. 2005) is likely to bring about the better understanding of plant chemical diversity and also assist the discovery of new compounds.

The proportion of pharmaceutically useful, biologically active ingredients is still rather limited. This could mean a huge reservoir of potential medical agents but only in a case when the chemical diversity MAPs is preserved.

References

Aggarwal BB, Kunnumakkara AB, Harikumar KB, Tharakan ST, Sung B, Anand P (2008) Potential of spice-derived phytochemicals for cancer prevention. Planta Med 74(13):1560

Baker JT, Borris RP, Carté B, Cordell GA, Soejarto DD, Cragg GM, Tyler VE (1995) Natural product drug discovery and development: new perspectives on international collaboration. J Nat Prod 58(9):1325–1357

Baloglu E, Kingston GI (1999) The taxane diterpenoids. J Nat Prod 62:1448–1472

Bates DM (1985) Plant utilization: patterns and prospects. Econ Bot 39(3):241–265

Bishop CT, Jennings J (1982) Immunology of Polysaccharides. In: Aspinall GO (ed) The polysaccharides, vol 1. Academic, New York, pp 291–330

Bruneton J (1999) Pharmacognosy phytochemistry medicinal plants, 2nd edn. Lavoisier Publishing, Londres/Paris/New York

Butler MS (2004) The role of natural product chemistry in drug discovery. J Nat Prod 67:2141–2153

Cardelina JH (2002) Challenges and opportunities confronting the botanical dietary supplement industry. J Nat Prod 65:1073–1084

Chen J-L, Fang H-M, Ji Y-P, Pu C-B, Guo Y-W, Huang L-L, Du Z-G, Liu B-Y, Ye H-C, Li G-F, Wang H (2011) Artemisinin biosynthesis enhancement in transgenic Artemisia annua plants by downregulation of the β-caryophyllene synthase gen. Planta Med 77:1759–1765

Cragg GM, Newman DJ, Yang SS (2006) Natural product extracts of plant and marine origin having antileukemia potential. The NCI experience. J Nat Prod 69:488–498

Csupor-Löffler B, Zs H, Réthy B, Zupkó I, Máthé I, Rédei T, Falkay G, Hohmann J (2009) Antiproliferative activity of Hungarian Asteraceae species against human cancer cell lines, Part II. Phytother Res 23:1109–1115

Dahlgren R (1977) Commentary on a diagrammatic presentation of the angiosperms in relation to the distribution of character states. Plant Syst Evol, Suppl. 1. Springer. 1. 253–283. Kubitzki J (eds) Flowering plants, evolution and classification of higher categories. Springer, Wien

De Smet PAGM (1993) Legislatory outlook on the safety of herbal remedies. In: De Smet PAGM (ed) Adverse effects of herbal drugs, vol 2. Springer, Berlin/Heidelberg/New York/London/Paris/Tokyo/Hong Kong/Barcelona/Budapest, pp 1–90

Dobson CM (2004) Chemical space and biology. Nature 432(7019):824–828

Evans WCE (ed) (2000) Trease and Evans pharmacognosy. Elsevier, Edinburgh/London/New York/Oxford/Philadelphia/St Louis/Sydney/Toronto. Reprinted in 2002, 2003 and 2004

Gertsch J (2008) Immunomodulatory lipids in plants: plant fatty acid amides and the human endocannabinoid system. Planta Med 74:638–650

Gibbons S (2008) Phytochemicals for bacterial resistance – strength, weaknesses and opportunities. Planta Med 74:594–602

Grabley S, Thiericke R (1999) Recent development in drug discovery technologies. In: Grabley E, Thiericke R (eds) Drug discovery from nature. Springer, Berlin, pp 38–48

Grayer RJ, Chase MW, Simmonds MSJ (1999) A comparison between chemical and molecular characters for the determination of phylogenetic relationships among plant families: an appreciation of Hegnauer's "Chemotaxonomie der Pflanzen". Biochem Syst Ecol 27:369–393

Hänsel R, Sticher O, Steineger E (1999) Pharmakognosie- phytopharmazie. Springer, Berlin/Heidelberg/New York/Barcelona/Budapest/Hongkong/London/Mailand/Paris/Singapur/Tokio

Hegnauer R (1962–1992) Chemotaxonomie der Pflanzen, vols 3–10. Bitkhauser Verlag, Basel/Stuttgart

Hegnauer R (1963) The taxonomic significance of alkaloids. In: Swain T (ed) Chemical plant taxonomy. Academic Press, London/New York, pp 389–427

Hegnauer R (1984) Bedeutung der Chemotaxonomie für die Pharmazeutische Biologie. In: Czygan FC (ed) Biogene Arzneistoffe, Entwicklung auf dem Gebiet der Pharmazeutischen Biologie, Phytochemie und Phytotherapie. Friedr. Vieweg and Sohn, Braunsweig/Wiesbaden, pp 154–175

Kreis W, Baron D, Stoll G (2001) Biotechnologie der Arzneistoffe. Deutsche Apotheker Verlag, Stuttgart

Lewis WR (2003) Pharmaceutical discoveries based on ethnomedicinal plants: 1985 to 2000 and beyond. Econ Bot 57(1):126–134

Lipinski C, Hopkins A (2004) Navigating chemical space for biology and medicine. Nature 432(7019):855–861

Luckner M (1984) Secondary metabolism in microorganisms, plants and animals. Springer, Berlin/Heidelberg/New York/Tokyo

Máthé I Jr, Kemertelidze EP, Máthé I, Sn VÁ, Mardaletshvili AA (1988) Comparative study of the iridoid production of Hungarian and Georgian Galium verum L. populations. Acta Botanica Hungarica 34(1–2):257–268

Máthé I, Csedő K (2007) Chemical differences and similarities in the family Lamiaceae. Revista de Medicina si Farmacie (Universitatea de Medicina si Farmacie din Targu-Mures) 53:1–14

Máthé I, Hohmann J, Máthé Á (2009) Adatok a hazai (magyarországi) flora kémiai ismertségéről (Data on the chemical knowledge of the Hungarian flora. Proceedings on VI. Kárpátmedencei Biológiai Szimpozium, Budapest, 12–13 Nov 2009, pp 21–30

Máthé I, Máthé I Jr (1973) Data to the European area of the chemical taxa of Solanum dulcamara L. Acta Bot Acad Sci Hung 19(1–4):441–451

Máthé I, Précsényi I (1966) Changing of vincamine agent in Vinca minor L. According to regional and year of growth. Acta Agron Acad Sci Hung 15(3–4):273–283

Mishra BB, Tiwari VK (2011) Natural products: an evolving role in future drug discovery. Eur J Med Chem 46(10):4769–4807

Newman DJ, Cragg GM (2007) Natural products as sources of new drugs over the last 25 years. J Nat Prod 70(3):461–477

Newman DJ, Cragg GM, Snader KM (2003) Natural products as sources of new drugs over the period 1981–2002. J Nat Prod 66(7):1022–1037

Nicolaus JS, Maria CC (2008) Genome-based approaches to the authentication of medicinal plants. Planta Med 74:603–623

Oberlies NH, Kroll DJ (2004) Camptothecin and taxol: historic achievements in natural products research. J Nat Prod 67:129–135

Ramallo IA, Salazar MO, Mendez L, Furian RLE (2011) Chemically engineered extracts: source of bioactive compounds. Acc Chem Res 44(4):241–250

Schippmann U, Cunningham AB, Leaman DJ (2003) Case study no 7. Impact of cultivation and gathering of medicinal plants on biodiversity: global trends and issues. (Biodiversity and the ecosystem approach in agriculture, forestry and fisheries FAO, Rome, 12–13 Oct 2002) http://www.fao.org/docrep/005/y4586e/y4586e08.htm

Spjut RW (1985) Limitations of a random screen: search for new anticancer drugs in higher plants. Econ Bot 39(3):266–288

Sucher NJ, Carles MC (2008) Genome-based approaches to the authentication of medicinal plants. Planta Med 74:603–623

Walker JM, Rapley R (eds) (2000) Molecular biology and biotechnology, 4th edn, Published by The Royal Society of Chemistry. Athenaeum Press, Cambridge, UK

Wang M, Lamers R-JAN, Korthout HAAJ, Nesselrooij JHJ, Witkamp RF, Heijden R, Voshol PJ, Havekes LM, Verporte R, Greef J (2005) Metabolomics in the context of systems biology: bridging traditional Chinese medicine and molecular pharmacognosy. Phytother Res 19:173–182

Xu H, Fabricant DS, Piersen CE, Bolton JL, Pezzuto JM, Fong H, Totura S, Farnsworth NR, Constantinou AI (2002) A preliminary RAPD-PCR analysis of Cimicifuga species and other botanicals used for women's health. Phytomedicine 9:757–762

Yang SS, Cragg GM, Newman DJ, Bader JP (2001) Natural product-based anti-HIV drug discovery and development facilitated by the NCI developmental therapeutics program. J Nat Prod 64(2):265–277

Chapter 4
Application of Spectroscopic Methods and Hyphenated Techniques to the Analysis of Complex Plant Extracts

Dejan Gođevac, Milka Jadranin, Ivana Aljančić, Vlatka Vajs, Vele Tešević, and Slobodan Milosavljević

Abstract Through the selected examples of different classes of secondary metabolites two general approaches in the analysis of complex mixtures of natural products are presented. The first one involves isolation of the constituents using optimized semi-prep LC in combination with classical column chromatography (CC) and the second one represents direct (on-line) analysis of complex plant extracts prior to isolation of the constituents. Thus the isolation of mg-quantities of jatrophanes the bioactive constituents of *Euphorbia dendroides,* with their structure determination on the basis of spectroscopic data interpretation (IR, NMR, MS), is described. The direct analysis of the plant extracts is demonstrated by the characterization of antioxidative polyphenols, i.e. oligomeric proanthocynidins from the MeOH extracts of grape *Vitis vinifera* seeds by the application of hyphenated methods (LC/UV/MS) in combination with ^{13}C NMR spectroscopy, as well as tandem mass spectrometry (MS/MS) and MS^n in combination with and without LC for identification of oligomeric proanthocynidins from other sources. The potential of another hyphenated instrumentation, LC/UV/SPE-NMR is shown by on-line identification of the various constituents of the crude extract of *Hypericum perforatum.* The possibilities and limitations of the NMR technique named diffusion ordered spectroscopy (DOSY) for direct analysis of the complex mixture, such as e.g. methanolic extract of *Gentiana lutea,* is discussed.

Keywords Spectroscopic methods • Hyphenated techniques • Plant extracts • Analysis • Possibilities • Limitations

D. Gođevac • M. Jadranin • I. Aljančić • V. Vajs
Center for Chemistry, Institute for Chemistry, Technology and Metallurgy, University of Belgrade, Studentski trg 12-16, Belgrade 11000, Serbia

V. Tešević • S. Milosavljević (✉)
Faculty of Chemistry, University of Belgrade, Studentski trg 12-16, Belgrade 11000, Serbia
e-mail: smilo@chem.bg.ac.rs

Á. Máthé (ed.), *Medicinal and Aromatic Plants of the World*, Medicinal and Aromatic Plants of the World 1, DOI 10.1007/978-94-017-9810-5_4

1 Introduction

Natural products (secondary metabolites) have always been among the most fascinating objects of the practicing chemist. In fact, in many definitions of chemistry the isolation, purification and structural elucidation of natural products play a central role. For centuries, drugs were entirely of natural origin and composed of herbs, animal products and inorganic materials. The chemistry of natural products has always excited the interest of the scientists and it provided the early stimulus for foundation of organic chemistry as a separate discipline. The serious study of natural products, lasting for a long time, has been greatly facilitated by the advent of modern spectroscopic techniques, namely nuclear magnetic resonance spectroscopy (NMR) and mass spectrometry (MS), as well as separation techniques, such as gas and liquid chromatography (GC and LC). Nowadays more rapid strategies for chemical characterizations of phytoconstituents of natural products as well as assessing the bioactivities of the natural products are available. The coupling of spectrometers to chromatographs resulted in very powerful so-called hyphenated (coupled) techniques enabling on-line direct analysis of complex mixtures of natural products without prior isolation.

The GC/MS, developed during the 1950s, after being originated by James and Martin in 1952 (James and Martin 1952) was practically the only heavily used hyphenated method for half a century. But its application is rather limited to thermally stable non-polar volatiles, e.g. essential oils, fatty acid methyl esters and similar samples. The breakthrough, responsible for introducing the combination LC/MS applicable to the variety of polar non-volatiles has occurred in the second half of the twentieth century by development of MS ionic sources compatible with LC: APCI (atmospheric pressure chemical ionization) by J. Henion (Henion et al. 1982; Covey et al. 1986) and ESI (electrospray ionization) by J. Fenn, (Fenn et al. 1989) the latter was awarded a share of the Nobel Prize in chemistry in 2002 for this achievement. Today, the various hyphenated methods involving coupling LC and MS are becoming routine, e.g. LC/UV- photodiode array detection/MS (LC/UV/MS), LC-tandem MS (LC/MS/MS) and LC-multiple stage MS (LC/MS^n).

The combination LC/UV/MS affords on-line UV and mass spectra of the eluted components, as well the liquid chromatograms measured as the variation in time of the absorbance (mAU, miliabsorbance units) at the selected wavelength (PDA) andor total ion current, TIC (%∑I) with MS detector (see the example presented in Fig. 4.3).

LC coupled to a NMR spectrometer (LC/NMR) has first appeared in the late 1970 through demonstration of both stop-flow and continuous-flow LC/NMR, and until recently, been little used mainly because of its lack of sensitivity. However, development of suitable flow cells and techniques to optimize NMR acquisition conditions, introduction of pulse field gradients (PFG) and solvent signal suppression sequences, together with improvements in probe technology (development of cryoprobe) and the construction of high field magnets have alleviated many of the

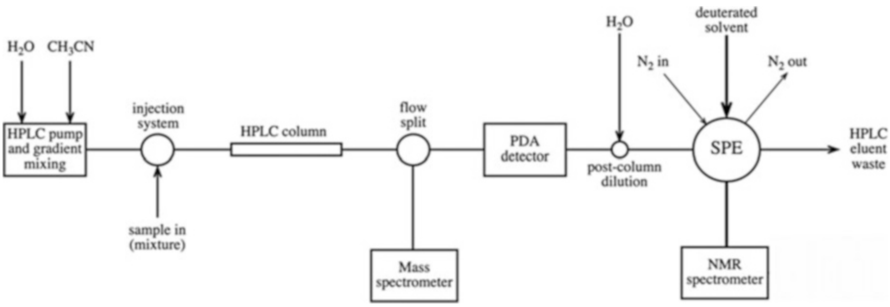


Fig. 4.1 LC/UV/MS/SPE NMR device (Ref. Jaroszewski (2005))

initial problems of this technique. LC/NMR has important potential for on-line structure identification of natural products (Wolfender et al. 2001). Indeed, NMR spectroscopy is by far the most powerful spectroscopic technique for obtaining detailed structural information about organic compounds in solution. The development of solid phase extraction (SPE) and the recent addition of an automated SPE unit to an LC/NMR system for peak trapping results in an improved NMR signal-to-noise ratio (S/N) and also has other practical advantages. This indirect hyphenation technique (LC/SPE NMR) involving multiple collections of the separated compounds on SPE cartridges, followed by drying with nitrogen and their subsequent transfer to the NMR flow cells using deuterated solvents (Fig. 4.1) is nowadays the most efficient technique in comparison with the previous ones (on-flow and stop-flow) leading to foundation for today's commercial HPLC-NMR instruments (Jaroszewski 2005). The advantages of LC/SPE NMR are the following:

- LC separation with cheap, non-deuterated solvents
- Additives non-compatible with NMR are possible.
- The complete peak can be trapped: concentration effect.
- This leads to a higher sensitivity. Multiple trapping feasible
- Desorption with 100 % deuterated solvents is possible.
- Choices! No or less solvent suppression is necessary
- Choice of peaks that should be analysed
- Easy comparison with literature spectra
- Measurements at another NMR spectrometer are possible.
- Easy 2D NMR mesurements (COSY, NOESY, HSQC, HMBC etc.)

At the same time, due to the high sensitivity of the mass spectrometry, a small portion of the effluent after LC can be transferred in the same run via splitter to the mass spectrometer (Fig. 4.1) thus affording online UV, MS and NMR spectra in the same experiment. Combining spectral data from MS, NMR and UV can considerably decrease the time for structural elucidation of secondary metabolites. Identification of a compound often is very difficult based on NMR spectral data only; hence, MS data also are essential. Therefore, LC based hyphenation of NMR and

MS detectors to combine sets of spectral information of secondary metabolites has already been used for several years.

In addition, other LC-combinations using different spectrometers as chromatographic detectors have also developed: LC-infrared spectroscopy (LC/IR), and LC-circular dichroism (LC/CD). The application of LC/CD enables chiroptical online stereoanalysis of natural products directly from stereoisomeric mixtures, thus giving the full absolute stereostructures of novel compounds without the necessity of isolation and purification. The combination of the high separation efficiency of LC with these different detectors has made possible the acquisition of on-line complementary spectroscopic data, e.g. LC-MS/MS-NMR-CD "triad", on an LC peak of interest within a complex mixture (Bringmann and Lang 2003).

In further text two general approaches to the analysis of the plant extracts are discussed: (*i*) the "traditional" one involving isolation of the components and (*ii*) identification of the components in the crude extracts without prior isolation.

2 Disscusion

2.1 The "Traditional" Procedure of the Analysis of the Plant Extracts

The rather simplified presentation of the "traditional" procedure being used in the identification of constituents of the plant is presented in Fig. 4.2. Unfortunately, extraction, which is usually the first step in the analysis of the plant constituents, rarely results in the form of pure compounds. More often rather complex mixtures containing quite a few components are obtained. The complete characterization of constituents has to rely on spectral analyses of their purified form. Therefore, rational and efficient isolation procedures have to be developed. Historically, separation and isolation of components from complex mixtures has occupied (and still does) a central role in the activity of researchers in the field of natural products research. Consequently, most of the working time is devoted to the separation/ purification steps and not to the structural determination.

The following drawbacks of this (traditional) methods are encountered:

- Large amounts of the rare biological material are sometimes needed.
- Expensive tools and supplies, like adsorbents and eluents
- Long lasting process (several days....)
- Unstable compounds may decompose already during the preparative separation and thus may escape the analysis.

Recently, a development of the very efficient automated LC semi-prep chromatographic methods, which could be performed on the analytical LC-devices made the preparative step much more efficient, enabling collecting mg quantities of the pure compounds ready for structure determination in one run. The versatile

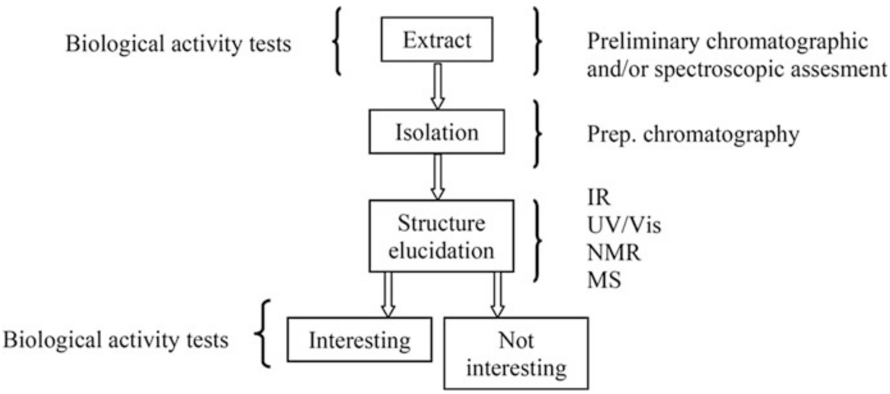


Fig. 4.2 Traditional method of analysis of the crude extracts

semi-prep systems perform sample injection, fraction collection and re-injection of the collected fractions. The difference between the analytical and preparative HPLC is the amount of sample applied to the column. Semi-preparative chromatography is the link between analytical HPLC and preparative LC. Even though the chromatographic systems used for semi-preparative LC do not reach the size of preparative LC systems, the objectives remain the same:

- Purification and isolation of maximum sample quantity
- Savings in time and costs.

In the analytical LC the applied sample amount is very small compared to the amount of stationary phase in the column (less than 1:10,000). Therefore, very good separations can be achieved. To purify higher amounts of sample in a single run the loadability of a column has to be increased. This can be done either by concentration or volume overloading, depending on the application. Consequently, the scale-up from analytical LC (microgram quantity) to semi-prep one (milligram quantities) requires the increase column dimensions from *ca.* 4–8 to 10–20 mm ID, as well as the flow rate from *ca.* 0.4–2 to 2–10 mL/min, respectively. In addition, in last two decades some of the inherent insensitivity of the NMR spectroscopy which is the major tool for structure elucidation, has been overcome by improvements in spectrometer hardware and development of new multipulse sequences, considerably reducing the required quantity of the sample to be analysed down to the sub-microgram range.

Example #1

Isolation of jatrophanes, bioactive diterpenoids from Euphorbia dendroides (Tree Spurge) using optimized semi-prep LC in combination with classical column chromatography (CC)

The application of this improved purification step involving semi-prep LC in combination with the classical chromatographic methods used for preliminary purification and enrichment of the interesting fractions in the analysis of the

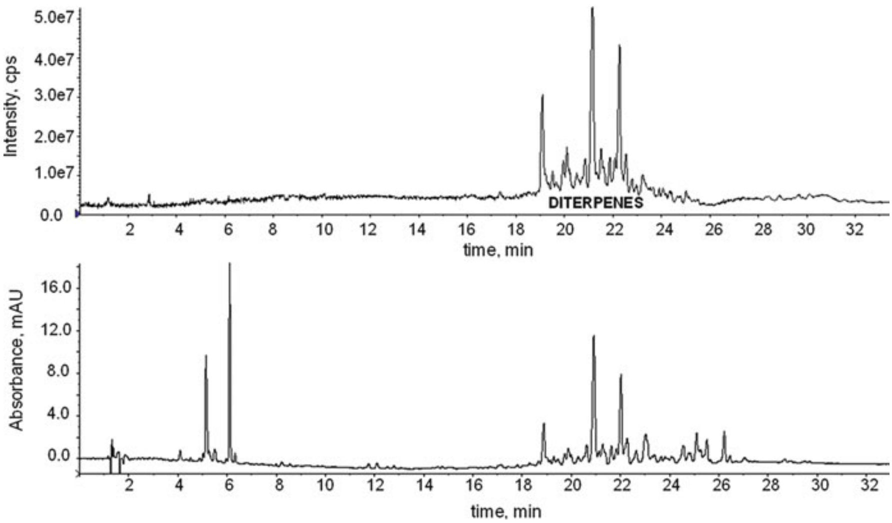


Fig. 4.3 LC/UV/ESI TOF MS (+ mode) chromatogram of the lyophilized crude latex of *Euphorbia dendroides*: LC/MS total ion current (TIC) (***upper trace***) and LC/PDA ($\lambda = 260$ nm) (***lower trace***); Experimental conditions: Zorbax Eclipse Plus C_{18} column (150×4.6 mm i.d.; 1.8 μm), mobile phase (0.6 mL/min): A (0.20 % formic acid in MilliQ water) and B (acetonitrile), gradient mode of elution, 5 % B (0–5 min), 5–95 % B (5–26 min), 95 % B, (26–35 min); HPLC instrument (Agilent 1200 Series) and a photodiode-array detector (PDA) coupled with a 6210 Time-of-Flight LC/MS system (Agilent Technologies)

bioactive constituents of the Montenegrin spurge *Euphorbia dendroides* as a part of the comprehensive study of this species has been carried out recently in our laboratory (Aljančić et al. 2011; Pesic et al. 2011; Jadranin et al. 2013). In course of these investigations, two types of samples were prepared: 60 % aqueous ethanolic extract of the aerial parts (Aljančić et al. 2011) and *n*-hexane fraction of the lyophilized latex (Jadranin et al. 2013). Before the preparative isolation both samples were subjected to LC/UV/ESI TOF MS as well as the ^{1}H NMR analysis (not shown), revealing rather complex mixtures with diterpenoids as the major constituents (see LC/UV/ESI MS chromatograms of the lyophilized latex, Fig. 4.3).

Before the semi-prep LC, the isolation of the extract constituents involved a combination of the classical prep chromatographic techniques on Si-gel, i.e. DCFC/prep TLC (Aljančić et al. 2011), or DCFC/CC (Jadranin et al. 2013) (see Fig. 4.4). The isolation scheme used for the purification of latex components (Fig. 4.4) involving in the first step DCFC on Si-gel (n-hexane/EtOAc in three different proportions, 60 %, 80 %, and 100 % EtOAc) afforded fractions **A** – **C**, respectively, containing, according to the ^{1}H NMR and LC/UV/ESI TOF MS spectra, jatrophane diterpenoids as the main constituents. In the second step these fractions were divided by CC on silica gel eluting with EtOAc/*n*-hexane (Fig. 4.4), into subfractions $\mathbf{A_{1-3}}$, $\mathbf{B_{1-2}}$ and $\mathbf{C_{1-2}}$ and subjected to the semi-prep LC. The example of semi-prep LC chromatogram of fraction $\mathbf{A_2}$ containing four diterpenes (**1**, **2**, **6** and **9**) is presented in Figs. 4.5 and 4.6.

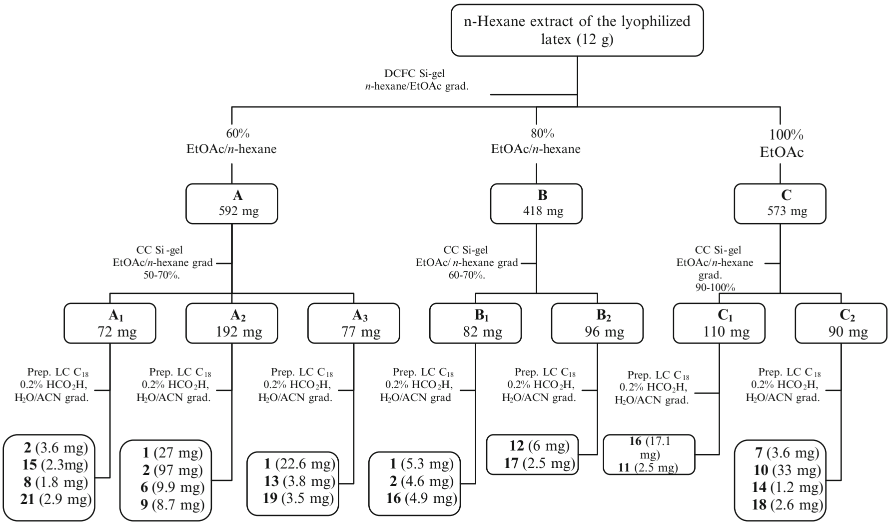


Fig. 4.4 Isolation scheme used in isolation of pure compounds from the liophilized crude latex of *Euphorbia dendroides* (Jadranin et al. 2013)

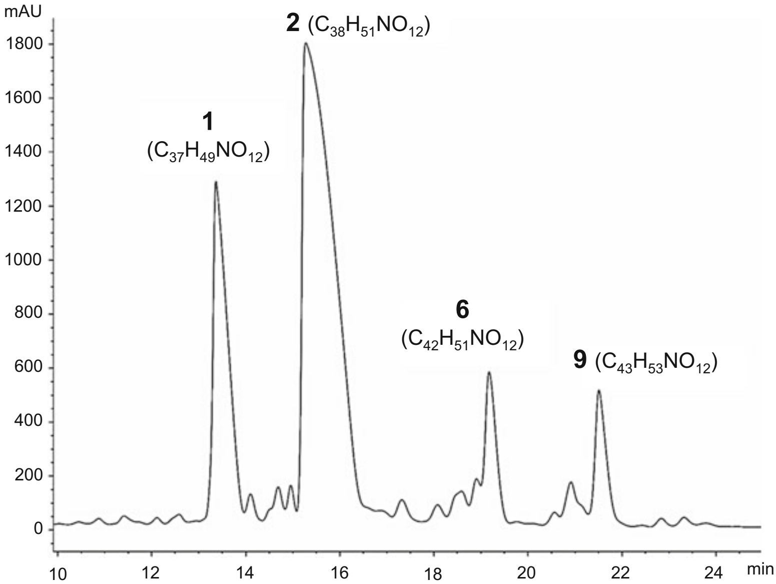
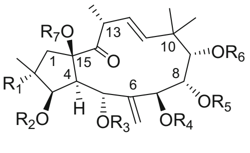
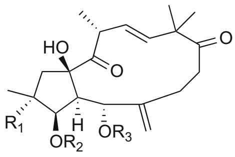


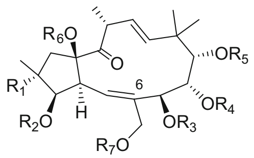
Fig. 4.5 Semi-Prep LC of fraction **A_2:** on the Agilent Technologies 1100 Series analytical instrument equipped with autosampler under the following conditions: injection volume 1,000 μL (c ~ 20 mg/mL, MeOH), Zorbax Eclipse XDB-C_{18} column (250 × 9.4 mm; 5 μm), column temp. 25 °C, mobile phase (4.00 mL/min): A (0.20 % formic acid in MilliQ water) and B (acetonitrile), gradient mode of elution, 55–90 % B (0–25 min), 90 % B (25–35 min). The molecular formula are based on the precise mass measurement on LC/ESI TOF MS



1 - 14



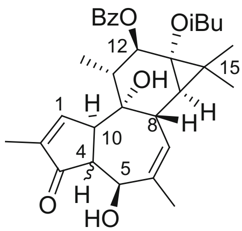
15



16 - 19

	R$_1$	R$_2$	R$_3$	R$_4$	R$_5$	R$_6$	R$_7$
1	H	Pr	Ac	iBu	Ac	Nic	H
2	H	iBu	Ac	iBu	Ac	Nic	H
3	H	Pr	Ac	iBu	Ac	Nic	Ac
4	H	iBu	Ac	Ac	Bz	Ac	H
5	H	Pr	Ac	iBu	Bz	Ac	H
6	H	Pr	Ac	iBu	Bz	Nic	H
7	H	Pr	Ac	iBu	Nic	Nic	H
8	H	Pr	Ac	iBu	iBu	Nic	H
9	H	iBu	Ac	iBu	Bz	Nic	H
10	H	iBu	Ac	iBu	Nic	Nic	H
11	H	iBu	Ac	Ac	Nic	Ac	H
12	H	Ac	Ac	iBu	Ac	Nic	H
13	OAc	iBu	Ac	iBu	Ac	Nic	H
14	OAc	iBu	Nic	iBu	Ac	Nic	H
15	OAc	iVal	Ac				
16	OAc	Ac	iBu	Ac	Nic	Ac	Ac
17	OAc	Pr	iBu	Ac	Nic	Ac	Ac
18	OAc	Ac	iBu	Nic	Nic	Ac	Ac
19	OAc	Ac	iBu	Ac	Bz	Ac	Ac

Ac–acetyl, Bz–benzoyl, iBu–isobutanoyl, iVal–isovaleroyl, Nic–nicotinoyl, Pr–*n*-propanoyl



20 (4αH)
21 (4βH)

Fig. 4.6 The structures of the diterpenoids isolated from the aerial parts and latex of *E. dendroides* (Aljančić et al. 2011; Pesic et al. 2011; Jadranin et al. 2013)

From both extracts (aerial parts and latex) nineteen jatrophane diterpenoids (**1**–**19**) and two epimeric tiglianes (**20**, **21**) have been isolated (Fig. 4.7). With exception of tigliane **21,** named Euphorbia factor Pr_2 by Wu et al. (1994), all isolated compounds were new and their structures were elucidated by spectroscopic methods, including HRESIMS, 1D and various 2D NMR techniques. Compounds **1**, **2** and **16** were found in both extracts. It also should be noted that the biological activity of compounds **1**–**5**, **16** and **20** was studied against four human cancer cell lines. The most effective jatrophane-type compound (**2**) and its structurally closely related derivative (**1**) were evaluated for their interactions with paclitaxel and doxorubicin using a multidrug-resistant cancer cell line. Both compounds exerted a strong reversal potential resulting from inhibition of P-glycoprotein transport (Aljančić et al. 2011; Pesic et al. 2011). Among the jatrophanes isolated from latex, the assessment of the P-glycoprotein (P-gp) inhibiting activities of the representative set of compounds **1**, **2**, **6**–**10** and **15**–**19** revealed the activity of jatrophanes **6** and **9** as the most powerful in inhibition of P-gp, even higher than R(+)-verapamil and tariquidar in colorectal multi-drug resistant (MDR) cells (DLD1-TxR) (Jadranin et al. 2013).

2.2 Identification of the Components in the Crude Extracts Without Prior Isolation

There are two general approaches in the analysis of the crude extracts without prior isolation: hyphenated (coupled) techniques involving the combination of chromato-

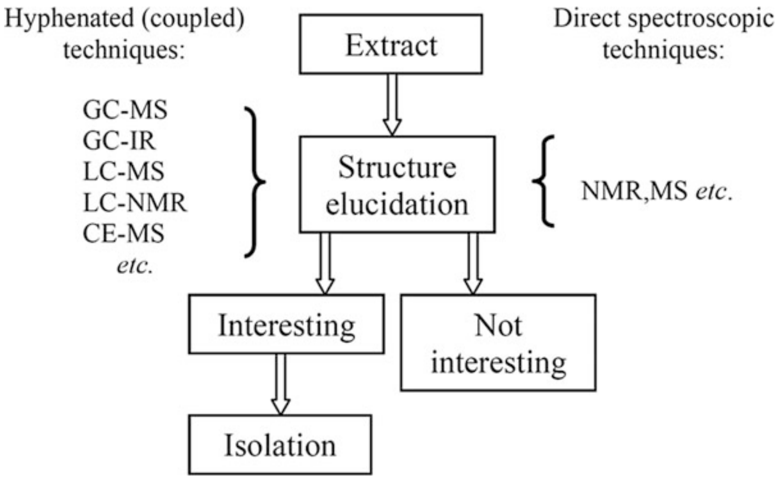


Fig. 4.7 Identification of the components in the crude extracts without prior isolation

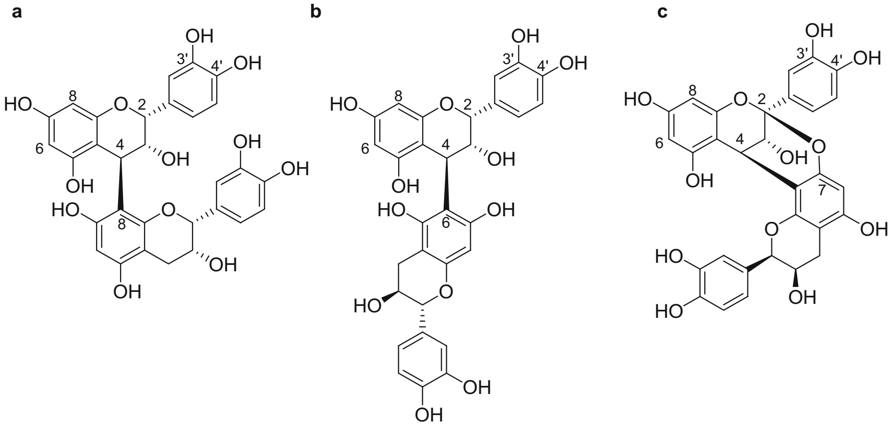


Fig. 4.8 Structures of dimeric B-type proanthocynidins: (**a**) epicatechin-(4β → 8)-catechin, (**b**) epicatechin-(4β → 6)-catechin and dimeric A-type proanthocynidin: (**c**) epicatechin-(2β → 7; 4β → 8)-epicatechin

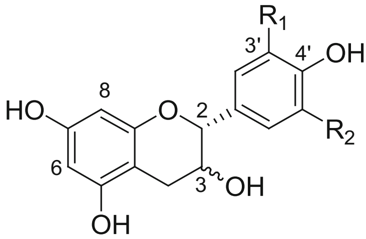
graphic and spectroscopic techniques and direct spectroscopic measurement of the extract, as well as their combination (Fig. 4.7).

Example #2
Proanthocynidins from grape (**Vitis vinifera*****) seed extracts***

The hyphenated techniques involving LC are now extensively used for investigation of various polar biologically active secondary metabolites such as, for example, antioxidant phytochemicals. Most of them are polyphenols widely present in the plant kingdom. Very important representatives of these natural products are proanthocyanidins (PAs), also known as condensed tannins. They are ubiquitous and one of the most abundant group of natural polyphenols and can be detectable in a wide variety of food sources, such as fruits, nuts, beans, apples, and red wine (Prior and Gu 2005). PAs in foods are of interest in human nutrition and medicine due to their antioxidant capacities and beneficial effects on human health in reducing the risk of chronic diseases, such as cardiovascular and cancer. They are oligomers and polymers composed of flavan-3-ol units linked mainly through C(4)-C(8) (Fig. 4.8a) and less frequent through C(4)-C(6) bond (Fig. 4.8b), both denoted as B-type. The flavan-3-ol units can also be doubly linked by an additional ether C (2)-O(7) which is assigned as A-type (Fig. 4.8c).

The monomeric units of the oligomeric and polymeric PAs are afzelechin, catechin, gallocatechin and their 3β-epimers (Fig. 4.9). The size of PAs is described by its degree of polymerization (DP). Oligomers are those with DP = 2 ~ 7, whereas polymers exhibit DP = 8 ~ 24 or more.

The most common classes of the proanthocyanidins are the procyanidins, which are chains of catechin, epicatechin, and their gallic acid esters, and the prodelphinidins, which consist of gallocatechin, epigallocatechin, and their



R_1 = H, R_2 = H: Afzelechin (3α-OH), Epiafzelechin (3β-OH)

R_1 = OH, R_2 = H: Catechin (3α-OH), Epicatechin (3β-OH)

R_1 = R_2 = OH: Gallocatechin (3α-OH), Epigallocatechin (3β-OH)

Fig. 4.9 Structure of the flavan-3-ol units in proanthocyanidins

galloylated derivatives as the monomeric units. The proanthocyanidins containing afzelechin as the subunits are named propelargonidins.

Several methods for the analysis of polyphenols have been presented in the literature (Gu 2012). Most of them are based on liquid chromatography (LC) coupled with either a photodiode array (PDA) detector and/or a mass spectrometer (MS). This is a very effective and highly sensitive method for characterizing procyanidins from complex matrices (Flamini 2003; Reed et al. 2005). In recent years, electrospray ionization (ESI) has been shown to be suitable for the analysis of such polar compounds in aqueous solutions without any previous sample derivatisation (Gaskell 1997). ESI permits the identification of the molecular weight of procyanidins with different degrees of polymerisation. Both normal and reverse phase LC techniques are applied. The normal- phase technique effects the separation of the proanthocyanidin oligomers according different degrees of polymerisation (DP) (Gu et al. 2003) whereas reversed-phase C_{18} columns have the ability to separate different isomeric oligomers with equivalent molecular mass. However reverse-phase analysis of higher oligomeric proanthocyanidins (i.e. $\geq$ tetramers) is not feasible due to the fact that, with increasing degrees of polymerization, the number of isomers also increases. This effect results in a retention time overlap of isomers containing differing degrees of polymerization, causing the large unresolved peak (Guyot et al. 1997). Catechins and proanthocyanidins are usually detected at 280 nm using a UV detector. However, peak intensity is rather low, and many other phenolic compounds also absorb light at 280 nm. Much better sensitivity is obtained using fluorescence with excitation and emission set to 230 and 321 nm, respectively (Gu 2012; Gómez-Alonsoa et al. 2007).

The application of the LC/MS method for the analysis of such compounds, together with ^{13}C NMR spectroscopy, is demonstrated in our laboratory by direct analysis of polyphenol constituents of grape seed MeOH extracts (GSE) (Stanković et al. 2008). Grape seeds are known as an abundant source of procyanidins

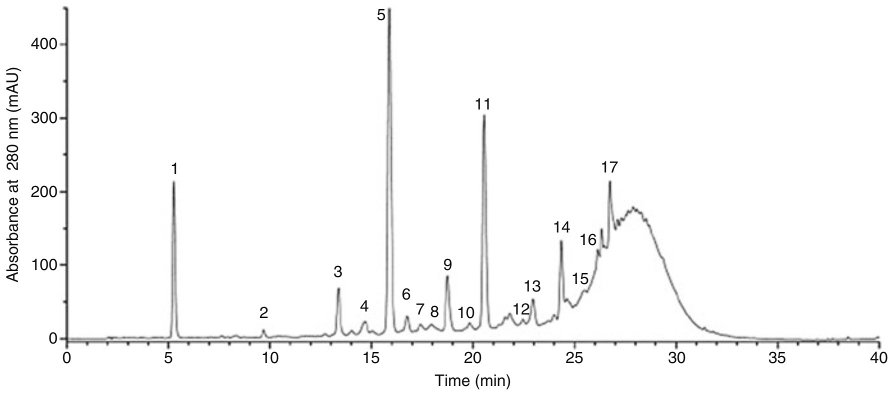
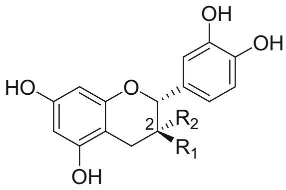


Fig. 4.10 LC PDA of GSE (*V. vinifera* L., cv. Cabernet Sauvignon, Montenegro) chromatograms ($\lambda = 280$ nm), measured using Zorbax Eclipse XDB-C_{18} column (5 μm, 150 × 4.6 mm). For the chromatographic conditions (see Ref. Stanković et al. (2008))

consisting of both (+)-catechin and (−)-epicatechin forming units that, in the particular case of (−)-epicatechin, can appear galloylated or not (Cantos et al. 2002). The use of a rapid resolution HPLC column and the appropriate gradient program afforded the separation of seventeen phenolic compounds in less than 30 min (see the example shown in Figs. 4.10 and 4.11) (Stanković et al. 2008). The exact mass measurements of the of molecular and fragment ions of analytes with a time-of-flight (TOF) mass spectrometer in positive and in negative polarity mode revealed the structures of seventeen polyphenols in our extract comprising catechin and epicatechin monomers, i.e. procyanidin oligomers, and their gallates (Fig. 4.11).

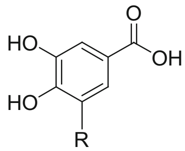
The procyanidin dimers, trimers, tetramers and pentamers (B-type) have molecular masses of 578, 866, 1,154 and 1,442, respectively and their gross structures were identified in the positive mode by the abundant $[M+H]^+$ and, in some cases by $[M+Na]^+$ and $[2M+Na]^+$ sodium adducts (positive mode). All identified compounds exhibited $[M\text{-}H]^-$ in the negative mode, as well as $[2M\text{-}H]^-$ ions for some of them, confirming molecular mass. In addition, procyanidin dimers and epicatechin gallate possessed high affinity to sodium ions, giving adducts such as $[M+Na\text{-}2H]^-$ and $[2M+2Na\text{-}3H]^-$. Doubly charged $[M\text{-}2H]^{2-}$ species were observed for procyanidin epicatechin gallate possessed high affinity to sodium ions, giving adducts such as $[M+Na\text{-}2H]^-$ and $[2M+2Na\text{-}3H]^-$. Doubly charged $[M\text{-}2H]^{2-}$ species were observed for procyanidin trimers.

It should also be noted that such identification, based solely on precise mass measurement of quasimolecular ions, of the gross structure of procyanidin oligomers is tentative. This method allows only elucidation of chain length, and the chemical constitution of the individual chains, but not the stereochemistry at the chiral centers C-2 and C-3. In order to get more information regarding the structure of procyanidin oligomers detected in the extract, the ^{13}C NMR spectroscopy was applied (Fig. 4.12). The ^{13}C NMR spectrum of the GSE was measured by a pulse



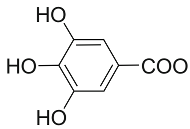
5: R_1= OH, R_2= H (+)-Catechin

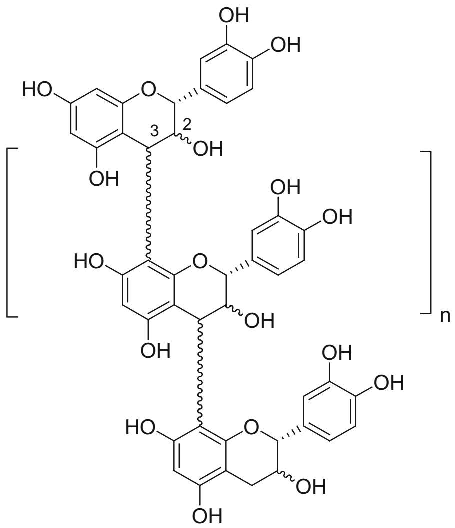
6: R_1= H, R_2= OH (-)-Epicatechin



1: R = OH Gallic acid

2: R = H Protocatechuic acid

17: R_1= H, R_2=  (-)-Epicatechin gallate



3, 4, 8, 9, 14 : n = 1, Proanthocyanidin dimers

6, **12**, **13** : n = 2, Proanthocyanidin trimers

7: n = 3, Proanthocyanidin tetramer

15: n = 4, Proanthocyanidin pentamer

Fig. 4.11 Gross structures of the identified compounds in GSE (numbered according to LC-elution order, Fig. 4.11). In addition, monogallates of proantocyanidin trimer (**10**) and dimer (**14**), (−) – epicatechin monogallate (**17**) and proantocynaidin dimer digallate (**16**) were detected

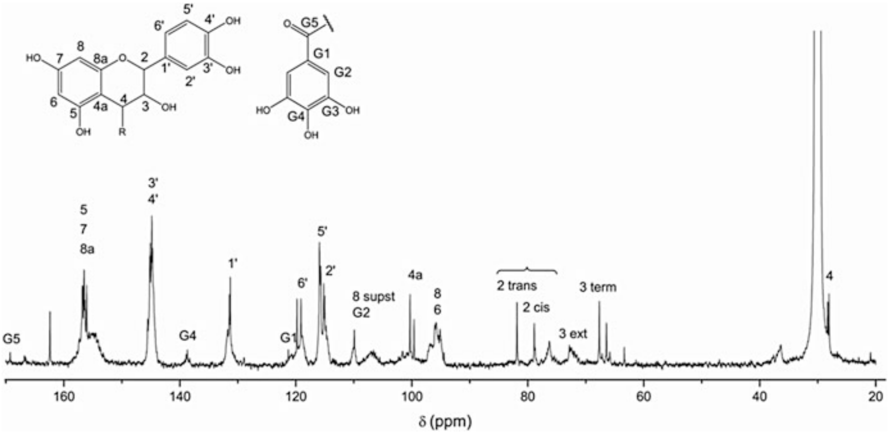


Fig. 4.12 ^{13}C 125 MHz NMR spectrum of the GSE in acetone-d_6/D_2O + tris(2,4-pentadionato) chromium(III) (CrAcac) at conc. 50 mM (relaxation reagent), recorded with inverse gated decoupling pulse sequence for NOE suppression, 8-supst – substituted position 8; 3ext-, 3term – position 3 at extender and terminal flavan-3-units (Ref. Stanković et al. (2008))

sequence allowing the total relaxation of all carbons, as well as the suppression of the nuclear Overhauser effect (NOE effect) which enables quantitative measurements based on peak intensities. Assignments of the resonances (Fig. 4.12) were based on comparison with those reported in the literature (Newman et al. 1987; Behrens et al. 2003).

This spectrum provided structural and stereochemical details on subunit composition. The signals of the ^{13}C-NMR spectrum indicated the presence of catechin, epicatechin and B-type linked procyanidins, with some traces of gallate unit. There were no other major signals present in GSE, ruling out the presence of some other types of flavonoid molecules, or proanthocyanidins based on other units. The signals at δ 82 and 79 ppm were diagnostic for the 2,3-*trans* and 2,3-*cis* stereochemistry of the heterocyclic ring respectively, indicating that both stereoisomers (catechin/epicatechin) are present. Comparison of the areas of these resonances, gave catechin/epicatechin ratio of 1:0.74.

The same HPLC UV/ESI MS method was also applied subsequently in screening of the GSE from eight grape cultivars (*Vitis vinifera*) growing in Serbia for their polyphenolic composition (Gođevac et al. 2010). The study revealed 34 phenolic compounds belonging to the following groups: flavan-3-ol monomers, procyanidin dimers and trimers, flavonoids, hydroxycinnamic acid and hydroxybenzoic acid derivatives. The quantities of the main constituents were determined using LC PDA. Qualitative and quantitative differences among the cultivars were observed.

In the above examples only oligomeric proanthocyanidin chains of B-type formed from the same monomeric flavanol building blocks, i.e. (+)-catechin and its (−)-epimer, i.e. procyanidins, were encountered. However, there are numerous examples of heterogeneous proanthocynidins containing in addition to (epi)catechin other monomer units such as (epi)afzelechin and/or gallocatechin, as well as

A-type linkage. Such type of mixed chains could be analysed by combination of LC with tandem mass spectrometry, e.g. (LC/MS/MS) or LC-multiple stage MS (LC/MS^n). Tandem mass spectrometry (MS/MS) can give more information about the structural details of the different molecules which is based on their characteristic fragmentation patterns. The MS/MS technique used for this analysis is mostly product ion scan involving selection of the quasi-molecular ion, such as $[M-H]^-$ or $[M+H]^+$ by the first analyzer, its fragmentation by the collisional activation, followed by the analysis of product ions. The proanthocynidin mixtures can also be analysed directly, without chromatographic separation using matrix-assisted laser desorption ionization in combination with time of flight mass spectrometry (MALDI/TOF MS) using the so-called post source decay (PSD) fragmentation (Behrens et al. 2003). Another example of direct analysis, without chromatographic separation of the condensed tannins is a direct flow injection electrospray ionization ion trap tandem mass spectrometry (ESI-IT-MS/MS) which was used to investigate the polyphenolic compounds present in an infusion from the barks of *Hancornia speciosa* Gom. (Apocynaceae), a native Brazilian plant popularly known as 'mangabeira', used as a source of nutrition and against gastric disorders (Rodrigues et al. 2007).

In the negative mode MS the proanthocynaidins exhibit three main MS fragmentation pathways assigned as quinone methide (QM), the heterocyclic ring fission (HFR) and the retro-Diels-Alder (RDA) mechanisms (Fig. 4.13), that can be useful in structure determination, namely the assignment of the connection sequence of different monomeric units (Gu 2012; Gu et al. 2003).

The QM, HFR and RDA fragmentation patterns are also encountered in the positive mode MS of proanthocyanidins and are also used for sequencing of the proanthocyanidins (Li and Deinzer 2007). The A-type linkage could be easily recognized from the molecular mass. Introduction of one A-type linkage leads to its reduction of mass by two mass units in comparison with that of the B-type. Thus, procyanidin trimer gave rise to $[M-H]^-$ *m/z* 865, whereas the corresponding trimer with one A-type linkage yielded $[M-H]^-$ *m/z* 863. Contrary to the B-type, A-type interflavan bond doesn't undergo QM cleavage, which an additional difference between them used for the sequencing.

Example #3

Major constituents of* Hypericum perforatum *(St. John's Wort) by LC/SPE-NMR and LC/MS (Tatsis et al. 2007)

The hyphenated instrumentation of LC/UV/SPE-NMR and LC/UV/ESI MS techniques have been applied for separation and structure verification of the major known constituents present in Greek *Hypericum perforatum* extracts. The chromatographic separation was performed on a C_{18} column (Fig. 4.14). Acetonitrile-water was used as a mobile phase.

For the on-line NMR detection, the analytes eluted from column were trapped one by one onto separate SPE cartridges, and hereafter transported into the NMR flow-cell. LC/UV/SPE-NMR and LC/UV/MS allowed the characterization of constituents of Greek *H. perforatum*, mainly naphtodianthrones (hypericin,

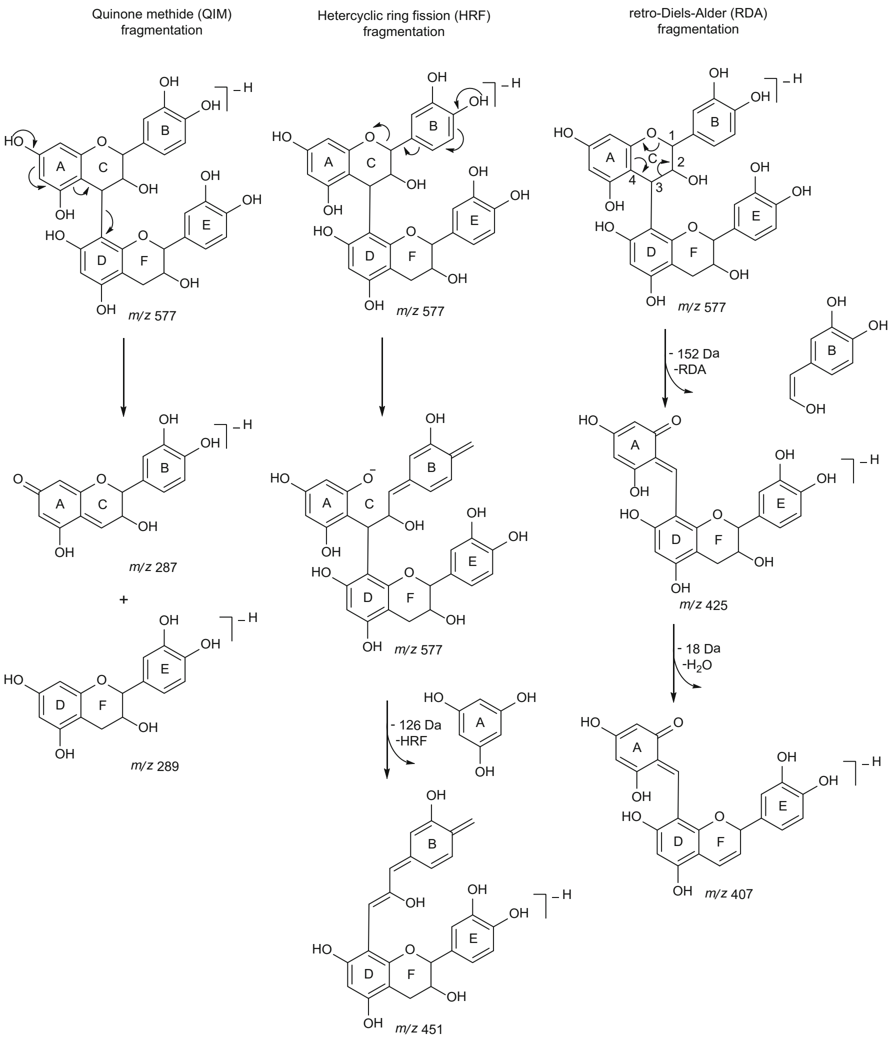


Fig. 4.13 Main fragmentation pathways of B-type proanthocyanidins illustrated for the dimer identified in the infusion from the barks of *Hancornia speciosa* (Ref. Rodrigues et al. (2007))

pseudohypericin, protohypericin, protopseudohypericin), phloroglucinols (hyperforin, adhyperforin), flavonoids (quercetin, quercitrin, isoquercitrin, hyperoside, astilbin, miquelianin, I3,II8-biapigenin) and phenolic acids (chlorogenic acid, 3-O-coumaroylquinic acid) (Fig. 4.15). Two phloroglucinols (hyperfirin and adhyperfirin) were detected for the first time, which have been previously reported to be precursors in the biosynthesis of hyperforin and adhyperforin (compounds **9** and **10**, Fig. 4.15).

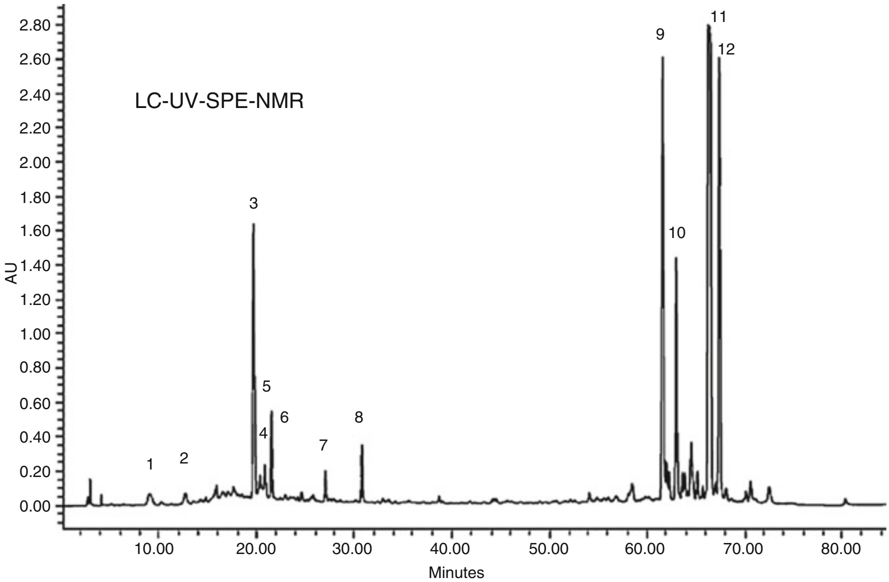


Fig. 4.14 Chromatographic profile of *Hypericum perforatum* extract at 270 nm as obtained by **LC-PDE/SPE-NMR** instrumentation. The peaks under study, which are denoted **1**–**12**, were trapped three times each in the same SPE cartridge (Ref. Tatsis et al. (2007))

The identification of novel phloroglucinols **9** and **10** (named hyperfirin and adhyperfirin, respectively) was based on measured *m*/*z* values of 467 and 481 of $[M\text{-}H]^-$ ions, respectively. The mass data and especially the MS/MS results showed that these phloroglucinols are homologues of hyperforin (**11**) and adhyperforin (**12**). The MS/MS fragmentation pattern analysis indicated the absence of a prenyl chain compared to those of **11** and **12**. The ^1H NMR spectra obtained by the LC/SPE-NMR (Fig. 4.16) were fully compatible with the structures of acylphloroglucinols **9** and **10**, which were reported as biosynthetic intermediates of hyperforin and adhyperforin (Adam et al. 2002; Bystrov et al. 1975).

The prenylated phloroglucinol hyperforin is thought to be an essential component for the anti-depressant activity of St. John's Wort (*Hypericum perforatum*), but this molecule is highly unstable undergoing to facile oxidative degradation (Trifunović et al. 1998; Verotta et al. 1999; Vugdelija et al. 2000; Vajs et al. 2003). The degradation products of hyperforin were the subject of numerous studies involving different LC-hyphenated techniques, mostly LC/MS (Wang et al. 2004; Ang et al. 2004; Fuzzati et al. 2001; Wolfender et al. 2003). In one of them (Wolfender et al. 2003) the application of on-flow and stop-flow LC/NMR and LC/MS/MS of the extract revealed the structures of few products of oxidative degradation of hyperforin.

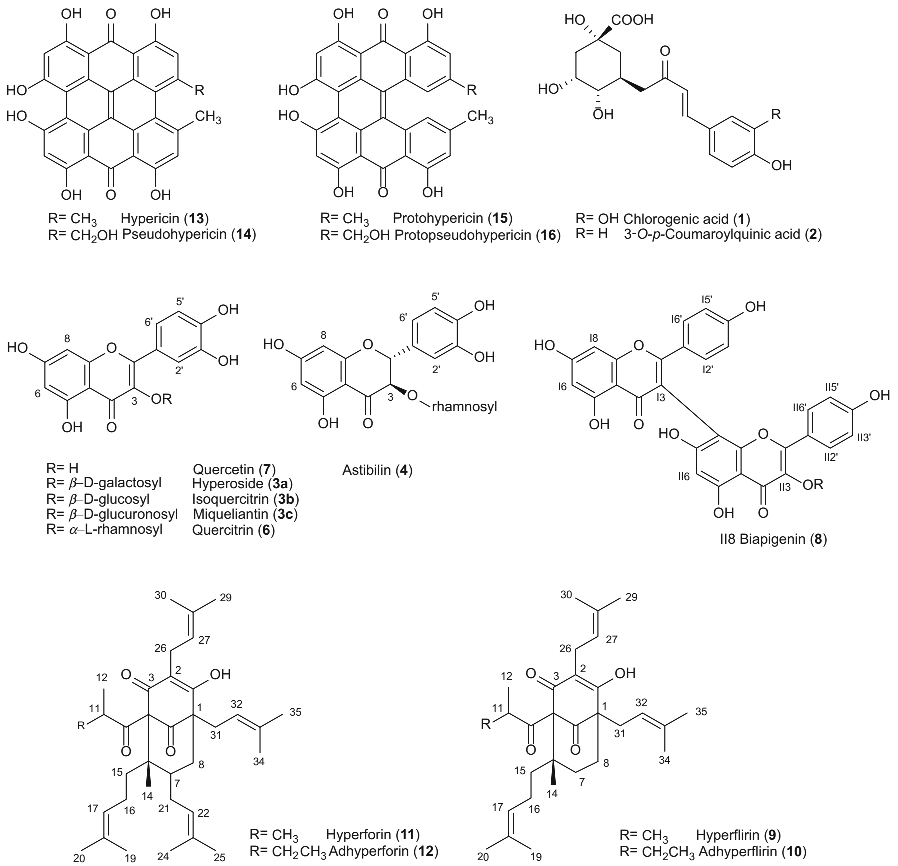


Fig. 4.15 Structures of the major compounds present in Greek *H. perforatum* extract identified by LC/UV/SPE-NMR and LC/UV/ESI MS (Ref. Tatsis et al. (2007))

Example #4

An attempt to analyze a mixture of secondary metabolites (secoiridoids, xanthones and* C*-glucoflavones) of Gentianceae species by application of diffusion-ordered spectroscopy (DOSY) (unpublished results)

Nowadays, and thanks to the developments of NMR, it is not always necessary to separate the components of a mixture in order to obtain spectroscopic information from its constituents. The mixture could be directly submitted to NMR and the acquisition is implemented in a way that allows the data from the components to be obtained with no previous separation. The most important procedures are based on differences in the translational diffusion and NMR (diffusion ordered sectroscopy, DOSY) and relaxation times (so called relaxation editing) of the components in the mixture. The survey of these methods is presented in a review article by R. Novoa-Carballal et al. (2011). Unlike other physical methods used for mixture analysis, such as LC/MS or LC/NMR, DOSY does not require a prior separation. DOSY has

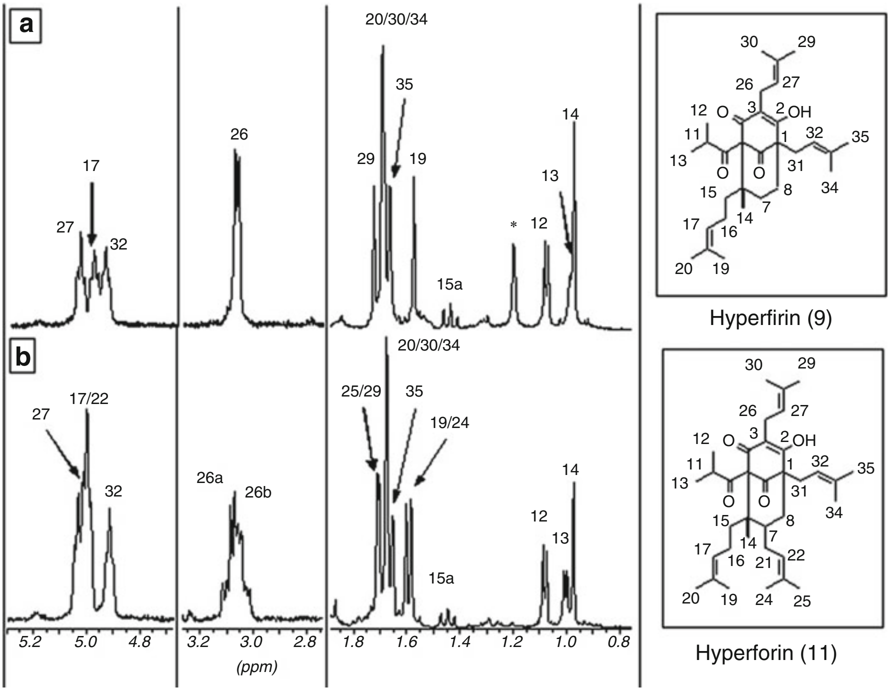


Fig. 4.16 Selected regions of the 400 MHz ^{1}H NMR after triple trapping on the same cartridge of (**a**) **9**, hyperfirin, and (**b**) **11**, hyperforin. The asterisk denotes an unknown peak (Ref. Tatsis et al. (2007))

been utilized as a non-invasive technique which involves the separation of the NMR peaks corresponding to individual components of mixtures based on differences of their diffusion coefficients, and therefore differences in the size and shape of the molecule, as well as physical properties of the surrounding environment such as viscosity, temperature, etc. (Morris 2002). The two dimensional DOSY spectrum represents chemical shifts and diffusion coefficients in two orthogonal directions, and the signals of the individual components should be effectively resolved in the diffusion dimension. One of the recent examples involving application of the technique in the field of the natural product chemistry is a flavonoid mixture analysis by DOSY (Cassani et al. 2012). However, as it was pointed out in this paper, the method struggles when diffusion coefficients are very similar and/or when spectra are highly overlapped. Nearly in all (except the simplest) mixtures, some signals overlap. In heavily overlapped spectra, interpretation of the data can be prevented (see Fig. 4.17).

In course of our studies of secondary metabolites of wild-growing plant species from the central part of Balkan belonging to Gentianaceae family three classes of compounds were encountered typical for the family: secoiridoids, C-glucoflavones and xanthones (Aljančić et al. 2008). In these studies the "tradititional" method involving the application of various preparative chromatographic techniques for

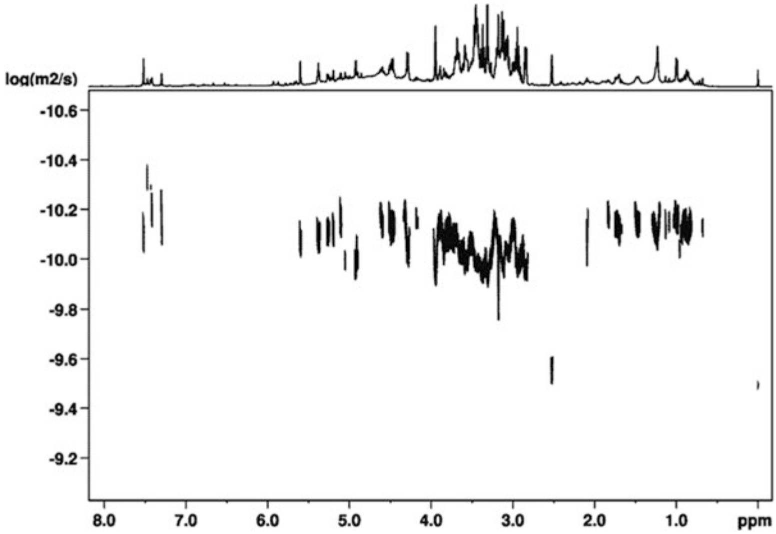


Fig. 4.17 DOSY (500 MHz) spectrum in DMSO-d_6 of the crude MeOH extract of leaves of *Gentiana lutea*

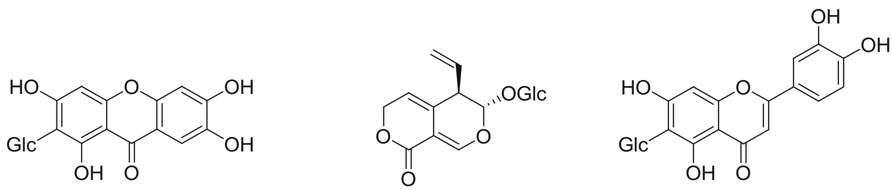
isolation of pure compounds was applied. In order to develop more efficient method for the investigation of such extracts, preliminary DOSY experiments were carried out on the MeOH extract of leaves of the very well known medicinal plant *Gentiana lutea* (Yellow Gentian), containing the above mentioned three classes of secondary metabolites (Šavikin et al. 2009). As it could be seen, the DOSY of this extract (Fig. 4.17) is the typical example of the severe overlap which doesn't allow the interpretation of the data.

At the same time, the DOSY spectrum of the artificial mixture of three constituents, mangiferin (*C*-glucoxanthone), gentiopicroside (secoiridoid) and isoorientin (*C*-glucoflavone) (Fig. 4.18), detected in the *G. lutea* extract using LC UV/ ESI MS (not shown), was recorded (Fig. 4.19).

With exception of the majority of glycosidic signals and a vinyl proton at δ 7.40 the resonances of gentiopicroside are well resolved on the diffusion axis. Due to the very similar diffusion coefficients, the signals of *C*-glucosides mangiferin and isoorientin are overlapped.

At the same in DOSY spectrum of a binary mixture of mangiferin and gentiopicroside, only some sugar signals are not resolved on the diffusion axis (Fig. 4.20).

There are some processing techniques for overcoming the problem of signals overlap, such as multi-exponential fitting (Nilsson et al. 2006) and multivariate methods (Nilsson and Morris 2008). Another approach to significantly reduce the overlap is extending the 2D DOSY experiment to 3D DOSY using HMQC, (Barjat et al. 1998) COSY, (Wu et al. 1996) TOCSY, (Viel and Caldarelli 2008) or NOESY (Gozansky and Gorenstein 1996). Unfortunately, much longer data acquisition



Mangiferin (*C*-glucoxanthone) Gentiopicroside (secoiridoid) Isoorientin (*C*-glucoflavone)

Fig. 4.18 The structure of three components of the MeOH extracts of the leaves of *G. lutea*, containing *ca*. 15 constituents (According to LC/UV/ESI MS)

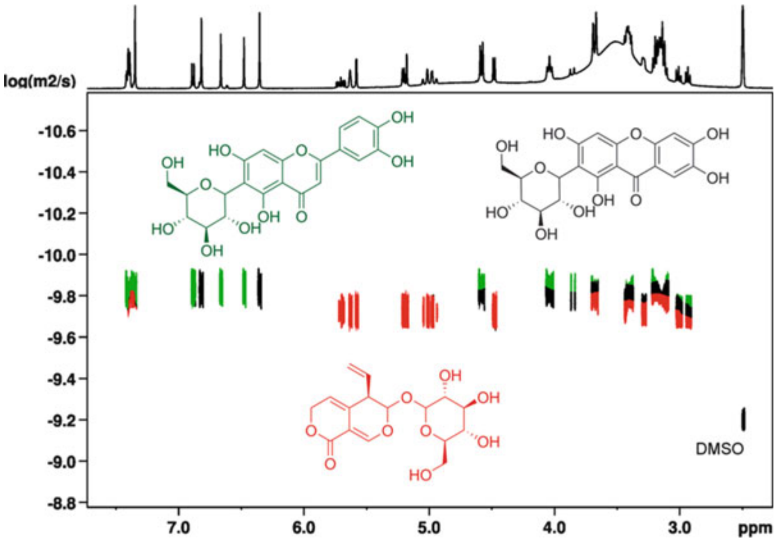


Fig. 4.19 DOSY (500 MHz) spectrum in DMSO-d$_6$ of the mixture three constituents of *G. lutea* extract, mangiferin (*black*) + gentiopicroside (*red*) + isoorientin (*green*)

times and more complicated processing are required for 3D experiments, which can still fail to resolve complex mixtures. Peak overlap can also be efficiently reduced by suppressing the multiplet structure, as in method called pure shift NMR (Nilsson and Morris 2007). It has been also shown that performing DOSY in a matrix with which the analytes interact differentially can resolve signals from similar compounds that would otherwise show the same diffusion. In this method, called matrix-assisted DOSY the interaction of the analytes with the matrix modulates the average diffusion coefficients, as different mixture components bind to the matrix to different extents.

Adding matrix to the mixture can also sometimes helps to resolve the spectral overlap by causing differential chemical shift changes (Tormena et al. 2010). It has been demonstrated that the component spectra of a mixture of isomers with nearly identical diffusion coefficients (catechol, resorcinol, and hydroquinone) can be

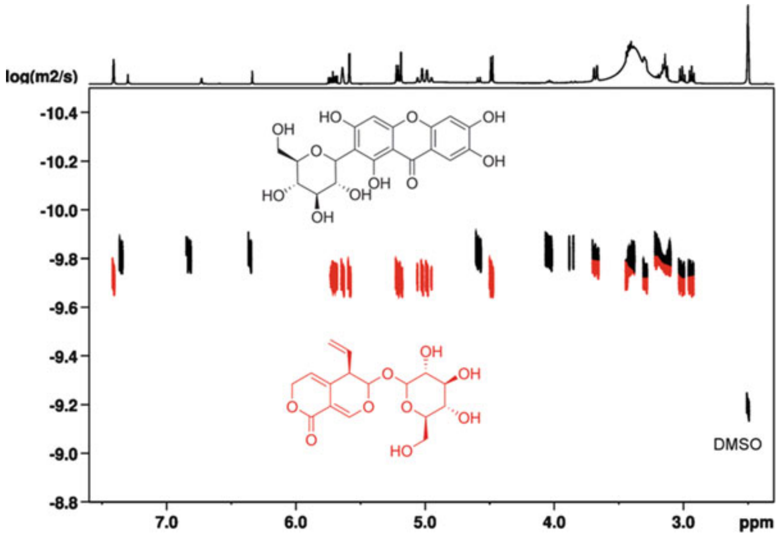


Fig. 4.20 DOSY experiments for mixture of mangiferin (*black*) + gentiopicroside (*red*) in DMSO-d_6

resolved using matrix-assisted DOSY, in which diffusion is perturbed by the addition of a surfactant. The isomeric species are then resolved due to their different degrees of interaction with micelles or reversed micelles formed by the addition of a surfactant (Evans et al. 2009).

Another example is dissolving the two naturally-occurring epimers of naringin in an aqueous solution of β-cyclodextrin which caused both shift and diffusion changes, allowing the signals of the epimers to be distinguished. Chiral matrix-assisted DOSY has the potential to allow simple resolution and assignment of the spectra of epimers and enantiomers (Adams et al. 2011).

According to this, matrix-assisted DOSY method has great potential for the analysis of complex mixtures, such as those common in natural product chemistry.

3 Conclusion

As shown in the examples given, hyphenated techniques – in which chromatographic separations are coupled with powerful spectroscopic techniques such as MS and NMR, as well as diffusion-ordered NMR (DOSY) experiments provide a great deal of information about the content and nature of constituents of the crude plant extracts. These methods are playing an increasingly important role as strategic tools for screening or secondary metabolite profiling aimed at distinguishing between already known compounds (dereplication) and new molecules directly in crude plant extracts. They are very useful when dealing with large numbers of extracts because unnecessary isolation of known compounds is avoided. At the same time, after the preliminary analyses using the hyphenated methods, the isolation of pure compounds, for complete structural elucidation and biological or pharmacological testing, is still needed.

Acknowledgements This research was supported by the Ministry of Education and Science of the Republic of Serbia (Grant no. 172053).

References

Adam P, Arigoni D, Bacher A, Eisenreich W (2002) Biosynthesis of hyperforin in *Hypericum perforatum*. J Med Chem 45:4786–4793

Adams RW, Aguilar JA, Cassani J, Morris GA, Nilsson M (2011) Resolving natural product epimer spectra by matrix-assisted DOSY. Org Biomol Chem 9:7062–7064

Aljančić IS, Vajs VE, Tešević VV, Milosavljević SM (2008) Some wild-growing plant species from Serbia and Montenegro as the potential sources of drugs. Curr Pharm Des 14:3089–3105, and references therein

Aljančić IS, Pešić M, Milosavljević SM, Todorović NM, Jadranin M, Milosavljević G, Povrenović D, Banković J, Tanić N, Marković ID, Ruždijić S, Vajs VE, Tešević VV (2011) Isolation and biological evaluation of jatrophane diterpenoids from *Euphorbia dendroides*. J Nat Prod 74:1613–1620

Ang CYW, Hu L, Heinze TM, Cui Y, Freeman JP, Kozak K, Luo W, Mattia A, Dinovi M (2004) Instability of St. John's Wort (*Hypericum perforatum* L.) and degradation of hyperforin in aqueous solutions and functional beverage. J Agric Food Chem 52:6156–6164

Barjat H, Morris GA, Swanson AG (1998) A three-dimensional DOSY-HMQC experiment for the high-resolution analysis of complex mixtures. J Magn Reson 131:131–138

Behrens A, Maie N, Knicker H, Kögel-Knabner I (2003) MALDI-TOF mass spectrometry and PSD fragmentation as means for the analysis of condensed tannins in plant leaves and needles. Phytochemistry 62:1159–1170

Bringmann G, Lang G (2003) Full absolute stereostructures of natural products directly from crude extracts: the HPLC-MS/MS-NMR-CD 'triad'. In: Müller WEG (ed) Marine molecular biotechnology. Springer, Berlin/Heidelberg, pp 89–116

Bystrov NS, Chernov BK, Dobrynin VN, Kolosov MN (1975) The structure of hyperforin. Tetrahedron Lett 32:2791–2794

Cantos E, Espin JC, Tomas-Barberan FA (2002) Varietal differences among the polyphenol profiles of seven table grape cultivars studied by LC-DAD-MS-MS. J Agric Food Chem 50:5691–5696

Cassani J, Nilsson M, Morris GA (2012) Flavonoid mixture analysis by matrix-assisted diffusion-ordered spectroscopy. J Nat Prod 75:131–134

Covey TR, Lee ED, Henion JD (1986) High-speed liquid chromatography/tandem mass spectrometry for the determination of drugs in biological samples. Anal Chem 58:2453–2460

Evans R, Haiber S, Nilsson M, Morris GA (2009) Isomer resolution by micelle-assisted diffusion-ordered spectroscopy. Anal Chem 81:4548–4550

Fenn JB, Mann M, Meng CK, Wong SF, Whitehouse CM (1989) Electrospray ionization for mass spectrometry of large biomolecules. Science 246(4926):64–71

Flamini R (2003) Mass spectrometry in grape and wine chemistry. Part I: polyphenols. Mass Spectrom Rev 22:218–250

Fuzzati N, Gabetta B, Strepponi I, Villa F (2001) High-performance liquid chromatography–electrospray ionization mass spectrometry and multiple mass spectrometry studies of hyperforin degradation products. J Chromatogr A 926:187–198

Gaskell SJ (1997) Electrospray: principles and practice. J Mass Spectrom 33:677–688

Gođevac D, Tešević V, Veličković M, Vajs V, Milosavljević S (2010) Polyphenolic compounds in seeds from some grape cultivars grown in Serbia. J Serb Chem Soc 75:1641–1652

Gómez-Alonsoa S, García-Romeroa E, Hermosín-Gutiérrez I (2007) HPLC analysis of diverse grape and wine phenolics using direct injection and multidetection by DAD and fluorescence. J Food Comp Anal 20:618–626

Gozansky EK, Gorenstein DG (1996) DOSY-NOESY: diffusion-ordered NOESY. J Magn Reson B 111:94–96

Gu L (2012) Analysis methods of proanthocyanidins. In: Xu Z, Howard LR (eds) Analysis of antioxidant-rich phytochemicals. Wiley-Blackwell, Chichester, pp 247–274, and references therein

Gu L, Kelm MA, Hammerstone JF, Zhang Z, Beecher G, Holden J, Haytowitz D, Prior RL (2003) Liquid chromatographic/electrospray ionization mass spectrometric studies of proanthocyanidins in foods. J Mass Spectrom 38:1272–1280

Guyot S, Doco T, Souquet JM, Moutounet M, Drilleau JF (1997) Characterization of highly polymerized procyanidins in cider apple (*Malus sylvestris* var. *Kermerrien*) skin and pulp. Phytochemistry 44:351–357

Henion JD, Thomson BA, Dawson PH (1982) Determination of sulfa drugs in biological fluids by liquid chromatography/mass spectrometry/mass spectrometry. Anal Chem 54:451–456

Jadranin M, Pešic M, Aljančić IS, Milosavljević SM, Todorović NM, Podolski-Renić A, Banković J, Tanić N, Marković I, Vajs VE, Tešević VV (2013) Jatrophane diterpenoids from the latex of *Euphorbia dendroides* and their anti-P-glycoprotein activity in human multi-drug resistant cancer cell lines. Phytochemistry 86:208–217

James AT, Martin AJP (1952) Gas–liquid partition chromatography: the separation and micro-estimation of volatile fatty acids from formic acid to dodecanoic acid. Biochem J 50:679–680

Jaroszewski JW (2005) Hyphenated NMR methods in natural products research, part 2: HPLC-SPE-NMR and other new trends in NMR hyphenation. Planta Med 71:795–802

Li H-J, Deinzer ML (2007) Tandem mass spectrometry for sequencing proanthocyanidins. Anal Chem 79:1739–1748

Morris GA (2002) Advances in NMR. In: Grant DM, Harris RK (eds) Encyclopedia of nuclear magnetic resonance, vol 9. Wiley, Chichester, p 35

Newman RH, Porter LJ, Foo LY (1987) High-resolution ^{13}C NMR studies of proanthocyanidin polymers (condensed tannins). Magn Reson Chem 25:118–124

Nilsson M, Morris GA (2007) Pure shift proton DOSY: diffusion-ordered ^{1}H spectra without multiplet structure. Chem Commun 933–935

Nilsson M, Morris GA (2008) Speedy component resolution: an improved tool for processing DOSY data. Anal Chem 80:3777–3782

Nilsson M, Connell MA, Davis AL, Morris GA (2006) Biexponential fitting of diffusion-ordered NMR data: practicalities and limitations. Anal Chem 78:3040–3045

Novoa-Carballal R, Fernandez-Megia E, Jiménez C, Riguera R (2011) NMR methods for unravelling the spectra of complex mixtures. Nat Prod Rep 28:78–98

Pesic MM, Bankovic JZ, Aljancic IS, Todorovic NM, Jadranin MB, Vajs VE, Tesevic VV, Vuckovic IM, Momcilovic MB, Markovic ID, Tanic NT, Ruzdijic SD (2011) New anti-cancer characteristics of jatrophane diterpenes from *Euphorbia dendroides*. Food Chem Toxicol 49:3165–3173

Prior RL, Gu L (2005) Occurrence and biological significance of proanthocyanidins in the American diet. Phytochemistry 66:2264–2280

Reed JD, Krueger CG, Vestling MM (2005) MALDI-TOF mass spectrometry of oligomeric food polyphenols. Phytochemistry 66:2248–2263

Rodrigues CM, Rinaldo D, dos Santos LC, Montoro P, Piacente S, Pizza C, Hiruma-Lima CA, Souza Brito Alba RM, Vilegas W (2007) Metabolic fingerprinting using direct flow injection electrospray ionization tandem mass spectrometry for the characterization of proanthocyanidins from the barks of *Hancornia speciosa*. Rapid Commun Mass Spectrom 21:1907–1914

Šavikin K, Menković N, Zdunicć G, Stević T, Radanović D, Janković T (2009) Antimicrobial activity of *Gentiana lutea* L. extracts. Z Naturforsch C 64:339–342

Stanković M, Tešević V, Vajs VE, Todorović N, Milosavljević SM, Godjevac DM (2008) Antioxidant properties of grape seed extract on human lymphocyte oxidative defence. Planta Med 74:730–735

Tatsis EC, Boeren S, Exarchou V, Troganis AN, Vervoort J, Gerothanassis IP (2007) Identification of the major constituents of *Hypericum perforatum* by LC/SPE/NMR and/or LC/MS. Phytochemistry 68:383–393

Tormena CF, Evans R, Haiber S, Nilsson M, Morris GA (2010) Matrix-assisted diffusion-ordered spectroscopy: mixture resolution by NMR using SDS micelles. Magn Reson Chem 48:550–553

Trifunović S, Vajs V, Macura S, Juranić N, Djarmati Z, Jankov R, Milosavljević S (1998) Oxidation products of hyperforin from *Hypericum perforatum*. Phytochemistry 49:1305–1310

Vajs V, Vugdelija S, Trifunović S, Karadžić I, Juranić N, Macura S, Milosavljević S (2003) Further degradation product of *Hypericum perforatum* (St. John's Wort). Fitoterapia 74:439–444

Verotta L, Appendino G, Belloro E, Jakupovic J, Bombardelli E (1999) Furohyperforin, a prenylated phloroglucinol from St. John's Wort (*Hypericum perforatum*). J Nat Prod 62:770–772

Viel S, Caldarelli S (2008) Improved 3D DOSY-TOCSY experiment for mixture analysis. Chem Commun 2013–2015

Vugdelija S, Vajs V, Trifunović S, Djoković D, Milosavljević S (2000) A new hetereocyclization product of Adhyperforin from *Hypericum perforatum* (St. John's Wort). Molecules 5:M158

Wang Z, Ashraf-Khorassani M, Taylor LT (2004) Air/light-free hyphenated extraction/analysis system: supercritical fluid extraction on-line coupled with liquid chromatography-UV absorbance/electrospray mass spectrometry for the determination of hyperforin and its degradation products in *Hypericum pertoratum*. Anal Chem 76:6771–6776

Wolfender JL, Ndjoko K, Hostettmann K (2001) The potential of LC-NMR in phytochemical analysis. Phytochem Anal 12:2–22

Wolfender J-L, Verotta L, Belvisi L, Fuzzati N, Hostettmann K (2003) Structural investigations of isomeric oxidised forms of hyperforin by HPLC-NMR and HPLC-MS. Phytochem Anal 14:290–297

Wu D, Sorg B, Hecker E (1994) Oligocyclic and macrocyclic diterpenes in Thymelaeaceae and Euphorbiaceae occurring and utilized in Yunnan (Southwest China) 6. Tigliane type diterpene esters from latex of *Euphorbia prolifera*. Phytother Res 8:95–99

Wu D, Chen A, Johnson CS (1996) Three-dimensional diffusion-ordered NMR spectroscopy: the homonuclear COSY–DOSY experiment. J Magn Reson Ser A 121:88–91

Chapter 5
Medicinal and Aromatic Plants (MAP): How Do They Adapt to the Environment?

A. Cristina Figueiredo and José G. Barroso

Abstract Taking the best out of plants, namely from medicinal and aromatic plants (MAP), involves understanding their structural and chemical features, in connection with their biological roles, and, no less important, realizing the principles behind their use by plants. Plants are exquisitely sensitive to biotic and abiotic environmental changes. Nevertheless, plants are unique in their renewal capacity, since they can produce meristems during their life time, and in showing developmental plasticity, i.e., the ability to change its development in response to diverse factors. This means that a plant can very often survive, despite having been partially eaten, and/or that a single plant genotype can be expressed under different phenotypes, and the one(s) expressed will depend on local environmental stimuli. From man point of view this may have importance, as these different phenotypes can be associated to different chemotypes, which may have a tremendous implication on the biological activity of plant man-valued products and on their commercial value. Some of the structural and chemical traits involved in plant-abiotic and plant-biotic defence, and in attraction interactions, with some examples on how man uses some of these traits and mechanisms to his benefit, are here revised. Apoplastic barriers, glandular or non-glandular structures, mimicry, plant, or plant parts, movements, abiotic environmental relationships, interaction with pathogens and phytophagous and attraction of pollinators and seed dispersers, are discussed. In each case, plants use both constitutive, as well as inducible traits. Nevertheless, it is noteworthy that constitutive traits are not always constant. Indeed, constitutive structural and chemical features can be permanent or transient, depending on the plant developmental stage. Despite all available traits, plants are endowed with self-defence mechanisms against their own toxins. The awareness of plant structural and chemical adaptative features purposes can complement the knowledge derived from evolutionary studies, and provide us the know-how to project and develop plants with improved traits, or procedures based on their *modus operandi* and/or better plant (nano-)products.

A.C. Figueiredo (✉) • J.G. Barroso
Centro de Estudos do Ambiente e do Mar Lisboa, Faculdade de Ciências, Universidade de Lisboa, CBV, DBV, Lisboa 1749-016, Portugal
e-mail: acsf@fc.ul.pt

Á. Máthé (ed.), *Medicinal and Aromatic Plants of the World*, Medicinal and Aromatic Plants of the World 1, DOI 10.1007/978-94-017-9810-5_5

Keywords Medicinal and aromatic plants • MAP • Abiotic • Biotic • Structural features • Chemical features • Self-defence features

1 Introduction

Since ancient times plants have played a major role as food providers and as a physical and mental healing source. For their ornamental value, or for their use in horticultural therapy (Adevi and Lieberg 2012), they are known to reduce stress and improve well-being. However, many plants are toxic and they are used in particular socio-cultural contexts, namely as a tool for hunting, or fishing, or in rituals of superstition and magic. But human beings rely on plants for many beneficial effects and commodities. Plants provide many food and non-food products, including cloths, wood, dyes, cosmetics, and medicines, among many others products, or they serve as raw material for diverse commercial and industrial fine chemicals.

Medicinal and aromatic plants (MAP) embrace a diversity of such species, that have in common producing a wide range of natural compounds important to man and animals. Although many plants combine both the aromatic and medicinal properties, not all possess this dual characteristic. There are numerous examples of non-aromatic plants that produce active principles having valuable pharmacological properties, such as, for instance, foxglove (*Digitalis* spp.), whereas others are essentially known and used for their valuable aroma, such as jasmine (*Jasminum* spp.) (Barata et al. 2011).

Throughout the world, there is an ever-increasing interest in MAP, regarding their use, development, cultivation, conservation and sustainable use, as they are of utmost economic and social importance. These plants are also targets for several studies on pharmacognosy, phytochemistry, ethnobotany and biology. In addition to their medicinal value and to being traded as regular commodities, new trends of their use for sauces or jam production, or for flavouring olive oil, vinegar, wine, chocolate, wood, honey, paper or soap, reveal their enormous importance for local and countries' economies (Barata et al. 2011). Nevertheless, the production of MAP, and derived products are not always stable. Being biological systems, they can be negatively affected by many factors occurring prior to- or after harvest, namely natural catastrophes, phytophagy, storage, extraction mechanisms, to name just a few (Figueiredo et al. 2008a).

Given, in most cases, the close relationship between the metabolites produced by-, or the structural adaptations present in, plants and their surroundings, the knowledge on how plants react has important practical applications. Let us not forget that many man used products derived from plants are used by the plant either to deter phytophagous or to attract pollinators. This means that several of these products have some level of toxicity and their action is very much dose-dependent. As a consequence they may, if not correctly used, have negative implications on those using-, or working with them, independently of the route of administration (local or systemic).

So as to know the factors that influence MAP productivity, it is thus important to understand how a plant reacts and adapts to environmental biotic and abiotic challenges. Several studies on the factors affecting volatile components and essential oils production in plants, showed that the response is species specific (Figueiredo et al. 2008a). This chapter deals with medicinal and aromatic plants strategies to adapt to the surrounding environment, and how man has taken, or can take, advantage from these mechanisms.

2 Biological Importance of MAP Adaptation

"We must consider the distinctive characters and the general nature of plants from the point of view of their morphology, their behaviour under external conditions, their mode of generation, and the whole course of their life" [Theophrastus (372–287 B.C.) (transl. A. G. Morton 1981)].

Indeed, as plants are unable to move, they must rely on their morphology and on a variety of strategies to tolerate adverse biotic or abiotic conditions and to interact with pollinators, or otherwise they will perish, either because they will not endure the hostile environment or because they will not be able to propagate.

Plants interact with the environment for one of two reasons: defence from biotic and abiotic factors and/or attraction of pollinators and seed dispersing animals, Table 5.1. In each case, plants use both (a) constitutive, i.e. always present, and (b) inducible, i.e. activated by external factor, physical and chemicals traits. Chemical constitutive traits, as well as some physical ones, can be permanent, or transient, that is, vary with flowering stage, plant age, or other physiological factors (Figueiredo et al. 2008a). Moreover, chemicals involved in plant interactions can both be derived from (a) primary-, i.e. directly involved in growth, development, and reproduction, and/or (b) secondary metabolism, i.e. mostly having an ecological role (Figueiredo et al. 2008a). Nevertheless, it is important to stress that plant interactions rely on all mechanisms, physical and chemical, combined; there is not real boundary that clearly separates them, and they together act as a whole in the plant response.

2.1 Defence from Abiotic and Biotic Factors

Plant biomass yield can be severely affected by high winds, flooding, drought, high or low temperatures, high salinity, inadequate light intensity or nutrients concentration, pollution, amid other abiotic factors (Figueiredo et al. 2008a). Moreover, plants are surrounded by mammals, birds, insects, as well as nematodes, bacteria, viruses, fungi, among others. But if plants depend, in some cases, on some of the living organisms surrounding them, for pollination and seed dispersal, they have also to protect themselves from phytophagous and pathogens. Although the plant

Table 5.1 Structural and chemical mechanisms involved in plant-abiotic and plant-biotic defence and attraction interactions

Defence from abiotic and biotic factors
Structural features
Apoplastic barriers
Glandular or non-glandular structures
Mimicry and plant, or plant parts, movements
Chemical features
Relationship with the abiotic environment
Interference in community development (allelopathy and semiochemicals)
Interaction with pathogens and phytophagous
Attraction of pollinators and seed dispersers
Structural features
Chemical features

resistance or sensitivity is very much plant-specific, depending, *inter alia*, on the genotype and plant physiological stage (Figueiredo et al. 2008a), there are several structural and chemical features that help plants in their abiotic and biotic interactions.

2.1.1 Structural Features

The effect of fire, over-radiation and heat, grazing, pathogen attack, herbivory, for instance, can be mitigated in several ways by physical barriers. Among these, both constitutive and inducible structural features, the (a) apoplastic barriers, (b) glandular and non-glandular structures, and (c) mimicry and movements will be point out.

Apoplastic Barriers

In addition to cellulose and lignin, other classes of compounds, such as cutin, waxes, suberin, silica or callose, provide major plant surface physical (as well as chemical) protection. The outer cell walls of the epidermis of plants aerial parts are coated with a multilayered structure, of variable thickness, the cuticle, mainly composed of the hydrophobic cutin and waxes. In addition to its natural hydrophobic nature, waxes may show a coarse microstructure that enhances water repellence. The cuticle regulates the degree of surface hydration, the emission of volatiles, and protects, for instance, from rainwater leaching, wind erosion, abrasive moving soil, the action of pollutants and infections.

Combining a hydrophobic wax coating with structural roughness (nanostructures), the water-repellent lotus (*Nelumbo nucifera*) leaves, are known to be superhydrophobic, self-cleaning, and antifouling, known as the Lotus-effect

(Hsu et al. 2011; Bhushan 2012). The cuticle from lotus leaf epidermal cells possesses epicuticular wax protuberances, papilla, responsible for the structural roughness. Water droplets stand on the top of these nanostructures surface because air bubbles fill the gaps between them. The self-cleaning effect is of great importance for plants as allows the non-dirty surface to be available for photosynthesis and avoids pathogen contamination.

Suberin can occur not only internally, at the Casparian strip of the root endodermis, but it is also associated with the cork cells of the periderm, forming the outer bark of stems and roots of many species. The presence of this very thick and insulating bark, the cork, has a protection role on *Quercus* suber (cork oak) buds, enabling them to resprout after fire (Pausas et al. 2008). The knowledge on the factors that enable fire resistance and resilience are extremely important in correct forest management policies.

Stinging nettle (*Urtica dioica*), sometimes used as medicinal plant, is an example of a higher plant that possess cells with silica mineralized cell walls. The stinging trichomes (definition below), consist of a single narrow and long cell that ends in small bulb tip and has a saclike base embedded in a multicellular outgrowth. Whereas the cells the of multicellular outgrowth at the base have a common cellulosic cell wall, the long cell shows high rigidity, and roughness, due to the silica cell wall reinforcement and arrangement, that allows it to work as a tiny needle that deters herbivory.

In response to mechanical injury or high temperatures, or just before dormancy, callose is a polysaccharide that is deposited between the cell wall and plasma membrane. Likewise, callose cell-wall appositions, the papillae, are large physical barriers induced at the sites of pathogen attack (Luna et al. 2011).

Non-glandular or Glandular Structures

Along with the obstacles provided by the chemical compounds mentioned above, that mainly reinforce or seal the cell wall, there are additional plant-environment interaction traits, provided by isolated cells or groups of specialized cells, occurring either internally or externally in the plant body. Moreover the external specialized cells can be either non-glandular or glandular, Fig. 5.1.

Among non-glandular external structures, trichomes (discussed below), thorns (modified branches such as those of *Citrus* spp.), spines (modified leaves such as those of *Ferocactus* spp.), and prickles (slender sharp outgrowths from the cortex and epidermis, such as those of *Rosa* spp.) serve as protective structures providing shade (trichomes), reflecting incident radiation or dissipating heat (trichomes, thorns, spines and prickles) and/or reducing plant herbivory (thorns, spines and prickles). According to Agrawal et al. (2000), spines of *Centaurea solstitialis* (yellow star thistle) may not only deter mammalian herbivory, but also deter lepidoptera which are illegitimate flower visitors. Noteworthy is the special plant-herbivore interaction involving the spines of the bull's-horn acacia (*Acacia cornigera*) and ants (*Pseudomyrmex ferruginea*) that protect acacia from

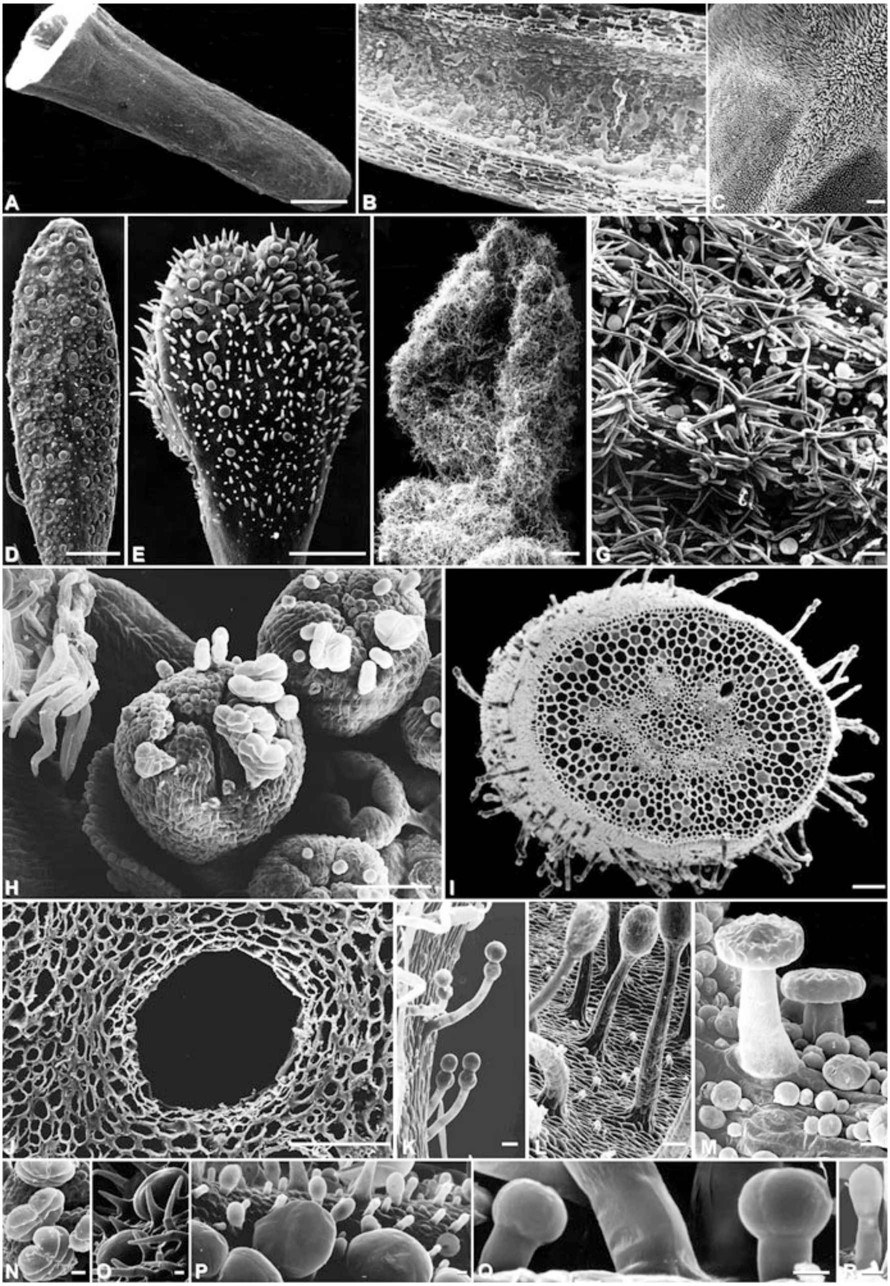


Fig. 5.1 Scanning electron micrographs (SEM) from different types of secretory structures (**a**–**j**) and details of different types of glandular trichomes (**k**–**r**) and non-glandular ones (**o**, **g**). (**a**). Young nectary spur and (**b**). Longitudinal section of the nectary spur of *Limodorum abortivum* (L.) Sw. (Orchidaceae). (**c**). Adaxial glandular epidermis of *Ophrys lutea* (Cav.) (Orchidaceae) labellum. (**D**–**I**). Glandular trichomes on vegetative and reproductive organs. (**d**). Adaxial surface of young leaf and (**e**). Flower bud of *Thymus caespititius* Brot. (Lamiaceae). (**f**). Young leaf and (**g**). Sepal of *Marrubium vulgar*e L. (Lamiaceae). (**h**). Initial stages of development of

herbivores, climbers, and competing vegetation. In return, ants live on the enlarged spine shelters and feed on the extrafloral nectar secreted by foliar nectaries (Heil et al. 2004).

The surface of the aerial parts of plants can also be covered by specialized epidermal cells outgrowths, either glandular or non-glandular trichomes, or other types of secretory cells (nectaries, hydathodes, emergences, osmophores, etc.). This type of external secretory structures are common in plant families such as Araceae, Asteraceae, Cannabaceae, Geraniaceae, Lamiaceae, Orchidaceae, Piperaceae, Plumbaginaceae, Rubiaceae, Rutaceae, Solanaceae, Verbenaceae (Figueiredo et al. 2008a).

Lato senso, trichomes are uni- or multicellular structures that have two types of functions: protective (non-glandular trichomes, or coverage trichomes) or secretory (glandular trichomes) (Tissier 2012). Non-glandular trichomes can form a dense indumentum that prevents water losses, protects against UV radiation and restricts access to insects. The velvety silvery-grey appearance of the surface of many leaf is due to the profusion of this indumentum (*Artemisia* spp., *Helichrysum* spp., *Santolina* spp., etc.). Some species, like common toxic garden plant, oleander (*Nerium oleander*), have stomata sunken in crypts covered with non-glandular trichomes, below the leaf surface. This feature enables reducing the drying effect of warm air and wind.

In some plants, such as the hummingbird plant (*Dicliptera suberecta*), lady's mantle (*Alchemilla vulgaris*), or tomato (*Solanum lycopersicum*) the profusion of non-glandular trichomes exhibits water-repellent properties similar to the Lotus-effect, defined by Hsu et al. (2011) as the Plastron-effect (shield-effect). In lady's mantle, water droplets lay on the trichomes as perfect spheres, without contacting the leaf cuticle, and run off the leaves very easily, without wetting them (Hsu et al. 2011).

The knowledge on both the plant structural features and their popular uses, may also be a source of inspiration for new applications. Based on popular knowledge that strewn kidney beans (*Phaseolus vulgaris*) leaf were used for trapping bed bugs, Szyndler et al. (2013) identified the structural features involved in the mechanical capture by these leaves, in order to design biomimetic surfaces for bed bug

Fig. 5.1 (continued) *Achillea millefolium* L. (Asteraceae) flower heads (Figueiredo and Pais 1994). **I**. Cross-section of *Pittosporum undulatum* Vent. (Pittosporaceae) stem. (**i–j**). Cross-sections of the canals in the (**i**) stem and (**j**) fruit of *Pittosporum undulatum* Vent. (Pittosporaceae). (**k–m**). Glandular trichomes on the leaf of (**k**) *Pittosporum undulatum* Vent. (Pittosporaceae), (**l**) *Drosera capensis* L. (Droseraceae) and (**m**) *Byblis* sp. (Byblidaceae). (**n**). Biseriate trichomes on young florets of *Achillea millefolium* L. (Asteraceae). (**o–p**). Non-glandular trichomes, and peltate and capitate glandular trichomes on the abaxial surface of *Thymus caespititius* Brot. (Lamiaceae) leaf. (**q–r**). Short- and long-stalked capitate trichomes on the abaxial surface of young *Marrubium vulgar*e L. (Lamiaceae) leaf. Bars = 500 μm (**a**, **c–f**); Bars = 100 μm (**b**, **g**, **h–j**, **l**); Bars = 25 μm (**k**, **m**). Bars = 10 μm (**n–r**) (Images reprinted with permission: (**b**). (Figueiredo and Pais 1992), (**h** and **n**). (Figueiredo and Pais 1994). (**f**, **g**, **q** and **r**). (Belhattab et al. 2006). (**i**, **j** and **k**) (Ferreira et al. 2007). (**e**, **o** and **p**). (Figueiredo et al. 2008b) trapping. The capture mechanism involved the physical impaling of bed bug feet (tarsi) by hooked trichomes, in a distinct way from the Velcro-like mechanism of non-piercing entanglement. Using bean leaves as templates, and plant cell walls-like polymers, Szyndler et al. (2013) microfabricated surfaces indistinguishable in geometry from the real leaves, including the trichomes. Despite snagging the bed bugs temporarily they did not hamper their locomotion as effectively as natural bean leaves.

Albeit the enormous diversity of glandular trichomes, in terms of number of cells, size, shape, secretory process, period of secretion and function, they can be divided in two main classes: capitate and peltate trichomes. The complex mixtures of secondary metabolites that accumulate within this structures and their role in plant adaptation are discussed under the chemical features section of this chapter.

Whereas glandular trichomes are examples of external secretory structures, the idioblasts, cavities, canals/ducts, salt glands and laticifers are internal secretory structures common, for instance in Anacardiaceae, Apiaceae, Araceae, Aristolochiaceae, Asteraceae, Calycanthaceae, Fabaceae, Hypericaceae, Lauraceae, Leguminosae, Magnoliaceae, Myoporaceae, Myrtaceae, Pinaceae, Piperaceae, Rutaceae and Saururaceae (Figueiredo et al. 2008a).

From the diversity of internal secretory structures, only one type of idioblast will be mentioned in this structural features section, since the other internal secretory structures accumulate different types of soluble chemical substances, detailed in the next section.

Idioblasts are isolated plant cells specialized in storage of different types of substances. Particularly common is calcium oxalate, which can assume several insoluble forms in the vacuole, depending on the species, the degree of hydration and crystallization conditions. Prismatic crystals are common in the vacuoles from the outer bulb scales of onions (*Allium cepa*); druses, the star-shaped compound crystals, are frequent in oleander (*Nerium oleander*) leaf cells, and on stem cells of hop (*Humulus lupulus*); and the bundles of needle-like crystals, the raphides, occur in banana (*Musa* spp.) pericarp cells, and aloe (*Aloe* spp.) leaf cells. As detailed by Franceschi and Nakata (2005), several studies demonstrated that this biomineralization process functions include, namely, calcium regulation, detoxification (e.g., heavy metals or oxalic acid), ion balance, plant protection and tissue support. Crystal's number, size, shape and placement may discourage herbivory. For example, some studies proposed that extensive crystal formation may provide resistance to bark-boring insects in conifers, and others demonstrated the aversion to oxalate-containing plants by goats. From man point of view, where in abundance, calcium oxalate crystals are released during harvesting and processing of plant tissues, having been correlated with contact dermatitis among field-, flower- and distillery industries workers (references *in* Franceschi and Nakata 2005).

Some plants have evolved specific structural characteristics to face particular stressful abiotic conditions, which are no less important. In conditions of limited water, some plants roll up the leaves, in order to reduce leaf surface and water loss. Other plants evolved taproots that grow deep to reach for water supply. Others accumulate the amino acid proline, or other solutes, creating a water imbalance that allows these plants to extract more water from the soil than other plants. Pneumatophores are special root systems that grow out of water-saturated soils, allowing oxygen to aerate the submerged parts of the root system. The aerenchyma is also a particular leaf parenchyma, developed by aquatic plants, that helps to store oxygen and impairs buoyancy. Pollution, either air pollution (ozone, acid rains, dust), or heavy metals pollution, such as near roadsides, cement factories, tanning industry, mining and quarries works, to name a few, determines gloom, stomata closure and diminishes CO_2 flux (Figueiredo et al. 2008a). Heavy metals tolerant plants tend to be heavy metal type specific, and only few mechanisms of tolerance are well understood. Nevertheless, phytoremediation (bioremediation) of heavy metal polluted soils can be potentially interesting (Sinha et al. 2013). Salty habitats, occurring in deserts, marshes or in seashore line, would cause water lost, wilting and death to the majority of plants, but some became adapted to these environments. These adapted halophytes plants, accumulate sodium and chloride ions in the vacuoles of leaf cells, creating a negative water potential that allows them to take up water more easily. Other halophytes have glandular structures named salt glands. These glands excrete salt that accumulates in the leaf surface and may reduce the loss of water by transpiration. From man point of view, there is a growing interest in not only using some halophytes as a *gourmet* salt substitute to avoid hypertension problems, such as salicornia (*Salicornia* spp.), but also in biosaline agriculture (Lu et al. 2010; Ksouri et al. 2012).

Salt glands, glandular trichomes, as well as other types of external and internal secretory structures, are good examples of combined physical and chemical response by plants. Glandular trichomes, for instance, are by themselves physical barriers, producing a vast array of natural products that the plant uses in its interaction with the environment and man takes profit from them, for instance as essential oils, pharmaceuticals, or ingredients for other applications.

Mimicry and Plant, or Plant Parts, Movements

From the structural point of view there are many other examples whereby plants react to living and non-living factors. Mimicry, although not so much studied in plants, is known for instance in members of the genus *Passiflora* (Passion flowers). Yellow egg-like structures develop in different *Passiflora* plant parts in what is known as *Heliconius* egg mimicry, because *Heliconius* butterflies are dissuaded to oviposit on host plants that possess these egg-like plant structures (Williams and Gilbert 1981; Bhushan 2012).

Also important, and in some cases in the borderline between structural and chemical reactions of plants to environment are plant, or plant parts, movements. Plant movement may occur in response to (a) general factors such as wind, raindrops or passing animals (plants swaying movements), to (b) sun, orienting plant parts either perpendicular or parallel to sun rays, as with sunflowers (*Helianthus annuus*) or cotton (*Gossypium* spp.) and/or to (c) touch as in the sensitive plant (*Mimosa pudica*), or in Venus flytrap (*Dionaea muscipula*). Some of the biochemical processes behind these movements are still not so well understood, but they involve both chemical and physical plant traits. The movement of the bracts in *Dalechampia* species as been interpreted as to avoid nocturnal florivory (Willmer et al. 2009).

2.1.2 Chemical Features

Plants also produce a large variety of organic compounds that help them in their adaptation to abiotic and biotic interactions. These compounds are known as secondary metabolites or natural products, and differ from the primary metabolites, as they have, among others, no direct role in primary metabolism and show a restricted distribution in plants (Figueiredo et al. 2008a). Despite their limited distribution, sometimes limited to a plant species or to a group of plant species, they are chemically very diverse. In a simple way, secondary metabolites can be grouped in terpenes, phenolics and nitrogen-containing compounds which can be characterized by histochemical and analytical procedures. These substances are sometimes stored in the vacuoles in a glycosidic form or accumulated in special secretory structures, Fig. 5.2.

Many MAP accumulate in internal structures, such as idioblasts, cavities, canals/ducts, or in external structures, like trichomes, a blend of monoterpenes, sesquiterpenes, as well as the phenolic phenylpropanoids, and/or sulphur compounds, polyacetyenes, fatty acids, etc., usually extracted from plant material in the form of essential oils, or other complex volatiles extracts. An essential oil is by definition a product obtained by (a) hydro-, steam- or dry-distillation of a plant or of some of its parts, or by (b) a suitable mechanical process without heating, as in the case of *Citrus* fruits (AFNOR 1998; Council of Europe 2010). In this sense, the complex blend of compounds that a plant accumulates in its secretory structures is much more than an essential oil, as the secretion may also contain non-volatile compounds. Nevertheless, for simplicity the terminology adopted will be essential oil. Basil (*Ocimum* spp.), chamomile (*Matricaria* spp.), eucalyptus (*Eucalyptus* spp.), lemon (*Citrus* spp.), peppermint (*Mentha* spp.) or thyme (*Thymus* spp.), are some examples of plants that contain these complex mixtures.

Many essential-oil producing plants were shown to possess a wide range of biological activities. Particularly studied are those properties mostly of man interest, either for health benefit, for agricultural assistance, or for food, and other commodities, preservation purposes (Adorján and Buchbauer 2010). Essentially based on in vitro studies, essential oils and volatile-containing plant extracts have shown several properties such as acaricide and larvicidal (Pohlit et al. 2011), allelopathic (Vokou 2007; Cummings et al. 2012), analgesic and against dementia and Alzheimer's disease (Dobetsberger and Buchbauer 2011), antimicrobial, immuno-modulatory, anti-tumour, anti-apoptotic and anti-angiogenic (Saad et al. 2013), insect repellent (Nerio et al. 2010; Boulogne et al. 2012; Nawrot and Harmatha 2012) and nematotoxic properties (Andrés et al. 2012; Ntalli and Caboni

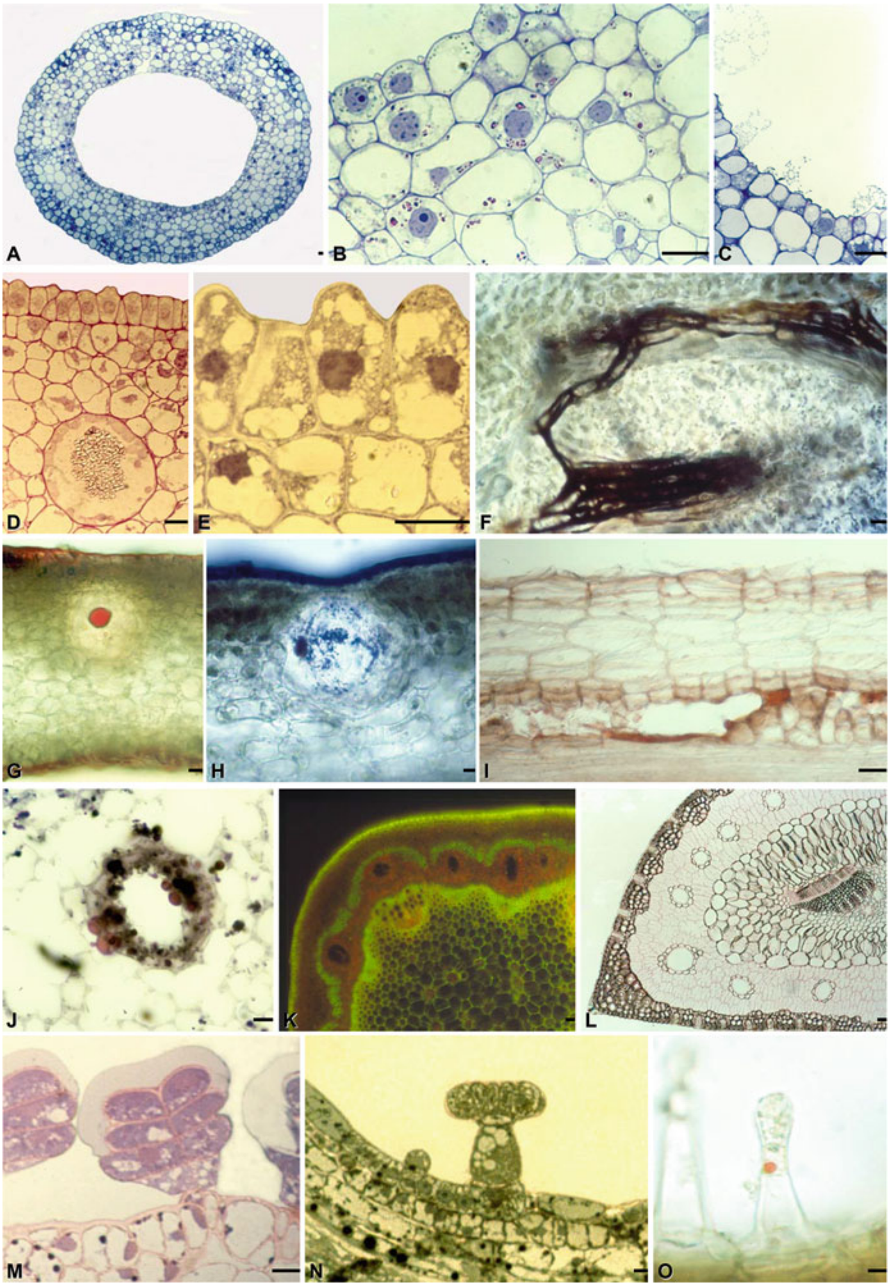


Fig. 5.2 Light microscopy micrographs from different types of secretory structures and histochemical characterization of the natural products present in the secretions (secondary metabolites). (**a**–**c**). Cross section of the nectary spur of *Limodorum abortivum* (L.) Sw. (Orchidaceae). (**d**). Adaxial glandular epidermis of *Ophrys lutea* (Cav.) and of (**e**). *O. fusca* Link (Orchidaceae). (**f**). Lacticifers in the capsules of *Papaver pinnatifidum* Moris (Papaveraceae). (**g**–**h**). Ducts in cross-sections of *Citrus* x *limon* (L.) Burm.f. (Rutaceae) leaf. (**i**–**l**). Canals in (**i**–**j**) longitudinal- and cross-sections of the stem of *Crithmum maritimum* L. (Apiaceae), (**k**) cross-section of

2012; Faria et al. 2013). In addition, essential oils are commercially important for man in flavouring foods and beverages, and making perfumes and cosmetics.

From the plant adaptive interactions point of view, there are an endless number of relations involving chemical communication between plants and other organisms as well as with the environment. Among these the (a) environmental abiotic interactions, (b) the interference in community development, and (c) defence against pathogens and phytophagous, will be highlighted. Indeed, in several cases this separation in different topics results artificial, as in any specific biotic or abiotic system they are all combined. For this reason several examples will fall within more than one topic, i.e., in several cases it is difficult to fix a clear border between the defence against phytophagous, from the interference in community development, as both are interlinked.

Environmental Abiotic Interactions

Albeit the qualitative and quantitative diversity and emission rates, many plant species emit terpenes, from internal or external specialized structures, occurring above (vegetative, flowering and fruit parts) or below ground. Less specialized systems include mesophyll cells occurring in some trees, and examples of more specialized systems are the internal resin ducts in *Pinus*, or the external trichomes of several Lamiaceae or Asteraceae species.

Loreto and Schnitzler (2010) reviewed the influence of abiotic stresses, such as temperature, drought and salt, irradiance, ozone and other oxidants, on the biosynthesis and emission of volatile terpenes. While stressing that the discussion is still enduring they showed examples of the thermoprotective and antioxidant physiological functions of volatile terpenes.

The simplest isoprenoids represent an important class of Volatile Organic Compounds (VOCs) emitted by plants. Not only deciduous trees emit isoprene, but also many plant species emit both isoprene and monoterpenes, such as eucalyptus (*Eucalyptus* spp.), fir (*Abies* spp.), myrtle (*Myrtus* spp.), oak (*Quercus* spp.), spruce (*Picea* spp.) or willow (*Salix* spp.) (Calfapietra et al. 2009).

Abiotic stresses do not only trigger the production of low molecular weight volatile terpenes, such as isoprene, mono- and sesquiterpenes. High-temperature stress induces also the production of heat shock proteins as well as the non-volatile carotenoids and phenolic compounds. Carotenoids are tetraterpenes, that along with

Fig. 5.2 (continued) *Schinus molle* L. (Anacardiaceae) stem and (**l**) cross-section on *Pinus pinaster* Aiton (Pinaceae) needles. (**m**–**o**). Glandular trichomes on (**m**) *Achillea millefolium* L. (Asteraceae) florets, (**n**) *Byblis* sp. (Byblidaceae) and (**o**) *Origanum vulgare* L. (Lamiaceae) leaf. Fresh material: (**f**–**k**, **o**). Historesin sections: (**a**–**e**, **l**–**n**). Staining and double-staining procedures: Toluidine blue: (**a**, **c**). Toluidine blue-periodic acid–Schiff's: (**b**, **m**). Paragon: (**d**). Alum carmine-green iodine (Mirande reagent): (**l**). Dittmar reagent for alkaloids: (**f**). Sudan III: (**g**, **i**, **o**), and Black Sudan B for lipids: (**n**). Nadi reagent for terpenoids: (**h**, **j**). UV autofluorescence: (**k**). Bars = 50 μm (**a**–**o**) other terpenes, such as isoprene or α-tocopherol, stabilize and photoprotect the lipidic phase of the thylakoid membranes (Wahid et al. 2007). In fact, carotenoids, and the diterpenes giberellins, or the triterpene derivatives, brassinosteroids, to mention just a few, have so well-characterized functions in plants that they, despite being products from the secondary metabolism, could be considered as primary metabolites.

In addition to their major attraction role, discussed below, flavonoids (anthocyanins, flavones and flavonols) protect the epidermal cells layers of flowers, fruits, leaves and stems from excessive UV-B. Along with their role as UV screen, the accumulation of anthocyanins observed under high temperature stress, seems to be implicated in increased uptake- and reduced water loss by transpiration (Wahid et al. 2007; Miller et al. 2011; Pallozzia et al. 2013).

Interference in Community Development (Allelopathy and Semiochemicals)

Healthy above and below-ground plants parts, as well as decaying plant material, release a huge diversity of secondary metabolites into the environment. The effect of such compounds in nearby community (other plants, microbes and phytophagous) is known as allelopathy. Although allelopathy often applies to the detrimental effects of plants on their neighbours, in a broader sense it includes also the beneficial interactions. Another commonly used term is that of semiochemicals to include the biologically active molecules that function as signalling compounds between organisms. Usually allelochemicals is mostly used for secondary metabolites, whereas semiochemicals include both primary- and secondary metabolites.

From the most common defensive point of view, allelopathy is known for controlling the vegetation composition, their spacing and speed of germination, and interfering in the decay process. If a plant can reduce the growth of neighbouring plants by releasing chemicals into the soil, it will increase its own access to light, water and nutrients, which means having an adaptive advantage. Allelochemicals, and/or semiochemicals, are also an important part of the defensive role against phytophagous.

The importance of allelopathy in natural systems is, very often, questioned, since it is difficult to prove that this phenomenon actually occurs in nature. If it is easy to show, in an *in lab* system, that plant extracts, or isolated compounds, inhibit the growth of other plant species, it is quite difficult to show that they occur in nature, in a concentration high enough to cause the same effect. In the field, the organic substances present in soil can be bound to soil particles, they can remain long or can be quickly degraded by microbes or lixiviated by rain water, both from above or below-ground plant parts. This is however a promising study area by its agricultural, forest and invasive plants management implications (Cummings et al. 2012; Cipollini et al. 2012).

A number of examples exists where microbes interact with plant allelochemicals, either by being their target, by facing community shifts, and/or by either inhibiting or by improving plants allelopathic effects (Cipollini et al. 2012). Some essential oils constituents, namely the monoterpenes camphene, β-myrcene and α-pinene, or sesquiterpenes such as α-longifolene, slow the growth of fungi that otherwise would promote the decomposition of some trees.

In addition to the most common examples of eucalyptus (*Eucalyptus* spp.) (Zhang et al. 2010) or walnut trees (*Juglans* spp.) (Weston and Duke 2003), the sensitivity of several plants to compounds produced by species of *Artemisia*, *Rosmarinus*, *Salvia*, *Sassafras*, *Satureja*, and *Thymus*, to name just a few, is also known. Camphene, camphor, 1,8-cineole, thujones and α- and β-pinene are some of the essential oil components that have marked seed germination and root respiration inhibitory effect, thus interfering with the spacing between plants and with the composition of the community. It is noteworthy that the toxicity of these compounds is quite often enhanced when they are in mixture, comparatively to the isolated compounds. The mechanism behind the synergistic effect is unknown but may involve the ability of a component of the mixture to inhibit the capacity of detoxification mechanisms of the target organism, or to enhance of absorption/penetration of the mixture.

Allelopathic interactions may be very complex, involving very often more than just one emitter and one receiver, as is the case of the tri-trophic system that includes the white-weed (*Ageratum conyzoides*). This weed often invades cultivated fields and is responsible for significant crop losses in several countries. However, according to Kong et al. (2005), intercropping this weed in citrus orchards leads to an effective suppression of other weeds growth and of several fungal pathogens spore germination, and to a population increase of the predatory mite *Amblyseius newsami*, an effective natural enemy of citrus red mite *Panonychus citri*, thus controlling this major citrus arthropod pest at low and non-harmful levels.

Many other examples exist whereby plant released semiochemicals provide some advantage to the plant, but in other cases the biological significance of the released semiochemicals remains elusive. Seeds of parasitic weeds, such as broomrapes (*Orobanche* spp. and *Phelipanche* spp.), and witchweed (*Striga* spp.), can only germinate in response to specific germination stimulants, present in the rhizosphere of both host- and some non-host plants (Zwanenburg and Pospíšil 2013). Almost all germination stimulants are the carotenoid derived terpene lactones, strigolactones. Strigolactones are considered nowadays to constitute a new class of plant hormones that stimulate the germination of parasitic weeds seeds, act also as branching factors for arbuscular mycorrhizal fungi, as well as repressors of lateral root formation and as inhibitors of shoot branching in order to optimize plant growth and development under diverse environmental conditions. Whether strigolactones developed as an adaptation of plants to life on land, by modifying growth and development in response to suboptimal conditions, is still a matter of debate (Brewer et al. 2013).

Interaction with Pathogens and Phytophagous

Virtually all ecosystems have bacteria, viruses, fungi, nematodes, ants, insects and other herbivores (phytophagous organisms in general), responsible for a number of plant diseases, injuries and/or depletion of plants, or plant parts. In all cases, interactions are two-way stories, that is, pathogens and phytophagous attack mechanisms have adapted together with plants defensive mechanisms.

Pathogen mechanisms of penetrating plant parts, either above or below-ground, involve direct access through the surface, wounds or stomata (Zhang et al. 2013). On the other hand, phytophagous can harm the plant in different ways due to diverse feeding habits. Although there are a number of peculiarities in plant-pathogen or plant-phytophagous interactions, it is currently considered that there are also a number of response similarities (Wondafrash et al. 2013; Zhang et al. 2013).

The different forms of cell wall thickening and strengthening, discussed above in reference to the apoplastic barriers, are one first line of pathogen- or phytophagous-induced defence. Because plants have the ability to replace wounded parts by growing new ones, they do no repair damaged tissues. Instead, they seal and induce death of the wounded area cells to restrict the damage. Cellulose, suberin, lignin and callose cell-wall appositions, are some strategies used by plants to intensify the apoplastic barriers and by this way enhancing mechanical and chemical barriers that block plasmodesmata limiting pathogen movement between adjacent cells.

If the pathogens, or phytophagous, are successful in penetrating all the apoplastic barriers, then an immense and complex array of defence mechanisms are triggered, which are discussed in detail by Wondafrash et al. (2013) and Zhang et al. (2013). Receptor-like protein kinases, defensins, phytoalexins, jasmonic acid and ethylene signalling molecules are implicated in nonspecific defence, but they do not all act at the same time, nor at the same speed, and they are not alone in plant response. Plant resistance "immune system" is triggered by membrane-anchored recognition receptors, followed by activation of mitogen activated protein kinases cascade and downstream transcription factors. The resistance genes (R gene)-mediated response to pathogens usually results in hypersensitive response, and activates salicylic acid-dependent signalling, leading to systemic acquired resistance (Wondafrash et al. 2013; Zhang et al. 2013).

As a part of plants defence systems, phytoalexins, low molecular weight antimicrobial compounds synthesized and accumulated by several plant species in response to their exposure to microorganisms, are of utmost importance. Isoflavonoids in Fabaceace (*Medicago* spp.) and sesquiterpenes and polyacetylenic compounds in Solanaceae (*Solanum* spp.) species are just some examples of phytoalexins. The stilbene resveratrol is an example of some plants induced phytoalexin which has gained attention by being connected with health improvement, namely as anti-aging, and having a positive effect on cardiovascular diseases, cancer and atherosclerosis. In addition to being a stress induced metabolite in several plant species, resveratrol is also constitutively accumulated in peanut (*Arachis hypogaea*). Several biotic and abiotic stimuli are being studied to increase its accumulation in peanut with commercial exploitation purposes (Hasan et al. 2013).

Interestingly, grazing is not always considered detrimental (Hegland et al. 2013). Long-term grazing exclusion may have a negative influence on species renewal and productivity (Jing et al. 2013). In many cases plants regrow quickly after grazing, even producing more branched plants than those that are not grazed. Nevertheless, most frequently plant species deter herbivory by using from simple amino acids, such as L-canavanine (Huang et al. 2011), signalling molecules like the systemin peptide (Pearce 2011) or jasmonates, along with many secondary metabolites, namely terpenes, phenolics, glucosinolates or alkaloids.

L-canavanine is a nitrogen storage compound, isolated originally from jack bean (*Canavalia ensiformis*), closely related to the protein amino acid, L-arginine. The toxic effect of L-canavanine to bacteria, fungi, yeast, algae, plants, insects, and mammals is considered to be due to its incorrect incorporation into proteins, in place of L-arginine. Relying on the structural similarity between L-canavanine and L-arginine, and on therefore the competition between these amino acids for various enzymatic reactions, L-canavanine is now being evaluated as a potential anti-cancer drug due to its toxicity to human cancer cells (Huang et al. 2011).

Semiochemicals, such as diverse type of terpenes (cardenolides from *Digitalis* spp., saponins from *Dioscoreae* spp., etc.), phenols (furanocumarins form *Citrus* spp., tannins from *Punica* spp., isoflavonoids from *Trifolium* spp. etc.) and alkaloids (*Lupinus* spp., *Senecio* spp., etc), play also an important defensive role as feeding deterrents. In conifers as, for instance, fir (*Abies* spp.), pine (*Pinus* spp.) or spruce (*Picea* spp.), a constitutive oleoresin comprising monoterpenes, sesquiterpenes, and diterpene resinic acids accumulates in resin ducts, found in the trunk, twigs and needles. These compounds are toxic to several insects, including the world wide conifer species pest, bark beetles. New, induced traumatic resin ducts are observed in the developing secondary xylem (wood) after insect attack, fungal elicitation, and mechanical wounding. In Norway spruce (*Picea abies*), Martin et al. (2002) showed that methyl jasmonate was able to induce these traumatic resin ducts and terpenes accumulation in the developing xylem, a tissue that, in this species, constitutively lacks axial resin ducts.

Some plants developed both structural and chemical strategies to control ant distribution in plant parts, restricting them to foliage rather than to flowers. These tactics involve physical barriers on or around flowers, foliage extrafloral nectaries, which act as chemical lures, and structural barriers located far from the flower, and/or, volatile organic compounds, used as signals to attract ants to leaves and/or deter them from flowers. In some acacia species, repellency involves at least some volatiles that are known components of ant alarm pheromones, and that are not repellent to beneficial bee pollinators (Willmer et al. 2009).

As a part of their defensive traits, plants do not only use direct defense mechanisms, that is, producing compounds that act directly on the phytophagous, but they also use indirect defence mechanisms, which may deter phytophagy and thus increase in reproductive ability (Quintero et al. 2013). This type of defence includes the release of volatile plant signals that attract natural enemies of the phytophagous, such as predators or parasitoids. *Inter alia*, indirect defence and has been reported in cabbage (*Brassica* spp.), cotton (*Gossypium* spp.), cucumber (*Cucumis* spp.), elm (*Ulmus* spp.), Lima bean (*Phaseolus lunatus*), maize (*Zea mays*) and pine (*Pinus* spp.) (van Poecke and Dicke 2004; Degenhardt 2009).

Interestingly, from a man applicability point of view, the knowledge on plant feeding deterrents may be of commercial importance. Actually, in what concerns food selection by insects, feeding deterrents seem to be more important than feeding attractants or stimulants. Thus, grain or seed protection could be achieved by adding some deterrent components to a commodity, as long as the deterrent satisfied a number of conditions, such as (a) absence of toxicity to people, animal and beneficial insects, (b) causing no taste, smell or other commodity characters changes, (c) showing broad inhibition feeding spectrum, (d) showing toxicity at low doses and long-lasting and (e) being stable, easy to apply, leaving no residues, compatible with other products and with low production cost (Nawrot and Harmatha 2012). Despite plants having so many natural defences, many studies, either by conventional breeding or metabolic engineering (Degenhardt 2009; Lange and Ahkami 2013), attempt to provide crop plants with commercial interesting improved traits against herbivores that do not diminish plant fitness.

2.2 Attraction of Pollinators and Seed Dispersers

Apart from the defensive role of plant structural and chemical traits, many of these also act in combination to attract, reward, and sometimes lure, pollinators and seed dispersers.

Each plant must display its best structural and chemical traits to divert the attention of pollinators and seed dispersers from the other plants, to itself. Visual, tactile and olfactory signals are in this sense particularly important to help attracting animals to flowers and fruits. Again it should be stressed that visual, tactile and olfactory features act in combination, probably potentiating each other in pollinators, and some seed dispersers, memory. Moreover, we should look at these interactions on a broader scale, keeping in mind that visual, tactile and olfactory signals will be able to attract both beneficial and opportunistic individuals, that is, the plant is, sometimes, under opposite pressures and a balance must be found between attraction and defence. Indeed, only for simplicity these mechanisms are dealt with separately.

2.2.1 Structural Features

From an adaptative point of view, pollination performed by animals is probably the most important in terms of evolution. Visual and tactile traits involved in plant–pollinator and plant–seed disperser relationships include, in addition to scent (discussed under chemical features), colour, particular structural features of the flower (corolla tubular or pendant, enclosed or open, with or without nectar guidelines, with or without spurs, with landing platform present or absent, etc.), and a reward which is usually present under the form of nectar and/or pollen. Bees consume both nectar and pollen; birds, butterflies and moths only nectar, and some pollinators are not rewarded at all, but deceived into pollinating a flower, as the example of the spider orchid, discussed below.

Pigments create visual signals and flowers, fruits, as well as other plant parts, use these coloured signals to attract pollinators and seed dispersers. For this reason, colour is more often observed in animal- rather than wind-pollinated plant species. Colour can help animals with plant species selection, flower location and ripeness, and nectar and pollen location within the flower. Petal colours often change over time both associated with flower development and pollination. Thus flowers pigmentation seems to play a major role in pollination process and success. In fruit, colours change as the seeds reach maturity and the fruit ripens, thus ensuring that animals will be attracted to mature fruit and will disperse mature seed (Miller et al. 2011).

Apart from chlorophyll, there are other three main types of pigments which are stored in different plant cells compartments. Carotenoids are terpenoid compounds that accumulate mostly in chromoplasts and are responsible for the yellow, orange and red colours. Flavonoids are phenolic compounds that accumulate in the vacuole. From these, anthocyanins are the most common pigmented flavonoids, responsible for most of the red, pink, purple and blue colours in different plant parts. Anthocyanin colour depends on several factors, such as the degree of hydroxylation and methoxylation groups of the anthocyanin B-ring, the number and type of linked sugars units and on the vacuole pH. Flavones and flavonols are flavonoids that absorb light at shorter wavelengths, invisible to the human eye, but that insects such as bees see as attraction cues. Betalains are nitrogen-containing water-soluble molecules, with restricted distribution, that contribute to red, purple and yellow of some plants.

Bees are able to see in the ultraviolet spectrum and do not perceive the primary colours in the same way as humans do. For instance, if a flower has a corolla with yellow carotenoids at the edge and a yellowish flavonoid in the centre, it will look yellow throughout in visible light. However, for bees, the corolla will be shiny yellow at the edge and with a darker centre. Moreover, many flowers have “honey guides”, some only seen in ultraviolet light, that direct the bees to the nectar.

Nature colour palette has become highly important from man point of view, as artificial dyes are being banned from market. Actually, some natural products are able to give colour without interfering with food taste, particularly carotenoids that are being used as natural colouring agents for food, generally for yellow and/or orange colours (Miller et al. 2011). Moreover, the full set of natural colours is also important in nature based colour charts (RSH 2007), which are standard reference for plant colour identification of landscape management, food colourings, fabric designers and chemical engineering.

As stated earlier, particular structural characteristics of the flower are also very important in plant-pollinator interaction. The flower epidermis, for instance, provides visual (discussed above) and tactile clues for pollinators. Floral epidermal surfaces, composed of conical or papillate cells protruding outwards from the plane of tissue, such as in heartsease (*Viola tricolor*), give a more velvety appearance than parallelepiped forms, as is the case in hibiscus (*Hibiscus* spp.), or than common leaf surfaces as in rubber tree (*Ficus elastica*) leaf. Conical petal epidermal cells vary greatly in overall size, and are usually associated with pollination. Whitney et al. (2011) reviewed the effects of conical petal cells on flower form, plant reproductive success and pollinator behaviour. The authors concluded that although the fitness benefits provided to plants were likely to vary with pollinator and habitat, conical petal cells seemed to play a role in enhancing colour by focusing light into petals. Moreover, the cone shape might have a light-scattering effect, generating a sparkling appearance. Also conical epidermal cells provide a grip surface, whereas flat-celled petals are more slippery, showing thus the importance of tactile cues in pollination.

Fruits and seeds have evolved a variety of dispersal adaptations by water, wind or animals. Also in the case of pollen, some flowers have developed amazing close-by structural relationships with certain animals. Stewart (2013), showed that *Orpheum frutescens* is a flower with an exclusive relationship with a particular bee, the carpenter bee (*Xylocopa* spp.). In order to get the pollen from the unusual type of curled stamens, the carpenter bee has to vibrate its wings at a particular frequency, around 261.6 Hz, corresponding to the musical note middle C (or Do in the fixed-Do solfeggio scale). The vibrations make the flower open the stamens, releasing the pollen, covering the bee with the fine yellow powder. This specific relationship ensures that the flowers' pollen is taken only by the carpenter bee to another flower of the same species.

2.2.2 Chemical Features

The capacity of plants to chemically interact with their environment has already ben pointed out with the examples in reference to the defensive role mechanisms. Nevertheless, several of the chemical substances illustrated earlier are also used in attraction purposes. Again these compounds fall down in the classes of terpenes, phenols, nitrogen-bearing compounds and other primary and/or secondary metabolites. However, these substances are often at reduced levels and/or different ratios.

Plant volatile organic compounds play a major role in pollinator's attraction (Farré-Armengol et al. 2013). Not surprisingly then, the volatiles emission is dynamic and very often coordinated with the pollinator activity cycle. The maximum volatiles emission during the night is usually associated with plants with night pollinators, such as bats, mice, or nocturnal moths. In contrast, in plants with diurnal pollinators, the volatiles emission attains its maximum during the day. The honeysuckle (*Lonicera japonica*), was found to synchronize the strongest odour emission with its nocturnal moth pollinator, and the spider orchid (*Ophrys sphegodes*), is a case of highly specialized system with pheromones-like production (references *in* Figueiredo et al. 2008a). This orchid produces the same type of volatile compounds, in similar relative amounts, as those found in the sexual pheromones of the virgin female pollinator cuticle, the bee *Andrena nigroaenea*. Male bees are attracted by the shape and odour of the spider orchid flower, which resembles the virgin female bee. During the so-called pseudocopula with the flower labella, pollinia are transferred to- and carried by the bee. The works of Schiestl et al. (1999, 2000) showed that the odour changes after the pollination is completed, and that farnesyl hexanoate, until then a trace compound, started to increase. This compound is known to inhibit the insect copula when it is present in large amounts on the female cuticle. After the pollination, the combined morphological changes and ending of the volatiles emission, not only saves plant resources but also redirects the pollinators to the still unpollinated spider orchid flowers.

Examples of particular and/or extreme specificity between floral scent and pollinator, can also be found in Canada thistle (*Cirsium arvense*), between figs (*Ficus* spp.) and fig wasp pollinators, or midst yucca (*Yucca* spp.) and yucca moth (*Tegeticula*). Canada thistle floral emissions do not follow ambient temperature fluctuation; it is maximal when pollinators are abundant, and reduced when florivores are active (Theis et al. 2007).

The yucca flowers open at night and attract the yucca moth. Inside the flower the female moth gathers balls of sticky pollen from the stamens of one plant and carries them to another plant. There the moth enters the flowers and pierces the ovary walls, laying eggs in the vicinity of the tiers of ovules. Depending on the species, the moth either packs the pollen balls into the stigma cavities or rubs the pollen across the stigma surfaces, thus effectively pollinating the flowers. As the ovules develop into seeds, the eggs hatch, and the larvae consume some of the seeds for food. When the larvae are fully grown, they bore their way out of the ovaries and after falling into the ground, they burrow into the soil until the next flowering season, when the adults moths emerge. In all but one species, all reproductive behaviours of moths, specifically mating inside flowers, pollination, and oviposition, take place after sunset when yucca flowers are open and fragrant. In the case of *Yucca filamentosa* volatiles, amongst the twenty-one scent compounds, some commonly released by injured plants (*E*-4,8-dimethylnona-1,3,7-triene), as well as characteristic compounds (two di-oxygenated compounds) were found (Svensson et al. 2005). The release of *E*-4,8-dimethylnona-1,3,7-triene in *Y. filamentosa* is apparently not induced by herbivory, because it is emitted constitutively from undamaged floral tissue. According to Svensson et al. (2005), the combination of unique compounds and low variation in the fragrance blend may reflect highly selective attraction of obligate pollinators to flowers.

The stage of development of the plant organ as well as the plant part (leaf, flower, fruit, etc.) can have a major influence in volatiles composition. As detailed in Figueiredo et al. (2008a), volatiles variability can be particularly obvious in entomophilous flowers, since they can act as orientation clues, and thus the flowers volatiles may be distinct from those of the other plant parts.

Pollen and nectar volatiles play also an equally important role in pollinators' attraction, despite their low abundance. Nevertheless, pollen and nectar have nutritional value to pollinators and seed dispersers. Opposite to bees and birds, ants have a limited potential as pollinators (Willmer et al. 2009), anyhow, they are good seed dispersers. The seed appendages from bleeding heart (*Lamprocapnos spectabilis*) or trillium (*Trillium* spp.) contain oils attractive to ants. After transporting the seeds to the nest, the ants remove the appendages for food and do not harm the seeds.

All the plant physiological variability, along with variations imposed by environment make it difficult to have a broad and integrated structural and chemical picture on the plant-animal interaction. In addition, most studies address just one particular topic of the relationship and not all combined. Parachnowitsch et al. (2012) evaluated the scent, floral morphology, corolla colour and life history traits in plants from three populations of the bee-pollinated *Penstemon digitalis*. The emitted scent differed among populations in a common garden, highlighting the possibility of scent being determined by differential selection pressures. Despite the importance of the flower number and display size, selection favoured scent rather than flower size or colour. Linalool was a direct target of selection and its high frequency in floral-scents pinpointed that further studies of both pollinator- and antagonist-mediated selection on this compound are required for understanding scent evolution.

3 Self-Defence in MAP Adaptation

Despite being able to produce a wide range of chemical substances that are toxic to pathogens and phytophagous, plants are able to protect themselves from these toxic chemicals.

In a way similar to that used by some phytophagous that avoid plant toxins, plant self-defence may include modified receptors or modified enzymes, which do not recognize the toxic compound, avoiding its metabolization. This is the case for L-canavanine producing plants, mentioned earlier (Huang et al. 2011).

Another mechanism of self-defence results from the fact that many plants accumulate compounds in a non-toxic form in specialized cells, and the enzymes that convert them into toxic forms in other cells. The active toxic compound is only produced if the plant is, in any way, wounded and the substrate and the enzyme come together. Moreover, only the phytophagous or mechanically damaged plant part will be injured by the toxic compound. This is the case of cyanogenic glycosides in cassava (*Manihot esculenta*), and glucosinolates in broccoli (*Brassica* spp.). Glucosinolates and their hydrolytic enzymes, myrosinases, are good examples of this type of self-defence, as they are stored in separate cell compartments in the intact plant tissue. However, when glucosinolates mix with myrosinases, as consequence of tissue damage, they are rapidly hydrolyzed to toxic isothiocyanates (mustard oils) and other derived products (Winde and Wittstock 2011).

Probably, the most common way of self-defence in plants is to produce and/or accumulate a panoply of toxins, deterrents, repellents either in the vacuole, in the waxes of the cuticle, or in the different glandular structures, such as trichomes, idioblasts, cavities, canals/ducts and laticifers. Some toxic compounds possessing low water solubility/miscibility are glycosylated prior to being translocated to the vacuole. This glycosylation process turns the otherwise toxic compounds into harmless compounds to the cell, by enabling their cellular transport and vacuole storage. Most commonly, the large majority of plant secondary metabolites such as alkaloids, terpenoids and phenolics accumulate in specialized glandular internal or external structures. This segregation process keeps substances away from the chloroplasts, mitochondria and other cellular enzymatic machinery.

4 Conclusion

In addition to the constitutive structural and chemical traits of the plant integrated whole, both structural and chemical characters can be rapidly induced when plant cells detect the presence of any type of biotic and abiotic stresses. Moreover, both constitutive and induced plant structural and chemical characters play a major role in attracting pollinators and seed dispersing animals.

The knowledge on, and understanding of-, these adaptative mechanisms (structure and biological function) can have positive practical implications from man point of view, when using plants in general, and MAP in particular. There are several examples of how man has used plant adaptative traits on his own benefit. Biomimetics, for example, by imitating biology or nature, allows synthesizing products by artificial mechanisms, which mimic the in nature occurring ones. Replication of the so-called Lotus effect has been used to develop a multiplicity of surfaces with superhydrophobicity, self-cleaning, decreased adhesion, and antifouling properties. Any pest, or disease, that is detrimental for plants will be an important threat to food, or other plant derived products production. In this sense, plant pathogen and/or phytophagous resistance is a field to explore by both conventional and genetic engineering plant breeding methods, to create plants with increased resistance, but that concomitantly do not lose plant fitness. On the other hand, the recognition of allelopathic interactions, and/or the response to elicitors, to name just a few examples, can be used in weed and pest integrated management measures in forestry, agriculture, agroforestry as well as in biodiversity conservation.

It should nevertheless be stressed that, when considering management strategies, it is highly important to keep in mind that changes in structural or chemical traits in a plant species may have restraining effects on the interaction with one agent while representing an attraction to other agents. It is thus of extreme importance considering plant interactions as an integrated whole.

Acknowledgments Partially funded by Fundação para a Ciência e a Tecnologia (FCT), under Pest-OE/EQB/LA0023/2011 and UID/AMB/50017/2013.

References

Adevi AA, Lieberg M (2012) Stress rehabilitation through garden therapy: a caregiver perspective on factors considered most essential to the recovery process. Urban For Urban Green 11:51–58

Adorján B, Buchbauer G (2010) Biological properties of essential oils: an updated review. Flavour Fragrance J 25:407–426

AFNOR (1998) Association Française de Normalisation NF T 75–006

Agrawal AA, Rudgers JA, Botsford LW, Cutler D, Gorin JB, Lundquist CJ, Spitzer BW, Swann A (2000) Benefits and constraints on plant defense against herbivores: spines influence the legitimate and illegitimate flower visitors of yellow star thistle, *Centaurea solstitialis* L. (Asteraceae). Southwest Nat 45:1–5

Andrés MF, Gonzalez-Coloma A, Sanz J, Burillo J, Sainz P (2012) Nematicidal activity of essential oils: a review. Phytochem Rev 11:371–390

Barata AM, Rocha F, Lopes V, Bettencourt E, Figueiredo AC (2011) Medicinal and aromatic plants – Portugal. In: Ozturk M, Ameenah G-FB (eds) Medicinal and aromatic plants of The World, Encyclopedia of Life Support Systems (EOLSS). Developed under the Auspices of the UNESCO, Eolss Publishers, Oxford, http://www.eolss.net

Belhattab R, Larous L, Figueiredo AC, Santos PAG, Costa MM, Barroso JG, Pedro LG (2006) Essential oil composition and glandular trichomes of *Marrubium vulgar*e L. growing wild in Algeria. J Essent Oil Res 18:369–373

Bhushan B (2012) Biomimetics, Biological and medical physics, biomedical engineering. Springer, Berlin/Heidelberg, pp 1–9

Boulogne I, Petit P, Ozier-Lafontaine H, Desfontaines L, Loranger-Merciris G (2012) Insecticidal and antifungal chemicals produced by plants: a review. Environ Chem Lett 10:325–347

Brewer PB, Koltai H, Beveridge CA (2013) Diverse roles of strigolactones in plant development. Mol Plant 6:18–28

Calfapietra C, Fares S, Loreto F (2009) Volatile organic compounds from Italian vegetation and their interaction with ozone. Environ Pollut 157:1478–1486

Cipollini D, Rigsby CM, Barto EK (2012) Microbes as targets and mediators of allelopathy in plants. J Chem Ecol 38:714–727

Council of Europe (2010) European Pharmacopoeia, 7th edn. European Directorate for the Quality of Medicines, Strasbourg, France

Cummings JA, Parker IM, Gilbert GS (2012) Allelopathy: a tool for weed management in forest restoration. Plant Ecol 213:1975–1989

Degenhardt J (2009) Indirect defense responses to herbivory in grasses. Plant Physiol 149:96–102

Dobetsberger C, Buchbauer G (2011) Actions of essential oils on the central nervous system: an updated review. Flavour Fragrance J 26:300–316

Faria JMS, Barbosa P, Bennett RN, Mota M, Figueiredo AC (2013) Bioactivity against *Bursaphelenchus xylophilus*: nematotoxics from essential oils, essential oils fractions and decoction waters. Phytochemistry 94:220–228

Farré-Armengol G, Filella I, Llusia J, Peñuelas J (2013) Floral volatile organic compounds: between attraction and deterrence of visitors under global change. Perspectiv Plant Ecol Evol Syst 15:56–67

Ferreira NJ, Meireles de Sousa IG, Cunha Luís T, Currais AJM, Figueiredo AC, Costa MM, Lima ASB, Santos PAG, Barroso JG, Pedro LG, Scheffer JJC (2007) *Pittosporum undulatum* Vent. grown in mainland Portugal: secretory structures, seasonal variation and enantiomeric composition of the essential oil. Flavour Fragr J 22:1–9

Figueiredo AC, Barroso JG, Pedro LG, Scheffer JJC (2008a) Factors affecting secondary metabolites production in plants: volatile components and essential oils. Flavour Fragrance J 23:213–226

Figueiredo AC, Barroso JG, Pedro LG, Salgueiro L, Miguel MG, Faleiro ML (2008b) Portuguese *Thymbra* and *Thymus* species volatiles: chemical composition and biological activities. Curr Pharm Des 14:3120–3140

Figueiredo AC, Pais MS (1992) Ultrastructural aspects of the nectary spur of *Limodorum abortivum* (L) Sw. (Orchidaceae). Ann Bot 70:325–331

Figueiredo AC, Pais MS (1994) Ultrastructural aspects of the glandular cells from the secretory trichomes and from the cell suspension cultures of *Achillea millefolium* L. ssp. *Millefolium*. Ann Bot 74:179–190

Franceschi VR, Nakata PA (2005) Calcium oxalate in plants: formation and function. Annu Rev Plant Biol 56:41–71

Hasan MM, Cha M, Bajpai VK, Baek K-H (2013) Production of a major stilbene phytoalexin, resveratrol in peanut (*Arachis hypogaea*) and peanut products: a mini review. Rev Environ Sci Biotechnol 12:209–221

Hegland SJ, Lilleeng MS, Moe SR (2013) Old-growth forest floor richness increases with red deer herbivory intensity. For Ecol Manage 310:267–274

Heil M, Baumann B, Kruger R, Linsenmair KE (2004) Main nutrient compounds in food bodies of Mexican *Acacia* ant-plants. Chemoecology 14:45–52

Hsu S-H, Woan K, Sigmund W (2011) Biologically inspired hairy structures for superhydrophobicity. Mater Sci Eng 72:189–201

Huang T, Jander G, de Vos M (2011) Non-protein amino acids in plant defense against insect herbivores: representative cases and opportunities for further functional analysis. Phytochemistry 72:1531–1537

Jing Z, Cheng J, Chen A (2013) Assessment of vegetative ecological characteristics and the succession process during three decades of grazing exclusion in a continental steppe. Ecol Eng 57:162–169

Kong CH, Hu F, Xu XH, Zhang MX, Liang WJ (2005) Volatile allelochemicals in the *Ageratum conyzoides* intercropped citrus orchard and their effects on mites *Amblyseius newsami* and *Panonychus citri*. J Chem Ecol 31:2193–2203

Ksouri R, Ksouri WM, Jallali I, Debez A, Magne C, Hiroko I, Abdelly C (2012) Medicinal halophytes: potent source of health promoting biomolecules with medical, nutraceutical and food applications. Crit Rev Biotechnol 32:289–326

Lange BM, Ahkami A (2013) Metabolic engineering of plant monoterpenes, sesquiterpenes and diterpenes—current status and future opportunities. Plant Biotechnol J 11:169–196

Loreto F, Schnitzler J-P (2010) Abiotic stresses and induced BVOCs. Trends Plant Sci 15:154–166

Lu D, Zhang M, Wang S, Cai J, Zhou X, Zhu C (2010) Nutritional characterization and changes in quality of salicornia bigelovii Torr. during storage. LWT – Food Sci Technol 43:519–524

Luna E, Pastor V, Robert J, Flors V, Mauch-Mani B, Ton J (2011) Callose deposition: a multifaceted plant defense response. Mol Plant Microbe Interact 4:183–193

Martin D, Tholl D, Gershenzon J, Bohlmann J (2002) Methyl Jasmonate induces traumatic resin ducts, terpenoid resin biosynthesis, and terpenoid accumulation in developing xylem of Norway spruce stems. Plant Physiol 129:1003–1018

Miller R, Owens SJ, Rørslett B (2011) Plants and colour: flowers and pollination. Opt Laser Technol 43:282–294

Nawrot J, Harmatha J (2012) Phytochemical feeding deterrents for stored product insect pests. Phytochem Rev 11:543–566

Nerio LS, Olivero-Verbel J, Stashenko E (2010) Repellent activity of essential oils: a review. Bioresour Technol 101:372–378

Ntalli NG, Caboni P (2012) Botanical nematicides in the mediterranean basin. Phytochem Rev 11:351–359

Pallozzia E, Tsonevc T, Marinoa G, Copolovicie L, Niinemetse Ü, Loreto F, Centritto M (2013) Isoprenoid emissions, photosynthesis and mesophyll diffusion conductance in response to blue light. Environ Exp Bot 95:50–58

Parachnowitsch AL, Raguso RA, Kessler A (2012) Phenotypic selection to increase floral scent emission, but not flower size or colour in bee-pollinated *Penstemon digitalis*. New Phytol 195:667–675

Pausas JG, Llovet J, Rodrigo A, Vallejo R (2008) Are wildfires a disaster in the mediterranean basin? –a review. Int J Wildland Fire 17:713–723
Pearce G (2011) Systemin, hydroxyproline-rich systemin and the induction of protease inhibitors. Curr Protein Pept Sci 12:399–408
Pohlit AM, Ribeiro AR, Baldin ELL, Lopes NP, Neto VFA (2011) Plant extracts, isolated phytochemicals, and plant-derived agents which are lethal to arthropod vectors of human tropical diseases – a review. Planta Med 77:618–630
Quintero C, Barton KE, Boeg K (2013) The ontogeny of plant indirect defenses. Perspectives in Plant Ecology, Evolution and Systematics 15:245–254
RSH (2007) Royal Horticultural Society Colour Chart
Saad NY, Muller CD, Lobstein A (2013) Major bioactivities and mechanism of action of essential oils and their components. Flavour Fragrance J 28:269–279
Schiestl FP, Ayasse M, Paulus HF, Löfstedt C, Hansson B, Ibarra F, Francke W (1999) Orchid pollination by sexual swindle. Nature 399:421–422
Schiestl FP, Ayasse M, Paulus HF, Löfstedt C, Hansson B, Ibarra F, Francke W (2000) Sex pheromone mimicry in the early spider-orchid (*Ophrys sphegodes*): patterns of hydrocarbons as the key mechanism for pollination by sexual deception. J Comp Physiol A 186:567–574
Sinha S, Mishra RK, Sinam G, Mallick S, Gupta AK (2013) Comparative evaluation of metal phytoremediation potential of trees, grasses, and flowering plants from tannery-wastewater-contaminated soil in relation with physicochemical properties. Soil Sediment Contam 22:958–983
Stewart I (2013) How to grow a planet. The bee that holds the key: good vibrations. BBC documentary (Series Producer: Andrew Thompson, Presenter: Iain Stewart, Director and Producer: Nigel Walk, Executive Producer: Mark Hedgecoe)
Svensson GP, Hickman MO, Bartram S, Boland W, Pellmyr O, Raguso RA (2005) Chemistry and geographic variation of floral scent in *Yucca filamentosa* (Agavaceae). Am J Bot 92:1624–1631
Szyndler MW, Haynes KF, Potter MF, Corn RM, Loudon C (2013) Entrapment of bed bugs by leaf trichomes inspires microfabrication of biomimetic surfaces. J R Soc Interface 10:20130174
Theis N, Lerdau M, Raguso RA (2007) The challenge of attracting pollinators while evading floral herbivores: patterns of fragrance emission in *Cirsium arvense* and *Cirsium repandium* (Asteraceae) *Int*. J Plant Sci 168:587–601
Tissier A (2012) Glandular trichomes: what comes after expressed sequence tags? Plant J 70:51–68
van Poecke RM, Dicke M (2004) Indirect defence of plants against herbivores: using *Arabidopsis thaliana* as a model plant. Plant Biol 6:387–401
Vokou D (2007) Allelochemicals, allelopathy and essential oils: a field in search of definitions and structure. Allelopath J 19:119–133
Wahid A, Gelani S, Ashraf M, Foolad MR (2007) Heat tolerance in plants: an overview. Environ Exp Bot 61:199–223
Weston LA, Duke SO (2003) Weed and crop allelopathy. Crit Rev Plant Sci 22:367–389
Whitney HM, Bennett KMV, Dorling M, Sandbach L, Prince D, Chittka L, Glover BJ (2011) Why do so many petals have conical epidermal cells? Ann Bot 108:609–616
Williams KS, Gilbert LE (1981) Insects as selective agents on plant vegetative morphology – egg mimicry reduces egg-laying by butterflies. Science 212:467–469
Willmer PG, Nuttman CV, Raine NE, Stone GN, Pattrick JG, Henson K, Stillman P, McIlroy L, Potts SG, Knudsen JT (2009) Floral volatiles controlling ant behaviour. Funct Ecol 23:888–900
Winde I, Wittstock U (2011) Insect herbivore counteradaptations to the plant glucosinolate–myrosinase system. Phytochemistry 72:1566–1575
Wondafrash M, Van Dam NM, Tytgat TOG (2013) Plant systemic induced responses mediate interactions between root parasitic nematodes and aboveground herbivorous insects. Front Plant Sci 4:1–15

Zhang J, An M, Wu H, Stanton R, Lemerle D (2010) Chemistry and bioactivity of *Eucalyptus* essential oils. Allelopath J 25:313–330

Zhang Y, Lubberstedt T, The MX (2013) Genetic and molecular basis of plant resistance to pathogens. J Genet Genomics 40:23–35

Zwanenburg B, Pospíšil T (2013) Structure and activity of strigolactones: new plant hormones with a rich future. Mol Plant 6:38–62

Chapter 6
Breeding and Germplasm Preservation

Christoph Carlen and Xavier Simonnet

Abstract With the increasing demand for plant based products, cultivation of medicinal and aromatic plants becomes more and more important. Breeding is one of the key technologies for the progress of the supply of high quality medicinal and aromatic plants. To react more rapidly to the requirements of the stakeholders, further improvement of breeding efficiency requires an intensification of (1) the characterisation of individual plants from accessions and conservation of well-defined genotypes, (2) the co-operation between genetic resource experts and breeders to use efficiently the available genetic potential, (3) the investigation on the reproduction biology of traditional and new medicinal plants to choose appropriate breeding strategies, (4) the exploration of molecular markers of important traits and (5) the development of tools to accelerate the breeding procedure.

Keywords Biodiversity • Biological active compounds • Breeding • Germplasm • Medicinal and aromatic plants • Molecular marker • Selection

1 Introduction

The plant kingdom offers a rich source of biologically active compounds. Especially plants rich in secondary metabolites are of interest for pharmaceutical, nutritional and cosmetic, as well as for phytosanitary and veterinary use. Secondary metabolites with therapeutic potential such as nitrogen containing substances (alkaloids, amines, glycosides, peptides, etc.), terpenes and phenolics are thought to help the plants (Pierce et al. 2012). Plants are producing biomass (i.e. energy), via photosynthesis. They are the primary producer of energy in most terrestrial

C. Carlen (✉)
Agroscope, Institute for plant production sciences, Route des vergers 18, Conthey 1964, Switzerland

Mediplant, Research Centre on Medicinal and Aromatic Plants, Route des vergers 18, Conthey 1964, Switzerland
e-mail: christoph.carlen@agroscope.admin.ch

X. Simonnet
Mediplant, Research Centre on Medicinal and Aromatic Plants, Route des vergers 18, Conthey 1964, Switzerland

Á. Máthé (ed.), *Medicinal and Aromatic Plants of the World*, Medicinal and Aromatic Plants of the World 1, DOI 10.1007/978-94-017-9810-5_6

ecosystems. Consequently, they have a strong controlling effect on the resources available to other organisms. However, plants have a sedentary lifestyle, they cannot flee their predators (insects, microbes, etc.) and must defend their tissues to retain the biomass. Therefore plants produce biological active compounds to resist against different organisms. Other functions of the biological active compounds are to promote growth as stress-protecting agents and activators of physiological processes. Secondary metabolites represent adaptive characters that have been subjected to natural selection during evolution when the presence of a particular biological active compound conferred an advantage to the species. Therefore, the variation in biological active compounds within a species might be caused by its adaptive potential to environments that vary in space and time.

The supply of biological active compounds is possible by harvesting plants form wild, from agricultural production and from biotechnology. Considerable efforts has been done in the past years to generate such metabolites in plant cell suspension cultures. Bioreactors offer possibilities for the large-scale synthesis and genetic transformation may be a powerful tool for enhancing the productivity of biological active compounds in such systems for the future. Nevertheless, collection from wild and agricultural production of medical plants still remain the most important supply for plant–derived bioactive compounds. A great number of medicinal plants species are collected from wild. These are mainly used for primary health care needs but they are also traded. However, harvesting from wild, especially for species with a high demand, can cause loss of genetic diversity and habitat destruction due to overharvesting. In addition, quality of the plants from wild is often neither accurate nor standardized. The shortage of raw material has lead seed companies, researchers and farmers to select cultivars that could substitute for the raw material that once came from the wild. The agricultural production of medicinal and aromatic plants offers several advantages: reliable botanical identification, less genetic, phenotypic and phytochemical diversity, availability of well-defined cultivars adapted to the requirements of the stakeholders, better guarantee for appropriate conservation, less extract variability and instability and a steadier source of raw material.

Agronomic research and development play an essential role to improve cultivation of medicinal plants. One of the most important processes for the progress of the medicinal and aromatic plant sector is breeding. The basis of the breeding work is the availability of a high diversity of well-defined accessions and genotypes within a species. New cultivars can be developed according to the needs of the stakeholders of the whole supply chain to enable high quality, profitable and sustainable product development. The different breeding approaches shown in this chapter were mainly realized in Switzerland by Agroscope and Mediplant.

2 Breeding

Breeding is a key technology for the improvement of medicinal and aromatic plants. Breeding for increased yield of valuable compounds, for elimination of unwanted compounds, for tolerance against abiotic and biotic stresses and for better homogeneity of the cultivars are important issues (Carlen 2012). The cultivars must also fit for sustainable production, extraction and purification processes, as well as for propagation by seed. Compared to traditional food crops, breeding of medicinal and aromatic plants is nowadays in the initial stages, with the advantage that breeders can exploit a high available natural variability within a species. This generally high natural variability within a species is one of the reasons that classical breeding approaches were mainly used till now. Other reasons are that these methods are relatively cheap and allow a return on investments even with low seed sales quantity. Furthermore, transgenic medicinal plants are not accepted on the European market (Canter et al. 2005; Pank 2010). Breeding a new cultivar needs 5–15 years according the species and the selection criteria. To react more quickly to the requirements of the stakeholders, methods to accelerate the breeding procedures must be taken into account such as the use of morphological, phytochemical and genetic markers at a very early stage in the reproduction cycle, increasing the number of generations per year, as well as rapid and cheap measurement methods of target traits. Different steps are considered for the breeding process: (1) analysis of the natural diversity within a species, (2) definition of the selection criteria and breeding strategies, (3) tools to improve the selection efficiency.

2.1 Variability Within a Species

2.1.1 Natural Variability

Successful breeding needs genetic variability. At the beginning of a breeding program it is important to get information about the natural variability of the target traits within the considered species. If such data are not available, seeds or vegetative propagation material have to be systematically collected form wild, from botanical gardens, gene-banks, research stations and private companies. The optimal way is to analyse these accessions in the same experimental fields under similar growing conditions as the future commercial production such as fertile conditions including irrigations, as well as similar soil and climatic conditions (Dudai 2012).

In a study in Switzerland the morphological and phytochemical variability was analysed within the *Asteraceae* species *Tanacetum vulgare* L.. *T. vulgare* is a perennial, herbaceous flowering plant, native to temperate Europe and Asia. Common tansy is often mentioned in the literature as a plant with anthelmintic properties for livestock (Waller et al. 2001). Its use in commercial products is also reported

Table 6.1 Mean composition of the essential oil of 27 accessions of common tansy and in brackets variability within an accession (min.–max.) at the harvest stage of beginning of flowering (– indicates no detection of the molecule)

Accessions	Origin of the seeds	α-thujone (%)	β-thujone (%)	Chrysanthenone (%)	lyratol (%)	Umbellulone (%)
TV-1	CH	–	**36** (0–70)	–	–	–
TV-2	CH	–	**49** (11–87)	–	–	–
TV-4	D	–	**88** (63–97)	–	–	–
TV-5	F	–	**13** (0–40)	**3** (0–16)	**5** (0–55)	**4** (0–12)
TV-6	DK	–	**20** (0–83)	–	–	**8** (0–32)
TV-7	HU	–	**11** (0–29)	–	–	–
TV-8	N	–	**19** (0–50)	–	–	**25** (12–36)
TV-9	B	–	**49** (11–92)	**25** (0–70)	–	**1** (0–6)
TV-10	D	–	**48** (0–80)	–	–	–
TV-11	CND	**2** (0–25)	**2** (0–23)	–	–	**7** (0–29)
TV-12	CND	–	**34** (0–93)	–	–	–
TV-13	D	–	**36** (0–67)	**5** (0–29)	–	–
TV-14	D	–	**20** (0–59)	–	–	–
TV-15	D	**19** (0–61)	**31** (0–71)	**9** (0–64)	–	–
TV-17	D	–	**11** (0–30)	**4** (0–26)	–	–
TV-19	RO	–	**5** (0–21)	**8** (0–82)	**5** (0–51)	–
TV-20	CH	–	**82** (27–94)	–	–	–
TV-23	D	–	**14** (0–93)	–	**22** (0–49)	–
TV-24	F	–	**45** (0–95)	–	–	–
TV-25	F	–	**24** (0–85)	–	**21** (0–42)	**4** (0–12)
TV-26	CH	–	**84** (11–96)	–	–	–
TV-28	CH	–	**31** (0–85)	–	–	–
TV-34	CND	–	**94** (89–96)	–	–	–
TV-35	CH	–	**92** (79–96)	–	–	–
TV-36	CH	**16** (0–55)	**1** (0–5)	–	–	–
TV-37	CH	**38** (0–86)	**17** (0–95)	–	–	–
TV-38	CH	–	**6** (0–18)	–	–	–

(Valchev et al. 2009). However, only few studies have been devoted to the domestication and cultivation of this perennial species. As a first step, 27 accessions from ten countries were evaluated by Mediplant in an experimental field under fertile conditions with irrigation, in Conthey (Table 6.1). Significant differences between the accessions were recorded, such as the essential oil content of the leaves and flowers (0.30–1.39 %) and high variations of the contents of several molecules in the essential oil such as α-thujone (0–84 %), β-thujone (0–96 %), chrysanthenone (10–83 %), lyratol (23–55 %) and umbellulone (6–36 %). This high variability, especially of the composition of the essential oil, is a valuable basis for a breeding program.

Another study on *Artemisia umbelliformis* Lam. also showed a high morphological and phytochemical variability (Rey and Slacanin 1997). The floral stems of *A. umbelliformis* have digestive properties and are mainly used to prepare a highly prized liqueur characterized by a bitter taste and a specific flavor. However, this species is protected in Switzerland and its collection is prohibited. To avoid collecting from wild, a domestication program was started by Agroscope. The variability of this species was analysed first. Plants were obtained from seeds from different alpine regions in Switzerland and grown in an experimental field of Agroscope in Bruson (1,100 m). The yield, the growth and the phytochemical composition, especially the thujone content of the essential oil were analysed as well as the resistance against different soil and airborne diseases. Thujones are considered as neurologically toxic in higher concentrations, in alcoholic beverages and total amounts of these compounds were limited to 35 ppm in European Union and Swiss legislation. Plants form the Saas valley region showed a relatively low mortality, high yield, mainly plants with erect growth and nearly no thujone, whereas the plants from Simplon region showed a very high content with about 70 % thujone in the essential oil (Rey and Slacanin 1997). Erect genotypes with high yield were selected from these two populations. The selected genotypes from Saas valley region were then isolated from the ones from Simplon. Even with the complex flower biology of this *Asteraceae* species with combined self-pollination of the hermaphrodite flowers and a cross pollination of the 'female' flowers (flowers without pollen formation) open pollination between the selected genotypes allowed to get relatively homogenous cultivars. The seeds obtained from the genotypes from Saas valley region gave the cultivar 'RAC 12' with nearly no thujones in the essential oil and from the Simplon genotypes the cultivar 'RAC 10' with 60–70 % thujones (Rey and Slacanin 1997; Carlen et al. 2012). This example shows that a great variability can exist in nature within one species concerning the phenotype and the phytochemical profile. Another interesting aspect of this species are the bitter compounds. The cultivar 'RAC 12' from Swiss Alps contains C-6 trans-sesquiterpene lactones, mainly costunolide and minor amounts of its related bitter compounds such as genepolide (Appendino et al. 2009; Rubiolo et al. 2009). In contrast, bitter compounds of the same species of the Western Alps such as in Piemont are characterized by the accumulation of C-8 cis-sesquiterpene lactones such as cis-8-eudesmanolide derivatives and by the absence of costunolide and genepolide.

2.1.2 Creation of Variability

When the natural variability does not provide the desired trait expression or trait combination, new variability can be created. The most common ways to generate new variability are the crossing of distinct genotypes from the same species or from two related species. Genotypes are crossbred to introduce traits/genes from one genotype to the other to get new trait combinations. For example, a disease resistant genotype may be crossed with a high-yielding but susceptible genotype to get both traits in some progenies.

Other approaches are the induction of mutations and of poliploidy, as well as biotechnological methods such as gene transfer (Bernáth 2002). Mutations are induced by chemicals or radiation. The mutants obtained are tested and further selected for desired traits. There some examples with induced mutation giving interesting results such as for *Mentha piperita* populations with increased menthol content (Zheljazkov et al. 1996) or for *Cymbopogon flexuosus* with modification of the essential oil accumulation pattern (Kulkarni et al. 1992). Another possibility to create variability and to improve the basis for breeding are the induced polyploidy. A high proportion of medicinal and aromatic plants are diploid. The increase of chromosomes sets per cell can be artificially induced by applying the molecule colchicine, which leads to a doubling of the chromosome number. In various plant species, induced polyploidy has proven to successfully increase the productivity of secondary metabolites and essential oil such as shown for *Acorus calamus* (Dhawan and Lavania 1996). Similarly, the essential oil content of *Lavandula angustifolia* (Raev et al. 1996) have been increased with polypolidy. Induced mutation and polyploidy were not frequently used in medicinal and aromatic plants due to extension of the breeding process and due to the generally high natural variability within a species.

Another way of increasing variability of some important traits is genetic modification. Genetic modification of plants is achieved by adding a specific gene or genes to a plant, or by knocking down a gene, to produce a desirable phenotype. It is expected that genetic modification can produce a plant with the desired trait or traits such as the resistance to pests and diseases faster than classical breeding because the majority of the plant's genome is not altered. However, it remains an open question, what role transgenic medicinal plants will have in the future, considering that currently such plants are not accepted on the European market.

Another issue for genetically modified crops are pharma plants (Kaiser 2008). There is great interest from pharmaceutical companies in using plants or plant cells as bioreactors for producing pharmaceuticals, such as vaccines, monoclonal antibodies, enzymes etc. The most important advantages of plant-made pharmaceuticals (PMPs) compared to other protein expression systems (especially bacterial systems) are: the low risk of contamination with animal or human pathogens, relatively low investment and production costs, and good possibilities for scale-up, processing and modification of pharmaceutical proteins. (Hoffmann-Sommergruber and Dorsch-Häsler 2012). The feasibility and potential efficacy have well been established. It is possible that the first PMPs in use will be animal vaccines, because the demonstration of efficacy is easier in animals.

2.2 Breeding Strategies

2.2.1 Species with Vegetative Propagation

Vegetative propagation can be used for most of the plant species to create a cultivar (clone). After a screening process, the most adapted genotype can be then propagated, in general by cuttings or in-vitro. The advantages of this approach is that the selection time is much shortened and that the plants are very homogenous. However, the disadvantage is that vegetative propagation is very expensive compared to propagation by seeds. This breeding strategy is mainly used to react very quickly to a demand of industry for supplying low quantities of plant material, to multiply perennial species such as bush and tree species (Quennoz et al. 2006a, b) and for species without seed development such as *Thymus x citriodorus* (Pers.) Schreb. and *Mentha x piperita* (Carron et al. 2009; Vouillamoz et al. 2013).

2.2.2 Species with Apomictic Reproduction

Apomictic reproduction means that seed formation occurs without fertilization of the egg cells by the sperm cells. Breeders can use the apomictic behavior for fast genetic fixation of aimed trait expression because seed progenies have the same genetic code as the mother plants (Pank et al. 2003). An important medicinal plant with this flower biology is *Hypericum perforatum* L.. *H. perforatum* is recommended in plant therapy for its antiviral, vulnerary and antidepressive properties. However this species badly suffered from a wilt disease, an anthracnose caused by *Colletotrichum gloeosporioides* (Debrunner et al. 2000). Therefore an important breeding aim is the resistance or the tolerance to this anthracnose. In a project of Mediplant, the commercial cultivars Topas, Hypermed and Elixir and 21 wild accessions collected in Switzerland, Germany, Italy and Canada were compared (Gaudin et al. 1999). The accessions were very different with regard to anthracnose resistance, flowering time, plant morphologies, flower and drug yields and phytochemical profiles. The accession P7 was selected because of its low susceptibility to this disease. Furthermore, yield and the phytochemical profile (hypericines and flavonoides) were similar to those of the standard cultivar Topas and met the industrial requirements. Thanks to the apomictic reproduction the accession P7 could easily be multiplied by seeds and is now registered as the cultivar ‘Hyperivo 7’ (Gaudin et al. 2003).

Another medicinal plant with apomictic reproduction is *Alchemilla xanthochlora* Rothm. In a breeding program of Agroscope different individual plants for Swiss mountain regions were collected. After a the selection process an adapted genotype was found, giving the cultivar ‘Aper’ (Rey and Slacanin 1999). Due to the apomictic reproduction, seed production was very simple.

2.2.3 Self-Pollinated Species

For self-pollinated species the first breeding step is to select single plants with high performance (such as yield, quality, resistance, . . .) within accessions from wild and to develop seed production and a cultivar by self-pollination. If no adapted genotype is found a forced crossing can be done between two adapted genotypes after removal of anthers from one of the genotypes and then the best single plants have to be selected from the progenies The next step is to produce progenies, by self-pollination, from each one of the selected plants, and again to choose only the best ones during 4–7 generations until a uniform line is obtained. Thus a new cultivar is obtained such as realized with Carum carvi and Ocimum basilicum (Putievsky et al. 1994; Dudai et al. 2002).

Another example is the *Hyssopus officinalis* L. cultivar Perlay. This cultivar is originated from a cross between two selected individual plants within populations of the subspecies officinalis (from an Hungarian accession) and the subspecies *canescens* (from an accession of Valais, Switzerland). As self-pollination is dominating in *H. officinalis*, the hybridization required manual castration of the male part of the flowers of the Hungarian genotype selected as female plant. Thereafter, seeds collected from this "female plant", were propagated and single plants with high performance were chosen. From these single plants, progenies were produced by self-pollination and again only the best ones were chosen during some generations until a uniform line was obtained. The characteristic traits of the *H. officinalis cultivar* 'Perlay' are an erected, vigorous, homogenous growth type, a mean yield of 6–7 t/ha of dry matter since the second year of production, and an essential oil content of 0.8–1.3 % mainly composed of pinocamphon (40–60 %), isopinocamphon (20–30 %), and ß-pinen (4–15 %). It is also a cultivar with a good winter hardiness and resistance to the disease Sclerotinia (Rey et al. 2004a).

2.2.4 Cross-Pollinated Species

Cultivars Based on a Population of Genotypes

A population cultivar consist of individual plants with open-pollination. Each plant in a population will have a unique set of genes and will be genetically different from all other plants in the population. Collectively, however, each new generation of a population will share certain characteristics inherited from the previous generation. Each population cultivar will have a set of characteristics that distinguishes it from other populations cultivars of the same species. Some common selection methods are positive and negative mass selection. Examples of population cultivars are the *Salvia officials* L. cultivar 'Extracta' or the *Thymus vulgaris* L. cultivar 'Deutscher Winter' (Carron et al. 2005; Carlen et al. 2010). Seed production of these varieties requires no controlled pollinations. In fact, populations cultivars were obtained by saving seed from the current year's crop.

Cultivars Based on Several Genotypes

Cultivars can be produced by several selected parents with subsequent crossing by open pollination under isolation. The potential of a genotype as constituent of a cultivar with several parents (synthetic cultivar) is indicated by its general combining ability tested with a polycross. Optimal parent number might be 4–6 as suggested for forage grasses (Piano et al. 2007). The parents are genotypes with generally vegetative multiplication and conservation (clones). Synthetic varieties are interesting for their better homogeneity compared to population cultivars.

A polycross test was made with *Carum carvi* L. var. *annuum* hort by crossing 40 potential parents (Pank et al. 2007). The parental lines were then selected according to the performance of their progenies to get information on the general combining ability of the parental lines. The best parental lines were then considered to produce the desired synthetic varieties.

An example of a cultivar based on several parental lines is the *Rhodiola rosea* L. cultivar Mattmark (Vouillamoz et al. 2012). Five populations from the Swiss Alps were screened for their salidroside and rosavins contents. With an average content of 1,49 % for salidroside and 1,57 % for rosavins, the accession Mattmark (Saas Fee, Valais) turned out to have the most productive and vigorous genotypes. 4 male and 4 female genotypes of the Mattmark accession with high contents of salidrosid and rosavins were chosen. A random polycross was performed with these 8 genotypes to produce seeds of the cultivar ‘Mattmark’ offering high rhizome production and high salidroside and rosavins contents.

Another example is the *Melissa officinalis* L. cultivar ‘Lorelei’. This synthetic cultivar is based on 6 genotypes used as parents. The cultivar ‘Lorelei’ is characterized by a high dry matter yield, a high yield in rosmarinic acid content in the leaves and relatively high yield in essential oil content (Carron et al. 2013).

Cultivars Based on Two Genotypes

A frequently employed plant breeding technique is hybridization. The aim of hybridization is to bring together desired traits found in different genotypes into one plant line via cross-pollination. The first step is to generate homozygous inbred lines. This is normally done by forcing isolated self-pollination where pollen from one flower fertilize the same flower or a flower of the same plant. Cultivars based on two homozygous parental lines are better for their homogeneity than synthetic varieties and can benefit of heterosis. Another approach to achieve homozygous breeding line is doubled haploidy. Haploid cells are generated from pollen or egg cells and chromosome-doubling produces doubled haploids. Doubled haploid plants, have been reported in some medicinal and nutraceutical species (Ferrie 2007). However, in general for medicinal and aromatic plants, it might be too expensive to make hybrid breeding with homozygous inbred lines in relation to the potential seed sales.

However, homogenous cultivars are very important for the whole supply chain allowing to standardize better the production and extraction. A promising and cheap approach is the utilisation of natural male sterility of cross-pollinating plant species. Rey (1993), Pank and Krüger (2003), Rey et al. (2004b) and Mewes et al. (2008) showed that crossing sterile male with fertile male plants to breed hybrids is an adequate approach to improve homogeneity of *T. vulagris* cultivars, for example. Therefore a breeding program was conducted by Agroscope to optimise the quality, the yield performance and the homogeneity of *T. vulgaris* by exploiting the natural gynodioecy of its flowers (male sterile and hermaphroditic plants). The breeding program yielded 56 new hybrids obtained by crossing sterile male and fertile male genotypes (Carlen et al. 2010). Some of them showed very promising results, in particular a hybrid, called cultivar 'Varico 3', obtained by crossing two accessions from the Agroscope breeding material, showed a high essential oil content with 4.4 % average over 5 harvests and a high leaf yield. In addition, the parents of this hybrid are well synchronised in their flowering period and have quite a good seed production potential. In addition, other breeding programs conducted by Agroscope with *Salvia officinalis* L. (cultivar 'Regula') (Rey et al. 2000), with *Origanum vulgare* L. (cultivar 'Carva') (Rey et al. 2002) and with *Leontopodium alpinum* Cass. (cultivar 'Helvetia) (Carron et al. 2007) showed that crossing sterile male (MS) with fertile male (MF) plants to breed hybrids is a well-adapted breeding strategy to improve homogeneity of a cultivar. The exploitation of male sterility allowing controlled pollination will become more and more important in the future for breeding medicinal and aromatic plants (Pank 2010; Carlen et al. 2010).

Another example for a cultivar based on two parental genotypes are the *Artemisia annua* L. cultivars Artemis and Apollon, created by Mediplant. *A. annua* is an important medicinal plant for the production of antimalarial drugs based on artemisinin. Only the distribution of cultivars with a high artemisinin production potential allows making this new culture attractive and this way answering to the increasing demand for low cost artemisinin (Ferreira et al. 2005). Since the self-pollination is insignificant for *A. annua* (Delabays 1997), the genotypes were isolated in groups of 2 to ensure the production of hybrid seeds. The two genotypes, are selected through their specific combining ability. The breeding work conducted by Mediplant since 1989 allowed to develop hybrids with over 1 % artemisinin in the dried leaves such as the cultivar 'Artemis' (Delabays et al. 1993, 2001). In the following years Mediplant tested hundreds of genotypes for their artemisinin content in the leaves, the leaf dry weight productivity and the flowering period under field conditions. The most promising genotypes were tested on their specific combing ability. Seeds of 45 combinations were obtained and the progenies were tested. One of the most promising new hybrid, the cultivar Apollon, showed a similar yield in dry leaves, but much higher content of artemisinin in the leaves and production of artemisinin compared to the cultivar 'Artemis' (Simonnet et al. 2008; Simonnet et al. 2010). The artemisinin contents in the leaves was very high, with up to 1.95 %. Although the artemisinin content is the first selection criterion retained *for A. annua*, other factors such as the aptitude for in vitro conservation, the productivity of leaves, the flowering requirements and the tolerance to pests and

Fig. 6.1 In-vitro preservation and reproduction of the most interesting genotypes of *Artemisia annua* L

diseases are also considered and, of course, the adaptability in various areas of the intertropical zone which appear to be the main production sites for artemisinin (Fig. 6.1).

2.3 Tools to Improve the Breeding Efficiency

Breeding a new cultivar needs 5–15 years according to the species and the breeding objectives from bioprospecting up to cultivar registration. This is a very long time for companies developing and trading plant based products. To react more quickly to the requirements of the stakeholders, methods to accelerate the breeding procedures must be taken into account. One of the possibilities is the acceleration of generation succession by vernalisation and cultivation in the greenhouse allowing to increase the number of generations per year (Pank and Schwarz 2005). Other approaches are the use of morphological, phytochemical and genetic markers at a very early stage in the reproduction cycle, as well as rapid and cheap measurement methods of target traits. Three methods to improve the selection efficacy will be described in the following chapters.

2.3.1 Molecular Marker Assisted Selection

Molecular breeding tries to discover plant's genes and their functions. The use of molecular markers allows plant breeders to screen large populations of plants and helps to select the genotypes possessing the trait of interest to be used to breed high-performing cultivars. The role of molecular markers in the characterization of medicinal plants was reviewed by Tharachand et al. (2012) and Sarwat et al. (2012). Several types of markers were used, including Randomly Amplified Polymorphic DNA (RAPD), Sequence Characterized Amplified Region (SCAR), Inter Simple Sequence Repeats' (ISSRs), Amplified Fragment Length Polymorphism (AFLPs), Single Nucleotide Polymorphisms (SNPs), Simple Sequence Repeats (SSRs, or microsatellites), Restriction Fragment Length Polymorphisms (RFLPs).

In a recent study of Graham et al. (2010), the molecular basis for marker-assisted breeding of *Artemisia annua* was established. Quantitative trait loci (QTL) related with key traits controlling artemisinin yield were identified. QTL analysis is a statistical method that links phenotypic data (specific traits) and genotypic data (molecular markers) to explain the genetic basis of complex traits. The presence of positive artemisinin yield QTL in parents will help to obtain new high-yielding hybrids for the future helping to answer more quickly to the increasing demand in artemisinin.

Molecular markers provide an independent and objective approach for the characterization of medicinal plant materials and can be used for an effective breeding. Furthermore, molecular markers can help to efficiently manage germplasm collections (in-situ/ex-situ) through monitoring and assessing genetic diversity, screening of germplasm collection for duplicates and formulating critical conservation strategies on what should be conserved on a priority basis. In the future it will be very fruitful if a concentrated effort was made to integrate existing molecular marker data and to co-ordinate projects of molecular characterization of medicinal plants.

2.3.2 Rapid Methods to Determine Valuable Compounds

One of the most important breeding aims is the improvement of the content of valuable compounds in the plants. However, the high expenses for phytochemical analysis are a limiting factor for breeding in medicinal and aromatic plants. Therefore, rapid and cheap analytical methods are essential to provide a high selection efficiency by analysing an appropriate amount of individual plants. In addition, analytical methods are required allowing to analyse small sample quantities such as young single plants or parts of them without preceding sample preparation. Furthermore, non-destructive methods are preferred to keep the investigated plant material alive for following breeding procedures.

A rapid, low cost analytical method is near infrared spectroscopy (NIRS) (Schulz et al. 1999; Camps et al. 2011; Camps et al. 2014). A NIRS-based method was developed to determine the artemisinin content in dry leaf powder of Artemisia annua (Camps et al. 2011). In another study, hand-held NIRS (1,000–1,800 nm) measurements and FT-NIRS (1,000–2,500 nm) measurements were tested to determine the essential oil content of oregano dried leaf powder. The FT-NIR approach allowed the development of an accurate model for the essential oil content prediction. Although the hand-held NIR approach is promising, it needs additional development before it can be used in practice. There are several other examples proving that near infrared and mid-infrared spectroscopy techniques are extremely helpful supporting very efficiently plant breeding programs and evaluation of plant resources (Schulz 2010).

Another method for rapid analysis is the device SMart Nose®. The discriminating power of the SMart Nose®, an electronic nose based on mass spectrometry of the gas phase, was tested on seven cultivars representing four chemotypes previously distinguished by HPLC-DAD (Vouillamoz et al. 2009). This experiment shows that the distinction between the cultivars with the SMart Nose® is fast, reproducible and inexpensive. The use of the SMart Nose® could be extended in the future for screening of genotypes from natural populations and progenies after crossing to detect a particular chemotype.

On-site measurements on living plants can be performed with SPME (Solid Phase Microextraction) by placing a suitable glass chamber containing sidearms closed with septa over the plants. Depending on the ambient temperature approximately 15–20 min are needed for sampling. Then the loaded fibre can be directly analysed or mailed to a remote laboratory. SPME-GC field sampling of living rose flowers could be easily performed (Schulz 2003) and allowed to discriminate the fragrance of different rose cultivars. This on-field non destructive method has been also successfully applied in breeding research to analyse numerous rose progenies of crossings with different smelling rose cultivars.

2.3.3 Rapid Determination of Resistance Against Biotic and Abiotic Factors

An analysis of 302 scientific publications on resistance research and breeding in medicinal and aromatic plants showed that the activities have been intensified in this field (Gabler 2002). The majority, i.e. 70 %, of the contributions was concerned with fungal diseases, reflecting the economic importance of this pathogen group as a damaging factor. Rapid screening methods for resistance against fungal diseases are necessary to improve the breeding efficiency. A methodology tested by Michel et al. (2014) was focused on Anthracnose, a major production constraint for *H. perforatum* caused by the fungus *Colletotrichum gloeosporioides* (Penz.). A greenhouse screening method based on seedlings mortality was developed to eliminate accessions susceptible to anthracnose in the early stage of breeding for resistant cultivars. The mortality of 22 accessions at the seedling stage artificially inoculated with a strain of C. gloeosporioides was positively correlated with the mortality in the field tests. Breeding of anthracnose resistance of *H. perforatum* can be based on a greenhouse screening of seedlings to eliminate accessions susceptible to anthracnose in the early stage of breeding.

Resistance breeding in medicinal and aromatic plants is also focused on other biotic factors such as pests (nematodes and arthropods), as well as abiotic factors such as cold, heat, drought, wetness, nutrition deficiency or oversupply, salinization. Improvements in abiotic stress tolerance are very complex and can rarely be selected for in the greenhouses at seedling stage of the plants (Gabler 2002).

3 Germplasm Preservation

For the future it is important to prevent the loss of genetic diversity, including the diversity within species. Being a repository of unforeseen potentialities, medicinal and aromatic plants genetic resources should be studied and preserved for the benefit of present and future generations. In this context, various policies, plans and interventions have been recommended worldwide at international, national and state levels such as the Convention on Biological Diversity (CBD) defined at the United Nations Conference on Environment and Development (UNCED) in 1992. The Convention on Biological Diversity was inspired by the world community's growing commitment to sustainable development. It represents a dramatic step forward in the conservation of biological diversity, the sustainable use of its components, and the fair and equitable sharing of benefits arising from the use of genetic resources.

Germplasm preservation includes, as mentioned by Janick and Whipkey (2007): (1) the conservation of germplasm of diverse medicinal plant species through collection, acquisition, and exploration; (2) conducting a variety of germplasm related research such as morphological, phytochemical and genetic evaluation; and (3) encouraging the use of germplasm collections and associated information for research, crop improvement, and product development. For example the complete German parsley germplasm collection, 220 accessions from 35 countries all over the world, contains modern and old cultivars as well as landraces (Lohwasser et al. 2010). Both morphological types, leaf parsley and root parsley, were under study to look for the variability within the collection, as well as the interaction of morphological, molecular and phytochemical characters.

Plant genetic resources conservation can be considered from two points of view: On the one hand, in-situ conservation involves the establishment and/or maintenance of natural reserves where species are allowed to remain in optimal ecosystems. On the other hand, ex-situ conservation involves the use of botanic gardens, field plantations, seed stores and gene banks. Plant in-vitro technology offers a potential solution to both the long term conservation of these presently difficult to store germplasm categories and in the mass production of species that are categorised as endangered. In-vitro technology has the distinct advantage that a variety of cells and organised tissues can be stored, e.g. single cells, meristems embryos. Cryopreservation of these tissues, i.e. conservation at temperatures below −80 °C and usually at liquid nitrogen temperature (−196 °C), is a powerful tool for germplasm preservation. For the future, efforts have to be made to preserve this genetic material in situ and ex situ. Such as realized in Portugal, it would be very fruitful to pull synergies and join efforts between researchers and to propose collaborative networks on ethnobotany and germplasm preservation, characterization and evaluation (Barata et al. 2011).

References

Appendino G, Taglialatela-Scafati O, Romano A, Pollastro F, Avonto C, Rubiolo P (2009) Genepolide, a sesterpene γ-lactone with a novel carbon skeleton from mountain wormwood (*Artemisia umbelliformis*). J Nat Prod 72(3):340–344

Barata AM, Rocha FA, Lopes VM, Morgado J, Maia J, Bettencourt E, Dias S, Delgado F, Costa M, Farinha N, Póvoa O, Salgueiro L, Figueiredo AC (2011) Networking on conservation and use of medicinal, aromatic and culinary plants genetic resources in Portugal. Acta Hort 925:21–35

Bernáth J (2002) Strategies and recent achievements in selection of medicinal and aromatic plants. Acta Hort 576:115–128

Camps C, Toussirot M, Quennoz M, Simonnet X (2011) Determination of artemisinin and moisture content of *Artemisia annua* L. dry powder using a hand-held near infrared spectroscopy device. J Near Infrared Spectrosc 19:191–198

Camps C, Gerard M, Quennoz M, Brabant C, Oberson C, Simonnet X (2014) Prediction of essential oil content of oregano by hand-held and fourier transform NIR spectroscopy. J Sci Food Agric 94(7):1397–402

Canter PH, Thomas H, Ernst E (2005) Bringing medicinal plants into cultivation: opportunities and challenges for biotechnology. Trends Biotechnol 23:180–185

Carlen C (2012) Breeding and cultivation of medicinal plants. In: Bagetta G, Cosentino M, Corasaniti MT, Sakurada S (eds) Herbal medicines for human health. Taylor & Francis, London, pp 79–91

Carlen C, Schaller M, Carron CA, Vouillamoz JF, Baroffio CA (2010) The new *Thymus vulgaris* L. hybrid cultivar 'Varico 3' compared to five established cultivars from Germany, France and Switzerland. Acta Hort 860:161–166

Carlen C, Carron CA, Simonnet X (2012) Optimising the cultivation of white genepi to achieve stable yields and high quality. Acta Hort 955:211–217

Carron CA, Previdoli S, Cottagnoud A, Rey C, Carlen C (2005) Salvia officinalis: productivity and quality of the new cultivar Regula. Revue suisse Vitic Arboric Hortic 37:235–239

Carron CA, Rey C, Previdoli S, Baroffio C (2007) Helvetia, a new hybrid clones Edelweiss cultivar. Revue suisse Vitic Arboric Hortic 39:125–130

Carron CA, Baroffio CA, Vouillamoz JF (2009) Lemon thyme: comparison of two clones and definition of the optimum harvest stage. Revue suisse Vitic Arboric Hortic 41(6):357–361

Carron CA, Vouillamoz J, Baroffio C (2013) Lemon balm: influence of agrotextile covering on the yield in dry matter, essential oil and rosmarinic acid. Revue suisse Vitic Arboric Hortic 45(5):276–282

Debrunner N, Rauber AL, Schwarz A, Michel V (2000) First report of St John's-wort anthracnose caused by *Colletotrichum gloeosporioides* in Switzerland. Plant Dis 84:203

Delabays N (1997) Biologie de la reproduction chez l'Artemisia annua et génétique de la production en artémisinine. Ph.D. thesis, University of Lausanne Suisse, p 169

Delabays N, Benakis A, Collet G (1993) Selection and breeding for high artemisinin (Qinghaosu) yielding strains of Artemisia annua. Acta Hort 330:203–207

Delabays N, Simonnet X, Gaudin M (2001) The genetics of artemisinin content in Artemisia annua L. and the breeding of high yielding cultivars. Curr Med Chem 8:1795–1801

Dhawan OP, Lavania UC (1996) Enhancing the productivity of secondary metabolites via induced polyploidy: a review. Euphytica 87(2):81–89

Dudai N (2012) Domestication and breeding of wild medicinal and aromatic plants – 30 years of experience in Israel. Acta Hort 955:175–183

Dudai N, Chaimovitsh D, Reuveni R, Ravid R, Larkov O, Putievsky E (2002) Breeding of sweet basil (*Ocimum basilicum*) resistant to Fusarium wilt caused by *Fusarium oxysporum* f. sp. *basilicum*. In: Johnson CB, Franz C (eds) Breeding research of aromatic and medicinal plants. The Haworth Press, New York, pp 45–51

Ferreira JFS, Laughlin JC, Delabays N, Magalhaes PM (2005) Cultivation and genetics of *Artemisia annua* L. for increased production of the antimalarial artemisinin. Plant Genet Res 3:206–229

Ferrie AMR (2007) Doubled haploid production in nutraceutical species: a review. Euphytica 158(3):347–357

Gabler J (2002) Breeding for resistance to biotic and abiotic factors in medicinal and aromatic plants: general situation and current results in annual caraway (*Carum carvi* L. var. *annum*). J Herbs Spices Med Plants 9(2–3):1–11

Gaudin M, Simonnet X, Debrunner N, Ryser A (1999) Breeding for a high-yielding, dietback tolerant variety of St. John's Wort. Resultas of two years of research. Revue suisse Vitic Arboric Hortic 31:335–341

Gaudin M, Simonnet X, Debrunner N (2003) *Colletricum gloeosporioides* as cause of St. John's wort (*Hypericum perfortum*) dieback in Switzerland and breeding for tolerant variety. In: Ernst E (ed) Hypericum – the genus Hypericum. Taylor and Francis, London/New York, pp 23–42

Graham IA, Besser K, Blumer S, Branigan CA, Czechowski T, Elias L, Guterman I, Harvey D, Isaac PD, Khan AW, Larson TR, Li Y, Pawson T, Penfield T, Rae AM, Rathbone DA, Reid S, Ross J, Smallwood MF, Segura V, Townsend T, Vyas D, Winzer T, Bowles D (2010) The genetic map of *Artemisia annua* L. identifies loci affecting yield of the antimalarial drug artemisinin. Science 327:328–331

Hoffmann-Sommergruber K, Dorsch-Häsler K (2012) Medical issues related to genetically modified plants of relevance to Switzerland. Vdf Hochschulverlag, Zürich, p 54

Janick J, Whipkey A (eds) (2007) Issues in new crops and new uses. ASHS Press, Alexandria, p 366

Kaiser J (2008) Is the drought over for pharming? Science 320:473–475

Kulkarni RN, Mallavarapu GR, Ramesh S (1992) The oil content and composition of new variants of Cymbopogon flexuosus. J Essent Oil Res 4:511–514

Lohwasser U, Declercq M, Börner A, Struckmeyer T, Budahn H, Krüger H, Ulrich D, Marthe F (2010) The German parsley germplasm collection – interaction of morphological, molecular and phytochemical characters. Acta Hort 860:235–240

Mewes S, Krüger H, Pank F (2008) Physiological, morphological, chemical and genomic diversities of different origins of thyme (Thymus vulgaris L.). Genet Resour Crop Evol 55:1303–1311

Michel V, Debrunner N, Simonnet X (2014) A rapid greenhouse screening method to identify St. John's Wort (*Hypericum perforatum*) accessions resistant to Colletotrichum gloeosporioide. Hortscience 49(1):31–34

Pank F (2010) Conventional breeding of medicinal and aromatic plants – fundamentals and examples. Acta Hort 860:135–146

Pank F, Krüger H (2003) Sources of variability of thyme populations (*Thymus vulgaris* L.) and conclusions for breeding. Z Arznei- Gewürzpflanzen 8:117–124

Pank F, Schwarz S (2005) Cultivation of annual caraway (*Carum carvi* L. var. *annuum* hort.) in the greenhouse during winter to accelerate the breeding progress. Z Arznei- Gewürzpflanzen 10:194–197

Pank F, Matzk F, Kästner U, Blüthner WD, Foltys de Garcia E, Meister A, Ryschka U, Schumann G (2003) Reproductive diversity and strategies for breeding in St. John's wort (*Hypericum perforatum* L.). Euphytica 134:77–84

Pank F, Krüger H, Quilitzsch R (2007) Results of a polycross-test with annual caraway (*Carum carvi* L. var. *annuum* hort). Z Arznei- Gewürzpflanzen 12(3):127–135

Piano E, Annicchiarico P, Romani M, Pecetti L (2007) Effect of the number of parents and their combining ability on the performance of synthetic varieties in tall fescue. Aust J Agr Res 58(11):1100–1105

Pierce S, Luzzaro A, Cerabolini BEL (2012) Taxonomy, morphology and ecology of medicinal plants: a botanical perspective. In: Bagetta G, Cosentino M, Corasaniti MT, Sakurada S (eds) Herbal medicines for human health. Taylor & Francis, London, pp 7–26

Putievsky E, Dudai N, Ravid U, Katzir I (1994) A new cultivar of caraway (*Carum carvi* L.) and its essential oil. J Herbs Spices Med Plants 2(2):81–84

Quennoz M, Simonnet X, Vergères C, Hausamann H (2006a) Sea buckthorn (*Hippophaë rhamnoides* L.), a species for cosmetics industry. Revue suisse vitic arboric hortic 38(4):215–217

Quennoz M, Vergères C, Hausamann H, Simonnet X (2006b) Rose bushes for cosmetic oil production. Revue suisse Vitic Arboric Hortic 38(4):219–223

Raev RT, Jordanov R, Zheljazkov V, Rabotyagov VD (1996) Induced polyploidity in lavender. Acta Hort 426:561–572

Rey C (1993) Hybrids de thyme (*Thymus vulgaris* L.) attractive for mountain cultivation. Revue suisse Vitic Arboric Hortic 25:269–275

Rey C, Slacanin I (1997) Domestication of white genepi (*Artemisia umbelliformis* Lam.). Revue suisse Vitic Arboric Hortic 29:I–VIII

Rey C, Slacanin I (1999) The 'Aper' yellowish lady's mantle: growing techniques and pharmacological basics. Revue suisse Vitic Arboric Hortic 31(6):309–316

Rey C, Carron CA, Nendaz B (2000) Very promising hybrids of sage. Revue suisse Vitic Arboric Hortic 32:I–VIII

Rey C, Carron CA, Bruttin B, Cottagnoud A (2002) The oregano cultivar 'Carva'. Revue suisse Vitic Arboric Hortic 34:I–VIII

Rey C, Carron CA, Cottagnoud A, Bruttin B, Carlen C (2004a) The hyssop (*Hyssopus officinalis*) cultivar 'Perlay'. Revue suisse Vitic Arboric Hortic 36(6):337–341

Rey C, Carron CA, Cottagnoud A, Schweizer N, Bruttin B, Carlen C (2004b) New hybrids in garden thyme. Revue suisse Vitic Arboric Hortic 36:297–301

Rubiolo P, Matteodo M, Bicchi C, Appendino G, Gnavi G, Bertea C, Maffeh M (2009) Chemical and biomolecular characterization of *Artemisia umbelliformis* Lam., an important ingredient of the alpine liquer "Genepi". J Agric Food Chem 57(9):3436–3443

Sarwat M, Nabi G, Das S, Srivastava PS (2012) Molecular markers in medicinal plant biotechnology: past and present. Crit Rev Biotechnol 32(1):74–92

Schulz H (2003) Odoriferous substances and pigments. In: Roberts AV, Debener T, Gudin S (eds) Encyclopedia of rose science, vol I. Elsevier, Oxford, pp 231–240

Schulz H (2010) Application of rapid analytical methods for efficient evaluation of plant genetic resources and breeding material. Acta Hort 860:225–234

Schulz H, Krüger H, Steuer B, Pank F (1999) Bestimmung von Inhaltsstoffen des Majorans (*Origanum majorana* L.) mittels Nahinfrarot-Spektroskopie. Z Arznei- Gewürzpflanzen 4:62–67

Simonnet X, Quennoz M, Carlen C (2008) New *Artemisia annua* hybrids with high artemisinin content. Acta Hort 769:371–373

Simonnet X, Quennoz M, Carlen C, Lopez O, Kowalyzyn D, Giccia G, Desmarchelier C (2010) Performance of *Artemisia annua* L. cultivars in different locations in Argentina. Acta Hort 860:187–192
Tharachand C, Immanuel Selvaraj C, Mythili MN (2012) Molecular markers in characterization of medicinal plants: an overview. Res Plant Biol 2(2):01–12
Valchev G, Popova-Ralcheva S, Bonovska M, Zaprianova I, Gudev D (2009) Effect of dietary supplements of herb extracts on performance in growing pigs. Biotechnol Anim Hus 25(5/6):859–870
Vouillamoz JF, Schaller M, Carron CA, Bozzi-Nising A (2009) Discrimination des chémotypes de thym vulgaire avec le 'nez électronique' SMart nose®. Revue suisse Vitic Arboric Hortic 41(6):345–348
Vouillamoz JF, Carron CA, Malnoë P, Baroffio CA, Carlen C (2012) *Rhodiola rosea* 'Mattmark', the first synthetic cultivar is launched in Switzerland. Acta Hort 955:185–189
Vouillamoz JF, D'Anna E, Carron CA, Baroffio C (2013) *Mentha* × *piperita* '541': DNA certification of peppermint in Switzerland by RAPD. Revue suisse Vitic Arboric Hortic 45(5):284–288
Waller P, Bernes G, Thamsborg S, Sukura A, Richter S, Ingebrigtsen K, Hoglund J (2001) Plants as de-worming agents of livestock in the Nordic countries: historical perspective, popular beliefs and prospects for the future. Acta Vet Scand 42:31–44
Zheljazkov V, Margina A, Stoeva T (1996) Effect of gamma irradiation on some quantitative and qualitative characteristics in mint and cormint. Acta Hort 426:381–388

Chapter 7
Conservation of Wild Crafted Medicinal and Aromatic Plants and Their Habitats

Dea Baričevič, Ákos Máthé, and Tomaž Bartol

Abstract The use of Medicinal and aromatic plants (MAPs) in the form of traditional medicine, is still the preferred method of medical treatment in developing countries, owing to the unavailability and/or high prices of modern medicines. In the developed economies, as a contract, the market sectors for plant products seem to show new potentials, like in the case of different processing industries. The market for herbal medicines and supplements is expected to reach 107 billion USD by 2017. Most of the MAPs supply required for meeting the global demand, however, still originates from natural sources. Overharvesting, in addition to the destruction of natural habitats, lead to serious losses in biodiversity. Many countries have introduced measures to protect species under the guidance of the CBD and GSPC targets. Nevertheless, the numbers of rare or threatened wild species have been on an increase, especially in recent years. Numerous in situ and ex situ conservation strategies to preserve rare or threatened MAP species have been proposed. Some complementary conservation measures include restrictions on wildcrafting, protection in botanical, ethnobotanical gardens or other areas (national parks, nature reserves). Involvement of local communities in development programs of conservation, for example on-farm cultivation instead of wildcrafting, is essential for preservation of valuable genetic resources for the future.

Keywords Wild-crafted medicinal plants • Overharvesting • Conservation strategies • Conservation measures

1 Introduction

Medicinal and Aromatic Plants (MAPs) play a valuable role in local communities all over the world. They are used in cosmetics, production of dyes, colorants and crop protection products (Lubbe and Verpoorte 2011). In addition to scientific

D. Baričevič (✉) • T. Bartol
University of Ljubljana, Ljubljana, Slovenia
e-mail: dea.baricevic@bf.uni-lj.si

Á. Máthé
Faculty of Agriculture and Food Science, University of West Hungary, Budapest, Hungary

Á. Máthé (ed.), *Medicinal and Aromatic Plants of the World*, Medicinal and Aromatic Plants of the World 1, DOI 10.1007/978-94-017-9810-5_7

research, the markets are also rapidly developing. This is accompanied by national and international regulations applicable to the industrial use of raw plant materials. Raw materials (botanicals) and herb-based products have to meet strict quality as well as safety/efficacy criteria (Máthé and Máthé 2006; Máthé 2011; Ncube et al. 2012).

The use of wild plants is an important component of local knowledge. Plants also play a significant role in modern lifestyle and trends in health foods and healthcare. This involves historical, geographical, cultural, economic and social aspects (Sõukand and Kalle 2010; Łuczaj et al. 2012). Ethnobotanical studies demonstrate the diversity of traditional uses. For example, in Southeast Asia, nearly 2000 different plant species are used in women's healthcare (De Boer and Cotingting 2014).

Inter-generational transfer of traditional knowledge has sharply declined in recent decades (Hamilton 2004; Kala 2005; Kala and Ratajc 2012), although it is one of the key elements in the conservation of species and habitats. Ethnobotanical surveys and confidence between indigenous peoples (knowledge holders) and researchers thus play a crucial conservational role on national level. Local communities are profoundly aware of the environment and ecological needs of particular species. Over the centuries they have developed sustainable ways of exploitation (Kala 2010).

During the past decades, growing global demand for plants and local demand for plant-based traditional medicines put a tremendous pressure on the existing populations (Baricevic and Kusar 2006). Historically, most wild plants represented a natural component of vegetation of a particular region. Uncontrolled over exploitation, habitat-loss and habitat-alteration as well as climate change, which all directly affect plants' ecosystems are the main global threats. However, the increasing demand for plants and products offers a marketing niche for developing countries through land rehabilitation and sustainable management of resources (Lambert et al. 2005; Booker et al. 2012), on condition that the quality of raw materials and/or processed products meet the requirements of international standards.

2 Wild Crafted MAPs in the World Market

The use of herbal preparations in healthcare in developing countries, an increasing variety of modern-day uses, new trends in nutrition and evolving markets, are all reasons for an increasing demand for plants-based raw materials. In addition, elderly people with complex health-care needs increasingly use herbal preparations (Dennis 2013).

After stable growth during the last decade, the global markets of these products will continue to flourish, the recent economic recession notwithstanding, according to the New Report by Global Industry Analysts, Inc.(GIA) (http://www.prweb.com/releases/herbal_supplements/herbal_remedies/prweb9260421.htm; Dennis 2013). Herb market is anticipated to reach 93 billion USD by 2015 and 107 billion by 2017. According to CBI (2013), it is expected to reach 71 billion USD in 2015.

According to Lange (2006), most of the market share in 1991–2003 was associated with 12 countries, globally. The reported annual global export amounted on average to 467,000 tones (value 1.2 billion USD).

In developing countries, it is necessary to differentiate between two kinds of wildcrafting:

1. **harvesting of wild plants for local use** (collectors supply health practitioners in order to improve their livelihood, or, practitioners collect materials for their own use),
2. large scale **wildcrafting for supplying the global markets** on a commercial basis.

Often, these two concepts (sustainable local marketing, and the marketing for exports) are incorrectly linked-up. Erroneous interpretation may have long-term consequences locally and bring about depletion of stocks through over exploitation. Maximizing profit by intermediaries or even by the local government representatives can obstruct prevention (e.g. as the ranking in the CITES list (Convention on International Trade in Endangered Speceis) Appendix II) (Abensperg-Traun 2009) which would have otherwise limited the trade of species at risk. In several countries, using of wild resources is a key to survival as it generates revenues and economic security (Hamilton 2004). This chapter thus highlights main issues related to the wildcrafting of MAPs with regard to marketing chains.

In **India**, with one of the oldest recorded uses of MAPs, some 7,500–8,000 species are in use (cited from: Sati 2013). Most of the recent demand has been met by gathering plants in the highlands. Although increasingly cultivated, the proportion of cultivated in comparison with the wild plants remains very low. In Pithoragarh alone (Himalayan region), more than 1,300 tons are collected annually. Over half a million tons of dry raw material is collected from the wild every year (Tandon 2006). The gap between the demand and supply (about 162 species) is estimated at about 200,000–400,000 tons (Sati 2013). Poor socio-economic conditions stimulate wild crafting which provides income.

In Yunnan Province in southwestern **China**, famous for its richness and diversity of medicinal plants, 216 medicinal species belonging to 194 genera in 98 families were recorded in the local markets (Lee et al. 2008). 173 species (80.1 %) are wild and 43 (19.9 %) are cultivated in gardens or semi-cultivated in wild habitats. Wild *Psammosilene tunicoides*, *Coptis quinquesecta*, species of Orchidaceae family (*Anthogonium gracile* Wall., *Bletilla formosana* Schltr., *Dendrobium aphyllum* C.E.C. Fisch., *Dendrobium capillipes* Rchb.f., *Dendrobium wardianum* Warner, *Goodyera procera* Hook., *Goodyera schlechtendaliana* Rchb.f., *Pleione yunnanensis* Rolfe, *Thunia alba* Rchb.f.) are included in the China Red List of endangered species, while *Cibotium baronets* and all species of Orchidaceae are included in CITES Appendix II (listing species which may threatened unless the trade is controlled).

Many wild species from **Africa** are exported (e.g. *Aloe faro*, *Aloe sinkatana*, *Aloe scabrifolia*, *Warburgia salutaris*, *Warburgia ugandensi*, *Prunus africana*, *Garcinia afzelii*, *Randia acuminata*). Some species have now become subject to international trade controls under CITES (Maundu et al. 2006). In **Ghana,** some

951 tons of medicinal plants are sold annually (7,8 million dollars) in addition to 6 tons used locally (Van Andel et al. 2012). The lists include some rainforest species, e.g. *Daniela ogea* (bark and resin, used mainly for ritual purposes) also harvested in officially protected areas, or *Pericopsis elata* (wood – main uses ritual) – vulnerable on the IUCN red list of Ghana.

In **South Africa**, nearly 4.000 species are used. About 700 indigenous species are traded locally. 20,000 tons of plant material are marketed yearly (McGaw et al. 2005). The lack of raw materials may affect traditional healthcare in the future.

The vast majority of plant materials in the markets in northern **Peru** are harvested in the wild. Cultivation is negligible (Bussmann and Sharon 2009). More than two-thirds of the species originate from the highlands. More than 40 % of the sales is represented by 7 native (*Croton lechleri*, *Uncaria tomentosa*, *Equisetum giganteum*, *Peumus boldus*, *Erythrina* spp., *Buddleja utilis* and *Piper aduncum*) and 3 exotic species (*Chamomilla recutita*, *Ruta graveolens*, *Eucalyptus globulus*). The total value of recorded sales in Trujillo and Chiclayo markets reached 1.2 million USD per year. This is only a part of income and does not include the fee charged by healers. These activities contribute strongly to the local economy. In **Ukraine**, such uses are declining, partly due to lower natural stocks because of excessive past exploitation (in 1980, 17,000 tons were used, mostly harvested in the wild) and because of the changes in the ecosystems. Minarchenko (2011) estimates that at present about 600 tons of wild materials and 400 tons of cultivated materials are in use. In **Turkey**, collection in protected areas (e.g. national parks), is still legal. Collectors pay a fee to General Directorate of Forestry which issues licenses (Cetinkaya 2010). On estimation, some 472 tons were collected from the wild (reportingly sustainable) in Köprülü Kanyon National Park in 2005 alone.

3 Threat to Wild Crafted MAPs and Their Habitats

Natural ecosystems play an important role in conservation of indigenous plants and habitats. Each species occupies a particular niche. According to Chase and Leibold (2003), an ecological niche is described as an association of all environmental factors that allow an organism to maintain fertility in a population at least equal, if not greater than mortality, together with all the effects of the organism on the environment. Populations have historically evolved in interaction with the environment. Some genotypes may possess unique genetic patterns of future research interest. Ecological diversity in the areas with native populations should be assessed, ecogeographically monitored (using state-of-the art global information systems – GIS) in order to describe the ecological conditions for populations in situ (Parra-Quijano et al. 2012; Baricevic et al. 2012; Cordell 2009).

Factors causing a particular species to become endangered (rare or rare with a threat of extinction or extinct) can be either natural (environmental) or anthropogenic (e.g. demographic explosion and intensified industrial and agricultural activities) (Zlobin 2012). In many cases the direct (e.g. overcollection, grazing) or indirect (tilling, forest cutting, swamp drainage, fires, change or complete destruction due to pollution or penetration of alien or even invasive species, global warming, climate change) (Hamilton 2008; Zlobin 2012) impacts are reported.

Even though many countries have already published Red Lists or Red Data Books it is often not clear how many and which species have become threatened (Heywood 2011). An estimation puts some 15,000 of species under threat. Primary causes are the destruction of habitats and overcollection (McKenzie et al. 2009).

Pharmaceutical and other industrial uses must urgently be limited, such as massive exploitation of *Harpagophytum* spp. in southern Africa, *Prunus africana* in montane sub-Saharan Africa and Madagascar, *Caesalpinia echinata* in Brazilian Atlantic rainforest, *Warburgia salutaris* in southern Africa and *Saussurea costus* in the Himalaya (Abensperg-Traun 2009; Schippmann et al. 2006). In the United States, a massive overcollection is reported for *Taxus brevifolia*, *Panax ginseng*, *Hydrastis canadensis*, *Cimicifuga racemosa*, *Panax quinquefolius*, *Sanguinaria canadensis* L., *Chamaelirium luteum*, *Piper methysticum*, *Cypripedium* spp., *Lomatium dissectum*, *Ligusticum porteri*, *Mitchella repens*, *Lophophora williamsii*, *Ulmus rubra*, *Drosera* spp., *Trillium* spp., *Aletris farinosa*, *Dionaea muscipula* and *Dioscorea villosa* L. (McKenzie et al. 2009; Burkhart et al. 2012).

Some typical threats are as follows:

In the **United States**, *Echinacea tennesseensis* (Beadle) Small is endemic to cedar glades of middle Tennessee and limited to five sites (Walck et al. 2002). Listed under the U.S. Endangered Species Act, it has been partly threatened by collecting for medicinal purposes (cit. from: Simberloff 2012). The largest threat, however, is constituted by the destruction of habitats. Some protection has been offered by an international establishment of populations. Seed storage can also reduce the extinction risk (Walck et al. 2002).

In **India**, there are some 17,000 known angiosperms among 47,000 known species. A total of 560 species of India have been included in the International Union for Conservation of Nature and Natural Resources (IUCN) Red List of Threatened species, 247 at risk (Prakash and Nirmalaa 2013). Exports have in the last 30 years seriously endangered many plants. It has brought about the extinction of, for example *Saussuria obvallata*, *Ceropegia bulbosa*, *Erymostachys superba, Rauwolfia serpentina* (Kumar 2006). *Begonia tessaricarpa*, *Fritillaria roylei*, *Lilium polyphyllum, Polygonatum verticillatum*, *Roscoea Purpurea*. Two species of the orchid's family (*Habenaria intermedi, Malaxis muscifera*) have become critically endangered (Prakash and Nirmalaa 2013). In the Central Himalayan Region about 95 % of plants are collected from the wild. The process frequently destroys the entire plant: roots (29.6 %), barks (13.5 %), wood (2.8 %), rhizome (4 %) and whole plants (24.3 %) (Sati 2013). Many floral species became threatened through over-exploitation and other factors. Over 350 species are vulnerable, 161 of which are rare and severely threatened.

In **Ukraine**, 2,223 species are classified as medicinal (Minarchenko 2011). A total of 102 species are rare (in all regions *Anemone sylvestris L.*, *Hypericum humifusum* L., *Polemonium caeruleum* L). Wild harvesting has been prohibited. Some species have been classified as "endangered" (*Acorus calamus, Ledum palustre, Menyanthes trifoliata, Oxycoccus palustris*). The third category of "disturbed" MAPs involves species with limited potentials on account of small habitats or low population productivity (*Convallaria majalis, Potentilla erecta, Origanum vulgare, Vaccinium uliginosum, Valeriana officinalis).* More abundant but limited species were categorized as a "limited" group (*Crataegus* spp., *Frangula alnus, Helichrysum arenarium, Hypericum perforatum, Thymus serpyllum*). The last group of "prospective" species is characterized by a more abundant distribution (*Sambucus nigra, Alnus glutinosa...*). Here, some other sustainable measures have been proposed. Collection is strictly monitored. The measures are governed by national legislation.

In **China**, habitat destruction represents a problem. Political and economic actions have encouraged exploitation with little regard to sustainability and traditional conservation practices (Pei et al. 2010).

In **Pakistan** between 600 and 700 species are used medicinally, 300 of which are traded (Shinwari 2010). Ali and Qaiser (2009) report on 7 taxa – *Aesculus indica, Allium barszczewskii, Anthemis cotula, Bunium persicum, Delphinium nordhagenii (*endemic to Chitral*), Ferula narthex* and *Paeonia emodi* – extensively exploited locally. Collectors have no awareness of the suitable season and seed collection methods of *Pinus gerardiana,* declared as threatened. Some 94 % of *Ferula narthex* has been reported destroyed in Chitral Gol National Park protected area (plants are cut just above the root for latex harvesting). These populations have alarmingly decreased. According to Shinwari (2010), in biodiversity hot spots of Pakistan, between 150 and 200 species are threatened. In the Lesser Himalayas, some 5,000 families harvest plants in summer (Abbasi et al. 2012). Seasonal vendors sell plants elsewhere. Due to overharvesting (often the whole plant – *Ajuga bracteosa*, *Berberis lyceum*, *Bergenia ciliata*, *Viola canescens*, and *Zanthoxylum armatum*) and population fragmentation, many species are likely to become endangered. *Carissa opaca, Myrsine africana*, *Mallotus phillipensis, Berberis lycium*, *Bergenia ciliata*, *Justicia adhatoda*, *Pistacia chinensis*, *Quercus leuctrichophora*, *Punica granatum* and *Viola canescens* are also at risk because of slow growth of populations, over consumption (also for fuel wood) and grazing.

In **Albania,** natural resources of MAPs are largely depleted. Pieroni et al. (2014) surveyed four mountainous villages of the Peshkopia region. Several endemic taxa have been documented, such as *Achillea corabensis, Crepis macedonica*, *Dianthus macedonicus*, *Erysimum korabense, Sesleria korabensis.* Among the approximately 30 wild plants (not used locally) the following are most frequently collected: *Gentiana lutea, Primula veris, Urtica dioca, Crataegus* spp., *Thymus pulegioides, Hypericum perforatum, Vaccinium myrtillus, Juniperus communis, Achillea millefolium, Orchis* spp. and *Sambucus nigra.*

In **Turkey**, at least 346 taxa of plants are traded commercially. Most geophytes and bulbous plants exported are endemic and considered as threatened (cited from: Şekercioglu et al. 2011). In **Serbia**, harvesting of plants is a widespread activity. An area of extreme floristic abundance, reported as an internationally important biodiversity "hot spot", is located in Carpatho–Balkan Mountains (Zlatković et al. 2014). *Centaurium erythraea*, *Arctostaphylos uva-ursi*, *Hypericum perforatum*, *Teucrium montanum* and *Allium ursinum* have been illegally collected and over-harvested. *Carlina acaulis*, *Gentiana asclepiadea* and *Orchis morio* are threatened because of harvesting underground parts. The endemic *Nepeta rtanjensis* is collected in native habitat although protected under the CITES convention (CITES 1973). It has been categorized among the critically endangered (CR) taxa of Serbia. In **Sierra Leone**, an extensive exploitation was reported for *Xylopia aethiopica*, *Piper guineense*, *Garcinia kola*, *Carapa procera*, *Cassia sieberiana*, *Massularia acuminata*, *Salacia chlorantha* subsp. *demeusei*, *Rhigiocarya racemifera* and *Sarcocephalus latifolius* (Jusu and Cuni Sanchez 2014). Three species are defined as vulnerable under the IUCN Red list: *Garcinia kola*, *Fleroya stipulosa* and *Nauclea diderrichii*, which is endemic to the Upper Guinean Forest of West Africa.

4 Conservation Strategies

The potentially useful genetic resources need to be evaluated with modern research and scientific methods (Johns and Eyzaguirre 2000; Johns and Eyzaguirre 2002; Johns 2002; Baricevic et al. 2012), in order to preserve natural and cultural heritage. Moreover, these resources represent heritable materials with many benefits (economic, scientific or social significance) (Ayyanar et al. 2013; Heinrich et al. 2005).

In the wake of the Chiang Mai Declaration (1988) (Máthé 2011), many strategic documents and programs, proceeding from the Convention on Biological Diversity (CBD 1992) such as the Global Strategy for Plant Conservation and European Plant Conservation Strategy, Joint (IUCN and WWF) Plants Conservation Program or guidelines, specifically relevant to medicinal plants, such as the Guidelines on the Conservation of Medicinal Plants (WHO, IUCN, WWF 1993), WHO Guidelines on Good Agricultural and Collection Practices (GACP) for Medicinal Plants (WHO 2003), stressed the need for an effective in situ and ex situ conservation of threatened plants including medicinal plants and their habitats (Secretariat of the Convention on Biological Diversity, 2009, http://www.plantlife.org.uk/international/plantlifepolicies-strategies-epcs.html, Heywood 1989).

Guidelines on the conservation by WHO, IUCN & WWF in 1993 outlined the role of international organizations and provide basic elements for the preparation of national strategies on:

- traditional knowledge of the use of plants in primary health care, including ethnobotanical surveys and plant identification,

- utilization, cultivation, harvest and storage, development of breeding programs, and the sustainable use of wild plants,
- conservation in situ and ex situ, public support for conservation through education and information/publicity campaigns.

The Convention on International Trade in Endangered Species (CITES 1973) introduced a measure of controls in the international trade and was the basis of worldwide policies on protection.

In the last decade there has been a gradual increase of the species on the IUCN Red List (Secretariat of the Convention on Biological Diversity 2009).

As the origins of threat are similar to those of other wild species (i.e. loss of habitat, over harvesting for medicinal or other purposes, invasion by alien species, pollution, climate change) (Hamilton 2008) we were able to assess the conservation of ecosystems in the frame of CBD and global conservation strategies available to the national and international policy makers. The targets of CBD and other international programs (like Global Strategy for Plant Conservation) in the frame of species biodiversity conservation seem to be too ambitious (Heywood 2011; Zlobin 2012; Maestre Andrés et al. 2012). The target agreed by the world's governments in 2002 "to achieve by 2010 a significant reduction of the current rate of biodiversity loss on the global, regional and national level as a contribution to poverty alleviation and to the benefit of all life on Earth", has not been met (cited from: Secretariat of the Convention on Biological Diversity 2010).

In 2010, the participating parties of the Convention on Biological Diversity (CBD) adopted the Strategic Plan for Biodiversity 2011–2020, a 10-year framework for action by all countries and stakeholders with the view of preserving biodiversity and the benefits to people (CBD 2010). The Convention called on the national governments (193 parties) to prepare the National Biodiversity Strategies and Action Plans (NBSAPs) in order to fulfill the objectives of the CBD and the concrete actions (20 targets under 5 strategic goals that cover also the reduction of the direct pressures on biodiversity, promotion of sustainable use and safeguarding ecosystems, species and genetic diversity) (http://www.cbd.int/nbsap/). As of February 2014, 179 parties have completed NBSAPs, 44 parties have revised their NBSAPs; in 14 parties the NBSAPs are currently under revision http://www.cbd.int/doc/nbsap/nbsap-status.doc.

Several strategies have been adopted such as in situ conservation, restrictions on harvesting, sustainable wild collection (Schippmann et al. 2002, 2006; Wolfgang 2011), artificial regeneration of damaged or unsustainably harvested MAPs, restoration of natural habitats, ex situ conservation and on-farm cultivation. These complementary strategies should not be applied separately (Heywood 2004; Prakash and Nirmalaa 2013). However, two strategies should clearly be differentiated: in situ conservation taking into account target species and their natural habitats, where genetic flows between populations allow the evolutionary process to continue, ex situ approach which essentially strives to protect genetic diversity of species providing a better protection of germplasm (Bhattacharyya et al. 2009; Prakash and Nirmalaa 2013).

In situ conservation measures can be applied to conservation and maintenance of species biodiversity in various types of habitats, recovery and restoration programs (Heywood 2004).

Ex situ conservation measures apply to institutional models (botanical gardens, ethnobotanical gardens, herbal gardens, home gardens, national parks and other protected areas, wildlife sanctuaries, biosphere reserve and reserve forests, farms or other agro systems, genebanks, DNA banks. . .), where sustainable management provides for germplasm conservation and, in the case of ethnobotanical gardens, promotes local ethnopharmacological knowledge (Anbarashan and Padmavathy 2010; Heywood et al. 1993; Hammer et al. 2003; Bhattacharyya et al. 2009; Heywood 2004; Innerhofer and Bernhardt 2011; Kala 2010; Prakash and Nirmalaa 2013; Reed et al. 2011; Swain 2010; Salako et al. 2014). Most *ex-situ* conservation approaches employ complementary tools (*in vitro* and other biotechnological techniques – to enhance the biomass of germplasm) and require basic ecological understanding of the MAP species in question, related to edaphic, agro-climatic and geographical conditions for proper maintenance (Kala 2010).

One of the first successful projects for *ex-situ* conservation started in the 80s in **India** by the Tropical Botanic Garden and Research Institute (TBGRI), and has been described by Pushpangadan et al. (1999). A field MAPs gene bank has been established by the reconstructing the original habitat. In the 10-year period, at least 25 % of the original flora had been collected from the still existing neighboring forests and brought to an experimental plot of 50 ha, which included about 1000 plant species, 100 of which were MAPs.

Local communities as holders of the knowledge should be informed on the state of natural resources. Conservation strategies as well as sustainable practices must thus also involve locals and their socio-economic interests, intellectual property rights including (Antons 2010; Hamilton 2011). Biodiversity and ecosystem conservation can significantly benefit human societies (local healthcare support, opportunities for improvement of livelihood, affirmation of local culture), and provide more sustainable livelihoods and well-being (Abensperg-Traun 2009; Hamilton 2011; Smith-Hall et al. 2012).

In **Europe**, the MAPs working group (represented by 33 members) of the European Cooperative Program for Plant Genetic Resources (ECPGR) represents a driving force for the conservation. It introduced complementary ex situ and in situ conservation strategies through on-farm cultivation (Baricevic and Kusar 2006). Such an approach safeguards development of small and medium-sized rural economies, maintain sufficient quantities of high quality herbal drugs and, consequently, promote conservation of natural habitats.

5 Discussion and Conclusions

The way in which people manage wild plant resources will significantly influence the sustainability of livelihoods and the conservation of plant diversity (Hamilton 2008). Unsustainable collection from the wild, rapid loss of habitats, indiscriminate over harvesting, global warming and climate change are threatening some 15,000 species, hampering future use.

Demographic growth may have a considerable impact on both human health and the availability of plants. Some of the species used in pharmaceutical or other markets could become extinct through population pressure, habitat change or global climatic change (Heywood 2011). By disrupting the ecosystem function, such ecosystems become less flexible and more vulnerable, and ultimately less capable to supply humans with.

Human health and well-being depend on the environment. The diversity of plant resources plays an essential role in providing nutritional, health and socio-cultural benefits (Johns 2002). The loss of biodiversity has been a subject of many debates (http://www.who.int/globalchange/ecosystems/biodiversity/en/, Millennium Ecosystem Assessment 2005; Alves and Rosa 2007). Many arguments assert that ecosystem depletion directly or indirectly impacts human health and survival.

The quality of raw materials depends on a variety of factors (genetic, environmental, developmental – morphogenetic). These influence the growth and development of a specific population (genetic source) in situ or ex situ. Some factors also affect the quality of raw materials after harvesting (during the processes of drying, transport, in storehouse). Professional guidelines, rules and regulations aim at improving quality of herbal raw materials (Máthé and Máthé 2006). Factors reducing the value of wild crafted medicinal plants are represented by inconsistent and unpredictable quality, unverified biological efficacy, possible misidentifications, heavy metal contamination and microbial risks. The exploitation of MAPs should be thus based on scientific principles which will guarantee high-quality of raw materials and should be considered as an essential component in sustainable strategies.

In conclusion, MAP biodiversity conservation strategies, that are based on the dynamic processes of natural ecosystems and involve both MAP cultivation and human society, could be viewed also as an opportunity to advance the progress of local human communities. As Hamilton (2008) says, projects concerned with the conservation of MAPs cannot concentrate solely on pure conservation but have to integrate local people, who are interested in actual or anticipated values of medicinal plants for healthcare, income and cultural identity. Such an approach acts as a motivating force for conservation and security – for medicinal plant species and their habitats, and also economic prosperity of people and human societies in general.

References

Abbasi AM, Khan MA, Ahmad M, Zafar M (2012) Ethnobotanical aspects. In: Medicinal plant biodiversity of lesser Himalayas-Pakistan. Springer

Abensperg-Traun M (2009) CITES, sustainable use of wild species and incentive-driven conservation in developing countries, with an emphasis on southern Africa. Biol Conserv 142:948–963

Ali H, Qaiser M (2009) The ethnobotany of Chitral Valley, Pakistan with particular reference to medicinal plants. Pak J Bot 41(4):2009–2041

Alves RRN, Rosa IML (2007) Biodiversity, traditional medicine and public health: where do they meet? J Ethnobiol Ethnomed 3(14):1–9

Anbarashan M, Padmavathy A (2010) Ethno-medicinal plants in five sacred groves in Cuddalore District, Tamilnadu, India. Ethnobot Leafl 14:774–780

Antons C (2010) The role of traditional knowledge and access to genetic resources in biodiversity conservation in Southeast Asia. Biodivers Conserv 19:1189–1204

Ayyanar M, Ignacimuthu S, Houghton PJ (2013) Threat status of medicinal plants used by the tribal people in Kalakad Mundanthurai Tiger Reserve, Southern Western Ghats, India Proc Natl Acad Sci India, Sect B Biol Sci, 11 p

Baricevic D, Kusar A (2006) Conservation of medicinal and aromatic plants in Europe – a review of current progress. In: 4th conference on medicinal and aromatic plants of South-East European Countries, Iaşi-România, 28–31 May 2006. Conference proceedings: Iaşi-România, 28–31 May 2006. Alma Mater Publishing House, [2006], pp 24–30

Baricevic D, Ratajc P, Zupan M, Turk B, Vres B, Seliskar A, Seliskar T (2012) Conservation and evaluation of genetic resources of medicinal plants. Acta Agric Slov 99(3):363–371, http://aas.bf.uni-lj.si/december2012/10Baricevic.pdf

Bhattacharyya R, Asokan A, Bhattacharya P, Prasad R (2009) The potential of certification for conservation and management of wild MAP resources. Biodivers Conserv 18:3441–3451

Booker A, Johnston D, Heinrich M (2012) Value chains of herbal medicines ndash research needs and key challenges in the context of ethnopharmacology. J Ethnopharmacol, http://eprints.pharmacy.ac.uk/2679/1/Booker-et-al-ValueChainsJEP2012.pdf

Burkhart EP, Jacobson MG, Finley J (2012) A case study of stakeholder perspective and experience with wild American ginseng (Panax quinquefolius) conservation efforts in Pennsylvania, U.S.A.: limitations to a CITES driven, top-down regulatory approach. Biodivers Conserv 21(14):3657–3679

Bussmann RW, Sharon D (2009) Markets, healers, vendors, collectors: The sustainability of medicinal plant use in Northern Peru. Mt Res Dev 29(2):128–134

CBD (1992) Convention on biological diversity. Secretariat of the Convention on Biological Diversity, Montreal

CBD (2010) Strategic plan for biodiversity 2011–2020. Secretariat of the Convention on Biological Diversity, Montreal

CBI (2013) Tradewatch for natural ingredients for pharmaceuticals, CBI Market Information Database; CBI

Cetinkaya G (2010) Conservation and sustainable wild-collection of medicinal and aromatic plants in Köprülü Kanyon National Park, Turkey. J Med Plant Res 4(12):1108–1114

Chase JM, Leibold MA (2003) Ecological niche: linking classical and contemporary approaches. The University of Chicago Press, Chicago

CITES (1973) Convention on international trade in endangered species of wild fauna and flora. CITES Secretariat, Washington, DC

Cordell GA (2009) Sustainable drugs and global health care. Quim Nova 32:1356–1364

De Boer HJ, Cotingting C (2014) Medicinal plants for women's healthcare in southeast Asia: a meta-analysis of their traditional use, chemical constituents, and pharmacology. J Ethnopharmacol 151:747–767

Dennis J (2013) Where are the opportunities for growth and what challenges stand in the way of long-term market success? International perspectives on herbs & botanicals, nutraceuticals world, 1 July 2013

Hamilton AC (2004) Medicinal plants, conservation and livelihoods. Biodivers Conserv 13:1477–1517

Hamilton AC (ed) (2008) Medicinal plants in conservation and development: case studies and lessons learnt. Plantlife International, Salisbury
Hamilton AC (2011) An evidence-based approach to conservation through medicinal plants. In: Medicinal plant conservation, medicinal plant specialist group of the IUCN species survival commission, pp 2–7
Hammer K, Gladis T, Diederichsen A (2003) In situ and on farm management of plant genetic resources. Eur J Agron 19:509–517
Heinrich M, Pieroni A, Bremner P (2005) Medicinal plants and phytomedicines. In: Consulting Editor: Ghillean Prance, Scientific Editor: M. Nesbitt. The cultural history of plants. Routledge (Taylor and Francis), New York, pp 205–238
Heywood VH (1989) The IUCN: its role in biological diversity. Part II: plant conservation. Diversity 5:2–3, 11–14
Heywood VH (2004) Conserving species in situ – a review of the issues. Master lesson. 4th European conference on the conservation of wild plants, Valencia, 17–20 Sept 2004, http://www.nerium.net/plantaeuropa/Proceedings.htm
Heywood VH (2011) Ethnopharmacology, food production, nutrition and biodiversity conservation: towards a sustainable future for indigenous peoples. J Ethnopharmacol 137:1–15
Heywood VH, Gustafson JP (ed), Appels R (ed), Raven P (1993) Broadening the basis of plant resource conservation. Gene conservation and exploitation. Proceedings of the 20th stadler genetics symposium held at the University of Missouri, Columbia, 30 Sept–1 Oct 1991. Stadler Genetics Symposia Series, 1993:1–13
Innerhofer S, Bernhardt KG (2011) Ethnobotanic garden design in the Ecuadorian Amazon. Biodivers Conserv 20:429–439
IUCN, WHO, WWF (1993) Guidelines on the conservation of medicinal plants, IUCN, Gland
Johns T (2002) Plant genetic diversity and malnutrition: practical steps in the development of a global strategy linking plant genetic resource conservation and nutrition. Afr J Food Nutr Sci 3:98–100
Johns T, Eyzaguirre PB (2000) Nutrition for sustainable environments. SCN News 21:24–29
Johns T, Eyzaguirre PB (2002) Nutrition and the environment. In: Nutrition: a foundation for development. ACC/SCN, Geneva
Jusu A, Cuni Sanchez A (2014) Medicinal plant trade in Sierra Leone: threats and opportunities for conservation. Econ Bot XX(X):1–14
Kala CP (2005) Ethnomedicinal botany of the Apatani in the Eastern Himalayan region of India. J Ethnobiol Ethnomed 1(11):1–12
Kala CP (2010) Ethnobotanical and ecological approaches for conservation of medicinal and aromatic plants. Acta Horticult 860:19–26
Kala CP, Ratajc P (2012) High altitude biodiversity of the Alps and the Himalayas: ethnobotany, plant distribution and conservation perspective. Biodivers Conserv 21(4):1115–1125
Kumar P (2006) The economics of medicinal plants: are high commercial values enough to ensure biodiversity conservation?, pp 9–15. In: Miththapala S (ed) Conserving medicinal species: securing a healthy future. IUCN: Ecosystems and Livelihoods Group, Asia, 184 pp
Lambert JDH, Ryden PA, Esikuri EE (2005) Capitalizing on the bioeconomic value of multi-purpose medicinal plants for rehabilitation of dry lands in sub-sahara Africa. The international Bank for reconstruction and development. The World Bank, Washington, DC, pp 11–14
Lange D (2006) International trade in medicinal and aromatic plants. Actors, volumes and commodities. In: Bogers RJ, Craker LE, Lange D (eds) Medicinal and aromatic plants. Springer, Dordrecht, The Netherlands, pp 155–170
Lee S, Xiao C, Pei S (2008) Ethnobotanical survey of medicinal plants at periodic markets of Honghe Prefecture in Yunnan Province, SW China. J Ethnopharmacol 117:362–377
Lubbe A, Verpoorte R (2011) Cultivation of medicinal and aromatic plants for specialty industrial materials. Ind Crop Prod 34:785–801

Łuczaj Ł, Pieroni A, Tardío J, Pardo-de-Santayana M, Sõukand R, Svanberg I, Kalle R (2012) Wild food plant use in 21st century Europe: the disappearance of old traditions and the search for new cuisines involving wild edibles. Acta Soc Bot Pol 81(4):359–370

Maestre Andrés S, Calvet Mir L, van den Bergh JCJM, Ring I, Verburg PH (2012) Ineffective biodiversity policy due to five rebound effects. Ecosyst Serv 1:101–110

Máthé Á (2011) A new look at medicinal and aromatic plants. Acta Horticult (ISHS) 925:13–20. http://www.actahort.org/books/925/925_1.htm

Máthé Á, Máthé I (2006) Quality assurance of cultivated and gathered medicinal plants. Acta Hort (ISHS) 765:67–76, http://www.actahort.org/books/765/765_8.htm

Maundu P, Kariuki P, Eyog-Matig O (2006) Threats to medicinal plant species – an African perspective, pp 47–63. In: Miththapala S (ed) Conserving medicinal species: securing a healthy future. IUCN: Ecosystems and Livelihoods Group, Asia, 184 pp

McGaw L, Jäger A, Grace O, Fennel C, Van Staden J (2005) Medicinal plants. In: van Niekerk A (ed) Ethics in agriculture – an African perspective. Springer Netherlands, pp 67–83

McKenzie M, Kirakosyan A, Kaufman PB (2009) Risks associated with overcollection of medicinal plants in natural habitats. In: Kirakosyan A, Kaufman PB (eds) Recent advances in plant biotechnology. Springer Science+Business Media New York, pp 363–387

Millennium Ecosystem Assessment (2005) Ecosystems and human well-being: biodiversity synthesis. World Resources Institute, Washington, DC

Minarchenko V (2011) Medicinal plants of Ukraine: diversity, resources, legislation. In: Medicinal plant conservation, medicinal plant specialist group of the IUCN species survival commission, pp 7–13

Ncube B, Finnie JF, Van Staden J (2012) Quality from the field: the impact of environmental factors as quality determinants in medicinal plants. S Afr J Bot 82:11–20

Parra-Quijano M, Iriondo JM, Torres E (2012) Ecogeographical land characterization maps as a tool for assessing plant adaptation and their implications in agrobiodiversity studies. Genet Resour Crop Evol 59:205–218

Pei S, Hamilton AC, Lixin Y, Huyin H, Zhiwei Y, Fu G, Quangxin Z (2010) Conservation and development through medicinal plants: a case study from Ludian (Northwest Yunnan, China) and presentation of a general model. Biodivers Conserv 19:2619–2636

Pieroni A, Nedelcheva A, Hajdari A, Mustafa B, Scaltriti B, Cianfaglione K, Quave CL (2014) Local knowledge on plants and domestic remedies in the mountain villages of Peshkopia (Eastern Albania). J Mt Sci 11(1):180–194

Prakash K, Nirmalaa A (2013) A review on conservation of endangered medicinal plants in India. Int J Ethnomed Pharmacol Res 1(1):21–33

Pushpangadan P, Thomas J, Caffini N (ed), Bernath J (ed), Craker L (ed), Jatisatienr A (ed), Giberti G (1999) A novel experiment in the ex-situ conservation of medicinal and aromatic plants genetic diversity. Proceedings of the second world congress on medicinal and aromatic plants, WOCMAP-2. Biological resources, sustainable use, conservation and ethnobotany, Mendoza, 10–15 Nov 1997. Acta-Horticulturae 1999, 500:177–182

Reed BM, Sarasan V, Kane M, Bunn E, Pence VC (2011) Biodiversity conservation and conservation biotechnology tools. In Vitro Cell Dev Biol Plant 47:1–4

Salako VK, Fandohan B, Kassa B, Assogbadjo AE, Idohou AFR, Gbedomon RC, Chakeredza S, Dulloo ME, Kakaı RG (2014) Home gardens: an assessment of their biodiversity and potential contribution to conservation of threatened species and crop wild relatives in Benin. Genet Resour Crop Evol 61:313–330

Sati VP (2013) Cultivation of medicinal plants and its contribution to livelihood enhancement in the Indian Central Himalayan Region. Adv Med Plant Res 1(1):17–23

Schippmann U, Leaman DJ, Cunningham AB (2002) Impact of cultivation and gathering of medicinal plants on biodiversity: global trends and issues. Biodiversity and the ecosystem approach in agriculture, forestry and fisheries. Satellite event on the occasion of the Ninth Regular Session of the Commission on Genetic Resources for Food and Agriculture. Inter-Departmental Working Group on Biological Diversity for Food and Agriculture, Rome

Schippmann U, Leaman D, Cunningham AB (2006) A comparison of cultivation and wild collection of medicinal and aromatic plants under sustainability aspects. In: Bogers RJ, Craker LE, Lange D (eds) Medicinal and aromatic plants. Springer, Dordrecht, The Netherlands, pp 75–95

Secretariat of the Convention on Biological Diversity (2009) The convention on biological diversity plant conservation report: a review of progress in implementing the global strategy of plant conservation (GSPC). Montréal, 48 p

Secretariat of the Convention on Biological Diversity (2010) Global biodiversity outlook 3. Montréal, 94 pp

Şekercioglu ÇH, Anderson S, Akçay E, Bilgin R, Emre Can Ö, Semiz G, Tavşanoğlu Ç, Baki Yokeş M, Soyumert A, İpekdal K, Sağlam İK, Yücel M, Dalfes HN (2011) Turkey's globally important biodiversity in crisis. Biol Conserv 144(12):2752–2769

Shinwari ZK (2010) Medicinal plants research in Pakistan. J Med Plant Res 4(3):161–176

Simberloff D (2012) Sustainability of biodiversity under global changes, with particular reference to biological invasions. In: Weinstein MP, Turner RE (eds) Sustainability science. Springer, New York, pp 139–157

Smith-Hall C, Larsen HO, Pouliot M (2012) People, plants and health: a conceptual framework for assessing changes in medicinal plant consumption. J Ethnobiol Ethnomed 8:43

Sõukand R, Kalle R (2010) Plant as object within herbal landscape: different kinds of perception. Biosemiotics 3(3):299–313

Swain S (2010) Involving tribal communities in plant conservation, BGCI. Roots 7(2):20–24

Tandon V (2006) The risks of the loss of medicinal plants for livelihood and health security in South Asia, pp 32–39. In: Miththapala S (ed) Conserving medicinal species: securing a healthy future. IUCN: Ecosystems and Livelihoods Group, Asia, 184 pp

Van Andel T, Myren B, Van Onselen S (2012) Ghana's herbal market. J Ethnopharmacol 140:368–378

Walck JL, Hemmerly TE, Hidayati SN (2002) The endangered Tennessee purple coneflower Echinacea tennesseensis (Asteraceae): its ecology and conservation. Native Plant J 3(1):54–64

WHO (2003) WHO Guidelines on Good Agricultural and Collection Practices (GACP) for medicinal plants. WHO, Geneva

Wolfgang K (2011) The new FairWild standard – a tool to ensure sustainable wild-collection of plants. In: Medicinal plant conservation, medicinal plant specialist group of the IUCN species survival commission, pp 14–17

Zlatković BK, Bogosavljević SS, Radivojević AR, Pavlović MA (2014) Traditional use of the native medicinal plant resource of Mt. Rtanj (Eastern Serbia): ethnobotanical evaluation and comparison. J Ethnopharmacol 151:704–713

Zlobin YA (2012) Rare plant species: floristic, phytocenotic and population approaches. Biol Bull Rev 2(3):226–237

Web Link

http://www.cbd.int/doc/nbsap/nbsap-status.doc. Accessed 28 Feb 2014

http://www.cbd.int/nbsap/. Accessed 28 Feb 2014

http://www.ecpgr.cgiar.org/working_groups/medicinal_plants.html. Accessed 28 Feb 2014

http://www.plantlife.org.uk/international/campaigns/policies_strategies/european_plant_conservation_strategy

http://www.prweb.com/releases/herbal_supplements/herbal_remedies/prweb9260421.htm. Accessed 28 Feb 2014

http://www.who.int/globalchange/ecosystems/biodiversity/en/. Accessed 28 Feb 2014

Chapter 8
Challenges and Decision Making in Cultivation of Medicinal and Aromatic Plants

Zora Dajic-Stevanovic and Dejan Pljevljakusic

Abstract Cultivation of medicinal and aromatic plants (MAP) today is not only a promising alternative and counterpoint to wild collection, enabling preservation of natural genetic variability and survival of rare, endemic, vulnerable and endangered species, but also represents a powerful economy branch providing the high class quality raw material for pharmaceutical, cosmetic and the food industry. Domestication and cultivation of most of medicinal plants, usually conceived as a minor crops, face with many challenges on small, medium and large scale production, relating both cultivation technologies and market and prices fluctuations. Cultivated MAP material is increasingly preferred by the herbal industry, because it is easier to predict plant yield, quality and drug composition, especially when compared with wild harvested raw materials. In case of cultivated MAP material, the possibility of plant misidentification and adulteration is excluded. The profitability of cultivation of medicinal plants compete with profit achievable for standard field crops for which already exist a specialized machinery and a standard procedure for application of fertilizers and agrochemicals to control weeds, pests and diseases. For successful large scale cultivation of MAP, the high quality raw material should be produced using low input cultivation methods to be competitive at the international market and with plants collected from the wild. The most common issues with which the producers of medicinal plants encountered are the market, abundance and accessibility of wild populations, agro-environmental conditions, labor availability and costs, investments in machinery, post-harvest processing, and profitability of production. Superior genotypes are very important for profitable production of the high quality medicinal plants' row material. Out of all cultivated medicinal plant species, only a small percentage is clearly genetically defined and represented on the seed market in term of variety. Similarly to the other crops, traditional breeding methods, as well as biotechnological procedures and selection assisted by molecular markers are applied in development of new varieties and

Z. Dajic-Stevanovic (✉)
Faculty of Agriculture, University of Belgrade, Belgrade, Republic of Serbia
e-mail: dajic@agrif.bg.ac.rs

D. Pljevljakusic
Institute for Medicinal Plants Research "Dr Josif Pancic", Belgrade, Republic of Serbia

Á. Máthé (ed.), *Medicinal and Aromatic Plants of the World*, Medicinal and Aromatic Plants of the World 1, DOI 10.1007/978-94-017-9810-5_8

cultivars of MAP, aiming at improvement of their desirable characteristics. This refers to increased drug yield and the content of required secondary metabolites. Mapping of genes and specific DNA sequences involved in biosynthesis of particular metabolite classes seems to be a future challenge in MAP breeding programs. Most of actual research is focused on genetic variability among different taxa of medicinal plants using several types of DNA markers, including restriction fragment length polymorphism (RFLP), amplified fragment length polymorphic DNA (AFLP), random amplified polymorphic DNA (RAPD), cleavage amplified polymorphic sequence (CAPS), simple sequence repeat (SSR), and sequence characterized amplified region (SCAR) markers. Although the primary target for trait manipulation in medicinal plants is the content of active compounds, for development as crops, basic agronomic characters related to uniformity, stability, growth and development, and resistance to biotic and abiotic stresses, must also be improved.

Keywords Cultivation versus collection • Decision making • Breeding approaches

1 Introduction: Wild Collection Versus Cultivation in Brief

Sustainable practice of traditional medicine and supply of plant materials for drug development are hinged heavily on deliberate and concerted efforts to conserve indigenous medicinal and aromatic plants (Oladele et al. 2011). Together with growth in global demand for medicinal plants, the pressure on their existing populations has rapidly increased during the last few decades. About 15,000 species of medicinal plants are globally threatened. The key grounds of endangerment of MAP species and populations include: loss of habitat and habitat fragmentation, over-harvesting, improper collecting practices and pollution (Dajic-Stevanovic et al. 2012). In order to stop further biodiversity loss of natural resources of medicinal plants, it is needed to evaluate the remaining stocks of MAP populations and perform their sustainable and continuous use to conserve this essential part of our natural and cultural heritage (Johns 2002).

Cultivation may reduce harvesting pressure on some wild plants, particularly rare and threatened species, supporting their conservation. Approximately two thirds of the 50,000 different medicinal plant species in use are collected from the wild, and in Europe, only 10 % of medicinal species used commercially are cultivated (Vines 2004). Many medicinal plants, especially the aromatic herbs, are grown in home gardens, some are cultivated as field crops, either in sole cropping or in intercropping systems and rarely as plantation crops (Padua et al. 1999). In general, the production of plants as raw material for fine chemicals is different and in some aspects specific comparing with cultivation of ornamental or food crops (Lubbe and Verpoorte 2011).

Cultivated MAP material is increasingly preferred by herbal industry, because it is easier to predict plant yield, quality and composition from farmed sources comparing with raw material gathered from the wild. Cultivation also reduces the possibility of plant misidentification and adulteration. There are ubiquitous advantages of MAP cultivation, such as: production of raw material of a standard quality, use of available machinery and of existing simple processing facilities, following with more rational utilization of soil resources and yield planning, achievement of financial effects, and preservation of species whose collection is prohibited (Dajic-Stevanovic and Ilic 2005). Medicinal plant production, especially of species with moderate or low ecological requirements, is totally justifiable at sites where is not possible or worthwhile to produce the standard crops, which refers to rocky, sandy, saline, waterlogged and wet habitats, as well as abandoned mountainous pastures, and other sites of low fertile soils (Dajic-Stevanovic et al. 2005).

Some health care practitioners and consumers, however, believe that cultivated species make less effective remedies than wild harvested plants. While cultivated material can in some cases result in more uniform, higher yielding forms of medicinal plants with improved characteristics of a plant drug, intensive production may result in increased biomass, but decreased content of a certain secondary metabolites. Finally, wild collection is an important activeness, generating income for many rural household in developing countries, strongly contributing to progress and development of the rural regions. Thus, there are still different opinions on cultivation versus wild collection of medicinal plants, especially having in mind complexity of problems linked to bringing of MAP into cultivation.

2 Decision Making Algorithm for Starting Cultivation of MAP

Idea of growing a new plant culture is ubiquitous among farmers. The dream of every farmer is to find a plant of an unlimited market demand. To achieve this goal many farmers, encouraged with texts from popular magazines, start an experimental cultivation of alternative crops such as medicinal plants. Economic feasibility is the main rationale for a decision to bring a species in cultivation, but it is also a substantial limitation as long as sufficient volumes of material can still be obtained at a lower price from wild harvest (Schippmann et al. 2003). The profitability of cultivation of medicinal plants compete with profit achievable for standard field crops for which exist a specialized machinery and standard procedure for application of fertilizers and agrochemicals to control weeds, pests and diseases. Since MAP are non-standard field crops, even in the first years of the experimental cultivation, it becomes obvious that these plants are labor intensive and that for their successful cultivation is necessary to allocate additional funds to recruit seasonal workers. For successful large scale cultivation of MAPs, high quality raw material should be produced using low input cultivation methods to be competitive at the international market and with plants collected from the wild;

alternatively, where much investment is needed to set up cultivation, plant material with a high value should be grown (Lubbe and Verpoorte 2011).

The second model for medicinal plants cultivation occurs among small producers who have arable capacity for which usually is not worthwhile investing into machinery and agrochemicals. This model often attracts people who are, due to transition and economic crisis, jobless looking for some extra sources of the income.

The future market for less known species is highly unpredictable and because many are perennials requiring several years to establish and become harvestable, investment in them could represent a considerable commercial risk (Canter et al. 2005). Some additional problems could occur in MAP cultivation, such as slow adaptation of the plant, heterogeneous seed material, pest/disease-susceptible plant populations, and low yields. In reaching the decision on the choice of medicinal plant and the technology of its cultivation, farmers are usually faced with a series of questions for which is difficult to get accurate answers. The most common issues with which the producers of medicinal plants encountered are: (a) market, (b) abundance and accessibility of wild populations, (c) agro-environmental conditions, (d) labor availability and costs, (e) investments in machinery, (f) post-harvest processing, and (g) rationality of production.

2.1 Market

Forecasting the market trends for herbs is always difficult, due to very large variability in reports and information concerning amount of material in natural populations and plantations. Annual fluctuations in the amount of plants that are normally placed on the market are usually affected by climatic factors, depression due to irrational collection, number of available collectors and profitability of farming (Small 2004). It often happens that a new medicinal plant species reach the sudden popularity in the world of herbal pharmacy, followed by dramatic increase in demand for its raw material, resulting in rise in prices on the market. The high price cause greater collection of natural populations. Since the amount of raw material that can be collected from the wild is limited and heterogeneous in quality, experimental cultivation gets more chances. Cunningham (2001) divided the trend of prices and quantities collected from natural populations of new medicinal plants on the market in four phases (Fig. 8.1). From presented figure can be seen that during the discovery of new medicinal plant and its expansion in pharmaceutical industry, simultaneously raising the amounts of its raw material collected from the wild. Increase in demand is followed by market stabilization, where the price is fixed as well as the offer. This state of the market lasts until the appearance of the offer depression caused by decrease in amounts that can be collected from the wild. Over-exploitation of plant population in nature inevitably leads to their depletion, and consequently to reduced offer. Since at this stage pharmaceutical product is developed assuming that the industry demands for raw

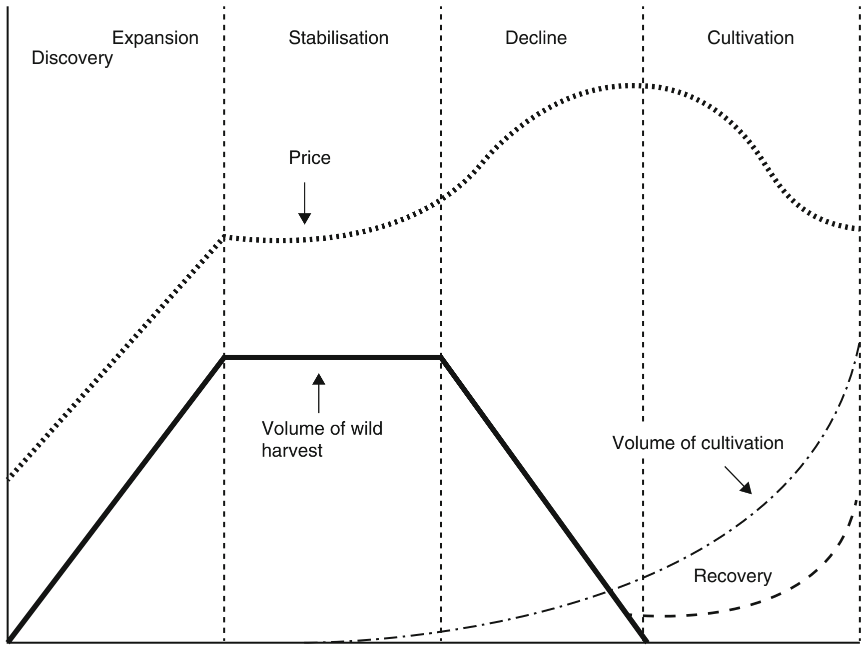


Fig. 8.1 Transition phases from wild harvesting to cultivation: after wild resources decline with over-harvesting, raw material prices increase and cultivation becomes economically feasible; more resilient species can recover (After Cunningham 2001)

material remain constant, its deficit will causes an increase in the market price, which encourages farmers to start cultivation of the certain plant species. Wiersum et al. (2006) recognized that two types of medicinal plant exploitation could be interrelated with two different conservation strategies: *in situ* and *in domo*. Ros-Tonen and Wiersum (2005) pointed that not only *in situ* conservation of vulnerable plant species should receive attention, but also *in domo*.

Good examples of conservation through cultivation would be mountain plants which circulate on market in larger quantities, such as *Gentiana lutea* (Franz and Fritz 1978; Bezzi and Aiello 1993; Radanović et al. 2007), *Arnica montana* (Bomme 1999; Galambosi 2004; Kathe 2006; Pljevljakusic et al. 2014), and *Sideritis spp*. (Pljevljakušić et al. 2011; Evstatieva and Alipieva 2012). Once raw material from cultivation entered, the market offer intends to stabilize, and the prices decline to the point of mutual interests, both of farmers and the pharmaceutical industry. At the same time, natural populations could recover through implementation of protection measures *in situ* conservation, reintroduction or spontaneous regeneration. Consequently, a balance between amounts of raw materials offered from the sustainable collection and from cultivation is reached satisfying market demand.

2.2 Abundance and Accessibility of Wild Populations

When choosing plant species for introduction into the cultivation, beside consideration of market issues, farmers usually have to take into account the abundance and accessibility of plant populations in the nature. If a plant species is widespread in the wild, then the interest of the farmer becomes questionable. The collector of wild plants has much lower production costs compared to investments that farmer has. In contrast to the cultivation of medicinal plants, a collector does not have to rent/farm the land and to perform necessary agricultural practices like: obtaining of good quality seeds, seedlings production, deep plowing, fertilizing, soil preparation, planting, watering, hoeing, etc.

In case of highly abundant natural populations, cultivation may be favored only by special requirements of the market, mainly related to strict request of a drug quality, i.e. relatively limited range of the content of particular secondary metabolite. When significant quantities of raw material are gathered from the wild, such material is mixture of populations differing in the content and composition of bioactive substances. In general, for the most traded species, cultivation is always an option. This is especially worthwhile for plants whose distribution in nature is linked to specific habitats, climate and/or geographic regions, as well as species whose medium- and long term collection could cause risks of endangerment (plants in which underground organs are collected, i.e. roots, tubers, and rhizomes). In case of collecting the underground plant organs, the whole plant is usually pulling out, whereas parts containing buds are not returned back into the soil, to ensure the further reproduction of the plant. This refers to many species, including *Gentiana lutea*, *G. punctata*, *Althaea officinalis*, *Carlina acaulis*, *Symphytum officinalis*, *Iris germanica*, *Inula helenium*, *Ononis spinosa*, *Petasites hybridus*, *Polygonum bistorta*, *Potentilla erecta*, *Primula veris*, *Sanicula europea*, *Sanguisorba officinalis*, *Saponaria officinalis*, and some other European plants.

Especially threatened are “root drug” species growing more or less solitary, i.e. those which not form abundant groups, such as: *Gentiana lutea*, *G. punctata*, or *Carlina acaulis*. Although recognized as internationally important species, many orchids are still harvested for their roots known as “salep” in SEE (mainly Macedonia, Kosovo, Albania).

In most of the countries there are nature protection laws and legislative on rare and endangered species, whose collection is controlled or in some cases completely forbidden. Naturally, for such species the best conservation strategy is cultivation (e.g. Bomme 1999; Galambosi 2004; Radanović et al. 2007; Pljevljakušić et al. 2011, 2014), although many elements of cultivation technology, especially the agro-environmental conditions, are still challenging.

2.3 Agro-environmental Conditions

Regional characteristics for growing a certain crop, depending on the climatic characteristics and soil type, might be treated as an additional key factor in selecting of medicinal plant species for cultivation. It is obvious that some alpine plants cannot tolerate long period of summer temperatures over 20 °C, and thus their cultivation is limited to higher altitudes which is especially true for southeast and southern EU countries, and other with a similar climate (yellow gentian, rhodiola, arnica). In some cases herbal industry seeks for a high level of active substances, which accumulate as a result of secondary metabolism, which is closely related to the strategy of survival under stress conditions induced by increased UV radiation i.e. rutin in buckwheat leaf (Kreft et al. 2002). Such plants should be grown at higher altitudes to achieve desired quality of the drug. As a general rule should be stressed that plant varieties which can be grown in the regions of a lower altitudes have a longer vegetation and higher yields than plants grown at higher altitudes, but, on the other hand the latter could have increased accumulation of secondary metabolites per unit mass of herbal drugs. It has been assumed that biosynthesis of most of secondary metabolites is induced by extra-optimal influence of various abiotic and biotic factors (e.g. Pavarini et al. 2012). Therefore, in optimal environmental conditions, a plant would tend to increase biomass of the photosynthetic organs and to invest into its reproduction, rather than to synthesize secondary metabolites usually needed to cope with stresses.

If this is the case, lower production cost and achievement of higher biomass may compete with the quality. Greater accumulation of secondary metabolites is sometimes clearly defined by the market quality criteria and therefore the cultivation of certain MAP species has to be adapted to the prescribed requirements.

The annual amount and distribution of rainfall is one of the key limiting factors in crop production, including MAP. Some medicinal plants for their undisturbed growth and development require a large amount of water (plantain, mint), while for the others too much water in the soil disturbs normal physiology, like for *Lavandula* spp., and *Melissa officinalis* for example (e.g. Hoffmann 1949).

Soil texture is the next limiting factor. For most medicinal plants, in which the drug is underground organ, it is impossible or difficult the cultivation on the soil of a ‘heavy’ texture, including clayey and rocky soils. Also, soils which have a lot of clay and/or silt often have poor water drainage of arable layer and high level of underground water, which could be a limiting factor in production of some medicinal plant species like *Sideritis* spp., *Helychrisum* spp., etc. (e.g. Chittendon 1956; Huxley 1992).

Moreover, chemical features of the soil, naturally determine selection of species for cultivation. This refers to soil pH, salinity level and the content of soil macro- and micronutrients. Some of plants successfully grow on low soil pH, while other prefer neutral or slightly-alkaline environment. The same situation is with the species that naturally inhabit the saline habitats, including chamomile, yarrow, pennyroyal and some others (Dajic-Stevanovic et al. 2008, 2014), being able for cultivation on slightly salt effected soils.

2.4 Labor Availability and Costs

Unlike conventional farming, cultivation of medicinal plants is carried out on smaller areas. For this reason, the interest of the agro-chemical industry to develop specific programs to protect these crops is small or nil. In the absence of selective agents to combat the weed flora plantation maintenance is usually performed by inter-row cultivation and manual hoeing. Weed pressure on the plot could be significantly reduced by combining agriculturally intensive field crops in the crop-rotation system with herbs and applying glyphosate based herbicide prior to plantation establishment (NMPB 2008). Regarding weed reduction, wheat is the best preceding crop in crop-rotation, since it has range of beneficial effects suitable for the next crop. Wheat has short vegetation, where after the harvest and the shallow plowing many weeds are brought in a good position for growth, which afterwards can be effectively treated with total herbicides (e.g. Dajic-Stevanovic et al. 2007). Despite the integral measures against weeds, producer of medicinal plants still must account for hiring of additional seasonal labor for this purpose. In addition, in most herbs some of the process of planting, harvesting and post-harvest processing is not fully automated and therefore this must be taken into account for the required number of seasonal workers. Considering that it has been estimated that seasonal labor in the total sum of production costs relates significant part, right after the cost of energy for drying (Qaas and Schiele 2001), proper planning of the number of seasonal workers and the amount of their allowances can be crucial for success of production. The problem of availability and motivation of labor for growing of alternative crops is usually the limiting factor in production of medicinal plants in hilly-mountain rural areas, due to general depopulation. Unstable situation in terms of availability and costs of labor for field farming should be taken into account when producer is choosing the plots for cultivation of medicinal plants. Since the problem of labor costs may limit even the best planned plant production, in organization of farm work starting from a seed towards the raw material, producer should strive to increase level of automation of the overall production process.

2.5 Investments in Machinery

Some processes in cultivation of medicinal plants can be facilitated by inclusion of specialized machinery. For some processes it is possible to use the machinery of major crops production in unmodified form, or with some minor modifications. Thus, for seed processing the existing sieves and cyclones for air-selection could be used; for direct sowing cereal or pneumatic drills are appropriate; for seedling production the soil block machinery and plastic containers from vegetable production could be recommended; for planting - planters could be used; harvesting could be done with harvesters or mowers, and for digging of roots potato diggers and ploughs could be a good option. For harvest of some herbs specialized harvesters are used, which could be operational with minor modifications for harvesting of some other medicinal plants. For example, the harvester specialized for chamomile flowers picking, could be used for harvesting of peppermint, lemon balm, and ribwort plantain. Among all herbs, the automation of the production process in case of chamomile has reached the furthest point (Zimmer and Müller 2004). Since investments in machinery, as well as in facilities for drying of the raw material, significantly raise production costs, these additional funds must be justified by the final price of raw materials. Therefore, it is evident that a large cultivation area more quickly justifies resources invested in automation, i.e. machinery. For example, a standard chamomile harvester can turn 3–4 ha flower in a day, which means that, if the harvest campaign lasts 15 days, it could turn theoretically 45–60 ha (Brkić S, personal communication). On the other hand, the capacity for industrial dryers of raw materials is the most common limiting factor, so in this case harvester will almost never work at its full capacity. Nevertheless, the same harvester, after a period of chamomile harvest, could be used with minor modifications to harvest herb in other medicinal crops. For any new investment in the automation of the medicinal plants production process, it is reasonable to carefully calculate pay off of such investment.

2.6 Postharvest Processing

There is a wide range of machinery for postharvest processing of fresh and dry medicinal plants raw material. In case of fresh plant material processing, the most common steps are lines of washing, chopping, and the separation by size, while the processing equipment for dry plant material are mainly used for the separation of the leaf from the stem, machine for flowering stalk cutting (e.g., chamomile), and vibrational or air separators. Some of the postharvest practices are necessary and their absence would cause an irreversible quality loss of raw material. Thus, for example, insufficiently washed roots can dry out in optimal conditions, but the soil residuum on root increases the critical quality parameter - the share of ash (%) and makes raw material useless for further industrial processing. Use of additional facilities, such as choppers and size separators enable more rational use of energy during further drying of raw material. Drying of medicinal and aromatic plants is crucial process in maintaining high quality of herbal drugs. Drying is one of the oldest ways of preserving and processing of food, and also the most important part of primary processing of medicinal plants. It is a process that facilitates the evaporation of water from plant tissues using the heated air flow. The heat may come from the sun or it could be generated by artificial sources, with additional consumption of some the energy (oil, fuel oil, coal, wood, gas, etc.). In most plants it is easy to gather a large amount of raw material for a short time, but it usually takes much more time to devote to post-harvest processing of plant material. Too fast drying, with applying the additional heat, could cause degradation of the active components in plant tissue. When plants are dried too slowly, this can lead to secondary microbiological infections and the initiation of certain enzyme mechanism effects, which may also degrade the desirable active metabolites. Drying costs, besides the cost of wages for seasonal workers, are the largest group of costs (30–50 %) in the production of medicinal plants (Qaas and Schiele 2001).

Modern production of high-quality raw medicinal herb, flower and root is inconceivable without the use of industrial dryers. Investing in the packaging does not usually significantly increases the cost of the production, but it is certainly the cost of which the producer should be aware. Warehouses are usually an essential element of infrastructure for the production of medicinal herbs, so when planning the production of large quantities of herbs their construction should be considered.

2.7 Rationality of Production

Taking into account all of the above listed parameters that must be considered carefully during decision making process about investing in the cultivation of medicinal plants, we assume that these parameters can be roughly evaluated according to the presented aspects.

After evaluation of the parameters proposed in of production rationality (I_R) could be calculated using the equation:

$$I_R = (P + V + AE + Q)/(L + M + PH + T),$$

Where P, V, AE, Q, L, M, PH, T, represent **P**rice, **V**ulnerability, **A**gro-**E**cological conditions, recognizable **Q**uality, **L**abor cost, **M**achinery purchase costs, **P**ost-**H**arvest (drying) costs and **T**ransport costs, respectively. Each parameter was scored from 1 to 5 (1 – low; 5 – high).

Calculated index values may vary in the range of 0.2–5.

Based on the authors' experience, an estimation have been made that the ratio of investment and the profit equalized when index of production rationality (I_R) is equal to 1, and moreover that most of the plantation of medicinal plants by the index rationality production ranges from 1.3 to 2. If the estimated index of production rationality is less than 1, it should be considered that the selection of MAP brought into the cultivation wasn't well planned and that some of the other factors that affect the success of the production have to be considered and better elaborated.

3 Challenges and Current Approaches in Selection and Breeding of Medicinal Plants

Similarly to the other crops, traditional breeding methods, as well as biotechnological procedures and selection assisted by molecular markers, are applied into development of new varieties and cultivars of medicinal plants, aiming at improvement of their desirable characteristics. This refers to both increased drug yield and the content of particular secondary metabolites. Superior genotypes are very important for profitable production of high quality medicinal plants' row material.

Out of all cultivated medicinal plants only a small percentage is clearly genetically defined and represented on seed market in term of variety (Pank 2002). Therefore, portion of varieties in medicinal plant cultivation practice is small in comparison with other crop groups. It is considered that seed production and viability are target traits in which considerable success can be expected simply by selecting vigorous and fertile genotypes, a process that also establishes a population adapted to the growing conditions provided (Canter et al. 2005).

Performance potential of varieties of MAP has yet to be developed by breeding this group of special crops. Precise pharmacognostic definition of medicinal plants for bringing into the cultivation is very important to achieve standardized products for pharmaceutical industry. Therefore, standardized medicinal plant raw material is essential for success in further steps of processing the herbal drugs.

For efficient plant breeding, knowledge on the genetic diversity within adapted germplasms and the relatedness of potential parental lines, as well as detailed information on the mode of inheritance of the trait of interest, is a prerequisite (Wagner et al. 2005). Mapping of genes and specific DNA sequences involved in biosynthesis of particular metabolite classes seem to be future challenge in MAP breeding programs.

Most of actual research is focused on genetic variability among different taxa of medicinal plants using several types of DNA markers, including restriction fragment length polymorphism (RFLP), amplified fragment length polymorphic DNA (AFLP), random amplified polymorphic DNA (RAPD), cleavage amplified polymorphic sequence (CAPS), simple sequence repeat (SSR), and sequence characterized amplified region (SCAR) markers (e.g. Bussell et al. 2005).

In such studies on medicinal and aromatic plants, AFLP and RAPD markers have been used to determine genetic variability in individual species, including *Agava angustifolia* (Sánchez-Teyer et al. 2009), *Rosa damascena* (Baydar et al. 2004), *Origanum vulgare* (Van Looy et al. 2009), and *Allium hirtifolium* (Ebrahimi et al. 2009), *Primula farinosa* (Reisch et al. 2005), *Chamomila recutita* (Solouki et al. 2008), *Hypericum perforatum* (Arnoldt-Schmitt 2002), etc. Moreover, different nuclear and plastid markers have been applied to evaluate phylogenetic relations and genetic variability within particular genera and tribes of MAP, including *Thymus* species of the section *Serphyllum* (Sostaric et al. 2012), *Echinacea* (Kapteyn et al. 2002), *Mentha* (Khanuja et al. 2000) and *Micromeria*

(Meimberg et al. 2006) species, as well as tribes *Rutae* (Salvo et al. 2008), *Malvae* (Garcia et al. 2009) and some others.

In addition, molecular markers were used in order to evaluate relations between genetic - and phytochemical variability. In most cases, including research performed on volatiles in *Juniperus* (Adams et al. 2003), *Angelica lignescens* and *Melanoselinum decipiens* (Mendes et al. 2009), *Ocimum gratissimum* (Vieira et al. 2001), *Thymus caespitosus* (Trindade et al. 2008), *Ocimum basilicum* cultivars (Labra et al. 2004), and some other MAP species, no significant correlation between genetic and phytochemical profiles were observed. Nevertheless, strong association between surveyed phytochemical variables (cichoric acid and tetraene) and DNA polymorphism was shown for cultivated *Echinacea purpurea* germplasm and some related wild species (Baum et al. 2001).

To date, there have been relatively few reports of molecular marker-based approaches to medicinal plant improvement, and not even the most skeletal of genetic maps is available for any of the important species (Canter et al. 2005). However, apart from AFLP studies, microsatellites were developed for *Cannabis* for breeding the species as a fibre crop (Gilmore and Peakall 2003) and for *Catharanthus roseus* (Shokeen et al. 2007) for example. The latter species has become an important model medicinal plant system for biotechnology and secondary metabolism studies, especially in regard of alkaloid biosynthesis, including mapping of genes responsible for this metabolic pathways (van der Heijden et al. 2004). Significant success was achieved in isolation and characterization of several structural genes related to flavonoid biosynthetic pathway in gentian (Nakatsuka et al. 2005, 2008; Shimada et al. 2009). Furthermore, segregation analysis of F2-individuals of chamomile revealed a monogenic recessive mode of inheritance of the (−)-α-bisabolol and chamazulene content, whereas by bulked segregant analysis three AFLP-markers linked to the (−)-α-bisabolol locus and one RAPD and 17 AFLP markers for the chamazulene content were detected, suggesting that the markers for the chamazulene content could be suited for pre-flowering marker based selection procedures (Wagner et al. 2005).

Pank (2002) reported that focus of breeding research in medicinal plants arise mostly in the fields of pharmacology, genetics, breeding methodology, reproductive biology, pathogen diagnostic, development of initial material for breeding, rationalization of trait determination methods, hybrid creation and biotechnology in general. So far, selection efforts have included the most popular medicinal and aromatic species on the market such as: chamomile, yarrow, artichoke, fennel, valerian, basil, dill, St. John's wort, caraway, marjoram, parsley, thyme, lemon balm and numerous niche cultures (Johnson and Franz 2002; Beschreibende Sortenlisten 2002). In the selection of species and their breeding objectives, the requirements of the market and society should be observed.

Although the primary target for trait manipulation in medicinal plants is the content of active compounds, for development as crops, major agronomic characters related to yield uniformity and stability, as well as tolerance to biotic and abiotic stresses should be improved (e.g. Dubey and Guerra 2002; Bernáth 2002).

Selection and breeding of medicinal plants follows the logic of genetic research in other crops. Unlike conventional field crops, where varieties are uniformed and classified by quality, in the selection of herbs more attention is given to specific target secondary metabolites. There are four main directions in the selection of medicinal plants and they are: yield, phenology, disease tolerance and chemical profile.

The yield of the most often quantitative property on which selection is based among all the crops. In many selection programs on medicinal plants best yielding wild populations have been characterized.

The aim of selection of the field of phenology of medicinal plants is equalization of crops according to the time of technological maturity and plants growth forms for easier and cheaper automated harvesting. Often, the content of active substance in plant tissue depends on the plant phenophase. Mechanized harvesting of plants in different maturation stages would lead to heterogeneous raw material whose quality in terms of medicinal properties could be compromised. Yarrow tetraploid proazulene variety 'Proa', for example, has very high quality of essential oil, but flowering time of whole variety population is very diverse. To overcome this problem, producer must perform two or three separated harvests (mainly manually) or to clonally propagate plants that bloom at the same time. Such a situation can not be imagined in conventional agriculture, where today all operations are mechanized, but breeding efforts in this plant species is directly proportional to areas in which it is grown. Recently, a group of researchers recognized this problem in populations of cultivated medicinal plants and tried to solve it through the production of double haploids (HAPLOTECH 2004–2006). They have pointed that in normal case conventional inbreeding procedures take six generations to achieve complete homozygosity. However, the method of doubled haploidy should achieve it in one generation. This group have produced haploid plant cells from pollen or egg cells or from other cells of the gametophyte; then, through induced chromosome doubling a doubled haploid cell is produced which can be grown into a doubled haploid plant. Although this project has not successfully produced new homozygous population and thereby justifies investment in this molecular manipulation, the idea is very good and the efforts in equalization of medicinal plots should continue, especially as seasonal labor becomes more expensive.

In some medicinal crops diseases could cause irreparable damage to the plantation. For example anthracnose is a major production constraint for St. John's wort (*Hypericum perforatum* L.) caused by the fungus *Colletotrichum gloeosporioides* (Penz.) (Michel et al. 2014). Plenty different research studies were conducted in this field, since active principles of this plant has been recognized as anti-depressant. Gaudina et al. (2002) screened 21 wild and 3 commercial varieties for anthracnose tolerance on three sites with distinct soil climates. As a result of this study the most tolerant population (P7) has been recognized, which later became a commercial cultivar Hyperivo 7. Similar screening has been done by Radanovic et al. (2005), where the most tolerant *H. perforatum* wild population (D4) has become a promising cultivar 'Maja' (Jevđović et al. 2005). German medicinal plant variety list includes 12 St. John's wort cultivars (Beschreibende Sortenlisten 2002).

Depending on the content of certain active components in the plant tissues many varieties of medicinal plants that exhibit different biological effects were formed. For example, in Europe the most popular medicinal plant in the cultivation is chamomile, whose natural populations have been extensively screened for decades (Pothke and Bulin 1969; Galambosi et al. 1988; Gasic et al. 1989; Lal et al. 1996; Salamon 2009; Singh et al. 2011). Selection from chamomiles' natural populations has been done primarily on chemical profiles of essential oil, where content of chamazulene and α-bisabolol was the main criteria. Later, populations which differ in flowering time were included in the selection programs in order to extend the harvesting period. The most recent trends in chamomile breeding are focused on improving homogeneity of flower insertion and flower development, increasing the flower yield as well as on increasing the yield and quality of essential oil (Honermeier et al. 2013).

The main problem of plant breeding research on medicinal plants is return of the investments. In conventional agriculture, there is a large and well-developed market of certified and hybrid seeds that exists because of regulations that protect the intellectual property breeder. Since medicinal plants are grown on a disproportionately small area, and that a large number of these species is perennial, commercial trade of their seeds is not profitable enough to justify the investment in the selection and breeding as commercial production branch. Pank (2002) has also pointed out that in general medicinal and aromatic plant breeding needs notably promotion in the field of pre breeding, because the reflux of breeders' expenses is insufficient due to the limited seed sales quantity. In most cases, the variety of medicinal plants has emerged as a result of screening a large number of populations for certain properties (the active ingredient) that is of interest of phyto-pharmaceutical industry (Gaudina et al. 2002; Radanovic et al. 2005; Janković et al. 2012; Jug-Dujaković et al. 2012; Salehi and Nazarian-Firouzabadi 2013; Seidler-Łozykowska et al. 2013; Okoń et al. 2013). Therefore, funding for this kind of pre breeding or chemical selection process came from research bodies or pharmaceutical industry, but additional funds for final trimming of selected populations into commercial cultivars, which specimens should be uniform in morphological characteristics, growth and flowering time is lacking. This is because the fact that such funding should refund through seed market, which is in the case of medicinal plants a long term option.

Finally, in regard to challenges in MAP breeding, problems of intellectual property rights should be stressed. In some cases, when selection and breeding seem to result in remedy of a high economic interest, naturally funding is not the issue. For instance selection programs in *Artemisia annua*, which contains sesquiterpene lactone artemisin, were funded by the Bill & Melinda Gates Foundation to develop and deliver new varieties. The World Health Organization has recommended artemisinin combination therapies (ACTs) for treating uncomplicated malaria caused by the parasite *Plasmodium falciparum*. The medicinal plant *A. annua* is currently the main source of artemisinin (Dalrymple 2012). Extensive genetic research on large number of populations has been performed searching for a population that has the highest content of artemisin (Graham et al. 2010) CNAP

Artemisia annua F1 hybrids have been developed over a period of 6 years at the University of York, UK (Townsend et al. 2013).

Apart from studies of "omics" in medicinal plants, for their breeding programs, tissue culture techniques are frequently and successfully used. As well as being essential elements of a system for rational engineering of the genetic makeup of medicinal plants, tissue culture and regeneration could make important contributions to other aspects of plant breeding, having a potentially significant part to play in establishing breeding material from wild populations and in mass-producing material for selection or engineering (e.g. Bourgaud et al. 2001; Canter et al. 2005).

4 Concluding Remarks

According to Schippmann et al. (2002) it was summarized that from the perspective of the market, domestication and cultivation of MAP provide a number of advantages over wild harvest for production of plant-based medicines, as following: (a) cultivation provides reliable botanical identification, (b) cultivation guarantees a steady source of raw material and yield planning, (c) wholesalers and pharmaceutical companies can agree on volumes and prices over time with the grower, (d) cultivation allows controlled post-harvest handling, (e) quality control can be assured and product standards can be adjusted to regulations and consumer preferences, (f) cultivated material can be easily certified, including organic or biodynamic, and g) selection and breeding with commercially desirable traits from the wild or managed populations may offer opportunities for the economic development of the medicinal plant species as a crop.

Many species are difficult to cultivate because of certain biological features or ecological requirements (slow growth rate, special soil requirements, low germination rates, susceptibility to pests, etc.). Although the primary target for trait manipulation in medicinal plants is the content of active compounds, for development as crops, basic agronomic characters related to uniformity, stability, growth and development, and resistance to biotic and abiotic stresses, must also be improved (e.g. Canter et al. 2005; Dubey and Guerra 2002; Bernáth 2002).

In EU, the Common Agricultural Policy (European Commission 2013) was issued to enable the agriculture to fulfil its multifunctional role in society: producing safe and healthy food, contributing to sustainable development of rural areas, and protecting and enhancing the status of the farmed environment and its biodiversity. Besides the GWP (Good Wildcrafting Practices), the EUROPAM (European Herb Growers Association) has issued the GAP (Good Agriculture Practices 2003), relevant for MAP cultivation.

However, cultivation must not be used as a reason for failing to safeguard viable wild populations of MAP species and their natural habitats or undertaken without consideration of the impact on local users and rural harvesters. In many cases a mixture of production systems will be needed to satisfy the local, regional and world's demands for herbal medicines.

Acknowledgement Authors are grateful for support of EU Commission project AREA, No 316004 and to Ministry of Education, Science and Technological Development of the Republic of Serbia for financing the Projects No. 31089 and 31057.

References

Adams RP, Schwarzbach AE, Pandey RN (2003) The concordance of terpenoid, ISSR and RAPD markers, and ITS sequence data sets among genotypes: an example from Juniperus. Biochem Syst Ecol 31:375–387

Arnoldt-Schmitt B (2002) Characterization of Hypericum perforatum plants from various accessions by RAPD fingerprinting. J Herbs Spices Med Plants 9:163–169

Baum BR, Mechanda S, Livesey JF, Binns SE, Arnason JT (2001) Predicting quantitative phytochemical markers in single Echinacea plants or clones from their DNA fingerprints. Phytochemistry 56:543–549

Baydar NG, Baydar H, Debener T (2004) Analysis of genetic relationships among Rosa damascena plants grown in Turkey by using AFLP and microsatellite markers. J Biotechnol 111:263–267

Bernáth J (2002) Strategies and recent achievements in selection of medicinal and aromatic plants. Acta Horticult 576:115–128

Beschreibende Sortenlisten (2002) Bundessortenamt: Arznei- und Gewürzpflanzen, Deutscher landwirtschaftsverlag GmbH

Bezzi A, Aiello N (1993) The cultivation of yellow gentian (*Gentiana lutea* L.) on pre-Alps and Apenninic mountains: results and application. Acta Horticult 331:19–25

Bomme U (1999) Anbau und Züchtung von *Arnica montana* L. Zeizschrift fur Arznei- und Gewürzpflanzen 4:202–203

Bourgaud F, Gravot A, Milesi S, Gontier E (2001) Production of plant secondary metabolites: a historical perspective. Plant Sci 161:839–851

Brkić S (pers. comm.) Agronomist, Head of Department of medicinal plant production in the Institute for Medicinal Plants Research "Dr Josif Pančić", Belgrade

Bussell JD, Waycott M, Chappill JA (2005) Arbitrarily amplified DNA markers as characters for phylogenetic inference. Perspect Plant Ecol Evol Syst 7:3–26

Canter PH, Howard T, Edzard E (2005) Bringing medicinal plants into cultivation: opportunities and challenges for biotechnology. Trends Biotechnol 23:180–185

Chittendon, F (1956) RHS dictionary of plants plus supplement. Oxford University Press, Oxford, U.K

Cunningham AB (2001) Applied ethnobotany. People, wild plant use and conservation. – London, Earthscan (People and Plants Conservation Manuals)

Dajić Stevanović Z, Ilić B (2005) Sustainable development of natural resources of medicinal and aromatic plants in the region of Serbia. Proceedings of papers of symposium with international participation "Environment for Europe", Belgrade, 5–8 June, pp 83–89

Dajić Stevanović Z, Vrbničanin S, Ilić B (2005) Ruralni razvoj u Srbiji i održivost prirodnih resursa lekovitog i aromatičnog bilja. Zbornik radova "Ruralni razvoj i zaštita životne sredine". Vlasotince, 1–3. 09. Jugoslovensko udruženje za sociologiju sela i poljoprivrede, Balkanska asocijacija za za sociologiju sela i poljoprivrede, Zavod za sociologiju sela, Beograd, Poljoprivredni fakultet, Beograd, pp 68–77

Dajic Stevanovic Z, Vrbnicanin S, Jevdjovic R, Salamon I (2007) Weeding of cultivated chamomile in Serbia. Acta Horticult (ISHS) 749:149–155

Dajic Stevanovic Z, Pecinar I, Kresovic M, Vrbnicanin S, Lj T (2008) Biodiversity, utilization and management of grasslands of salt affected soils in Serbia. Community Ecol 9:107–114

Dajic Stevanovic Z, Acic S, Petrovic M (2012) Conservation of diversity of medicinal and aromatic plants in Southeast Europe: current state and future challenges. Proceedings of the 8th conference on medicinal and aromatic plants of Southeast European countries, 27–31 May, Subotica, R. of Serbia, pp 4–13

Dajic Stevanovic Z, Pljevljakusic D, Ristic M, Sostaric I, Kresovic M, Simic I, Vrbnicanin S (2014) Essential oil composition of *Achillea millefolium* Agg. populations collected from saline habitats in Serbia. J Essent Oil Bear Plants, ID: 884952. doi: 10.1080/0972060X.2014.884952)

Dalrymple DG (2012) *Artemisia annua*, artemisinin, ACTs & malaria control in Africa: tradition, science and public policy. Politics and Prose Bookstore, Washington, DC

Dubey T, Guerra DJ (2002) Use of biotechnology for growing medicinal plants. Recent Prog Med Plants 5:47–61

Ebrahimi R, Zamani Z, Kashi A (2009) Genetic diversity evaluation of wild Persian shallot (*Allium hirtifolium* Boiss.) using morphological and RAPD markers. Sci Hortic 119:345–351

European Commission (2013) Memo: CAP Reform – an explanation of the main elements, Brussels, 25 Oct 2013. http://europa.eu/rapid/press-release_MEMO-13-621_en.htm. Accessed 27 Apr 2015

Evstatieva LN, Alipieva KI (2012) Conservation and sustainable use of threatened medicinal plant *Sideritis scardica* in Bulgaria. Acta Horticult 955:89–92

Franz C, Fritz D (1978) Cultivation aspects of *Gentiana lutea* L. Acta Horticult 73:307–314

Galambosi B (2004) Introduction of *Arnica montana* L. in Finland. Zeizschrift fur Arznei- und Gewurzpflanzen 4:174–180

Galambosi B, Marczal G, Litkey K, Sváb J, Petri G (1988) Comparative examination of chamomile varieties grown in Finland and Hungary. Herba Hungarica 27:45–55

García PE, Schönswetter P, Aguilar JF, Feliner GN, Schneeweiss GM (2009) Five molecular markers reveal extensive morphological homoplasy and reticulate evolution in the *Malva* alliance (Malvaceae). Mol Phylogenet Evol 50:226–239

Gasic O, Lukic V, Adamovic R, Durkovic R (1989) Variability of content and composition of essential oil in various chamomile cultivators (*Matricaria chamomilla* L.). Herba Hungarica 28:21–28

Gaudina M, Simonneta X, Debrunnerab N, Ryserc A (2002) Breeding for a *Hypericum perforatum* L. variety both productive and *Colletotrichum gloeosporioides* (Penz.) tolerant. J Herbs Spices Med Plants 9:107–120

Gilmore S, Peakall R (2003) Isolation of microsatellite markers in *Cannabis sativa* L. (marijuana) in fibre crop varieties. Mol Ecol Notes 3:105–107

Graham IA, Besser K, Blumer S, Branigan CA, Czechowski T, Elias L et al (2010) The genetic map of *Artemisia annua* L. identifies loci affecting yield of the antimalarial drug artemisinin. Science 327:328–331

HAPLOTECH (2004–2006) FP6-SME: Novel innovative doubled haploid technology for ornamental and medical plant breeding, Project reference: 508210, Project result in brief available on-line at: http://cordis.europa.eu/result/brief/rcn/9289_en.html

Hoffmann FH (1949) Über den Einfluß einiger Bodenarten auf Wachstum und Gehalt von Arzneipflanzen. Büchler, Bern

Honermeier B, Ali S, Leschhorn B, Mahmood A, Ijaz M, Russo M, Shafiee-Hajiabad M, Ullah H, Zeller S (2013) Cultivation of medicinal and spice plants in Germany – a review. Int J Agric Biol 15:1379–1388

Huxley A (1992) The new royal horticultural society dictionary of gardening

Janković T, Zdunić G, Beara I, Balog K, Pljevljakušić D, Stešević D, Šavikin K (2012) Comparative study of some polyphenols in *Plantago* species. Biochem Syst Ecol 42:69–74

Jevđović R, Ivanović M, Radanović D (2005) Novostvorena sorta kantariona 'Maja', priznata Rešenjem Ministarstva poljoprivrede, šumarstva i vodoprivrede Vlade Republike Srbije. Beograd, broj 320-09-39/134/3-2005-06 od 21.09

Johns T (2002) Plant genetic diversity and malnutrition: practical steps in the development of a global strategy linking plant genetic resource conservation and nutrition. Afr J Food Nutr Sci 3:98–100

Johnson CB, Franz C (2002) Breeding research on aromatic and medicinal plants. The Haworth Press, New York

Jug-Dujaković M, Ristić M, Pljevljakušić D, Dajić-Stevanović Z, Liber Z, Hančević K, Radić T, Šatović Z (2012) High diversity of indigenous populations of dalmatian sage (*Salvia officinalis* L.) in essential-oil composition. Chem Biodiversity 9:2309–2323

Kapteyn J, Goldsbrough PB, Simon JE (2002) Genetic relationships and diversity of commercially relevant *Echinaceae* species. Theor Appl Genet 105:369–376

Kathe W (2006) Conservation of Eastern-European medicinal plants: *Arnica montana* in Romania. In: RJ Bogers, Craker LE, Lange D (eds) Medicinal and aromatic plants. Springer, Netherlands, pp 203–211

Khanuja SPS, Shasany AK, Srivaststava A, Kumar S (2000) Assessment of genetic relationships in *Mentha* species. Euphytica 111:121–125

Kreft S, Strukelj B, Gaberscik A, Kreft I (2002) Rutin in buckwheat herbs grown at different UV-B radiation levels: comparison of two UV spectrophotometric and an HPLC method. J Exp Bot 53:1801–1804

Labra M, Miele M, Ledda B, Grassi F, Mazzei M, Sala F (2004) Morphological characterization, essential oil composition and DNA genotyping of *Ocimum basilicum* L. cultivars. Plant Sci 167:725–731

Lal RK, Sharma JR, Misra HO (1996) Vallary: an improved variety of German chamomile. Pafai J 18:17–20

Lubbe A, Verpoorte R (2011) Cultivation of medicinal and aromatic plants for specialty industrial materials. Ind Crop Prod 34. Macmillan, London, pp 785–801

Meimberg H, Abele T, Bräuchler C, McKay JK, Pérez de Paz PL, Heubl G (2006) Molecular evidence for adaptive radiation of Micromeria Benth. (Lamiaceae) on the Canary Islands as inferred from chloroplast and nuclear DNA sequences and ISSR fingerprint data. Mol Phylogenet Evol 4:566–578

Mendes MD, Trindade H, Figueiredo AC, Barroso JG, Fontinha SS, Pedro LG (2009) Volatile and molecular characterization of two Portuguese endemic species: *Angelica lignescens* and *Melanoselinum decipiens*. Biochem Syst Ecol 37:98–105

Michel V, Debrunner N, Simonnet X (2014) A rapid greenhouse screening method to identify St. John's wort (*Hypericum perforatum*) accessions resistant to *Colletotrichum gloeosporioides*. Hort Sci 4(49):31–34

Nakatsuka T, Nishihara M, Mishiba K, Yamamura S (2005) Temporal expression of flavonoid biosynthesis-related genes regulates flower pigmentation in gentian plants. Plant Sci 168:1309–1318

Nakatsuka T, Sato K, Takahashi H, Yamamura S, Nishihara M (2008) Cloning and characterization of the UDP-glucose: anthocyanin 5-O-glucosyltransferase gene from blue-flowered gentian. J Exp Bot 59:1241–1252

NMPB (2008) Agro-techniques of Selected Medicinal Plants (vol 1), National Medicinal Plants Board, Department of AYUSH, Ministry of Health and Family Welfare, Government of India

Okoń S, Surmacz-Magdziak A, Paczos-Grzęda E (2013) Genetic diversity among cultivated and wild chamomile germplasm based on ISSR analysis, Acta Sci. Pol. Hortorum Cultus 12:43–50

Oladele AT, Alade GO, Omobuwajo OR (2011) Medicinal plants conservation and cultivation by traditional medicine practitioners (TMPs) in Aiyedaade Local Government Area of Osun State, Nigeria. Agric Biol J N Am 2:476–487

Padua LS, Bunyapraphatsara de N, Lemmens RHMJ (1999) Medicinal and poisonous plants, vol 1. – Leiden, Backhuys (Plants Resources of South-East Asia 12/1)

Pank F (2002) Aktueller Stand der Arznei- und Gewürzpflanzenzüchtung und Ableitung des Bedarfes in den Bereichen Züchtungsforschung und Züchtung. Zeitschrift für Arznei- und Gewürzpflanzen 7:406–415

Pavarini DP, Pavarini SP, Niehues M, Lopes NP (2012) Exogenous influences on plant secondary metabolite levels. Anim Feed Sci Technol 176:5–16

Pljevljakušić D, Šavikin K, Janković T, Zdunić G, Ristić M, Godjevac D, Konić-Ristić A (2011) Chemical properties of the cultivated *Sideritis raeseri* Boiss. & Heldr. subsp. raeseri. Food Chem 124:226–233

Pljevljakusic D, Jankovic T, Jelacic S, Novakovic M, Menkovic N, Beatovic D, Dajic-Stevanovic Z (2014) Morphological and chemical characterization of *Arnica montana* L. under different cultivation models. Ind Crop Prod 52:233–244

Pothke W, Bulin P (1969) Phytochemical study of a new hybrid chamomile variety. II. Essential oil. Pharm Zentralle 108:813–823

Qaas F, Schiele E (2001) Einfluss der Energiekosten auf die Rentabilität im Trocknungsbetrieb. Zeitschrift für Arznei- und Gewürzpflanzen 6:144–145

Radanovic D, Nastovski T, Pljevljakusic D (2005) Comparative investigation of cultivated *Hypericum perforatum* L. local populations in Serbia. Acta Fytotechica et Zootechnica 4:107–112

Radanović D, Nastovski T, Janković T, Šavikin K, Menković N, Zdunić G (2007) The amount of secondary metabolites in cultivated *Gentiana lutea* L. Planta Med 73:300

Reisch C, Anke A, Röhl M (2005) Molecular variation within and between ten populations of *Primula farinosa* (Primulaceae) along an altitudinal gradient in the northern Alps. Basic Appl Ecol 6:35–45

Ros-Tonen MAF, Wiersum KF (2005) The scope for improving rural livelihoods through non-timber forest products: an evolving research agenda. For Trees Livelihoods 15(2):17–36

Salamon I (2009) Chamomile biodiversity of the essential oil qualitative-quantitative characteristics. In: SENER B (ed) Innovations in chemical biology. Springer, pp 83–90 http://www.springer.com/us/book/9781402069543; http://link.springer.com/chapter/10.1007%2F978-1-4020-6955-0_7

Salehi K, Nazarian-Firouzabadi F (2013) Assessment of genetic diversity among and within Iranian chamomile populations using semi random intron-exon splice junction (ISJ) markers. J Plant Mol Breed 1:40–53

Salvo G, Bacchetta G, Ghahremaninejad F, Conti E (2008) Phylogenetic relationships of Ruteae (Rutaceae): new evidence from the chloroplast genome and comparisons with non-molecular data. Mol Phylogenet Evol 49:736–748

Sánchez-Teyer F, Moreno-Salazar S, Esqueda M, Barraza A, Robert ML (2009) Genetic variability of wild *Agave angustifolia* populations based on AFLP: a basic study for conservation. J Arid Environ 73:611–616

Schippmann U, Leaman DJ, Cunningham AB (2003) Biodiversity and the ecosystem approach in agriculture, forestry and fisheries. FAO satellite event on the occasion of the ninth regular session of the Commission on Genetic Resources for Food and Agriculture. Inter-Departmental Working Group on Biological Diversity for Food and Agriculture, Rome, 12–13 Oct 2002

Seidler-Łożykowska K, Bocianowski J, Król D (2013) The evaluation of the variability of morphological and chemical traits of the selected lemon balm (*Melissa officinalis* L.) genotypes. Ind Crop Prod 49:515–520

Shimada N, Nakatsuka T, Nakano Y, Kakizaki Y, Abe Y, Hikage T, Nishihara M (2009) Identification of gentian cultivars using SCAR markers based on intron-length polymorphisms of flavonoid biosynthetic genes. Sci Hortic 119:292–296

Shokeen B, Kumar Sethy N, Kumar S, Bhatia S (2007) Isolation and characterization of microsatellite markers for analysis of molecular variation in the medicinal plant Madagascar periwinkle (*Catharanthus roseus* (L.) G. Don). Plant Sci 172:441–451

Singh O, Khanam Z, Misra N, Srivastava MK (2011) Chamomile (*Matricaria chamomilla* L.): an overview. Pharmacogn Rev 5:82–95

Small E (2004) The economic potential of medicinal plant production with particular reference to Quebec, 4e colloque de la filière des plantes médicinales du Québec 26 mars 2004, Programme national d'hygiène de l'environnement - Biodiversité. Agriculture et Agroalimentaire Canada.

[on English], available on-line at: http://www.plantesmedicinales.qc.ca/colloques/2004/QuebecMedPlant.pdf

Solouki M, Mehdikhani H, Zeinali H, Emamjomeh AA (2008) Study of genetic diversity in Chamomile (*Matricaria chamomilla*) based on morphological traits and molecular markers. Sci Hortic 117:281–287

Sostaric I, Liber Z, Grdisa M, Marin PD, Dajic Stevanovic Z, Satovic Z (2012) Genetic diversity and relationships among species of the genus *Thymus* L. (section Serpyllum). Flora 207:654–661

Townsend T, Segura V, Chigeza G, Penfield T, Rae A, Harvey D, Bowles D, Graham IA (2013) The use of combining ability analysis to identify elite parents for *Artemisia annua* F1 hybrid production. PLoS One 8(4):e61989. doi:10.1371/journal.pone.0061989. Print 2013

Trindade H, Costa MM, Lima B, Sofia A, Pedro LG, Figueiredo AC, Barroso JG (2008) Genetic diversity and chemical polymorphism of *Thymus caespititius* from Pico, Sao Jorge and Terceira islands (Azores). Biochem Syst Ecol 36:790–797

van der Heijden R, Jacobs DI, Snoeijer W, Hallard D, Verpoorte R (2004) The *Catharanthus* alkaloids: pharmacognosy and biotechnology (a review). Curr Med Chem 11:607–628

Van Looy K, Jacquemyn H, Breyne P, Honnay O (2009) Effects of flood events on the genetic structure of riparian populations of the grassland plant *Origanum vulgare*. Biol Conserv 42:870–878

Vieira R, Grayer RJ, Paton A, Simon JE (2001) Genetic diversity of *Ocimum gratissimum* L. based on volatile oil constituents, favonoids and RAPD markers. Biochem Syst Ecol 29:287–304

Vines G (2004) Herbal harvests with a future: towards sustainable sources for medicinal plants, Plantlife International; www.plantlife.org.uk

Wagner C, Friedt W, Marquard RA, Ordon F (2005) Molecular analyses on the genetic diversity and inheritance of (−)-a-bisabolol and chamazulene content in tetraploid chamomile (*Chamomilla recutita* (L.) Rausch.). Plant Sci 169:917–927

Wiersum KF, Dold AP, Husselman M, Cocks M (2006) Cultivation of medicinal plants as a tool for biodiversity conservation and poverty alleviation in the Amatola region, South Africa. In: Bogers RJ, Craker LE, Lange D (eds) Medicinal and aromatic plants: agricultural, commercial, ecological, legal, pharmacological and social aspects, vol 17, Wageningen UR frontis series. Springer, Wageningen, pp 43–57. http://library.wur.nl/ojs/index.php/frontis/article/view/1222

Zimmer S, Müller J (2004) Erntetechnologie von Arznei- und Gewürzpflanzen, Gülzower Fachgespräche Bd.22, Fachagentur Nachwachsende Rohstoffe e.V., Gülzow

Chapter 9
Cultivation and Breeding of *Cannabis sativa* L. for Preparation of Standardized Extracts for Medicinal Purposes

Sayed Farag and Oliver Kayser

Abstract *Cannabis sativa* L. (marijuana; Cannabaceae) is a plant with worldwide distribution, yielding fiber and food, as well as a psychoactive drug. Cannabinoids and in particular the main psychoactive Δ^9-THC are promising substances for the development of new drugs and are of high therapeutic potential. This review gives an overview of *Cannabis* classification, the current verities, botanical features, genomics, chemicals constituents, cellular site and biosynthesis of cannabinoids. Furthermore, the different cultivation and breeding forms, changes in cannabinoids over time, method of harvesting, drying and processing of *Cannabis* are extensively described in addition to the analytical procedures for standardization of Cannabis based medicinal extracts. Finally, some aspects of current approved *Cannabis* based medicine and its ways of administration are described.

Keywords Cannabis sativa • Cultivation • Breeding • Standardized extracts • Medicinal uses

1 Introduction

Cannabis sativa L. (marijuana; Cannabaceae) is an annual dioecious flowering plant (Fig. 9.1a) and generally believed to be originated in the Northwest Himalayas and may have been cultivated as much as 10,000 years ago (Li 1974). Many evidences suggested that *C. sativa* has been long used in Chinese medicine during the reign of the Emperor Chen Nung around 5000 B.C. (Li 1974). Another important document of *Cannabis* use dates back to 2350 B.C. on the stone of the Egyptian Old Kingdom in Memphis at the end of the Fifth Dynasty indicating its medicinal uses as anti-inflammatory and antimicrobial herbal drug. Later, the medicinal plant appears in a number of Egyptian Papyrus of Ramesseum III (circa 1700 B.C.) and Ebers Papyrus (circa 1600 B.C.) has been found (Ebers

S. Farag • O. Kayser (✉)
Technische Biochemie, Fakultät Bio- und Chemieingenieurwesen, Technische Universität Dortmund, Emil-Figge-Str. 66, 44227 Dortmund, Germany
e-mail: oliver.kayser@bci.tu-dortmund.de

Á. Máthé (ed.), *Medicinal and Aromatic Plants of the World*, Medicinal and Aromatic Plants of the World 1, DOI 10.1007/978-94-017-9810-5_9

1875; Ebbell 1937; Russo 2007; Russo et al. 2008). Subsequently, the Ancient Greeks and Romans noted the medicinal value of *Cannabis*. The Greek Physician Diocorides in the first century BCE he published his *De Materia Medica*, in which *Cannabis* seeds was recommended for the treatment of otalgia (Dioscorides 1968). Bausanius and Glen (second century BCE) was apparently the first Roman to note the medicinal effects of *Cannabis* (Brunner 1973). In the ninth century, *Cannabis* resin was better known as hashish and widely used for its medicinal attributes, mainly in the treatment of pain. The Arabic physician of the early tenth century, Ibn Wahshiyah, warned of possible complications resulting from use of "hashish". In his book *On Poisons* he claimed that the plant extract might cause death when mixed with other drugs (Nahas 1982). Herbal drugs based on *C. sativa* appeared in the European literature at the end of the nineteenth century; the first report on medical utilization of *Cannabis* was in 1840 by William O'Shaughnessy, who had observed its uses in the treatment of rabies, rheumatism, epilepsy, and tetanus (Fankhauser 2002; Waldo 2006). In the following decades the medicinal use of *Cannabis* was widely spread in most European countries and USA, and *C. sativa* was taken up as monographic in national pharmacopeia (Russo 2011). After isolation and structure elucidation of the first pure substances from *Cannabis* in the 1960s, smoking of *Cannabis* as a recreational drug has become wildly known phenomenon in the western world (Gaoni and Mechoulam 1964). Currently, human intervention has focused on *Cannabis* plant to be grown under more controlled conditions and in areas where the plant would not have had a previous history.

Undoubtedly, the chemistry of *C. sativa* is still extremely complex and it's belonging to classes of polyketides, terpenoids, sugars, alkaloids, flavonoids, stilbenoids and quinones (ElSohly and Slade 2005; Appendino et al. 2011) more than 100 cannabinoids are known which belong to a combined biosynthesis of polyketids and terpenoids. The chemical skeleton of a tetrahydrobenzochromen ring system remains special in nature, because with the exception of the moss *Radula marginata* only in the genus *Cannabis* these compounds have been detected so far (Toyota et al. 2002). Whereas cannabinoids-like obtained from non-*Cannabis* sources, two cannabinoids; cannabigerol (CBG) and its corresponding acid have been reported from the South African *Helichrysum umbraculigerum* (Bohlmann and Hoffmann 1979). In *C. sativa* cannabinoids are present in all parts of the plants, but highest concentration can be found in glandular trichomes on the surfaces of leaves and flowers (Turner et al. 1979; Grotenhermen and Russo 2002). Owing to the presence of the highly potent psychotropic agent Δ^9-tetrahydrocannabinol (Δ^9-THC); biomedical attention on *C. sativa* has been focused on varieties of this plant used pharmacological activities (Elbatsh et al. 2012). After discovery of human endocannabinoids and their receptors CB(1) and CB(2) receptors about a decade ago, scientists have noted that our own body produces several cell signals that mimic the actions of *Cannabis*-derived chemicals in our brain, immune system and other organs (Lynn and Herkenham 1994). Despite the fact that chemical synthesis of Δ^9-THC can be accomplished (Tius and Kannangara 1992), but high cost, complexity and stability are problematic and hinders economic production (Beresford 1993; Flemming et al. 2007). On the other hand, micropropagation of

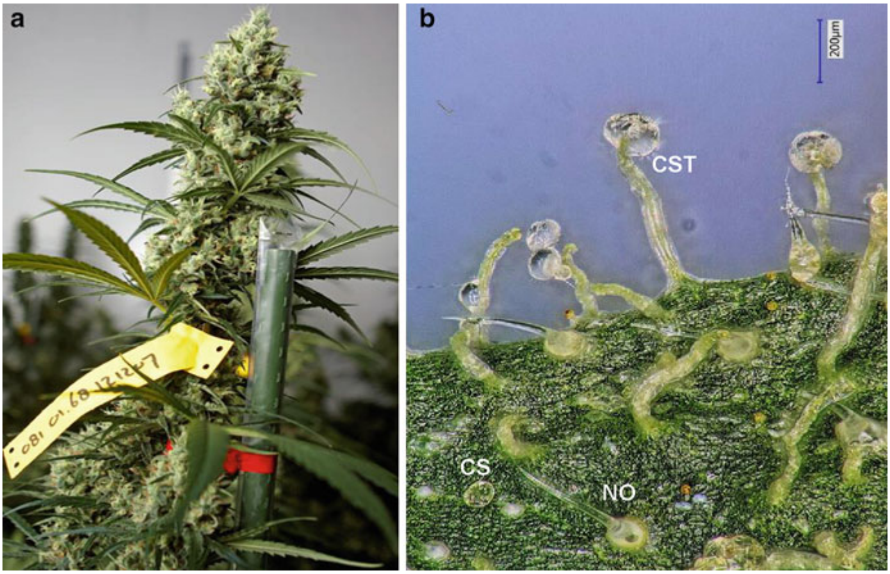


Fig. 9.1 (**a**) Female flowers of medicinal *C. sativa* at full bloom grown in Bedrocan BV (The Netherlands). (**b**) Microscopic photograph of *C. sativa* trichomes on the leaf; *CST* capitate-stalked trichome, *CSE* capitate-sessile trichome, *NOG* non-glandular trichome

C. sativa can be easier achieved (Lata et al. 2012; Lata et al. 2010b), but unlikely in vitro production of cannabinoids using callus and cell cultures has not been reported yet or insufficient production of the desired cannabinoids (Pec et al. 2010). Besides of progress in the field of organic synthesis and biotechnology, cultivation will be the preferred (Beresford 1993) option for the foreseeable future. Nowadays, despite its illegality several breeding and pharmaceutical programs are in progress around the world to grow medicinal *Cannabis* for pharmaceutical preparation with high standardized (de Meijer et al. 2003; Vanhove et al. 2011). However this chapter will focus on medicinal *C. sativa* including current *Cannabis* classification, varieties, botanical features, genomics, chemicals constituents, site and biosynthesis of cannabinoids, changes in phytocannabinoids over time, different cultivation and breeding forms, method of harvest, and analytical approaches for quality control of the crude extracts.

2 Natural Life Cycle

Cannabis sativa propagated from seeds, and grows vigorously in open sunny environments with light, well-drained soil and ample nutrients and water, and reaches up to 5 m (16-feet) in height in a 4–6 month grown season (Raman 1998;

Clarke and Watson 2002). Hermaphroditic varieties have been bred for industrial hemp production, as this allows more uniform crops (Leggett 2006).

- **Germination**: the seeds usually germinate within 3–7 days (Clarke and Watson 2002), no significant cannabinoid biosynthesis can be detected and therefor the medicinal value during this stage is low.
- **Juvenile stage:** During the 2–3 months of growth juvenile *Cannabis* plant exhibits marked phototropism, and extending towards whatever light source offering the appropriate wavelengths (Clarke and Watson 2002). It is difficult to recognize the male and female sex in this stage. However, Mandolino et al. (1999) reported that RAPD analysis to identify male-specific DNA markers is possible. The medicinal value during this stage is still low.
- **Vegetative (blooming) stage:** *Cannabis* characterized by increased biomass and total growth under long day length (more light per day than darkness). Cannabinoids start to be biosynthesized during this stage.
- **Flowering stage:** The reproductive phase of *Cannabis* begins when the plant is exposed to short day length (less light per day than darkness) of 12–14 h or less depending on its latitude and genetic origin (Brenneisen 1983). The development of male and female plants varies greatly; once the male flowers ripened, they require air currents to carry pollen grains to the female flowers, which results in fertilization and consequently seed formation, after that the male plant die directly before the seeds in the female plants ripen. Pollen has been frozen and successfully used for seed production up to 3 years later. The female seeds ripen in about 3–10 weeks and a large female plant can produce about one Kilogram of seeds (Clarke and Watson 2002). The maturation time for *Cannabis* varies from 2 to 10 months, and the *Cannabis* seeds would be sown in May and the plants harvested in September (Raman 1998).

3 Different Cultivation and Breeding Forms

3.1 Germplasm Collection

Cannabis populations are known to react strongly to environmental conditions, either as a direct environmental effect on the phenotype or during multiplication as a result of genetic drift in the population. Rapid changes in narcotic potency and plant habit after a few generations under altered conditions have been reported (Demeijer and Vansoest 1992). *Cannabis* germplasm is maintained ex situ in a few genebanks. Collections mainly occur in connection with recently abandoned or current breeding activities. The largest collection is stored at the Vavilov Institute (St. Petersburg, Russia) with about 200 accessions; the Hungarian genebank maintains about 70 accessions. Collections of up to 20 accessions are preserved in other depositories in Germany, Turkey, and Japan. In comparison with other crops the available number of well-documented *Cannabis* accessions is limited (Demeijer and Vansoest 1992). Nowadays, several accessions are maintained by the U.S. Department of Agriculture's (USDA) Agricultural Research Service for the National Plant Germplasm System of the United States and are now available through the Germplasm Resources Information Network (GRIN).

3.2 Indoor Cultivation

Cultivation of *Cannabis* is prohibited in most of countries except by permission for purposes of research and pharmaceutical uses. For this reason Bedrocan BV and GW Pharmaceuticals Ltd, grow medicinal *Cannabis* under strictly controlled conditions. Indoor vegetative propagation of high yielding *C. sativa* clones has been described by Rosenthal (1984), which reduces the nature allogamous (cross fertilization) of the species (Chandra et al. 2010), solves the problem of poor resin potency due to low outdoor temperatures, and minimizes the risk of detection by law-enforcement agencies (Stamler et al. 1985). Thus, the successful hydroponic *Cannabis* crop production requires an effective system to deliver nutrients and oxygen and support plants growth. However, there is a number of techniques have been proposed for growing *Cannabis* hydroponically, e.g. standing aerated technique (SAT), nutrient film technique (NFT), and aeroponics technique (AT). The nutrient solution, consist of all the essential elements for plant growth and development: (1) the concentration of cations, (2) the concentration of anions, (3) the total ionic concentration, and (4) pH (Steiner 1961). The essential macronutrients in solution are three cations (potassium, calcium, and magnesium) and three anions (nitrate, dihydrogen phosphate, and sulphate). Relative proportions of these cations and anions should be balanced in nutrient solution to avoid ionic imbalances by monitoring the pH within a certain range (5.5–6.5) for maximum uptake and good plant growth (Steiner 1961; Argo and Fischer 2002). On the other hand, many hydroponic growers use special formulations "recipes" based on management conditions (Le Bot et al. 1998). Oxygenation of either a nutrient solution or the rooting medium is an important factor in hydroponics and will affect the rate of root activity and function, particularly the rate of water and nutrient element uptake (Porterfield and Musgrave 1998). Indoor *Cannabis* crop cultivation needs artificial light for: photosynthetic, utilizing light sources to provide part or all that necessary for normal growth, and photoperiodic, that required for controlling flowering and plant shape (Coene 1995). The types of lamps available for plant use are described by van Patten (1992), focusing on high intensity discharge (HID), metal halide (MH), and high-pressure-sodium (HPS) lamps. MH lamps produce a complete spectrum similar to that of natural sunlight. One type of HID lamp is the sulfur lamp, light coming from a hot gas or plasma within a transparent envelope (Jones 1997; Zheng et al. 2005). However, the photosynthetic rate is positively correlated to the carbon dioxide (CO_2) concentration surrounding the plant and it exists in the ambient atmosphere at between 300 and 400 parts per million (ppm) (Sicher and Bunce 1997). Compressed CO_2 gas (cCO_2) is required for indoor cultivation which increases the plant size and speed the growth up 100 % (Jones 1997). Nowadays, numerous controlled environment agriculture (CEA) are available for scheduling the air and root temperature; atmospheric humidity; atmospheric gas composition; light intensity, wavelength composition and duration; water supply and quality; growing medium; and plant nutrition (Kubota and Thomson 2006). In this section we will focus on indoor breeding of medicinal *Cannabis* for uniform production of cannabinoids (Fig. 9.5a–c) as fellow:

3.2.1 Seed Selecting and Germination

High potency *Cannabis* seeds planted in small jiffy pots to standardize hydroponic indoor cultivation. Germination initiated in 3–7 days. Well-developed seedlings were transferred to small pot for their better vegetative growth. After enough development of roots and biomass plants transferred in a multi-flow hydroponics system equipped with nutrient reservoir and electronic timer to control the flow of nutrient (Chandra et al. 2010). Selecting *Cannabis* females that exhibits the most vitality to become mothers should be kept under constant vegetative light, usually HID lamps for 24 h and hydroponic solution in order to supply you with the needed propagation stock.

3.2.2 Selecting and Cloning

Potter et al. (2004), described the use of “clones” to maintain the genotype genetically identically for each subsequent generation of plants as its mother plant. The rooted cuttings termed “clones” are the result of asexual or vegetative propagation. These clones transferred into seedling tray contain small culture medium and fed with hydroponic solution nutrients. Initially, all clones exposed to uniform vegetative light, usually HID lamps for 24 h, 95–100 % relative humidity (RH) and temperatures 27 °C for 1 month. Root formation started in 2–3 weeks and once roots grown to a sufficient size can be transplanted into bigger hydroponic system with new conditions for accelerated growth (Cervantes 2006; Chandra et al. 2010).

3.2.3 Vegetative Period

In this stage all clones kept under similar environmental conditions (light, temperature, RH and CO_2 concentration) in a grow room for the indoor cultivation throughout the growing period. Light was provided to these plants with full spectrum 1,000 watts HID lamps hung above the plants. A hot air suction fan attached about 3–4 feet distance between plants and bulb was always maintained to avoid heating due to HID bulbs. By adjusting the distance between plant and light source, photosynthetically active radiation (PAR) of about $700 \pm 25\,\mu$mol m-^{2} s^{-1}

maintained at the pot level. Using an automatic electric timer photoperiod regulated to maintain vegetative (18 h/day) stage. Grow room temperature and humidity kept nearly constant at ~ 60 °F and ~ 60 % respectively throughout the growing period. Plants kept using a vegetative fertilizer formula for their acclimatization and vegetative growth and during this period plant reaches 50 cm with healthy root system (Potter 2004; Chandra et al. 2010).

3.2.4 Flowering Period

Flowering induced after 12 h photoperiod provided with desirable nutrient medium. During this period, female pistils flowers will start to appear on the plant in 7–14 days, the inflorescences increased in size and cannabinoids increased gradually. After 8 weeks of flowering the stigma and trichomes start to senesce and change into an organ/brown color and the rate of cannabinoids biosynthesis slow rapidly (Potter 2004; Chandra et al. 2010).

3.2.5 In vitro Micropropagation: Direct and Indirect Organogenesis

Direct organogenesis is regarded as the most reliable method for clonal propagation as it upholds genetic uniformity among the progenies (Chandra et al. 2010). Although, few reports have been published describing the regeneration protocols developed for different *Cannabis* genotypes and explant sources has been reported. Fisse et al. (1981) developed a method for inducing from *Cannabis* calli. However, these cultures were reported to be unreceptive to shoot formation. Shoot regeneration from calli was reported by Mandolino and Ranalli (1999), whereas Feeney and Punja (2003) failed to regenerate hemp plantlets, either directly or indirectly from callus or suspension cultures. Lata et al. (2008; 2010a, b) developed a high frequency plant regeneration system from leaf tissue derived callus and nodal segments and were maintained in a temporary immersion bioreactor for high yield shoot multiplication.

3.2.6 Synthetic Seed Technology

Synthetic seeds production and plantlet regeneration have been reported for some plants (Sicurani et al. 2001; Brischia et al. 2002; Hao and Deng 2003). Some reports describe the encapsulation of vegetative propagates such as axillary buds or shoot tips which could be used for mass clonal propagation as well as in long-term conservation of germplasm (Mathur et al. 1989; Pattnaik and Chand 2000). Synthetic seed technology could be a valuable aid for economical large-scale clonal propagation and germplasm conservation of the screened and selected elite germplasm of *C. sativa* (Chandra et al. 2010). Nodal segments containing axillary buds of *C. sativa* isolated from multiple shoot cultures were encapsulated in sodium alginate with calcium chloride dihydrate ($CaCl_2{\cdot}2H_2O$) Regrowth and conversion after encapsulation was evaluated both under in vitro and in vivo conditions for different planting substrates (Lata et al. 2008; 2009).

4 Phytochemistry

The phytochemistry of *C. sativa* has received considerable attention and several classes of compounds have been reported. More than 535 constituents have been reported (Zulfiqar et al. 2012), including constituents belonging to the classes of simple polyketides, terpenoids, polyphenolics, modified sugars, alkaloids, flavonoids, stilbenoids, quinones and terpenophenolics (Formukong et al. 1989). We can divide its constituents in following natural product groups:

4.1 Cannabinoids

Cannabinoids, a class of terpenophenolic C_{21} (or C_{22} for neutral form) compounds were found until now uniquely in *C. sativa* (Page and Nagel 2006). In *C. sativa* about 100 meroterpenoids (prenylated polyketides) are known mostly accumulated in glandular trichomes (Appendino et al. 2011). Cannabinoids are split into eleven different types based on their core structures:

- CBG-type, like cannabigerol
- CBC-type, like cannabichromene
- CBD-type, like cannabidiol
- THC-type, like Δ^9-tetrahydrocannabinol
- Δ^8-THC-type, like cannabicyclol
- CBL type, like cannabielsoin
- CBE type, like cannabinol (CBN) and cannabinodiol
- CBND type, like cannabitriol
- CBT type, like cannabitriol

All other compounds that do not fit into the main types are grouped as miscellaneous cannabinoids (Mechoulam and Shvo 1963). Cannabinoids have been detected in stem, pollen, seeds and roots by immunoassays and chemical analysis (Ross et al. 2000).

4.1.1 Cannabinoids Degradation Products

Cannabinoids are enzymatically biosynthesized in the plant as their corresponding carboxylic acid forms (Taura et al. 2007). Neutral cannabinoids are formed via decarboxylation of the acidic cannabinoids during exposure to light, heat, or as a result of prolonged storage (Thakur et al. 2005), oxidative degradation product (Fig. 9.3) are formed as a result of various influences, such as UV-light, oxidation or isomerization (Razdan et al. 1972; Trofin et al. 2012).

4.1.2 Site of Synthesis

Histochemical (Andre and Vercruysse 1976; Petri et al. 1988), immunochemical (Kim and Mahlberg 1997) and chemical studies (Happyana et al. 2013) confirmed that glandular trichomes (Fig. 9.1b) are the main site of cannabinoid production in *C. sativa*. Trichomes production and density reaches its peak in the late flowering stage, this gives an indication of the optimum total cannabinoid content and time to harvest the plant (Potter 2004; Page and Nagel 2006).

4.1.3 Biosynthesis of Cannabinoids

The biochemical pathway of cannabinoids is not well understood and the factors that control biosynthesis and distribution of cannabinoids within the plant are unknown, although the site of synthesis of most of these molecules has been investigated during the last two decades (Mahlberg and Kim 2004). The first step in the synthesis of cannabinoids is the condensation reaction (prenylation) of geranylpyrophosphate (GPP) with olivetolic acid (OA) (Fig. 9.2). The resulting product cannabigerolic acid (CBGA) is a central branch-point intermediate for the biosynthesis of the different major classes of cannabinoids; tetrahydrocannabinolic acid (THCA) (Fellermeier et al. 2001), cannabidiolic acid (CBDA) (Taura et al. 1996) and cannabichromenic acid (CBCA) (Gaoni and Mechoula 1966). The enzymes responsible for these cyclization and synthesis of these three cannabinoids are named THCA synthase, CBDA synthase and CBCA synthase respectively (Taura et al. 1995; Morimoto et al. 1998; Taguchi et al. 2008) (Fig. 9.3).

4.1.4 Changes in Cannabinoids Profile Overtime

From sequential harvesting studies on *C. sativa* chemotype it is evident that the concentration of cannabinoids in the flowers increases as the plant develops in the full flowering phase (Barnicomparini et al. 1984; Vogelmann et al. 1988; Pacifico et al. 2008; Muntendam et al. 2012). In basic experiments from the past it is obvious that the overall cannabinoid levels are significantly influenced by other factors: the plant's sex (Fetterma et al. 1971; Fairbairn and Rowan 1975), the strain type (Mandolino et al. 2003), phytogeographic area (Hillig and Mahlberg 2004a, b), light intensity (Mahlberg and Hemphill 1983), UV-B radiation (Zhang and Bjorn 2009), dry and windy conditions can raise cannabinoids content, and indoor cultivation conditions and infrastructure (Vanhove et al. 2011). On the other hand, cool summer and nitrogen fertilization can reduce the cannabinoids contents of the plant

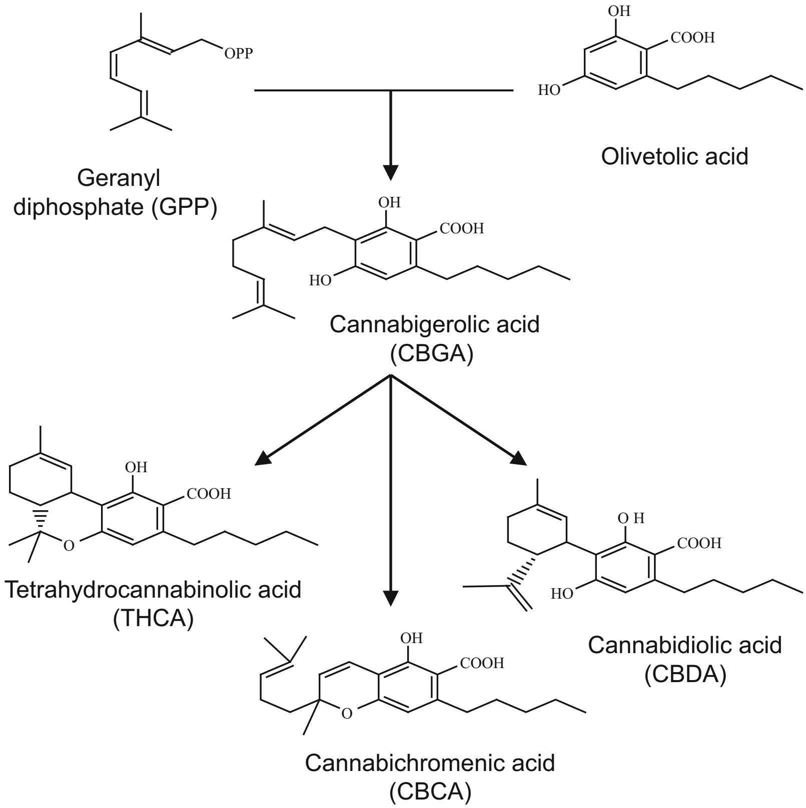


Fig. 9.2 Biosynthetic pathway of cannabinoids

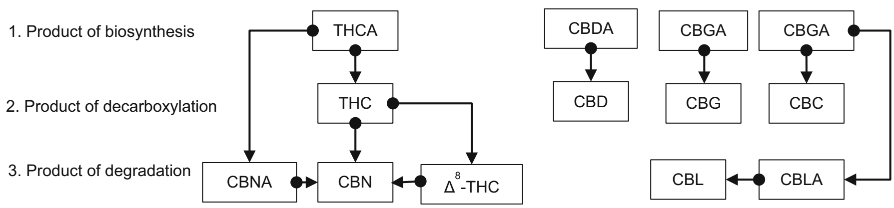


Fig. 9.3 Relationships between the major cannabinoids found in *Cannabis* plant materials. Three different groups are distinguished: cannabinoids produced by biosynthesis of the plant; cannabinoids resulting from natural decarboxylation of acidic cannabinoids; degradation products resulting from various influences, such as UV-light, oxidation or isomerization. *Arrows* indicate the routes of conversion (Adapted from Hazekamp et al. 2010)

(Latta and Easton 1975; Staquet et al. 1978). However, changes of cannabinoid levels were studied in *Cannabis* plants at different growth stages (Muntendam et al. 2009; Muntendam et al. 2012).

4.2 Non Cannabinoid Constituents

4.2.1 Terpenoids

Over 200 different terpenoids have been reported in *C. sativa* and most of them have been isolated from the essential oil from flowers (Ross and ElSohly 1996), from roots (Slatkin et al. 1971), leaves (Hendriks et al. 1975), and glandular trichomes (Kim and Mahlberg 2003). The volatile mono- and sesquiterpenoids are responsible for the various flavours found in *Cannabis* (Russo 2011). The most abundant terpenes in the *C. sativa* (chemo-type) are β-myrcene, *trans*-caryophyllen, α-pinen, *trans*-ocimen, and α-terpinolen (Malingre et al. 1975). The sesquiterpenoid β-caryophyllen-epoxid (Fig. 9.4) is the main volatile compound detected by narcotic dogs (Stahl and Kunde 1973). The variation and yield of terpenoids depends on the *Cannabis* verity, pollination, part of the plant, the method of cultivation, harvest time and processing (Brenneisen 2007; Fischedick et al. 2010).

4.2.2 Flavonoids

More than 20 flavonoids have been reported from the plant *C. sativa* and some of them can be glycosylated, prenylated or methylated (Vanhoenacker et al. 2002). These flavonoids have been isolated from the leaves (Barrett et al. 1986), flowers (Choi et al. 2004) and pollen (Paris et al. 1975; Ross et al. 2005). McPartland and Mediavilla (2002), isolated the conjugated *O*-glycosides of apigenin, luteolin, quercetin, and kaempferol. Vanhoenacker et al. (2002) additionally reported *C*-glycosides of Orientin, vitexin, luteolin-7-*O*-glucoside, and apigenin-7-*O*-glucoside. Prenylated flavonoids unique to *Cannabis* called cannaflavin A and B were reported as well and are typically for the family Cannabaceae and are found in *C. sativa* but also in *Humulus lupulus* as well (Barrett et al. 1986).

4.2.3 Alkaloids

Nitrogenous compounds in *C. sativa* have been investigated and only a minor number of ten alkaloids have been identified including some interesting pseudo-alkaloids and related precursors such as choline, trigonelline (a pyridine), muscarine (protoalkaloids), isoleucine betaine, and neurine (Turner et al. 1980a; Ross and ElSohly 1995). These alkaloids have been isolated from the leaves, stems, pollen, root and seeds (ElSohly et al. 1978; Mechoulam 1988).

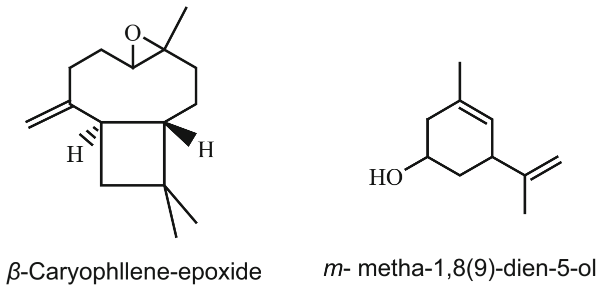


Fig. 9.4 Two special constituents of *Cannabis* plant

4.2.4 Others

Other compounds have been reported including non-cannabinoid phenols (stilbenoids, lignanamides and phenolic amides), sugars and related compounds, amino acids, proteins, enzymes, glycoproteins, sugars and related compounds, hydrocarbons, simple alcohols, simple aldehydes, simple ketones, simple acids, fatty acids, simple esters, lactones, steroids, vitamins, pigments and elements (ElSohly and Slade 2005; Brenneisen 2007).

5 Harvest and Processing

5.1 Harvest and Drying

On the assumption that production of Δ^9-THC is the main objective then harvesting is done when the crop is in full bloom (Vogelmann et al. 1988; Pacifico et al. 2008; De Backer et al. 2012). There minor published information on the methods of harvesting *C. sativa*. In Bedrocan bv (Netherlands), where the plant have been indoor grown, it is reported that the plants are cut at the base, left to dry in the dark under continuous dehumidified air for 7 days, afterwards leaves and inflorescences removed manually (Fig. 9.5d–f). Under ideal environmental conditions and expert management, yields of dried flowers commonly reach 400 g/m^2 per crop cycle. As a result of multiple cropping four or five times per year, total annual yields can add up to more than 2 kg of dried flowers per square meter (m^2) (Clarke and Watson 2002).

5.2 Processing

In *Cannabis* plants, THC is typically being present in low amounts in leaves and flowers in the form of carboxylic acid and it is necessary to decarboxylate THCA in the plant material before extraction. The conditions for efficient decarboxylation should be maximizing the chemical and non-enzymatic process avoiding or

Fig. 9.5 (**a**): *C. sativa* mother plants for vegetative propagation; (**b**) two week old plants of cutting; (**c**) female flowers of medicinal *C. sativa* at full bloom grown under a complex array of high-pressure sodium lamps; (**d**) drying racks, (**e**): trim buds; (**f**) bagging buds (Photos courtesy of Bedrocan BV, Netherlands)

minimizing oxidation in parallel (Veress et al. 1990; Guy and Stott 2005). A number of solvents can be used to extract the cannabinoids, ranging from polar solvents such as methanol and ethanol, to the less polar solvents such as benzene, petroleum ether, and *n*-hexane. Petroleum ether is widely used in *Cannabis* analysis since it extracts not only neutral cannabinoids but also their acid forms (Raharjo and Verpoorte 2004). In GW Pharmaceuticals Ltd., the whole *Cannabis* crude extract is subjected to further processing to remove unwanted byproducts from the extract for standardized enriched *Cannabis* secondary extract which is transferred to sealed, stainless steel containers and stored at -20 ± 5 °C to maintain stability (Guy and Stott 2005).

6 Diseases and Pest Control

Very few significant plant diseases or pests of *C. sativa* have been reported in the literature to date. Indoor grown *Cannabis sativa* is at risk to be attacked by certain insect pests and fungi mainly sucking insects (aphids, whiteflies and thrips), powdery mildew and grey mould fungus (Botrytis cinerea) (Mcpartland 1983; McPartland 1991; McPartland et al. 2000). To avoid indoor pests and diseases breeder can choose for insect- and fungus-resistant seed strains. But first line of prevention is proper cultivation under Good Agricultural Practice (GAP) conditions, like breeders and workers should work with separated clothing, visitors have to wear protective clothing as well (Potter 2004), keeping floors and air condition clean, use of High Efficiency Particulate Air filter (HEPA-filter) for ventilation, remove decomposed and dead biomass. Heavy populations of pests may need systemic insecticide, fungicide or miticide. Here, the use is critical and must respect approved chemicals because of the future utilization by patients. To work effectively, biocontrols as in process controls must be introduced as a monitoring system to warn for appearance of pests or early in a pest infestation (Heinz and Nelson 1996). Whenever, pests and diseases do strike, the affected plants have to be removed and destroyed before damage spreads (Potter 2004).

7 Quality Control of *Cannabis* Based Medicinal Extracts

For converting *Cannabis* based medicinal extracts (CBME) into herbal medicinal products (HMP) or as bulk material for THC extraction, a license must be issued by the regulatory authority around the world including regulatory standards of Good Laboratory Practice (GLP), Good Manufacturing Practice (GMP), Good Clinical Practice (GCP), and Good Agricultural Practice (GAP) standards according to the guidance documents provided by the International Conference on Harmonization (ICH). As a result, quality control is required throughout the whole manufacturing process, including cultivation, delivery and production of raw materials. For HMPs produced from plants, the regulatory authorities like FDA and EMA have released own guidelines on the production of HMPs (Guy and Stott 2005). For the analysis of cannabinoids, the analytical methods that are available have been reviewed by Raharjo and Verpoorte (2004). The most widely used chromatographic methods gas chromatography (GC) and high-performance liquid chromatography (HPLC).

7.1 Gas Chromatography–Mass Spectral Analysis

The use of gas chromatography (GC) commonly couples flame ionization detection (FID) or mass spectroscopy (MS) and allows the analysis of a large variety of cannabinoids with high resolution (De Backer et al. 2009). However, it cannot be used directly to analyze all cannabinoids owing to limitations in volatility of the compounds. Therefore, derivatisation is necessary when information about acidic cannabinoids is required (Lercker et al. 1992). GC/MS is the method of choice for creating profiles and fingerprints, a tool for attributing the country origin, the conditions of cultivation (indoor, outdoor), identity, content and purity (Fischedick et al. 2010).

7.2 High Performance Liquid Chromatography (HPLC)

In contrast to GC, no decomposition of the cannabinoids occurs by HPLC analysis and hence the cannabinoid acid forms can be analyzed directly for phenotype determination. Reversed phase HPLC has been used successfully in cannabinoid fingerprinting for identification purposes (Rustichelli et al. 1996; Rustichelli et al. 1998; Ferioli et al. 2000; De Backer et al. 2009). For quantitative analysis by HPLC, internal standards are required. Synthetic Δ^8-THC was proposed as the internal standard for the determination of Δ^9-THC (Rustichelli et al. 1998) since UV at 220 nm can be employed to detect both of them. However, analysis of all major cannabinoids in a typical *Cannabis* extract is not easily achieved, because of the complex composition resulting in chromatographic overlap of peaks. To overcome this problem, the use of mass detection (LC-MS) to distinguish between overlapping chromatographic peaks is today the analytical standard (Hazekamp et al. 2010). More recently, fully validated pharmacopeia method have been developed for the quality control of authentic cannabinoids in herbal *Cannabis* using Ultra-Performance LC (UPLC) (Hazekamp 2006).

8 Pharmaceutical Preparations Approved

In Canada, Sativex® (*Cannabis* extract, 27 mg/ml Δ^9-THC and 25 mg/ml CBD) is approved for the relief of pain due to disease of the nervous system, of pain and spasticity (muscular stiffness), multiple sclerosis, and for severe pain due to advanced cancer. Sativex® is undergoing clinical trials in the United States and is available on a limited basis by prescription in the United Kingdom, Germany and Spain (Whittle and Guy 2004; Whittle 2007). Recently, Bedrocan Company (The Netherlands) registered three *Cannabis* drugs from the dried flowers

- Bedrocan® containing 18 % Δ^9-THC and <1 % CBD,
- Bedrobinol® containing 11 % Δ^9-THC and <1 % CBD, and
- Bediol® containing 6 % Δ^9-THC and 7 % CBD

Occasionally, Cannador® is a new oral capsule containing *Cannabis* extract, where the THC to CBD ratio does not appear to be fully standardized. It has been clinically tested for disabling of tremor in patients with multiple sclerosis (MS) (Fox et al. 2004; Holdcroft et al. 2006; Rahn and Hohmann 2009).

9 Recommendations for Future Actions

Cannabis is accepted as a medicinal plant due to the impressive amount of therapeutic and pharmacological properties of cannabinoids specially THC and CBD. Even though grown technique's and analytical methods used for testing and detecting the ingredient of *C. sativa* are well known. Paradoxically, the actual impacts of biotechnical approaches in *Cannabis*-breeding via protoplast hybridization or gene transformation are still limited and should receive more attention in the future, especially as a result of the increasing psychotropic properties. However, one fascinating question is how to grow *C. sativa* under standardized condition to have a medicinal plant with high therapeutic properties? Is the standardized protocol of Bedrocan BV and GW Pharmaceuticals Ltd valid for the large scale? What are the general requirements to standardized protocol for *C. sativa* cultivation? In addition, we should not forget whether or not it is wise decision to use produce pharmaceuticals raw material from genetically modified (GM) *Cannabis*. However, although initial regulatory guidance for GLP, GMP and GCP guidelines specific for purification and processing of GM plants have to be clearly defined. Validation of specific facilities, protocols and analytical methods could be mentioned as a main purpose. Furthermore, public acceptance of GM based therapeutic products, and definitions of real final costs of plant-derived pharmaceuticals are critical aspects under consideration.

References

Appendino G, Chianese G, Taglialatela-Scafati O (2011) Cannabinoids: occurrence and medicinal chemistry. Curr Med Chem 18(7):1085–1099

Argo WR, Fischer PR (2002) Understanding pH management for container-grown crops. Meister, Willoughby

Barnicomparini I, Ferri S, Centini F (1984) Cannabinoid level in the leaves as a tool for the early discrimination of *Cannabis* chemovariants. Forensic Sci Int 24(1):37–42

Barrett ML, Scutt AM, Evans FJ (1986) Cannflavin-A and Cannflavin-B, prenylated flavones from *Cannabis sativa* L. Experientia 42(4):452–453

Beresford TP (1993) Marijuana the forbidden medicine. Grinspoon, L, Bakalar, Jb. Psychosomatics 34(4):368–369

Bohlmann F, Hoffmann E (1979) Compounds from *Helichrysum umbraculigerum* related to cannabigerol. Phytochemistry 18(8):1371–1374

Brenneisen R (1983) Psychotropic drugs. I. *Cannabis sativa* L. (Cannabinaceae). Pharm Acta Helv 58(11):314–320

Brenneisen R (2007) Chemistry and analysis of phytocannabinoids and other *Cannabis* constituents. In: Elsohly MA (ed) Marijuana and the cannabinoids. Humana Press, Totowa, pp 17–49

Brischia R, Piccioni E, Standardi A (2002) Micropropagation and synthetic seed in M.26 apple rootstock (II): a new protocol for production of encapsulated differentiating propagules. Plant Cell Tiss Org 68(2):137–141

Brunner TE (1973) Marijuana in Ancient Greece and Rome? The literary evidence. Bull Hist Med 47(4):344–355

Cervantes J (2006) Marijuana horticulture: The indoor/outdoor medical grower's bible. Van Patten, Vancouver, p 41

Chandra SH, Lata I, Khan A, ElSohly MA (2010) Propagation of elite *Cannabis sativa* for the production of Δ^9-Tetrahydrocannabinol (THC) using biotechnological tools. In: Rajesh A (ed) Medicinal plant biotechnology. CABI, Cambridge, MA, pp 98–114

Choi YH, Hazekamp A, Peltenburg-Looman AMG, Frederich M, Erkelens C, Lefeber AWM, Verpoorte R (2004) NMR assignments of the major cannabinoids and cannabiflavonoids isolated from flowers of *Cannabis sativa*. Phytochem Anal 15(6):345–354

Clarke RC, Watson DP (2002) Botany of natural *Cannabis* medicines. In: Grotenhermen F, Russo E (eds) *Cannabis* and Cannabinoids: pharmacology, toxicology, and therapeutic potential. The Haworth Press, New York, pp 3–13

Coene T (1995) Greenhouse coverings uncovered. The Growing Edge 6(3):66–72

De Backer B, Debrus B, Lebrun P, Theunis L, Dubois N, Decock L, Verstraete A, Hubert P, Charlier C (2009) Innovative development and validation of an HPLC/DAD method for the qualitative and quantitative determination of major cannabinoids in *cannabis* plant material. J Chromatogr B 877(32):4115–4124

De Backer B, Maebe K, Verstraete AG, Charlier C (2012) Evolution of the content of THC and other major cannabinoids in drug-type *Cannabis* cuttings and seedlings during growth of plants. J Forensic Sci 57(4):918–922

de Meijer EP, Bagatta M, Carboni A, Crucitti P, Moliterni VM, Ranalli P, Mandolino G (2003) The inheritance of chemical phenotype in *Cannabis sativa* L. Genetics 163(1):335–346

Demeijer EPM, Vansoest LJM (1992) The CPRO *Cannabis* germplasm collection. Euphytica 62(3):201–211

Dioscorides P (1968) The Greek herbal of Dioscorides, AD 1655. Hafner, London

Ebbell B (1937) The papyrus Ebers, the greatest Egyptian medical document. Levin & Munksgaard/H. Milford/ Oxford University Press, Copenhagen/London

Ebers G (1875) Das hermetische buch über die arzneimittel der alten Ägypten in heratischer schrift. 2Bde., Leipzing

Elbatsh MM, Moklas MA, Marsden CA, Kendall DA (2012) Antidepressant-like effects of delta (9)-tetrahydrocannabinol and rimonabant in the olfactory bulbectomised rat model of depression. Pharmacol Biochem Behav 102(2):357–365

ElSohly MA, Slade D (2005) Chemical constituents of marijuana: the complex mixture of natural cannabinoids. Life Sci 78(5):539–548

ElSohly MA, Turner CE, Phoebe CH, Knapp JE, Schiff PL, Slatkin DJ (1978) Anhydro*cannabisativine*, a new alkaloid from *Cannabis sativa*. J Pharm Sci 67:124

Fairbairn JW, Rowan MG (1975) Cannabinoid pattern in *Cannabis sativa* L seedlings as an indication of chemical race. J Pharmacy Pharmacol 27:P90–P90

Fankhauser M (2002) History of *cannabis* in Western Medicine. In: *Cannabis* and Cannabinoids. The Haworth Integrative Healing Press, New York

Feeney M, Punja ZK (2003) Tissue culture and *Agrobacterium*-mediated transformation of hemp (*Cannabis sativa* L.). In Vitro Cell Dev-Pl 39(6):578–585

Fellermeier M, Eisenreich W, Bacher A, Zenk MH (2001) Biosynthesis of cannabinoids – incorporation experiments with C-13-labeled glucoses. Eur J Biochem 268(6):1596–1604

Ferioli V, Rustichelli C, Pavesi G, Gamberini G (2000) Analytical characterisation of Hashish samples. Chromatographia 52(1–2):39–44

Fetterma P, Keith ES, Waller CW, Guerrero O, Doorenbo NJ, Quimby MW (1971) Mississippi-grown *Cannabis sativa* L- preliminary observation on chemical definition of phenotype and variations in tetrahydrocannabinol content versus age, sex, and plant part. J Pharm Sci 60 (8):1246–1249

Fischedick JT, Hazekamp A, Erkelens T, Choi YH, Verpoorte R (2010) Metabolic fingerprinting of *Cannabis sativa* L, cannabinoids and terpenoids for chemotaxonomic and drug standardization purposes. Phytochemistry 71(17–18):2058–2073

Fisse J, Braut F, Cosson L, Paris M (1981) Étude in vitro des capacités organogénétiques de tissus de *Cannabis sativa* L.; Effet de différentes substances de croissance. Plantes Médicinales et Phytotherapie 15:217–223

Flemming T, Muntendam R, Steup C, Kayser O (2007) Chemistry and biological activity of tetrahydrocannabinol and its derivatives. In: Khan MTH (ed) Topics in heterocyclic chemistry: bioactive heterocycles IV, vol 10. Springer, Berlin, pp 1–42

Formukong EA, Evans AT, Evans FJ (1989) The medicinal uses of *Cannabis* and its constituents. Phytother Res 3(6):219–231

Fox P, Bain PG, Glickman S, Carroll C, Zajicek J (2004) The effect of *Cannabis* on tremor in patients with multiple sclerosis. Neurology 62(7):1105–1109

Gaoni Y, Mechoula R (1966) Structure of cannabichromene a new active constituent of Hashish. J Chem Soc Chem Commun 1:20–21

Gaoni Y, Mechoulam R (1964) Isolation, structure, and partial synthesis of an active constituent of Hashish. J Am Chem Soc 86(8):1646–1647

Grotenhermen F, Russo E (2002) Chemical constituents of *Cannabis*: pharmacology, toxicology, and therapeutic potential. In: ElSohly MA (ed) *Cannabis* and Cannabinoids. Haworth Press, New York, pp 27–36

Guy GW, Stott C (2005) The development of Sativex®- a natural *cannabis* based medicine. In: Mechoulam R, Bruinrels J (eds) Cannabinoids as therapeutics milestones in drug therapy. Springer, Berlin, pp 231–263

Hao YJ, Deng XX (2003) Genetically stable regeneration of apple plants from slow growth. Plant Cell Tiss Org 72(3):253–260

Happyana N, Agnolet S, Muntendam R, Van Dam A, Schneider B, Kayser O (2013) Analysis of cannabinoids in laser-microdissected trichomes of medicinal *Cannabis sativa* using LCMS and cryogenic NMR. Phytochemistry 87:51–59

Hazekamp A (2006) An evaluation of the quality of medicinal grade *cannabis* in the Netherlands. Cannabinoids 1(1):1–9

Hazekamp A, Justin TF, Díez ML, Lubbe A, Ruhaak RL (2010) 3.24 – Chemistry of *Cannabis*. Comprehensive Natural Products II 3:1033–1084

Heinz KM, Nelson JM (1996) Interspecific interactions among natural enemies of Bemisia in an inundative biological control program. Biol Control 6(3):384–393

Hendriks H, Malingre TM, Batterman S, Bos R (1975) Monoterpene and sesquiterpene hydrocarbons of essential oil of *Cannabis sativa*. Phytochemistry 14(3):814–815

Hillig KW, Mahlberg PG (2004a) A chemotaxonomic analysis of cannabinoid variation in *Cannabis* (Cannabaceae). Am J Bot 91(6):966–975

Hillig KW, Mahlberg PG (2004b) A chemotaxonomic analysis of cannabinoid variation in *Cannabis* (Cannabaceae). Am J Bot 91(6):966–975

Holdcroft A, Maze M, Dore C, Tebbs S, Thompson S (2006) A multicenter dose-escalation study of the analgesic and adverse effects of an oral *Cannabis* extract (Cannador) for postoperative pain management. Anesthesiology 104(5):1040–1046

Jones JB (1997) Hydroponics: a practical guide for the soilless grower. Lucie Press, Bocan Raton

Kim ES, Mahlberg PG (1997) Immunochemical localization of tetrahydrocannabinol (THC) in cryofixed glandular trichomes of *Cannabis* (Cannabaceae). Am J Bot 84(3):336–342

Kim ES, Mahlberg PG (2003) Secretory vesicle formation in the secretory cavity of glandular trichomes of *Cannabis sativa* L. (Cannabaceae). Mol Cells 15(3):387–395

Kubota C, Thomson CA (2006) Controlled environments for production of value-added food crops with high phytochemical concentrations: lycopene in tomato as an example. Hortscience 41(3):522–525

Lata H, Chandra S, Khan IA, ElSohly MA (2008) Propagation of *Cannabis sativa* L. using synthetic seed technology. Planta Med 74(3):328–328

Lata H, Chandra S, Khan IA, ElSohly MA (2009) Propagation through alginate encapsulation of axillary buds of *Cannabis sativa* L. an important medicinal plant. Physiol Mol Biol Plants 15(1):79–86

Lata H, Chandra S, Khan I, ElSohly M (2010a) *Cannabis sativa* L. Micropropagation in temporary immersion bioreactor system. Planta Med 76 (P9)

Lata H, Chandra S, Khan IA, ElSohly MA (2010b) High frequency plant regeneration from leaf derived callus of high delta(9)-tetrahydrocannabinol yielding *Cannabis sativa* L. Planta Med 76(14):1629–1633

Lata H, Chandra S, Mehmedic Z, Khan IA, ElSohly MA (2012) In vitro germplasm conservation of high Delta(9)-tetrahydrocannabinol yielding elite clones of *Cannabis sativa* L. under slow growth conditions. Acta Physiol Plant 34(2):743–750

Latta R, Easton B (1975) Seasonal fluctuations in cannabinoid in cannabinoid content of Kansas marijuana. Econ Bot 29:153–163

Le Bot J, Adamowicz S, Robin P (1998) Modelling plant nutrition of horticultural crops: a review. Sci Hortic Amsterdam 74(1–2):47–82

Leggett T (2006) A review of the world *cannabis* situation. B Narcotics 58(1–2):1–155

Lercker G, Bocci F, Frega N, Bortolomeazzi R (1992) Cannabinoid acids analysis. Farmaco 47(3):367–378

Li HL (1974) Archeological and historical account of *Cannabis* in China. Econ Bot 28(4):437–448

Lynn AB, Herkenham M (1994) Localization of cannabinoid receptors and nonsaturable high-density cannabinoid binding sites in peripheral tissues of the rat: implications for receptor-mediated immune modulation by cannabinoids. J Pharmacol Exp Ther 268(3):1612–1623

Mahlberg PG, Hemphill JK (1983) Effect of light quality on cannabinoid content of *Cannabis sativa* L (Cannabaceae). Bot Gaz 144(1):43–48

Mahlberg PG, Kim ES (2004) Accumulation of cannabinoids in glandular trichomes of *Cannabis* (Cannabaceae). J Indust Hemp 9:15–36

Malingre T, Hendriks H, Batterman S, Bos R, Visser J (1975) The essential oil of *Cannabis sativa*. Planta Med 28(1):56–61

Mandolino G, Ranalli P (1999) Advances in biotechnological approaches for hemp breeding and industry. In: Ranalli P (ed) Advances in hemp research. The Haworth Press, New York, pp 185–212

Mandolino G, Carboni A, Forapani S, Faeti V, Ranalli P (1999) Identification of DNA markers linked to the male sex in dioecious hemp (*Cannabis sativa* L.). Theor Appl Genet 98(1):86–92

Mandolino G, Bagatta M, Carboni A, Ranalli P, de Meijer EPM (2003) Qualitative and quantitative aspects of the inheritance of chemical phenotype in *Cannabis*. J Ind Hemp 8:51–72

Mathur J, Ahuja PS, Lal N, Mathur AK (1989) Propagation of Valeriana-Wallichii Dc using encapsulated apical and axial shoot buds. Plant Sci 60(1):111–116

Mcpartland JM (1983) Fungal pathogens of *Cannabis sativa* in central illinois. Phytopathology 73(5):797–797

McPartland JM (1991) Common names for diseases of *Cannabis sativa* L. Plant Disease 75:226–227

McPartland J, Mediavilla V (2002) Noncannabinoid components. In: Grotenhermen F, Russo E (eds) *Cannabis* and Cannabinoids pharmacology, toxicology, and therapeutic. Potential Haworth Press, New York, pp 401–409

McPartland JM, Clarke RC, Watson DP (2000) Hemp diseases and pests: management and biological control: an advanced treatise. CABI Pub, New York

Mechoulam R (1988) Alkaloids in *Cannabis sativa* L. In: Brossi A (ed) The Alkaloids. Academic, San Diego, pp 77–93

Mechoulam R, Shvo Y (1963) Hashish-I. The structure of cannabidiol. Tetrahedron Lett 19:2073–2078

Morimoto S, Komatsu K, Taura F, Shoyama Y (1998) Purification and characterization of cannabichromenic acid synthase from *Cannabis sativa*. Phytochemistry 49(6):1525–1529

Muntendam R, Erkelens T, Kayser O (2009) Genetic and metabolic studies of cannabinoids in standardized medicinal *Cannabis sativa*. Planta Med 75(4):417–417

Muntendam R, Happyana N, Erkelens T, Bruining F, Kayser O (2012) Time dependent metabolomics and transcriptional analysis of cannabinoid biosynthesis in *Cannabis sativa*

var. bedrobinol and bediol grown under standardized condition and with genetic homogeneity. Online Int J Med Plant Res 1(2):31–40
Nahas GG (1982) Hashish in Islam 9th century to 18th century. B New York Acad Med 58(9):814–831
Pacifico D, Miselli F, Carboni A, Moschella A, Mandolino G (2008) Time course of cannabinoid accumulation and chemotype development during the growth of *Cannabis sativa* L. Euphytica 160(2):231–240
Page JE, Nagel J (2006) Biosynthesis of terpenophenolic metabolites in Hop and *Cannabis*. J Recent Adv Phytochem 40:179–210
Paris M, Boucher F, Cosson L (1975) Constituents of *Cannabis sativa* Pollen. Econ Bot 29(3):245–253
Pattnaik S, Chand PK (2000) Morphogenic response of the alginate encapsulated axillary buds from in vitro shoot cultures of six mulberries. Plant Cell Tiss Org 60(3):177–185
Pec J, Flores-Sanchez IJ, Choi YH, Verpoorte R (2010) Metabolic analysis of elicited cell suspension cultures of *Cannabis sativa* L. by (1)H-NMR spectroscopy. Biotechnol Lett 32(7):935–941
Petri G, Oroszlan P, Fridvalszky L (1988) Histochemical detection of hemp trichomes and their correlation with the THC content. Acta Biol Hung 39(1):59–73
Porterfield DM, Musgrave ME (1998) The tropic response of plant root to oxygen: oxitropism in *Pisum sativum* L. Planta Med 206:1–6
Potter DJ (2004) Growth and morphology of medicinal *Cannabis*. In: Guy GW, Whittle BA, Robson PJ (eds) The medicinal uses of *Cannabis* and Cannabinoids. Pharmaceutical Press, London, pp 17–54
Raharjo TJ, Verpoorte R (2004) Methods for the analysis of cannabinoids in biological materials: a review. Phytochem Analy 15(2):79–94
Rahn EJ, Hohmann AG (2009) Cannabinoids as pharmacotherapies for neuropathic pain: from the bench to the bedside. Neurotherapeutics 6(4):713–737
Raman A (ed) (1998) The *Cannabis* plant: botany, cultivation, and processing for use. *Cannabis*: the Genus *Cannabis*. Harwood Academic Publishers, Amsterdam
Razdan RK, Puttick AJ, Zitko BA, Handrick GR (1972) Hashish.6. Conversion of (−)-delta1(6)-tetrahydrocannabinol to (−)-delta1(7)-tetrahydrocannabinol – stability of (−)-delta1- and ″(−)-delta1(6)-tetrahydrocannabinols. Experientia 28(2):121–123
Rosenthal E (ed) (1984) Marijuana growers handbook; indoor/greenhouse. Quick American Publishing Company, San Fransisco
Ross SA, ElSohly MA (1995) Constituents of *Cannabis sativa* L. XXVIII-A review of the natural constituents: 1980–1994. Zagazig J Pharm Sci 4:1–10
Ross SA, ElSohly MA (1996) The volatile oil composition of fresh and air-dried buds of *Cannabis* sativa. J Nat Prod 59(1):49–51
Ross SA, Mehmedic Z, Murphy TP, Elsohly MA (2000) GC-MS analysis of the total delta9-THC content of both drug and fiber type *cannabis* seeds. J Anal Toxicol 24(8):715–717
Ross SA, ElSohly MA, Sultana GNN, Mehmedic Z, Hossain CF, Chandra S (2005) Flavonoid glycosides and cannabinoids from the pollen of *Cannabis sativa* L. Phytochem Anal 16(1):45–48
Russo EB (2007) History of *cannabis* and its preparations in saga, science, and sobriquet. Chem Biodivers 4(8):1614–1648
Russo EB (2011) Taming THC: potential *cannabis* synergy and phytocannabinoid-terpenoid entourage effects. Br J Pharmacol 163(7):1344–1364
Russo EB, Jiang HE, Li X, Sutton A, Carboni A, del Bianco F, Mandolino G, Potter DJ, Zhao YX, Bera S, Zhang YB, Lu EG, Ferguson DK, Hueber F, Zhao LC, Liu CJ, Wang YF, Li CS (2008) Phytochemical and genetic analyses of ancient *Cannabis* from Central Asia. J Exp Bot 59 (15):4171–4182

Rustichelli C, Ferioli V, Vezzalini F, Gamberini G (1996) Simultaneous separation and identification of hashish constituents by coupled liquid chromatography mass spectrometry (HPLC-MS). Chromatographia 43(3–4):129–134

Rustichelli C, Ferioli V, Baraldi M, Zanoli P, Gamberini G (1998) Analysis of cannabinoids in fiber hemp plant varieties (*Cannabis sativa* L.) by high performance liquid chromatography. Chromatographia 48(3–4):215–222

Sicher RC, Bunce JA (1997) Relationship of photosynthetic acclimation to changes of Rubisco activity in field-grown winter wheat and barley during growth in elevated carbon dioxide. Photosynth Res 52(1):27–38

Sicurani M, Piccioni E, Standardi A (2001) Micropropagation and preparation of synthetic seed in M.26 apple rootstock I: attempts towards saving labor in the production of adventitious shoot tips suitable for encapsulation. Plant Cell Tiss Org 66(3):207–216

Slatkin DJ, Doorenbo NJ, Harris LS, Masoud AN, Quimby MW, Schiff PL (1971) Chemical constituents of *Cannabis* sativa L root. J Pharm Sci 60(12):1891–1892

Stahl E, Kunde R (1973) Neue Inhaltsstoffe aus dem atherischen 01 von *Cannabis* sativa. Tetrahedron Lett 30:2841–2844

Stamler RT, Fahlman RC, Vigeant H (1985) Illicit traffic and abuse of *cannabis* in Canada. B Narcotics 37(4):37–49

Staquet M, Gantt C, Machin D (1978) Effect of a nitrogen analog of tetrahydrocannabinol on cancer pain. Clin Pharmacol Ther 23(4):397–401

Steiner AA (1961) A universal method for preparing nutrient solutions of certain desired composition. Plant and Soil 15(2):134–154

Taguchi C, Taura F, Tamada T, Shoyama Y, Shoyama Y, Tanaka H, Kuroki R, Morimoto S (2008) Crystallization and preliminary X-ray diffraction studies of polyketide synthase-1 (PKS-1) from *Cannabis sativa*. Acta Crystallogr F 64:217–220

Taura F, Morimoto S, Shoyama Y, Mechoulam R (1995) First direct evidence for the mechanism of delta(1)-tetrahydricannabinolic acid biosynthesis. J Am Chem Soc 117(38):9766–9767

Taura F, Morimoto S, Shoyama Y (1996) Purification and characterization of cannabidiolic-acid synthase from *Cannabis sativa* L. Biochemical analysis of a novel enzyme that catalyzes the oxidocyclization of cannabigerolic acid to cannabidiolic acid. J Biol Chem 271(29):17411–17416

Taura F, Sirikantaramas S, Shoyama Y, Shoyama Y, Morimoto S (2007) Phytocannabinoids in *Cannabis sativa*: Recent studies on biosynthetic enzymes. Chem Biodivers 4(8):1649–1663

Thakur GA, Duclos RI, Makriyannis A (2005) Natural cannabinoids: templates for drug discovery. Life Sci 78(5):454–466

Tius MA, Kannangara GSK (1992) Synthesis of 5′-(2h3)-(−)-11-nor-9-carboxy-delta-9-tetrahydrocannabinol methyl-ester methyl-ether. Tetrahedron 48(42):9173–9186

Toyota M, Shimamura T, Ishii H, Renner M, Braggins J, Asakawa Y (2002) New bibenzyl cannabinoid from the New Zealand liverwort *Radula marginata*. Chem Pharm Bull 50 (10):1390–1392

Trofin IG, Dabija G, Vaireanu DI, Filipescu L (2012) The influence of long term storage conditions on the stability of cannabinoids derived from *Cannabis* resin. Rev Chim-Bucharest 63(4):422–427

Turner CE, Cheng PC, Lewis GS, Russell MH, Sharma GK (1979) Constituents of *Cannabis* sativa. 15. Botanical and chemical profile of Indian variants. Planta Med 37(3):217–225

Van Patten GF (1992) Hydroponics for the rest of us. Growing Edge 3(3):24–51

Vanhoenacker G, Van Rompaey P, De Keukeleire D, Sandra P (2002) Chemotaxonomic features associated with flavonoids of cannabinoid-free *cannabis* (*Cannabis sativa* subsp *sativa* L.) in relation to hops (*Humulus lupulus* L.). Nat Prod Lett 16(1):57–63

Vanhove W, Van Damme P, Meert N (2011) Factors determining yield and quality of illicit indoor *cannabis* (*Cannabis* spp.) production. Forensic Sci Int 212(1–3):158–163

Veress T, Szanto JI, Leisztner L (1990) Determination of cannabinoid acids by high performance liquid chromatography of their neutral derivatives formed by thermal decarboxylation. 1. Study of the decarboxylation process in open reactors. J Chromatogr 520:339–347

Vogelmann AF, Turner JC, Mahlberg PG (1988) Cannabinoid composition in seedlings compared to adult plants of *Cannabis sativa*. J Nat Prod 51(6):1075–1079

Waldo AZ (2006) History of *cannabis* as a medicine: a review. Rev Bras Psiquiatr 28(2):153–157

Whittle BA (2007) *Cannabis* as a medicine (benefits and risks associated with phytomedicines). J Pharm Pharmacol 59:A69–69

Whittle BA, Guy GW (2004) Development of *cannabis* based medicines: risk, benefit and serendipity. In: Guy GW, Robson PJ, Whittle BA (eds) The medicinal uses of *Cannabis* and Cannabinoids. Pharmaceutical Press, London, pp 427–463

Zhang WJ, Bjorn LO (2009) The effect of ultraviolet radiation on the accumulation of medicinal compounds in plants. Fitoterapia 80(4):207–218

Zheng YB, Zhang P, Dixon M (2005) Evaluation of four lamp types for the production of tomato plants in controlled environments. Horttechnology 15(3):646–652

Zulfiqar F, Ross SA, Slade D, Ahmed SA, Radwan MM, Ali Z, Khan IA, ElSohly MA (2012) *Cannabis*ol, a novel delta(9)-THC dimer possessing a unique methylene bridge, isolated from *Cannabis sativa*. Tetrahedron Lett 53(28):3560–3562

Chapter 10
Sustainable Fertilization in Medicinal and Aromatic Plants

Alessandra Carrubba

Abstract The nutrient level in the soil is one of the most investigated aspects of agricultural research, also including research into Medicinal and Aromatic plants. The effect of fertilization has been studied in detail for many species, with contrasting results as concerns above all the qualitative aspects of production. Generally speaking, an increased level of nutrients induces an enhancement of plant biomass, but when the goal of cultivation is different from herbage yield, i.e. when a special plant part (seeds, or roots, or flowers) is of interest, or when the quality features are especially important, the outcome of fertilization may be dramatically different. A fine-tuned fertilization practice is therefore necessary, and forms, rates and times of distribution of fertilizers must be accurately planned and managed.

Keywords Crop nutrition • Nitrogen • Phosphor • Potassium • Cropping techniques • Sustainable management

1 Introduction

In ordinary agricultural management, the integration and/or enrichment of soil nutrients reserves is one of the most important tools to obtain satisfactory productions. The opportunity of such practice also in growing MAPs, besides being well-known to practitioners, has been recognized by WHO (2003), in that the use of fertilizers was defined "often indispensable in order to obtain large yields". At the same time, however, the use of correct types and quantities of fertilizers was termed necessary.

Nutrient availability in soils is related to several soil characters, both physical and chemical. Soil reaction (pH) and parent material are the major factors moderating the content and availability of mineral elements in the soil. In soils with high pH reaction and high content of lime, availability of most mineral elements

A. Carrubba (✉)
Department of Agricultural and Forest Sciences, Università degli Studi di Palermo, Palermo, Italy
e-mail: alessandra.carrubba@unipa.it

Á. Máthé (ed.), *Medicinal and Aromatic Plants of the World*, Medicinal and Aromatic Plants of the World 1, DOI 10.1007/978-94-017-9810-5_10

decreases, what usually causes chlorosis and retards development of certain plants (arnica) (Catizone et al. 1986). Oppositely, in strong acid soils, the availability of mineral elements increases, what can cause harmful accumulation of heavy metals (Cd, Ni, etc.), especially in certain plant species that are prone to such phenomenon (St. John's wort, yarrow, and others) (Radanović et al. 2004).

Moisture plays a determinant role as well. Most crops have shown significant interactions between nutrients and water, in that water deficiency conditions are often claimed to influence negatively plant's use of nutrients. As a consequence, in the arid and semi-arid areas of the world, more fertilizer is used when water for irrigation is available, and under rainfed Mediterranean conditions, fertilizer recommendations are tuned to the average rainfall incidence (Sivakumar and Huda 1984). A close interaction between nutrients and water availability was stated for nitrogen (a highly soluble element), and e.g. in coriander, Nitrogen Use Efficiency was found to increase with enhancing irrigation rate (Singh and Rao 1994). Nevertheless, some evidence exists that a moderate N-P supply may mitigate the detrimental effect of drought on biomass yield in some MAPs such as *Bupleurum chinense* DC (Zhu et al. 2009).

Decisions concerning fertilization, actually, involve many consequences. An improper nutrients supply may provoke a number of disadvantages on plants, including lodging, pest attacks, cycle delay and so on. A few elements may exert a negative effect on the quality features of product (quantity and quality of active compounds) (Radanović et al. 2004). Finally, environmental issues linked to fertilization practice are of concern. Indeed, for fertilization efficiency, elements must be in a soluble form, that plants may easily recover from soil, and when soluble elements are not picked up by plants, their excess in soil may generate pollution problems.

Many aspects of fertilization technique have been deepened in MAPs management. In most cases, the advised fertilizer amounts are tuned to obtaining high herbage yields, and the quality features are of concern only in few very evident occurrences. Table 10.1 summarizes a few information obtained from literature on this topic. The so-called "three elements of fertility", namely nitrogen (N), phosphor (P) and potassium (K) are taken into account, since they are usually the most used in agricultural practice. Of course, the high variability of environments involved in the various trials carried out worldwide makes somehow difficult to generalize the outcomes of experimental results. The available information allows however some general consideration. A first differentiation comes out between annuals and perennials: many perennials, such as sage, gentian or lavender, have reduced fertilizer needs in the first cropping year due to their small initial size, but higher amounts are required in following years (Catizone et al. 1986; Karamanos 2000). This is especially true for nitrogen, that in most cases is top-dressed in 2 or 3 fractions, allowing plants to extract it from soil in concordance with their needs along the growth cycle. Otherwise, P and K, claimed to have a scarce mobility throughout soil profile, are usually supplied before transplant (at the time of soil preparation) and distributed deeply enough to be absorbed by the roots during their elongation. Of course, in decision-making concerning fertilization, factors related

Table 10.1 Fertilizer amounts required by some selected MAPs (Data from Catizone et al. 1986; Singh and Rao 1994; Rangappa and Bhardwaj 1998; Karamanos 2000; Joy et al. 2001, 2001a; Rajamani 2001; Beyaert 2006; Basso 2009; Karkanis et al. 2011; Verma et al. 2011)

Species	N (kg ha^{-1})	P_2O_5 (kg ha^{-1})	K_2O (kg ha^{-1})
Achillea millefolium L. (Yarrow)	100	80–100	100–120
Althaea officinalis L. (Marsh mallow)			
1st year	100 (in 2 splits)	70	120
2nd year and following	100 (in 2 splits)	0	0
Anethum graveolens L. (Dill)	90–120 (50% at sowing, 50% 20–40 dd after emergence)	50–70	50–70
Angelica archangelica L. (Angelica)			
1st year	50–70	100–120	100–120
2nd year and following	50–70 (in spring)	0	0
Apium graveolens L. (Celery)	200	40	40
Artemisia spp. (Wormwood)			
Annual (*Artemisia annua* L.)	50	50	50
Absinthe (*A. absinthium* L.)	150, in 2–3 splits	100	150
Roman wormwood (*A. pontica* L.)	120–160 (50 % at sowing, 50 % after emergence)	100–120	100–120
Tarragon (*A. dracunculus* L.)			
1st year	40–50	60–70	120–150
2nd year and following	40–50	0	0
Atropa bella-donna L. (Deadly nightshade)			
1st year	100 (at transplant)	100–120	0 (only if needed)
2nd year and following	100 (before vegetative regrowing)	0	
Calendula officinalis L. (Marigold)	40–50	70–80 to 100	70–80 to 50–100
Carum carvi L. (Carvi)	100–120 (50 % at sowing, 50 % before vegetative regrowing of 2nd year)	60–80	60–80
Colchicum autumnale L. (Meadow saffron)			
1st year	0	80–100	80–100
2nd year and following	40–50 (spring)	0	0
Coriandrum sativum L. (Coriander)	40–50 to 80–90	40 to 80–100	40
Crocus sativus L. (Saffron)	Only if needed	60–70	60–70
Cuminum cyminum L. (Cumin)	30–40	30–40	30–40
Cymbopogon spp. (Lemongrass)	90 (1/3 at sowing, 2/3 after harvest of each year)	30	30

(continued)

Table 10.1 (continued)

Species	N (kg ha^{-1})	P_2O_5 (kg ha^{-1})	K_2O (kg ha^{-1})
Digitalis lanata L. (Grecian foxglove)	150 (1/3 at sowing; 2/3 at vegetative regrowing of 2nd year)	200 0	80–90 0
Echinacea angustifolia DC; *E. pallida* Nutt; *E. purpurea* Moench (Cone flower)	120, in 3 splits, each year	70	150
Foeniculum vulgare Mill. (Fennel)	100–120 (50 % at crop emergence, 50 % at vegetative regrowing in the 2nd year)	30 to 100–150	30 to 80–100
Gentiana lutea L. (Yellow gentian)			
1st year	0 or 40 at transplant	100	100
2nd year and following	50–70 to 40–100 (in spring)	0	0
Helichrysum italicum (Roth) Don. (Helichrysum)			
1st year	60–100 (50 % at sowing, 50 % 40–50 dd after emergence)	80–100	80–100
2nd year and following	60–100 (50 % at vegetative regrowing; 50 % after cutting)	0	0
Hypericum perforatum L. (St. John's wort)	0 to 100–150 in 3 Splits	70–100	180–200
Hyssopus officinalis L. (Hyssop)	30–40 to 50–70 (before spring each year)	60–80 to 70–90	70–90 to 100–120
Lavandula spp. (Lavender)			
1st year	50	50	50
2nd year and following	60–70 (spring)	60–70	60–70
Majorana hortensis L. (Marjoram)			
1st year	60–70	60–70	60–70
2nd year and following	40–50	0	0
Malva sylvestris L. (Common mallow)			
1st year	70–80	70–80	70–80
2nd year	50–60	0	0
Matricaria chamomilla L. (German chamomile)	30–50 to 80 (in spring)	30–50	20
Melissa officinalis L. (Lemon balm)			
1st year	0 to 70, in 2 splits	70 to 100–120	100–120
2nd year and following	70 to 100–130 to 250, in 2–3 splits	0	0
Mentha spp. (Mints)			
Japanese mint (*Mentha arvensis* L.)	150–160	40–50	30–40

(continued)

Table 10.1 (continued)

Species	N (kg ha^{-1})	P_2O_5 (kg ha^{-1})	K_2O (kg ha^{-1})
Peppermint (*M. x piperita*) L.	100–120 to 150–200	50–80 to 100–120	50 to 150–200
Ocimum basilicum L. (Basil)	40	40	40
Origanum heracleoticum L. (Oregano)			
1st year	40–60	100–120	100–120
2nd year and following	40–60 (spring)	0	0
Panax quinquefolius L. (Ginseng)	40 (spring, each year)	20	20
Papaver somniferum L. (Opium poppy)	60–120	70–100	70–100
Plantago ovata Forsk (Isabgol)	25	25	25
Pimpinella anisum L. (Anise)	50–60	60–70	60–70
Rosmarinus officinalis L. (Rosemary)			
1st year	60–80 to 100, in 3–4 splits	40 to 60–80	40 to 80–100
2nd year and following	60–80 to 100, in 3–4 splits	0	0
Salvia spp (Sage)			
Common sage (*Salvia officinalis* L.)			
1st year	70–80 to 40–100	30–80 to 100–150	30–100 to 100–150
2nd year and following	70–80 to 40–80 (50 % before vegetative regrowing, 50 % after the first cutting)	30–50 (if needed)	30–70 (if needed)
Clary sage (*Salvia sclarea* L.)			
1st year	50–80 to 100 at sowing/ transplant	30 to 100	30 to 100
2nd year and following	50–80 to 120 in spring		
Satureja hortensis L. (Winter savory)			
1st year	50	50	50
2nd year and following	50–80 (before vegetative regrowing)	0	0
Silybum marianum Gaertn. (Milk thistle)	50 (at sowing)	30.5 to 140	50 to 150
Thymus spp. (Thyme)			
1st year	40–50 to 100	40–50 to 50–60	40–50 to 100–120
2nd year and following	40–50 (spring)	0	0
Valeriana officinalis L. (Valerian)	100–110 to 130–150 (50 % at transplant, 50 % at vegetative regrowing)	60 to 70–90	100 to 200–220

to environment, plant genotype, goals of cultivation, and farm, must be kept into account. Total fertilizer rate is expected to be generally less important to plants than distribution method and timing, in that the availability of elements along the development of crops, above all in the physiological stages when it is especially important for them, is more important than their overall amount (Arnon 1992).

Moreover, it must be stressed that the commercial and industrial interest of MAPs is mainly due to their ability to produce and accumulate some special secondary metabolites; hence, besides the bare aspect of yield enhancement, the planning and management of fertilization must pay attention to all aspects related to their quality features, and the nutrients supply must be carefully tuned to the goal of cultivation (Franz 1983; Carrubba and Catalano 2009).

2 N Fertilization

As a matter of fact, N is the element that exerts the greatest and most evident effect on crops, in this explaining the farmers' tendency to spread high, and sometimes excessive, doses (Arnon 1992; Neeteson et al. 1999). In this case, the fate of this highly soluble element, and the consequent risks for health and environment, are of major concern (Yadav et al. 1997). Of course, many possibilities are available to minimize those risks, and N fertilization may be supplied to plants in various moments, rates, and forms, with different effects on health and environment.

The specific effects of N fertilization have been studied in detail on many MAPs, including peppermint (Dellacecca 1994), sage (Bezzi et al. 1992), fennel (Omidbaigi and Hornok 1992), *Origanum majorana* (Trivino and Johnson 2000), coriander (Carrubba 2009). Generally speaking, the primary effect of N fertilization is to promote plant development. Literature shows many examples of this: herbage yield was found to increase with increased N applications in coriander (Lenardis et al. 2000), Scotch spearmint *(Mentha gracilis* Sole) (Kothari and Singh 1995), peppermint (Piccaglia et al. 1993), Java citronella *(Cymbopogon winterianus* Jowitt) (Singh et al. 1996b), *Salvia fruticosa* (Karamanos 2000), St. John's Wort *(Hypericum perforatum* L.) (Berti et al. 2000), geranium (*Pelargonium* sp.) (Singh et al. 1996a), lavender (Biesiada et al. 2008), basil *(Ocimum basilicum* L.) (Rangappa and Bhardwaj 1998), and many others. This effect is striking at low fertilizer rates but becomes less and less evident with increasing N doses, until a ceiling value that may be considered the optimal N supply for a given crop and condition (Arnon 1992). The relationship between root and shoot dry matter accumulation per year and the N fertilization rate often takes the shape of a quadratic curve, as e.g. shown in ginseng (Beyaert 2006), where plant dry weight increases to a maximum and then decreases with increasing N application rate with a maximum at about 40 kg N ha^{-1}. In coriander, N fertilization enhanced seed yields by 10.7 % in comparison with the untreated plots, and generally speaking, every 10 kg ha^{-1} of N-fertilizer supply (until the optimal rate is reached) is able to generate an increase in seed yield ranging from about 20 to 70 kg ha^{-1}, depending on environmental conditions and crop management (Carrubba 2009). However, when the marketable part is not represented by the whole plant, e.g. when seeds, roots or inflorescences are of interest, crop response to N fertilization may be different. In Roman chamomile, N fertilization stimulates the formation of smaller flowers, hence reduces their commercial value (Catizone et al. 1986). Lower N amounts are generally required for crops addressed to the production of roots, such as valerian, gentian and echinacea, since N favors the development of the aerial part of plant and obstacles the formation of roots, that are the valuable part of plant (Catizone et al. 1986; Basso 2009). Moreover, the optimal N rate for herbage development is frequently higher than the optimal N rate for the reproductive part of plant. E.g. in dill, 80–90 kg ha^{-1} are suggested when plants are grown to obtain herbage yield, whereas a lower supply (60–70 kg ha^{-1}) is enough for a satisfactory seeds production (Basso 2009). In this case, a crucial importance is taken by those mechanisms that underlie to the partitioning of dry matter towards the diverse parts of plant. This different response to N fertilization of the various plant organs explains why in some cases variations of Harvest Index (HI; percentage of seeds yield/total plant biomass) are more evident than the absolute variations of both components, that was e.g. found in borage, where HI tended to decrease with increasing nitrogen application rate whereas seed yields and total dry biomass were rather not affected (El Hafid et al. 2002). It is possible therefore to find cases in which N fertilization has been found to affect negatively crop results, for example, allowing leaves to develop instead of other desired plant parts, or delaying flowering, or causing lodging of flowers (lavender and lavandin) and entire plants (anise) (Catizone et al. 1986).

3 P-K Fertilization

Recommendations for phosphor (P) and potassium (K) supplies as found in the literature for several MAPs are reported in Table 10.1. It is worth however to underline that, when making decisions regarding fertilizers application, to account for their availability in the soil is especially important. Moreover, the dosage of P-K fertilization is also determined by the uptake of plants from soil, which on its turn is dependent upon the expected yields (Pank 1993). Hence, soil testing is always to recommend, and the opportunity to add fertilizers should be carefully evaluated based on the goals of farmers.

In the field practice, as P and K are scarcely mobile along soil's profile, they are generally applied to perennials with the first soil works, allowing roots to find them as long as they grow (Arnon 1992). Very often, a local distribution of these elements at sowing time ("band" fertilization) is suggested (Kothari et al. 1987) but a great care is needed in the regulation of width and depth of the band, since in sensitive plants (e.g. anise), crop emergence may be injured (Catizone et al. 1986).

Phosphor was proven to have a positive influence on development of generative organs and stimulation of flowering (Radanović et al. 2004), and it was demonstrated that it has the capability to anticipate maturity in grain crops, conferring to them an important "drought escape" mechanism (Cooper 1984). A good P availability in soil was claimed to be important for glycosides production in foxglove (Catizone et al. 1986), and in sage, moderate P supplies led to significant increment of plant height, number of branches, fresh and dry weights (Naguib 2011). In this last species, the peak in phosphor demand was observed at the seed formation stage, i.e. later than that detected for nitrogen (Karamanos 2000).

Several studies have been devoted to study the effects of an excess in P, that generally was found to be detrimental for various aspects of MAPs performance. For example, in sage it causes a suppression in growth and development (Karamanos 2000), whereas in opium poppy it has been suspected to favor fungal infections on capsules (Catizone et al. 1986). Plants sensitivity is of course of relevance, and species as vetiver have shown an overall noticeable sensitivity to excess P. However, it should be noted that the optimal P concentration in the vetiver foliage was only slightly lower than the toxic levels (Edelstein et al. 2009). Different responses were finally observed about the effect of P supplies on essential oil yield in MAPs, since an enhancement in lemon balm (Sharafzadeh et al. 2011) and Japanese mint (Kothari et al. 1987), a decrease in lavender (Catizone et al. 1986) and no effect in sage (Karamanos 2000) were respectively detected.

Potassium is claimed to exert a positive effect on roots development and elongation, that is useful for several MAPs such as valerian or *Echinacea angustifolia* (Radanović et al. 2004; Basso 2009). In the field cultivation, K applications were found to exert a positive effect on stem differentiation in thyme (Basso 2009) and remarkable effects on the mass of roots and on the content of phenolic acids of *Salvia miltiorrhiza* Bunge (Buchwald 2004). A few negative effects of excess K have been however pointed out in many MAPs: in lavender, excess K promotes the formation of camphor, an unwelcome oil component (Catizone et al. 1986); in Java citronella it shows a depressive effect on oil yields (Malwatkar et al. 1984); in deadly nightshade, its availability is inversely correlated with alkaloids content (Catizone et al. 1986).

Nevertheless, many evidences exist that a balanced ratio between nutrients may be more important in determining several yield traits, than the absolute quantities of the elements themselves. In chamazulene-yielding species (chamomile), when N:K ratio is too low (unbalanced towards K), flowers are bigger but oil quality is reduced due to an increase in bisabololoxide; otherwise, proportionally high N and low K lead to a high content in bisabolole (Catizone et al. 1986). Similarly, the mutual interference of nutrients is probably the reason why P fertilization was found to have the maximum effect when P was applied with N, as seen e.g. in lemon balm *(Melissa officinalis* L.) (Sharafzadeh et al. 2011).

4 Fertilization with Other Elements

Although decision-making for fertilization usually claims attention only on nitrogen, phosphor and potassium, it is well acknowledged that a far larger number of elements exert some effect on plant life and performance; nevertheless, only some elements (such as B, Mg, Fe, Ca and others) are recognized as "essential", others (such as Cl and Na) are recognized as toxic, but the majority has been largely ignored, simply because their deficiency mostly does not give visible and apparent symptoms (Carrubba and Scalenghe 2012).

From the sparse research about mineral nutrition and MAPs, some interesting information may however be driven: a good calcium supply was found useful for seeds yield in anise (Catizone et al. 1986), and applications of calcium and magnesium were effective in enhancing herbage yield in *Origanum heracleoticum* L. (Dordas 2008). Iron (Fe) is especially important in calcareous soils, where Fe applications are often necessary in crops that are particularly prone to chlorosis, such as gentian (Catizone et al. 1986) or Java citronella (Sangwan et al. 2001); another noticeable effect of Fe seems the increase of gland density of *S. fruticosa* seedlings, an especially interesting feature in that it could bring to a significant (ca. 20 %) increase in essential oil concentration (Karamanos 2000). In palmarosa, iron application in form of ferric sulphate before the onset of flowering, resulted in significant increases in herbage yields (Pareek et al. 1981).

5 Effects on Quality Features

In MAPs, besides a deeper knowledge of the effect of each element on biomass yields, an additional emphasis is given on its relevance on qualitative features. In this respect, two alternative viewpoints should be considered: (1) the influence eventually exerted by each element on plant production of secondary metabolites, which would have a significant effect on the market value of the products on sale; and (2) the amount of such elements that might be retrieved in the marketable products by virtue of a plant's capability to selectively recover them from the soil. This second feature could be especially important in relation to the emphasis on ecological safety of herbal products, above all when grown on polluted soils (Del Río-Celestino et al. 2006).

As concerns the first issue, it should be reasonably expected that a more developed and healthy plant would also produce a higher quantity of secondary metabolites. This is true for many species, and it is the reason why a positive effect of N fertilization is often recognizable. A number of studies has concerned volatile oil-yielding plants: enhanced levels in essential oil production with increasing N supply were found in sage (Karamanos 2000), basil (Morakis et al. 2008), *Mentha gracilis* Sole (Kothari and Singh 1995), *Pelargonium* sp. (Singh et al. 1996a), although in more than one case, such results are probably due to a higher herbage yield rather than an enhancement of essential oil yield in plants. Otherwise, in other species contrasting results were evidenced: N fertilization was found to interfere with the production of essential oil in *Lavandula spica* and in anise (Catizone et al. 1986), whereas no effect at all was found in small-seeded coriander (Carrubba et al. 2010), *Salvia fruticosa* (Karamanos 2000) or *Salvia officinalis* (Bezzi et al. 1992).

Besides the bare quantitative effect, a deeper detail of volatiles profile of plants offers some interesting outcomes as well: in some Chinese aromatic rice ecotypes total soil nitrogen was one of the key factors in producing the aroma of traditional regional strains (Yang et al. 2012), whereas N supply was found to increase content of menthol and menthone in mint essential oil (Catizone et al. 1986), and to decrease carvone and increase limonene in Scotch spearmint (Kothari and Singh 1995). Oppositely, no fertilizer effects were detected on essential oil composition in basil (Morakis et al. 2008) and sage (Piccaglia and Marotti 1993). Also in secondary compounds different from essential oils, such as alkaloids in Papaveraceae or Solanaceae (but others as well), nutrient supply is just one in many different environmental factors involved in biosynthesis and storage processes, and to make general statements it is a bit difficult (Bernáth 1992). In liquorice, N fertilization was supposed to lead to the formation of roots and rhizomes scarcely endowed in glycyrrhizin (Catizone et al. 1986), whereas no effect was detected on ginsenoside content in ginseng roots (Beyaert 2006). Evidences exist, otherwise, of a direct and positive correlation of the alkaloids content with N content in plant in lupines, barley, *Datura, Atropa, Papaver,* periwinkle and deadly nightshade (Sreevalli et al. 2004; Basso 2009). In the last species, this occurrence was found to be more striking under water stress conditions (Baričevič et al. 1999; Baričevič and Zupančič 2002). The authors suggest the hypothesis that under stress conditions alkaloid plants shift their metabolism towards accelerated synthesis of nontoxic secondary products (alkaloids), which might therefore represent the form of nitrogen storage in suffering plants.

Finally, the effect of nutrients on quality parameters of MAPs was proven to interact with environmental and genetic factors. Among environmental factors, growth conditions may be mentioned: increasing the amount of fertilizer caused a significant concentration-dependent increase in antioxidant activity of cultivated *Teucrium polium* compared with wild type. In contrast, increasing the amount of fertilizer caused a significant concentration-dependent reduction in the antioxidant activity of powders prepared from cultivated *Eryngium creticum* when compared with wild plants (Azaizeh et al. 2005). The role of genetic factors was otherwise recalled by Sangwan et al. (2001) when quoting the differentiated response of Java citronella to nitrogen application, accounting for a variability in essential oil yield up to 36 % among cultivars, at the same N levels.

Actually, it seems possible to conclude that plant responses may vary according to the active principle, or group of active principles, that are of interest, and depending upon cases, there are at least three factors that may modify the response of plant. They are: (1) a change in dry matter production; (2) a change in the proportions of organs; (3) a modification in the accumulation level (Bernáth 1992).

As an example, several studies on coriander stated that, especially in certain genotypes, a high N supply addressed plant metabolism towards the production of biomass rather than to essential oil yield. Hence, a distinction was possible between "source-limited" and "sink-limited" genotypes, in which N availability (and therefore N supply) could be pushed (in the first case) or could be limited (the second case) (Lenardis et al. 2000). Environments in which the starting productivity levels are higher, also seem capable of gaining maximum advantages from supplementary N fertilization. Otherwise, in low-yielding environments, the advantages from such practice are much lower, and plants reach their maximum yield level with a comparatively reduced N supply. This result is probably the reason why the contemporary recourse to irrigation enhances the probability to reach higher seed yields (Carrubba 2009).

As seen, most of the available literature on this argument is concerned with nitrogen effect, whereas a reduced number of experiments was devoted to study other elements; manganese (Mn) was found to be very effective in enhancing oil production in Java citronella (Sangwan et al. 2001), in deadly nightshade an early supply of Co seems to enhance plant content in alkaloids (Catizone et al. 1986), and a number of elements (B, Mo, Zn, Mn and Co) stimulate the flower production and the carotinoids content in marigold (Basso 2009).

Actually, a few elements are accumulated into plant's tissues as a result of current plant metabolism, as they are absorbed from soil. In MAPs, their occurrence may be considered both a disadvantage, when such elements are toxic or somehow harmful, and an advantage when their role as dietary supplement is considered (Ansari et al. 2006; Musa Özcan et al. 2008; Zengin et al. 2008). In any case, when MAPs are used for human consumption as spices or herbal teas, the presence of minerals cannot be underestimated. Hence, many trials throughout the world have been concerned in detecting mineral elements in MAPs, or MAPs-derived products (Ansari et al. 2006; Sheded et al. 2006; Musa Özcan et al. 2008; Zengin et al. 2008).

The contents of heavy metals in MAPs depend on several factors, namely chemical and physical characteristics of soil, locality of plant cultivation/growth and contamination of region, and finally, the specific ability of some species to overaccumulate various toxic heavy metals (Súströková and Hecl 2004).

In years, many soils have been additionally loaded with exceptionally high occurrence of some minerals from anthropogenic source, both as an effect of the largest recourse to fertilization, and (especially for heavy metals such as Pb, Cd, Cr, Ni, Hg), under the influence of big industrial centers, highways, Cu, Zn and Pb smelters, steam power plants and communal sludge. The World Health Organization has repeatedly stressed the importance of ensuring adequate quality standard of MAPs by avoiding their contamination with harmful elements, above all arsenic and heavy metals (WHO 1998, 2003).

A high concentration of special elements in MAPs may be however originate from other sources. It was reported that sometimes metals are intentionally added to Asian herbal preparations, because the traditional Indian (Ayurvedic) and Chinese medicine believe in their therapeutic properties (Gjorgieva et al. 2010).

In some species, additionally, the possibility was stated that they act as hyperaccumulators of heavy metals, as found in Clary sage that concentrated Pb, Cu, Zn and Cd in all parts of plant, above all leaves (Angelova et al. 2005).

6 Organic Fertilization

An increased interest in safety and quality of MAPs is of evidence worldwide, and whatever their final destination of use, MAPs must achieve an increasingly large number of quality issues. In this sense, organic certification is a common requirement (Lubbe and Verpoorte 2011), and, although from some point of view the real differences between organic and conventional products are still matter of debate (Brandt and Mølgaard 2001), an increasing number of MAPs growers is moving on this crop management (Joy et al. 2001; Malik et al. 2011), as e.g. in Italy, where more than 60 % of the farms involved in MAPs cultivation employs organic or biodynamic methods (Vender 2001).

Organic management however requires a special approach to fertilization issues, in that fertility conditions are maintained and increased by means of proper agronomical practices, such as multiannual crop rotation including legumes and other green manure crops, and by the application of few allowed items. Regulations do not allow the use of synthetically-derived fertilizers, and where external inputs are required, these shall be limited to inputs from organic production (livestock manure or organic material, both preferably composted), natural or naturally-derived substances, and low solubility mineral fertilizers (EU Reg. n. 834/2007). The restriction to natural or naturally-derived substances for fertilization poses however on table a few questions concerning plant nutrition, mainly because a change in release rate (and therefore in availability to plants) of elements in organic form is expected. Prasad et al. (2004) demonstrated that actually only N has a slow release from organic fertilizers, whereas in P and K this process develops rapidly. These general statements may explain the well-known issue that, in organic farming, one of the main factors affecting crop yield is nitrogen availability in soil (Schmid et al. 1994), and justify the fact that a very large number of technical notes concerning crop nutrition in organic management are mostly concerned with N. Very often the slow release of N from organic fertilizers is claimed to be beneficial with regard to environmental protection; it is true, however, that in many experiments the N released from organic fertilizers was not sufficient for economic production, thus evoking the opportunity to submit commercial organic fertilizers to a test for N mineralization ability (Jakše and Mihelič 1999). Determination of C/N ratio, e.g., could be useful in such sense, since in some organic fertilizer, such as manure, the C/N ratio was found to be an important factor in determining the variations in the amount of N mineralized from the starting organic fertilizer (Qian and Schoenau 2002).

However, organic fertilization brings to a number of advantages that overpass the bare nutritional effect, and some benefit should also be attributed to a positive action on certain soil characteristics, namely water-holding capacity, cation exchange capacity and microbial activity. Studies about organic fertilization in MAPs, hence, are mostly concerned with the overall effect on yields, whereas less attention is paid to the specific effect of each single nutrient. Yield enhancements due to organic fertilization have been claimed for biomass and oil in geranium (Araya et al. 2006) and for seeds in coriander (Ursulino Alves et al. 2005). Such results might be linked however with soil moisture content, an aspect that was taken into consideration in some experiments on Goji *(Lycium barbarum* L.), where a higher plant dry weight value after organic rather than conventional fertilization was shown, provided soil moisture was always kept at optimum level (Chung et al. 2010). In coriander grown in Mediterranean environments, otherwise, organic fertilization gave lower seed yields than conventional (chemical) fertilization, probably due to the low rate of nitrification processes under those climatic conditions (Carrubba and Ascolillo 2009). Generally speaking, N fertilizers seem to give the strongest impulse to yield when applied in the faster available forms, and consequently used by the plants since the very first moments after their application. The application of N in organic form, on the contrary, generates much less evident effects; it is possible that the fertilizer units applied in this form show a delayed effect, since they need to undergo mineralization in order to free in the soil mineral nitrogen to be directly used by plants, and dry conditions do not allow a proper microbiological activity (Chiang et al. 1984).

Finally, a few authors suggest as best practice the fertilization by means of the recourse to both chemical and organic fertilizers (Joy et al. 2001; Ursulino Alves et al. 2005; Naguib 2011), with the goal to avoid the disadvantages and enhance the advantages of both. E.g. in the case of nitrogen, such "mixed" treatment could take into account the above-mentioned different release rate of N from the two sources, in this allowing fertilization to meet crop demand throughout its biological cycle. Various combinations of fertilizers belonging to the different typologies (sometimes also including mineral elements, yeast, amminoacids, PGPR – Plant Growth Promoting Rhizobacteria – and so on) were tried on coriander, dill, fennel, *Tagetes erecta* L., *Hyocyamus muticus* L. and others, but because of the variability of the obtained results, it sounds reasonable the recommendation by Naguib (2011), who suggests a careful preliminary evaluation of plant, soil and environmental conditions.

References

Angelova V, Ivanov K, Ivanova R (2005) Heavy metal content in plants from family Lamiaceae cultivated in an industrially polluted region. J Herbs Spices Med Plants 4(11):37–46. doi:10.1300/J044v11n04_05

Ansari TM, Ikram N, Najam-ul-Haq M, Fayyaz I, Fayyaz Q, Ghafoor I, Khalid N (2006) Essential trace metal (Zinc, Manganese, Copper and Iron) levels in plants of medicinal importance. J Biol Sci 4(2):95–99. doi:10.3923/jbs.2004.95.99

Araya HT, Soundy P, Steyn JM, Teubes C, Learmonth RA, Mojela N (2006) Response of herbage yield, essential oil yield and composition of South African rose-scented geranium *(Pelargonium* sp.) to conventional and organic nitrogen. JEOR 18:111–115

Arnon I (1992) Agriculture in drylands principles and practices. Elsevier, Amsterdam, p 975.

Azaizeh H, Ljubuncic P, Portnaya I, Said O, Cogan U, Bomzon A (2005) Fertilization-induced changes in growth parameters and antioxidant activity of medicinal plants used in traditional Arab medicine. eCAM 4(2):549–556. doi:10.1093/ecam/neh131

Baričevič D, Zupančič A (2002) The impact of drought stress and/or nitrogen fertilization in some medicinal plants. J Herbs Spices Med Plants 2–3(9):53–64. doi:10.1300/J044v09n02_08

Baričevič D, Umek A, Kreft S, Matičič B, Zupančič A (1999) Effect of water stress and nitrogen fertilization on the content of hyoscyamine and scopolamine in the roots of deadly nightshade *(Atropa belladonna)*. EEB 42:17–24. doi:10.1016/S0098-8472(99)00014-3

Basso F (2009) Piante officinali aromatiche e medicinali. Aspetti bioagronomici, aromatici e fitoterapeutici. Pitagora Editrice, Bologna, p 381 (in Italian)

Bernáth J (1992) Production ecology of secondary plant products. In: Craker LE, Simon JE (eds) Herbs, spices and medicinal plants: recent advances in botany, horticulture and pharmacology, vol 1. Food Product Press, Binghamton, pp 185–234

Berti M, Hevia F, Wilckens R, Joublan JP, Serri H, Allende J (2000) Fertilizacion nitrogenada del cultivo de Hierba de San Juan *(Hypericum perforatum* L.) en Chillan, provincia de Ñuble, Chile. Cien Investig Agr 27(2):107–116

Beyaert RP (2006) Influence of nitrogen fertilization on the growth and yield of North American Ginseng. J Herbs Spices Med Plants 4(11):65–80. doi:10.1300/J044v11n04_08

Bezzi A, Aiello N, Albasini A, Landi R, Marzi V, Melegari M, Ventrelli A, Zanzucchi C (1992) Salvia *(Salvia officinalis* L.): lavori effettuati nell'ambito dei progetti finalizzati del Ministero dell'Agricoltura e delle foreste: risultati e prospettive. Agricoltura Ricerca, March: pp 97–104 (In Italian)

Biesiada A, Sokół-Łetowska A, Kucharska A (2008) The effect of nitrogen fertilization on yielding and antioxidant activity of lavender *(Lavandula angustifolia* Mill.). Acta Sci Pol Hortoru 7(2):33–40

Brandt K, Mølgaard JP (2001) Organic agriculture: does it enhance or reduce the nutritional value of plant foods? J Sci Food Agric 9(81):924–931. doi:10.1002/jsfa.903

Buchwald W. (2004) The research on the biology of germination and cultivation of *Salvia miltiorrhiza* Bunge. Proceedings of the 3rd conference on medicinal and aromatic plants of Southeast European countries, Nitra, Slovak Republic, 5–8 Sept 2004, pp 65–66

Carrubba A (2009) Nitrogen fertilisation in coriander (Coriandrum sativum L.): a review and meta-analysis. J Sci Food Agric 6(89):921–926. doi:10.1002/jsfa.3535

Carrubba A, Ascolillo V (2009) Effects of organic and chemical N-fertilization on yield and morpho-biological features in coriander (Coriandrum sativum L.). Acta Hort 826:35–42

Carrubba A, Catalano C (2009) Essential oil crops for sustainable agriculture – a review. In: Lichtfouse E (ed) Climate change, intercropping, pest control and beneficial microorganisms. Springer, Dijon, pp 137–188. doi:10.1007/978-90-481-2716-0_8

Carrubba A, Scalenghe R (2012) The scent of Mare Nostrum: medicinal and aromatic plants in Mediterranean soils. J Sci Food Agric 6(92):1150–1170. doi:10.1002/jsfa.5630

Carrubba A, Catalano C, Militello M (2010) Effects of organic and conventional N-fertilization on quality traits in coriander (Coriandrum sativum L.). In: More sustainability in agriculture: new fertilizers and fertilization management. Fertilitas Agrorum 1:174–179

Catizone P, Marotti M, Toderi G, Tetenyi P (1986) Coltivazione delle piante medicinali e aromatiche. Patron publ, Bologna, p 379 (In Italian)

Chiang C, Soudi B, Moreno A (1984) Soil nitrogen mineralization and nitrification under Moroccan conditions. In Nutrient balances and the need for fertilizers in semi-arid regions. Proceedings of the 17th colloquium of the international Potash institute, Rabat/Marrakesh, Morocco: pp 129–139

Chung R-S, Chen C-C, Ng L-T (2010) Nitrogen fertilization affects the growth performance, betaine and polysaccharide concentrations of *Lycium barbarum*. Ind Crop Prod 3(32):650–655. doi:10.1016/j.indcrop.2010.07.021

Cooper PJM (1984) Crop management in rainfed agriculture with special reference to water use efficiency. In Nutrient balances and the need for fertilizers in semi-arid and arid regions. Proceedings of the 17th colloquium of the international Potash institute. Rabat/Marrakech, Morocco: pp 63–79.

Del Río-Celestino M, Font R, Moreno-Rojas R, De Haro-Bailón A (2006) Uptake of lead and zinc by wild plants growing on contaminated soils. Ind Crop Prod 3(24):230–237. doi:10.1016/j.indcrop.2006.06.013

Dellacecca V (1994) Ricerche sulla menta piperita *(Mentha* x *piperita* L.). Proceedings of the international meeting coltivazione e miglioramento delle piante officinali, Trento, 2–3 June 1994: pp 201–212 (In Italian, with English abstract)

Dordas C (2008) Application of calcium and magnesium improves yield and essential oil yield of Oregano *(Origanum vulgare* ssp. *hirtum*). IJA 3(3):417–418

Edelstein M, Plaut Z, Dudai N, Ben-Hur M (2009) Vetiver *(Vetiveria zizanioides)* responses to fertilization and salinity under irrigation conditions. J Environ Manage 1(91):215–221. doi:10.1016/j.jenvman.2009.08.006

El Hafid R, Blade SF, Hoyano Y (2002) Seeding date and nitrogen fertilization effects on the performance of borage *(Borago officinalis* L.). Ind Crop Prod 3(16):193–199. doi:10.1016/S0926-6690(02)00047-X

Franz C (1983) Nutrient and water management for medicinal and aromatic plants. Acta Hort 132:203–215

Gjorgieva D, Kadifkova-Panovska T, Bačeva K, Stafilov T (2010) Content of toxic and essential metals in medicinal herbs growing in polluted and unpolluted areas of Macedonia. Arh Hig Rada Toksikol 61:297–303. doi:10.2478/10004-1254-61-2010-2022

Jakše M, Mihelič R (1999) The influence of organic and mineral fertilization on vegetable growth and N availability in soil: preliminary results. Acta Hort 506:69–75

Joy PP, Thomas J, Mathew S, Jose G, Joseph J (2001) Aromatic plants. In: Bose TK, Kabir J, Das P, Joy PP (eds) Tropical horticulture Vol. 2. Naya Prokash, Calcutta, pp 633–733

Joy PP, Thomas J, Mathew S, Skaria BP (2001a) Medicinal plants. In: Bose TK, Kabir J, Das P, Joy PP (eds) Tropical horticulture, vol 2. Naya Prokash, Calcutta, pp 449–632

Karamanos AJ (2000) 7. The cultivation of sage. In: Kintzios SE (ed) Chap. IV. Cultivation and breeding; sage. The genus salvia, OPA (Overseas Publishers Association), Amsterdam, pp 93–108

Karkanis A, Bilalis D, Efthimiadou A (2011) Cultivation of milk thistle *(Silybum marianum* L. Gaertn.), a medicinal weed. Ind Crop Prod 1(34):825–830. doi:10.1016/j.indcrop.2011.03.027

Kothari SK, Singh UB (1995) The effect of row spacing and nitrogen fertilization on scotch spearmint *(Mentha gracilis* Sole). JEOR 3(7):287–297

Kothari SK, Singh V, Singh K (1987) Effect of rates and methods of phosphorus application on herb and oil yields and nutrient concentrations in Japanese mint (Mentha arvensis L.). JAS 3(108):691–693

Lenardis A, de la Fuente E, Gil A, Tubia A (2000) Response of coriander *(Coriandrum sativum* L.) to nitrogen availability. J Herbs Spices Med Plants 7(4):47–58. doi:10.1300/J044v07n04_06

Lubbe A, Verpoorte R (2011) Cultivation of medicinal and aromatic plants for specialty industrial materials. Ind Crop Prod 1(34):785–801. doi:10.1016/j.indcrop.2011.01.019

Malik AA, Suryapani S, Ahmad J (2011) Chemical Vs organic cultivation of medicinal and aromatic plants: the choice is clear. Int J Med Arom Plants 1(1):5–13

Malwatkar GM, Kokaji BA, Kelkar GD (1984) Seasonal variation in aldehyde content in oil, leaf browning and crinkling in Java citronella. Indian Perfumer 28:17–23

Morakis G, Eleftheriadou E, Karamanoli K, Dordas C, Bosabalidis A, Radoglou K, Costantinidou H-IA (2008) Nitrogen fertilization on basil: effects on physiology, oil gland morphology and on essential oils. IJA 3(3):459–460

Musa Özcan M, Ünver A, Uçar T, Arslan D (2008) Mineral content of some herbs and herbal teas by infusion and decoction. Food Chem 3(106):1120–1127. doi:10.1016/j.foodchem.2007.07.042

Naguib NYM (2011) Organic vs chemical fertilization of medicinal plants: a concise review of researches. Adv Environ Biol 2(5):394–400

Neeteson JJ, Booij R, Whitmore AP (1999) A review on sustainable nitrogen management in intensive vegetable production systems. Acta Hort 506:17–26

Omidbaigi R, Hornok L (1992) Effect of N-fertilization on the production of fennel (Foeniculum vulgare Mill.). Acta Hort 306:149–252

Pank F (1993) Methods of contemporary large scale cultivation of medicinal and aromatic plants. Acta Hort 331:89–108

Pareek SK, Saxena BB, Maheshwari MC, Gupta R (1981) Study of palmarosa oil grown for higher yield of oil and its quality under cultivation. Indian Perfumer 25:64–70

Piccaglia R, Marotti M (1993) Characterization of several aromatic plants grown in northern Italy. Flavour Fragr J 2(8):115–122. doi:10.1002/ffj.2730080208

Piccaglia R, Dellacecca V, Marotti M, Giovanelli E (1993) Agronomic factors affecting the yields and the essential oil composition of peppermint *(Mentha x piperita* L.). Acta Hort 344:29–40

Prasad M, Simmons P, Maher MJ (2004) Release characteristics of organic fertilizers. Acta Hort 644:163–170

Qian P, Schoenau JJ (2002) Availability of nitrose in solid manure amendments with different C:N ratios. Can J Soil Sci 2(82):219–225. doi:10.4141/S01-018

Radanović D, Antić MS, Sekulić P, Nastovski T (2004) Influence of soil characteristics and nutrient supply on medicinal and aromatic plants. Proceedings of the 3rd conference on medicinal and aromatic plants of Southeast European countries, 5–8 Sept 2004, Nitra, Slovak Republic, p 15

Rajamani K (2001) Effect of NPK levels on yield of *Plantago ovata* Forsk. under Shevroy Hills of Tamil Nadu. In: Sahoo S, Ramesh DB, Rao YR, Debata BK, Misra VN (eds) Conservation and utilization of medicinal plants. Allied Publishers, New Delhi, p 192

Rangappa M, Bhardwaj HL (1998) Nitrogen fertilization of Basil. HortScience 33(3):481

Sangwan NS, Farooqi AHA, Shabih F, Sangwan RS (2001) Regulation of essential oil production in plants. Plant Growth Regu 1(34):3–21. doi:10.1023/A:1013386921596

Schmid D, Strasser F, Gilomen R, Meili E, Wollesen J (1994) Agricoltura biologica (Manuali pratici di eco-agricoltura) Edagricole. Bologna (in Italian)

Sharafzadeh S, Khosh-Khui M, Javidnia K (2011) Effects of nutrients on growth and active substances of lemon balm (Melissa officinalis). Acta Hort 925:229–232

Sheded MG, Pulford ID, Hamed AI (2006) Presence of major and trace elements in seven medicinal plants growing in the South-Eastern Desert, Egypt. J Arid Envir 66:210–217. doi:10.1016/j.jaridenv.2005.10.022

Singh SD, Rao JS (1994) Yield-water-nitrogen response analysis in coriander. Ann Arid Zone 3(33):239–243

Singh M, Chandrashekhara GD, Ganesha Rao RS, Prakasa Rao EVS (1996a) Effect of irrigation and levels of nitrogen on herb and oil yield of Geranium *(Pelargonium* sp) under semiarid tropical India. JEOR 6(8):653–656. doi:10.1080/10412905.1996.9701035

Singh M, Chandrashekhara GD, Prakasa Rao EVS (1996b) Oil and herb yields of Java citronella *(Cymbopogon winterianus* Jowitt.) in relation to nitrogen and irrigation regimes. JEOR 5(8):531–534

Sivakumar MVK, Huda AKS (1984) Potential agricultural productivity in summer and winter rainfall areas. In: Nutrient balances and the need for fertilizers in semi-arid and arid regions. Proceedings of the 17th colloquium of the international Potash institute, Rabat – Marrakech (Morocco), pp 23–48

Sreevalli Y, Kulkarni RN, Baskaran K, Chandrashekara RS (2004) Increasing the content of leaf and root alkaloids of high alkaloid content mutants of periwinkle through nitrogen fertilization. Ind Crop Prod 2(19):191–195. doi:10.1016/j.indcrop.2003.09.005

Sústriková A, Hecl J (2004) Influence of the environmental factors on the heavy metals content in some medicinal plants. In: Proceedings of the 3rd conference on medicinal and aromatic plants of Southeast European countries, 5–8 Sept 2004, Nitra, Slovak Republic, p 107

Trivino MG, Johnson CB (2000) Season has a major effect on the essential oil yield response to nutrient supply in *Origanum majorana*. J Hortic Sci Biotechnol 5(75):520–527

Ursulino Alves E, Pereira De Oliveira A, De Lucena Alcântara BR, Sader R, Ursulino Alves A (2005) Rendimento e qualidade fisiológica de sementes de coentro cultivado com adubação orgânica e mineral. Revista Brasileira de Sementes 1(27):132–137, In Portuguese, with English abstract

Vender C (2001) Indagine sulla consistenza della produzione di piante officinali in Italia – Dati 1999. Comunicazioni di ricerca ISAFA 2001/3, CRA-MPF, pp 5–72 (In Italian)

Verma RK, Chauhan A, Verma RS, Gupta AK (2011) Influence of planting date on growth, artemisinin yield, seed and oil yield of Artemisia annua L. under temperate climatic conditions. Ind Crop Prod 1(34):860–864. doi:10.1016/j.indcrop.2011.02.004

WHO (1998) Quality control methods for medicinal plant materials. World Health Organization, Geneva

WHO (2003) WHO guidelines on good agricultural and collection practices (GACP) for medicinal plants. World Health Organization, Geneva

Yadav SN, Peterson W, William Easter K (1997) Do farmers overuse nitrogen fertilizer to the detriment of the environment? Environ Resour Econ 3(9):323–340

Yang S, Zou Y, Liang Y, Xia B, Liu S, Md I, Li D, Li Y, Chen L, Zeng Y, Liu L, Chen Y, Li P, Zhu J (2012) Role of soil total nitrogen in aroma synthesis of traditional regional aromatic rice in China. Field Crop Res 125:151–160. doi:10.1016/j.fcr.2011.09.002

Zengin M, Musa Özcan M, Çetin Ü, Gezgin S (2008) Mineral contents of some aromatic plants, their growth soils and infusions. J Sci Food Agric 4(88):581–589. doi:10.1002/jsfa.3120

Zhu Z, Liang Z, Han R, Wang X (2009) Impact of fertilization on drought response in the medicinal herb *Bupleurum chinense* DC.: growth and saikosaponin production. Ind Crop Prod 2–3(29):629–633. doi:10.1016/j.indcrop.2008.08.002

Chapter 11
Sustainable Weed, Disease and Pest Management in Medicinal and Aromatic Plants

Alessandra Carrubba, Gabriella Lo Verde, and Adele Salamone

Abstract As for all other crops, in MAPs as well, weeds, diseases and pests are important yield-reducing factors, which may severely curtain biomass production and, that is maybe more important, may affect several qualitative aspects of production. Research about this topic is generally lacking, for two main reasons: the first is that MAPs are generally grown on rather limited areas, and the incidence of specific pests and diseases rarely takes a relevance outside rather narrow boundaries. The second reason is that the economical importance of MAPs is much lower than that ascribed to the "major" crops, which the bigger efforts of research are addressed to. In the changing scenario of latter years, however, MAPs are taking an increasing relevance, and there is the necessity to draw proper guidelines for their cultivation technique, also including the advisable strategies for their sustainable protection.

Keywords Crop protection • Pathogenic agents • Insects • Mites • Nematodes • Sustainable management

1 Introduction

Until a few years ago, a widespread idea was that most Medicinal and Aromatic Plants (MAPs) had no serious pests or diseases (Simon et al. 1984). Arguably, the lack of information regarding this issue was mostly due to the limited cropping area of such crops. In fact, the sources of information related to the diseases and pests of

A. Carrubba (✉) • G. Lo Verde
Department of Agricultural and Forest Sciences, Università degli Studi di Palermo, Palermo, Italy
e-mail: alessandra.carrubba@unipa.it; gabriella.loverde@unipa.it

A. Salamone (✉)
Consiglio per la Ricerca e la sperimentazione in Agricoltura (C.R.A.), Unità di Ricerca per il recupero e la valorizzazione delle Specie Floricole Mediterranee (SFM), Bagheria, PA, Italy
e-mail: adele.salamone@entecra.it

Á. Máthé (ed.), *Medicinal and Aromatic Plants of the World*, Medicinal and Aromatic Plants of the World 1, DOI 10.1007/978-94-017-9810-5_11

MAPs were mostly limited to areas in which their cultivation reached appreciable levels. Nowadays, in view of the increasing interest on MAPs cultivation throughout the world, such an issue is gaining a deeper attention, and the pests and diseases control techniques have taken a great relevance inside the recommended growing protocols for MAPs. It is worth to notice, moreover, that such yield-reducing factors take a special relevance for MAPs, since their presence not only may lead to a decrease in yields, but also in the quality of production.

Quality features of MAPs, actually, are primarily determined by their content in essential oils or other secondary metabolites (Gil et al. 1998). Many harmful organisms negatively affect assimilation rate through changes in physiological parameters, as it happens when leaves or roots or vessels are attacked (Lövenstein et al. 1993), but also competition may exert major effects on plant metabolism when a shared use of environmental resources is of concern (Kegge and Pierik 2010). The type and severity of impact of weeds, pests and diseases on crop performance depends on the mode of interaction, but also on environmental and erratic factors (Arnon 1992). A deeper knowledge of the relationships between crops and harmful organisms should therefore be achieved, in order to identify effective control measures (Lövenstein et al. 1993).

2 Weeds

Weeds are responsible for problems for mechanized harvest, when mixed with the harvested product they may alter its end quality, and since they function as crop competitors, they may significantly diminish the yield and growth of crops (Lövenstein et al. 1993). Most experiments carried out on MAPs suitability to field conditions have confirmed the importance of a weeds removal as complete as possible: de la Fuente et al. (2003) stated that on coriander, especially under poor soil conditions, weeding had a greater effect than did N fertilization, whereas weeds were found to lower seed yields (40–90 %) in coriander, fennel and psyllium (Carrubba and Militello 2013), herbage and oil yields (30–50 %) in mint (Singhania 2001), herb yield and oil content (60–70 %) in rose-scented geranium (Kothari et al. 2002).

Competition with weeds is detrimental for MAPs production for two main reasons. The first is that, in acting as an important stress factor, the interference of weeds is supposed to generate variations in photosynthesis rate and direction, pushing plants to allocate more carbon to roots (competition for nutrients or water) or shoots (competition for light) (Kegge and Pierik 2010). Not so many works have been expressly addressed to evaluate this aspect: in Milk thistle, weed control increased the content of silymarin and decreased the amount of seed oil (Zheljazkov et al. 2006). Similarly, a significant lowering (from 0.7 to 0.4 %) of camphor content in seeds of fennel was found with varying the interspecific competition level (Carrubba et al. 2008), whereas none of the other components showed any variation (Carrubba et al. 2010).

Secondly, many MAPs have often been termed “cultivated wild crops”, in that in many cases they are spontaneous plants that are cultivated after they are discovered to be of some economic value. Thus, they are often characterized by not very competitive genotypes with features found in wild plants, such as a prolonged flowering period, a tendency to scatter seeds and a low harvest index (Schippmann et al. 2002; Carrubba and Catalano 2009). Consequently, the seed yield and biomass of MAPs are seriously affected by interspecific competition, meaning proper weed management becomes crucial. Not so many experiments have been however conducted about the details of such interference, that reasonably will show different outcomes according to the different crop growth stages when it takes place. It should be expected that the periods of marginal crop cover, i.e. the period after planting and those following harvests, are more critical in terms of weed control; in a few species this is a well-known topic, e.g. in mint, it was suggested that the field should be kept totally weed free, particularly during the initial stages of growth, till proper establishment and coverage of the ground area (Joy et al. 2001b). The research about this topic should however be deepened, and more aspects should be taken into consideration, especially in relation with the expected goals of cultivation. Additional information is required about, for instance, the mechanisms that underlie competition in MAPs in order to determine the intervention thresholds for each species and product, also taking into account the additional effects of environmental parameters (such as soil water availability and average and extreme temperatures).

More attention has been paid worldwide about the technical means for weeding, generally addressed to a removal of weeds as complete as possible, and sometimes to the effects of weeds on MAPs yields and quality. Trials carried out in many countries where MAPs are widely cultivated (such as India, Pakistan, Turkey and others) have been concerned about chemical weeds management, offering a comprehensive insight of this special aspect of MAPs cultivation (Kothari et al. 1989; Chaudhary 2000a, b). Because of the wide interest in using organic management techniques to cultivate MAPs, however, nonchemical methods of weed control are gaining an increased attention. It is worth to notice that, in view of keeping fields free from weeds for as long as possible, a combination of chemical, biological and/or mechanical methods for weed control may be recommended in many cases (Karamanos 2000).

2.1 *Chemical Weed Control*

Optimal solutions for chemical control must be tuned keeping in mind environmental conditions (e.g. temperature and moisture of soil, weed population, crop health and population density), law restrictions (e.g. in organic management but also as concerns specific restrictions of use for some special active ingredients) and goals of the farmer.

However, many references are quoting a large number of advantages of chemical weed control, alone or in combination with other methods, e.g. mechanical weeding (Karamanos 2000). The first reason is that it allows a significant reduction in manpower with respect to traditional weeding methods, a significant advantage in that manual weeding in many MAPs requires more than 90 % of the total number of hours required for cropping (Pank 1992). Additionally, chemical weed control could allow to sow MAPs at higher plant population, since it would be no longer necessary to arrange inter-row crop distances in order to allow mechanical operations, and at least in one case (Catizone et al. 1986) it has been suggested for MAPs in mixed crop (clary sage and dill).

Due to the high variability of local conditions, few experiments come into details about the botanical composition of weed flora, taking into account the effect of specific herbicides against specific weed species. E.g. in sage crops, a number of chemicals was tested, and, in comparison with the unweeded control, all of them significantly reduced the population of *Chenopodium murale* L. Other weeds of concern have been some *Oxalis* species (Karamanos 2000) in sage, annual mercury (*Mercurialis annua* L.) or black nightshade (*Solanum nigrum* L.) in chamomile, *Chenopodium album, C. murale, Heliotropium ellipticum, Melilotus indica, Tribulus terrestris* and *Portulaca* sp. in coriander (Chaudhary 2000a), but of course such results rarely bear a general interest, and a considerable number of works make only reference to larger groups of weeds, e.g. roughly breaking up weed flora into mono- or dicotyledonous species (Kristiansen 2003).

In years, chemical weed control has been tested for many species, including annuals such as coriander (*Coriandrum sativum* L.), milk thistle (*Silybum marianum* Gaertn.) and fennel (*Foeniculum vulgare* Mill.) (Catizone et al. 1986; Chaudhary 2000a; Joy et al. 2001b; Zheljazkov et al. 2006; Týr and Vereš 2011), and perennials as oregano (*Origanum vulgare* L.), sage (*Salvia officinalis* L.), savory (*Satureja officinalis* L.) and thyme (*Thymus officinalis* L.) (Catizone et al. 1986; Karamanos 2000; Zumelzú et al. 2001). An overview of the available literature shows that, especially in perennials, the use of certain herbicides is very effective, particularly in the first year of plant establishment, when MAPs are more sensitive to competition with weeds (Catizone et al. 1986). According to the severity of weeds infestation, the duration of crop, and the chosen chemical product, chemical control may be applied before emergence/transplant or after crop establishment. As a general rule, for a sustainable management of crops, post-emergence treatments should be preferable, in that they allow to address to a specific weed flora, avoiding indiscriminate and generalized treatments (Hall et al. 2000). In intensive cropping conditions, pre-emergence treatments may also be applied, but using active ingredients of short persistence.

Many active compounds suitable to the different conditions have been suggested in time; however, the legislation on herbicides, particularly the regulatory status of individual active ingredients, is different throughout the diverse world areas, and even in the same area, law restrictions are continuously updating. A special issue about this, above all in post-emergence treatments, is related to phytotoxicity, i.e. the capacity of a compound to cause temporary or long-lasting damage to crops, a problem of relevance both in post-emergence but also in pre-emergence treatments (James et al. 1991; OEPP/EPPO 2007). As it has known for years, phytotoxicity mainly depends upon the sensitivity of the crop, but also on the doses, the distribution moments and distribution methods of herbicide, and the prevailing environmental conditions such as temperature or moisture (Blackman et al. 1951). Phytotoxic effect of a few herbicides were registered in some species such as German chamomile (Tóth and Danilovič 2004), a species that in other experiments as well was found to be especially sensitive to chemical treatments (James et al. 1991). Other injuries, such as reduced plant development and seeds yield in comparison to the untreated check, were noted in milk thistle (Zheljazkov et al. 2006). No visible phytotoxicity was otherwise recorded on sage, treated at various crop development stages with a number of chemicals, a few of which are however banned inside the EU (James et al. 1991; Pank 1992; Hartley 1993; Mitchell and Aberneth 1993).

Attention was also given on possible effects of the applied herbicides on quality characteristics. Pank (1992), analyzing results from numerous field experiments (22 chemicals on 16 species of aromatic and medicinal plants), found that in *Salvia officinalis* (provided phytotoxic effects were avoided), the use of herbicides tended to increase plant leafiness, to reduce dry matter content and increase the thujone content of the essential oil. Other trials on sage and mint (*Mentha x piperita* L. and *M. arvensis* var. *piperascens* Malinv.) also reached the same conclusion, and in most cases the use of selected chemicals did not affect seed or oil yields, nor led to any variations in the quality of the oil (Kothari et al. 1989; Zheljazkov and Topalov 1992; Zheljazkov et al. 1996). Finally, some concern is linked to the safety of the end products (especially when the production is addressed to direct human consumption) as affected by the use of chemical herbicides, since herbicide residuals have been detected on some herbal products (Catizone et al. 1986; Kosalec et al. 2009).

2.2 *Non-chemical Weed Management*

Production of MAPs is increasingly involving “organic” cultivation, which, according to EU regulations (EU Reg. 834/2007), prohibits the use of chemicals. Hence, interest in chemical weed control in MAPs tends to be low (Demarco et al. 1999), and conversely, there is a growing interest in developing effective “alternative” nonchemical methods (agronomic, biological and physical) for weed control. In organic management, many approaches have been suggested, both direct and indirect, involving a range of mechanical equipment, biological means and agronomic strategies (Smith et al. 2000; Bond and Grundy 2001). Most, however, have not been adequately tested in MAPs cultivation, and their use is not common among farmers, who, without convenient alternatives, tend to resort to hand weeding. Hand-weeding is the most labor requiring though the most effective mean of control (Mitchell and Aberneth 1993). The labor-intensive nature of manual weed control is considered an important limiting factor, albeit not the only one, to the more widespread organic cultivation of MAPs. Therefore, finding suitable methods for nonchemical weed control in MAPs would be useful for farmers, and research into the effect of various methods on quantitative and qualitative production traits takes an additional relevance.

2.3 Mechanical Weeding

Mechanical weeding is certainly the most immediately applicable method for weed management when the use of chemicals is undesirable (Chicouene 2007), although in weedy fields it can be expensive because of the numerous treatments required. Generally speaking, especially in case of organic crop management, soil disturbance should be kept at the minimum level, e.g. avoiding deep ploughing in order to maintain fertility and biological activity of the soil (Demarco et al. 1999). When performed before crop emergence, mechanical weeding gives the crop an earlier advantage on weeds (Bond and Grundy 2001), but the utility of such intervention may be low when MAPs have a slow establishment rate, as for many perennials. In this case, weeding operation will have to be carried on after crop emergence, in this enhancing risks of crop injury and therefore requiring a greater attention in carrying out the operation. Many methods and tools may be used, addressed to inter-row cultivation (i.e. hoeing, harrowing, brushing) and intra-row cultivation (i.e. finger weeder, torsion weeder, split hoe, steering hoe) (Bond and Grundy 2001). All of them have the goal to cut (and eventually bury) weeds before than competition with crops will reach dangerous levels, and this of course will vary greatly with crops, weeds and environmental conditions. Mechanical weeding gave good results in enhancing rhizome length and fresh herbage yield in *Mentha* x *piperita* and *M. arvensis* var. *piperascens* (Zheljazkov et al. 1996). Other works, additionally, emphasize the variability of MAPs response to mechanical weeding in dependence on environmental factors, as found e.g. in *Echinacea* (Kristiansen 2003), coriander, fennel and psyllium (Carrubba and Militello 2013). Hence, generalizing the outcomes of the various trials is a bit difficult. Sometimes, mechanical weeding is supported by the adoption of special techniques, such as sowing in double instead of single rows, as successfully tried for oregano (Carrubba et al. 2002). Actually, the greatest difficulty in mechanical weed control is planning crop settlement, that is, considering from the outset the kind of equipment that will be used for weeding and then setting appropriate inter-row distances. Many failures of mechanical weeding are linked to neglect of this aspect of management (Carrubba and Catalano 2009).

2.4 Heating and Flaming

Thermal control has often been suggested as a convenient nonchemical method in organic cropping management (Bond and Grundy 2001); it is performed with special equipment that, when passed over and around weeds, quickly boils the water in their cells, causing wilting of the apex and ultimately death. Thermal treatments, both direct (by means of direct flame) and indirect (through infrared heating), have several important advantages, including the low labor requirement, the minimal soil disturbance and the possibility to be applied also when a high soil moisture does not allow other types of intervention (Ascard 1995). Moreover, unlike mechanical control, this method allows a good management of weeds growing in the crop row, that are normally more difficult to control otherwise (Sivesind et al. 2009). Anyway, exact timing of the intervention is crucial for effective weed control, because flamers should be used when weeds are still young and tender. Flaming kills annual weeds completely (although more will reappear), but it does not kill the roots of perennial weeds, which may send up new shoots within a few days after flaming; therefore, additional treatments are often required. Flaming was tried on some annual MAPs such as coriander, fennel and psyllium (Carrubba et al. 2009; Carrubba and Militello 2012), and perennial such as sage and lavender (Martini 1996), and the results seemed to depend upon the seasonal climatic patterns and the competition degree between the crop and the weeds.

2.5 Mulching

An alternative environmentally friendly technique is mulching. Besides weed control, mulch is claimed to have many advantages for crops, including a better water use efficiency and an earlier plant development due to the higher soil temperatures (Anzalone et al. 2010). Mulching acts against weeds in two ways: by preventing seeds from germinating and by killing the young plantlets soon after emergence; scarce effectiveness was claimed, however, against perennial well-established weeds (Bond et al. 2003). Of course, its efficiency will vary with the mulching material. In experiments about MAPs, mulching increased the yield of basil (*Ocimum basilicum* L.) but not parsley (*Petroselinum crispum* (Mill.) Fuss) compared with some other treatments (Bond et al. 2003). In cultivation trials of *Artemisia absinthium* L., a perennial herb, mulching resulted in a 5 % increase in average plant weight (Giorgi et al. 2006). Other perennials such as lavender (*Lavandula angustifolia* Mill), thyme (*Thymus vulgaris* L.) and rosemary (*Rosmarinus officinalis* L.) have often shown significant increases in average plant height and diameter with mulching (Bond et al. 2003; Fontana et al. 2006). Many MAPs growers have obtained good results using polyethylene mulch or black porous plastic (Galambosi and Szebeni-Galambosi 1992; Carrubba and Militello

2013), but natural materials such as cereal straw, flax straw, nonwoven wool or pine needles have been tried as well, with success varying according to species, environmental conditions and the nature of the organic materials used (Kothari et al. 2002; Carrubba and la Torre 2005; Duppong et al. 2004). Furthermore, the position of the plastic mulch may vary; it may be placed between crop rows after emergence or transplant (before the emergence of weeds), or introduced at the time of sowing with the crop seeds (or plants) inserted through apposite holes in the plastic film. These management choices will affect weed populations quite differently, because the first option leaves more room available for weeds, and the method must be chosen according to the acceptable level of weed infestation. In decision-making process about this technique, however, not only weeds and crop features play a role, but also different aspects including the cost and availability of the mulching material, the expertise level inside farm, and the easiness in eliminating mulch after cultivation, especially when plastic films are used (Bond et al. 2003). A further complication is that in some cases, mulching was found to lead to some variation in plant chemical traits (Duppong et al. 2004), a feature that in MAPs could have a crucial importance, deserving therefore additional investigations.

2.6 False Seeding

A widely advised method for organic field management is the stale or false seedbed technique (Bond and Grundy 2001; Barberi 2002; Bond et al. 2003), with which weed seedling emergence is first promoted with a shallow soil work, and then the seedlings are destroyed with a subsequent soil tillage. In this way, potential weed flora is affected, and the depletion of weed seed bank in the germination zone (the shallow soil layers from which weeds can emerge) reduces weed pressure in the crop (Boyd et al. 2006). This technique was proven especially effective in decreasing the density of annual weeds, in this increasing the chance of crop settlement. Many trials, however, have found that the efficacy of the stale seedbed technique is mainly determined by the moisture level of soil, since dry conditions may delay the flush of weed emergence until adequate soil moisture is available (Bond et al. 2003). This inconvenience could be solved by the recourse to irrigation (Bond et al. 2003), a solution that however cannot be suggested in all those areas where facilities for irrigation are lacking. This is probably the reason why in coriander, fennel and psyllium grown in a Mediterranean environment, the stale seedbed technique gave unsatisfactory results: weeds did not emerge until the first rainfalls in autumn, and the necessity to wait for a complete weeds emergence before destroying them, forced crops sowing time to an excessive delay, carrying out negative effects on seeds yield (Carrubba and Militello 2013).

3 Diseases of Medicinal and Aromatic Plants

MAPs are considered minor crops and for this reason there are not many chemicals registered for use on them. Only a limited number of products is specifically addressed for MAPs, also reporting the maximum safe amount of residues registered for each species. An Integrated Disease Management, by means of combined cultural, biological and chemical practices should be preferred, because it allows to obtain a high quality product. Furthermore, an effective diseases management should include an accurate identification of diseases caused by pathogenic microorganisms; besides fungi, they can include bacteria, viruses and protozoa, that affect plants showing various types of symptoms, including leaf spots, blights, cankers, root rots, damping-off, basal stem rot, scabs, anthracnose, powdery and downy mildew, vascular wilt and rust. An early diagnosis is essential for an appropriate use of chemical products. Their continuous application, in fact, could induce selection of pesticide-resistant strains and a general loss of efficacy. Moreover, chemical treatments, especially in MAPs, could cause severe problems, reducing yields and affecting the quality of the essential oils and the biologically active substances. Plant pathogenic agents spend part of their life on host plants and part in the soil or in plant debris (Parbery 1996). Hence, the soil disinfestation to control soilborne pathogens remains a big concern, that can be solved adopting accurate cultural practices (solarization), and whenever possible, choosing resistant varieties. Actually, an environmentally-friendly alternative to the use of chemicals seems to come from MAPs themselves. Many essential oils have been tested with appreciable results and could represent a promising solution, in the perspective of reducing the environmental impact through a more sustainable disease management (Salamone et al. 2009).

3.1 Fungal Diseases

Fungi are the primary source of most plant diseases and cause not only local or general necrosis but also the death of parts or whole plants. The powdery mildews are the most commonly widespread plant diseases, affecting all plant species including MAPs. They are responsible for the most recurrent epidemics because their spores are released into the air and can be dispersed by wind over great distances. The powdery mildew disease is caused by a range of different fungi and the symptoms on one species are usually different from those on another species (Agrios 1997). They show the presence of white to grayish mycelia covering young plant tissues (leaves, young shoots, stems, flowers and young fruits). Powdery mildew symptoms caused by a fungus of the genus *Oidium* (Erysiphales) were observed on leaves and stems of *Salvia officinalis* L. growing in greenhouse where, as disease progressed, the spots coalesced and the entire leaves turned necrotic (Cabrera et al. 2010). Several notes report some fungal genera from Erisiphales on MAPs belonging to different families, among them Lamiaceae, Apiaceae, Asteraceae, where mycelium becomes denser with age and sometimes leads to loss foliage (Catizone et al. 1986; Farooqi and Sreeramu 2004; Glawe et al. 2005; Madia and Gaetán 2005; Frużyńska-Jóźwiak and Andrzejak 2007; Liberato and Cunnington 2007; Bertetti et al. 2010, 2012; Kassai-Jáger and Kiss 2010; Wichura et al. 2012, see Table 11.1). The disease can be controlled by application of sulfur, both as spray and powder formulations. Recently, also sodium bicarbonates and essential oils have been successfully tested on rose plants (Salamone et al. 2009).

Among the most destructive diseases affecting MAPs, the rusts may be listed as well, caused by Basidiomycetes of the order Uredinales. The rust fungi mostly attack leaves and stems, and are highly specialized parasites with a complex life cycle. The infections are easy to identify because usually show up as numerous rusty, orange-yellow or white colored spots on the undersides of leaves (Agrios 1997). Cool temperatures and free moisture on leaves favor their development. Rust infections (Table 11.1) can greatly decrease the yield and quality of essential oils (Catizone et al. 1986; Koike et al. 1998; Edwards et al. 1999; Fruźyńska and Andrzejak 2007; Bokor 2011). Also in this case, it is important to destroy the infested plants and use more resistant varieties.

The soilborne diseases are caused by a group of fungi that grow parasitically on the host plant, continuing to live and multiply on its dead tissues and moving out of the host debris into the soil. These fungi have a wide host range and can survive in the soil for many years also in the absence of their specific hosts (Agrios 1997). They include fungal-like pathogens from Oomycetes, among them some of the most important recognized plant pathogens, as the genera *Pythium*, that is widespread and causes seed rot, seedling dumping-off and root rot, *Phytophthora* that causes root rots and blights of many host plants, *Plasmopara* and *Peronospora* causing a severe group of symptoms known as downy mildew (Table 11.1). *Phytophthora nicotianae*, a very dangerous species capable to cause great yield losses on crops worldwide, was isolated from symptomatic plants of lavender and rosemary showing delayed growth, general absence vigor, off-color of the foliage and wilting (Álvarez et al. 2007). The same species was found also on *Thymus vulgaris* L. and *T.* x *citriodorus* (Pers.) Schreb. ex Scwei (Martini et al. 2006). *Phytophthora* spp. were also detected on several MAPs (Catizone et al. 1986; Farooqi and Sreeramu 2004, see Table 11.1). Moreover, fennel, anise and angelica were also found affected by downy mildew caused by *Plasmopara nivea* (Unger) J. Schröt. The success of control of *Phytophthora* species, largely depends on choosing less susceptible crops; the use of resistant varieties is recommended, and all planting stock should be free of infection. The use of systemic fungicides applied as seed treatments may represent a good solution, but also soil application of a copper solution or Bordeaux mixture close to the plants can inhibit fungal activity and control the infections (Catizone et al. 1986).

Among the soilborne fungi, *Fusarium* spp. and *Verticillium* spp. are the causal agents of vascular wilts in numerous MAPs, often causing very destructive plant diseases. Different hosts plants are attacked by special forms or race of *Fusarium*;

Table 11.1 Association among pathogenic plant fungi and MAPs species

Fungal diseases and causal agents	MAPs	Affected organs and symptoms
Powdery mildew		
Erysiphe cichoracearum	Caraway (*Carum carvi* L.) Lovage (*Levisticum officinale* Koch) Marigold (*Calendula officinalis* L.) Milk-thistle (*Silybum marianum* Gaertn.) Peppermint (*Mentha* x *piperita* L.) Sage (*Salvia officinalis* L.) St. John's wort (*Hypericum perforatum* L.) Valerian (*Valeriana officinalis* L.)	Leaves, stems and fruits covered with whitish mycelium
Erysiphe heraclei	Caraway (*C. carvi* L.) Fennel (*Foeniculum vulgare* Mill.) Parsley (*Petroselinum crispum* (Mill.) Fuss)	
Erysiphe poligoni	Coriander (*Coriandrum sativum* L.)	
Golovinomyces biocellatus	Lemon balm (*Melissa officinalis* L.) Spearmint (*Mentha spicata* L.) Oregano (*Origanum vulgare* L.) Rosemary (*Rosmarinus officinalis* L.)	
Golovinomyces orontii	Rosemary (*R. officinalis* L.)	
Oidium erysiphoides	Coriander (*C. sativum* L.) Hyssop (*Hyssopus officinalis* L.)	
Downy mildew		
Peronospora lamii	Clary sage (*Salvia sclarea* L.)	Yellow angular spots, fine white to grayish downy growth on the lower leaf surface (flowers on chamomile)
Peronospora leptosperma	German chamomile (*Matricaria chamomilla* L.)	
Plasmopara nivea	Anise (*Pimpinella anisum* L.) Angelica (*Angelica archangelica* L.) Fennel (*F. vulgare* Mill.)	
Rusts		
Puccinia malvacearum	Hollyhock (*Althaea rosea* var. *nigra*) Common mallow (*Malva sylvestris* L.)	Leaves, petioles and stems with small lesions of a rusty color
Puccinia menthae	Lemon balm (*M. officinalis* L.) Japanese mint (*Mentha arvensis* L.) Oregano (*O. vulgare* L.) Peppermint (*M.* x *piperita* L.) Spearmint (*M. spicata* L.) Thyme (*Thymus* spp.) Winter savory (*Satureja montana* L.)	
Puccinia petroselini	Dill (*Anethum graveolens* L.) Coriander (*C. sativum* L.)	
Puccinia bullata	Angelica (*A. archangelica* L.)	

(continued)

Table 11.1 (continued)

Fungal diseases and causal agents	MAPs	Affected organs and symptoms
Puccinia kglechomae	Hyssop (*H. officinalis* L.)	
Puccinia salviae	Sage (*S. officinalis* L.)	
Uromyces graminis	Fennel (*F. vulgare* Mill.)	
Uromyces glycyrrhizae	Licorice (*Glycyrrhiza glabra* L.)	
Uromyces valerianae	Valerian (*V. officinalis* L.)	
Alternaria blight		
Alternaria daci *Alternaria radicina*	Parsley *(P. crispum* (Mill.) Fuss)	Seedling death and dark brown lesions on leaves leading to chlorotic margin
Leaf, stem and fruit spots		
Alternaria alternata	Caraway (*C. carvi* L.) Coriander (*C. sativum* L.) Lavender (*Lavandula angustifolia* Mill.) Lovage (*L. officinale* Koch) St. John's wort (*H. perforatum* L.) Thyme (*Thymus vulgaris* L.)	Localized lesions on host leaves consisting of dead and collapsed cells
Alternaria calendulae	Marigold (*C. officinalis* L.)	
Alternaria oleracea	Thyme (*T. vulgaris* L.)	
Alternaria solani	Deadly nightshade (*Atropa bella-donna* L.)	
Alternaria tenuissima	Caraway (*C. carvi* L.)	Lesions on fruits
Ascochyta malvicola	Common mallow (*M. sylvestris* L.)	Lesions on leaves
Botrytis cinerea	Caraway (*C. carvi* L.) Basil (*Ocimum basilicum* L.) Lemon balm (*M. officinalis* L.) Lovage (*L. officinale* Koch) Milk thistle (*S. marianum* Gaertn.)	
Botrytis parasitica	Caraway (*C. carvi* L.)	
Cercospora bieticola	Deadly nightshade (*A. bella-donna* L.)	
Cercospora cavarea	Licorice (*G. glabra* L.)	
Cercospora calendulae	Marigold (*C. officinalis* L.)	
Cercospora petroselini	Parsley *(P. crispum* (Mill.) Fuss)	
Cercosporidium punctum	Parsley *(P. crispum* (Mill.) Fuss) Fennel (*F. vulgare* Mill.)	Lesions on leaves (also on umbels in fennel plants)

(continued)

Table 11.1 (continued)

Fungal diseases and causal agents	MAPs	Affected organs and symptoms
Entiloma calendulae	Marigold (*C. officinalis* L.)	Lesions on leaves
Ramularia atropae	Deadly nightshade (*A. bella-donna* L.)	
Ramularia coriandri	Coriander (*C. sativum* L.)	
Ramularia foeniculi	Fennel (*F. vulgare* Mill.)	
Ramularia menthicola	Peppermint (*M. x piperita* L.)	
Ramularia valerianae	Valerian (*V. officinalis* L.)	
Septoria carvi	Caraway (*C. carvi* L.)	
Septoria colchici	Meadow saffron (*Colchicum autumnale* L.)	
Septoria heterochroa	Common mallow (*M. sylvestris* L.)	
Septoria hiperici	St. John's wort (*H. perforatum* L.)	
Septoria melissae	Lemon balm (*M. officinalis* L.)	
Cercospora foeniculi	Fennel (*F. vulgare* Mill.)	Leaf blight
Ascochyta hortorum	Deadly nightshade (*A. bella-donna* L.)	
Ascochyta lamiorum	Basil (*O. basilicum* L.)	
Ascochyta leonuri	*Mentha* spp.	
Ascochyta scalreae	Clary sage (*S. sclarea* L.)	
Cercospora carvi	Caraway (*C. carvi* L.)	Lesions on stems
Mycocentrospora acerina		
Septoria lavandulae	Lavender (*L. angustifolia* Mill.)	Lesions on seedlings
Antrachnose		
Colletotrichum gloesporioides	Basil (*O. basilicum* L.) St. John's wort (*H. perforatum* L.) Caraway (*C. carvi* L.)	Necrotic and sunken lesion on stems, leaves, fruits or flowers
Colletotrichum dematium	Caraway (*C. carvi* L.)	
Colletotrichum malvarum	Common mallow (*M. sylvestris* L.)	
Mycrocentrospora acerina	Caraway (*C. carvi* L.)	

(continued)

Table 11.1 (continued)

Fungal diseases and causal agents	MAPs	Affected organs and symptoms
Scab		
Fusicladium depressum	Angelica (*A. archangelica* L.) Dill (*A. graveolens* L.) Fennel (*F. vulgare* Mill.)	Localized lesions on leaves, fruits and tubers giving a scabby appearance
Canker		
Armillaria mellea	Lavender (*L. angustifolia* Mill.)	Localized necrotic lesions on stem
Rosellinia necatrix		
Phomopsis diachenii	Caraway (*C. carvi* L.)	
Phomopsis foeniculi	Fennel (*F. vulgare* Mill.)	
Phomopsis lavandulae	Lavender (*L. angustifolia* Mill.)	
Phomopsis sclareae	Clary sage (*S. sclarea* L.)	
Basal stem rot		
Conyothyrium lavandulae *Phoma lavandulae*	Lavander (*L. angustifolia* Mill.)	Disintegration of the lower part of the stem (bulbs in *Crocus*)
Rhizoctonia solani	Basil (*O. basilicum* L.) Coriander (*C. sativum* L.) Fennel (*F. vulgare* Mill.) Parsley (*P. crispum* (Mill.) Fuss) Peppermint (*M.* x *piperita* L.) Rosemary (*R. officinalis* L.) Sage (*S. officinalis* L.) St John's wort (*H. perforatum* L.) Thyme (*Thymus* sp.)	
Rhizoctonia violacea	Fennel (*F. vulgare* Mill.)	
Sclerotinia bulborum	Saffron (*Crocus sativus* L.)	
Sclerotinia minor	Basil (*O. basilicum* L.)	
Sclerotinia sclerotiorum	Anise (*P. anisum* L.) Basil (*O. basilicum* L.) Caraway (*C. carvi* L.) Coriander (*C. sativum* L.) Fennel (*F. vulgare* Mill.)	
Sclerotium rolfsii	Licorice (*G. glabra* L.)	
Phoma crocophila	Saffron (*C. sativus* L.)	
Phoma longissima	Fennel (*F. vulgare* Mill.)	
Phyllosticta atropae	Deadly nightshade (*A. bella-donna* L.)	

(continued)

Table 11.1 (continued)

Fungal diseases and causal agents	MAPs	Affected organs and symptoms
Phyllosticta destructiva	Common mallow (*M. sylvestris* L.)	Black rot
Phoma strasseri	Peppermint (*M* x *piperita* L.) Caraway (*C. carvi* L.)	Black stem rot
Phoma exigua	Valerian (*V. officinalis* L.)	
Root rot		
Macrophomina lavandulae	Lavender (*L. angustifolia* Mill.)	Decay of the root system
Macrophomina phaseoli	Peppermint (*M. x piperita* L.)	
Helicobasidium purpureum	Fennel (*F. vulgare* Mill.)	
Phytophthora cactorum	Roman wormwood (*Artemisia pontica* L.)	
Phytophthora erytroseptica	Deadly nightshade (*A. bella-donna* L.)	
Phytophthora nicotianae	Lavender (*L. angustifolia* Mill.) Rosemary (*R. officinalis* L.) Thyme (*T. vulgaris* L. and *T.* x *citriodorus* (Pers.) Schreb.)	
Thielaviopsis basicola	Deadly nightshade *(A. bella-donna* L.) Sage (*S. officinalis* L.)	
Phytophthora primulae	Parsley *(P. crispum* (Mill.) Fuss)	Root and crown rot
Phytophthora syringae	Fennel (*F. vulgare* Mill.)	Leaf blight
Pythium debaryanum	Dill (*A. graveolens* L.) Clary sage *(S. sclarea* L.)	Seedling damping-off
Pythium irregular	Coriander (*C. sativum* L.)	
Pythium ultimum	Saffron (*C. sativus* L.)	
Wilt and dieback		
Coniothyrium lavandulae	Lavender (*L. angustifolia* Mill.)	Generalized loss of turgidity and drooping of leaves or shoots
Fusarium avenaceum	Coriander (*C. sativum* L.) St. John's wort (*H. perforatum* L.) Peppermint (*M.* x *piperita* L.) Sage (*S. officinalis* L.)	

(continued)

Table 11.1 (continued)

Fungal diseases and causal agents	MAPs	Affected organs and symptoms
Fusarium bulbigerum	Saffron (*C. sativus* L.)	
Fusarium culmorum	St. John's wort (*H. perforatum* L.) Marjoram (*Origanum majorana* L.) Sage (*S. officinalis* L.)	
Fusarium oxysporum	Caraway (*C. carvi* L.) Coriander (*C. sativum* L.) Lemon balm (*M. officinalis* L.) Peppermint (*M.* x *piperita* L.) Sage (*S. officinalis* L.) St. John wort (*H. perforatum* L.) Thyme (*Thymus* sp.)	
Fusarium oxysporum f.sp. *basilici*	Basil (*O. basilicum* L.)	
Verticillium dahliae	Mint (*Mentha longifolia* (L.) L.) Peppermint (*M.* x *piperita* L.) Mint (*Mentha arvensis* L.)	
Verticillium albo-atrum	Peppermint (*M.* x *piperita* L.)	
Black knot		
Trichothecium rosei	Anise (*P. anisum* L.)	Irregular swelling on fruits

Data from Catizone et al.(1986), Garibaldi et al. (1997), Reuveni et al. (1997), Evenhuis (1998), Koike et al. (1998), Farooqi and Sreeramu (2004), Rodeva and Gabler (2004), Glawe et al. (2005), Martini et al. (2006), Álvarez et al. (2007), Frużyńska-Jóźwiak and Andrzejak (2007), Machowicz-Stefaniak et al. (2008), Zimowska (2008, 2011), Cabrera et al. (2010), Dung et al. (2010), Kassai-Jáger and Kiss (2010), Bertetti et al. (2010, 2012), Zalewska (2010), Bokor (2011), Wichura et al. (2012)

among them *F. oxysporum* was found on wilted stems of lemon balm, caraway, sage, peppermint, coriander, thyme and St John's wort; *F. avenaceum* on sage, peppermint, coriander and St John's wort and *F. culmorum* was isolated from marjoram, sage and St John's wort (Catizone et al. 1986; Frużyńska-Jóźwiak and Andrzejak 2007; Bokor 2011). A special form designated as *F. oxysporum* f. sp. *basilici* was found on basil plants (Garibaldi et al. 1997; Reuveni et al. 1997). The genus *Verticillium* includes the two common species *V. albo-atrum* Reinke & Berth. and *V. dahliae* Kleb., both found *M. x piperita* while the latter was isolated also from plants of *Mentha arvensis* and *M. longifolia* (Catizone et al. 1986; Dung et al. 2010).

Fusarium and *Verticillium* wilt are very difficult to manage. Since they are soilborne pathogens, crop rotation should exclude plants recognized to be specific host for a certain pathogen species, and it is indispensable to use disease-free seed. Nowadays, in the frame of a sustainable diseases management, the solarization method seems the best solution in order to reduce the plant pathogens living in soil.

Moreover, few laboratory tests have been carried out for evaluating the use of some essential oils to control such soilborne diseases, and the same solution can be adopted for other soilborne fungi like *Rhizoctonia solani* Kühn, causal agent of basal rots. It affects basil plants at all growth stages causing damping-off (Garibaldi et al. 1997), but also other MAPs have been found susceptible to this disease, such as mints, parsley, thyme, rosemary, coriander, St John's wort, peppermint, fennel and sage (Machowicz-Stefaniak et al. 2008; Frużyńska-Jóźwiak and Andrzejak 2007).

3.2 Bacterial Diseases

Bacterial diseases occur in every place and under favorable environmental conditions may be extremely destructive. They cause leaf spots and blight, soft rot of fruits, roots, wilts, scab and canker, symptoms that are often indistinguishable from those caused by fungi. Generally they survive in infected plants and their debris, and in the soil. Bacteria require a wound or natural opening in the plants to gain entry. Martini et al. (2009) report leaf spot caused by *Pseudomonas* spp. on sage and rosemary. The same bacterial disease was observed on coriander and fennel showing brown necrotic lesions on leaves, petioles and young shoots (Farooqi and Sreeramu 2004). *Erwinia carotovora* var. *carotovora* (Jones) Holl. was detected on fennel and caraway, and the last is also susceptible to *Xanthomonas* genus, causing soft rot of fruits (Catizone et al. 1986). Their management can be achieved by removal of any plant debris and a proper use of irrigation to maintain the foliage dry. A fair control can also be provided by copper treatments.

3.3 Viral Diseases

All plant viruses are parasitic in cells and cause many different diseases. Plant viruses enter cells only through mechanically produced wounds or by vectors. They cause dwarfing or stunting of entire plant, but the more easily recognizable symptoms appear on leaves (Agrios 1997). Some viral diseases have been detected also in MAPs (Catizone et al. 1986; Joy et al. 2001a, b). In India, the occurrence of abnormal light green spotting on the leaves of *Salvia splendens* Ker-Gawl., that in the most severe form were distorted, was reported; the agent was identified as a virus, that resulted easily transmissible by mechanical sap inoculation and by the two aphids *Myzus persicae* Sulz. and *Aphis gossypii* Glov. (Joshi and Dubey 1972). Another rod-shaped virus probably belonging to the *Tobamovirus* genus, was reported in Italy on *Salvia officinalis* L. "Maxifolia". Observed symptoms were chlorotic mosaic, yellow rings on the leaves and stunting (Bellardi et al. 2006). An association between variegation in lemon balm and the presence of Tulip Virus X (TVX) was also observed (Tzanetakis et al. 2005). On saffron plants a Bean Yellow Mosaic Virus (BYMV) was reported, whereas the alpha mosaic virus was observed on lavender (Catizone et al. 1986).

4 Pests of MAPs

A scarce amount of specialized literature about insects, mites and nematodes affecting MAPs and their management is available by far, probably due to the limited cropping area and to the fact that some allelochemicals produced by many of these plants showed a repellent, antifeedant or toxic action against many pests (Regnault-Roger 1997; Isman 2000; Miresmailli et al. 2006; Akhtar et al. 2012; Cavoski et al. 2012; Benelli et al. 2012; Faraone et al. 2012), leading to consider MAPs as less susceptible.

Direct damages caused by pests depend mainly on their biology, but also the plant conditions, such as water stress, nutritional factors, soil and climate, may play an important role (Catizone et al. 1986; Pillonel et al. 2012). Furthermore, some insects have a well-known ability to transmit dangerous viruses and viroids that in the specific case of essential oil crops could reduce both the quantitative and qualitative levels of yield. Although in most cultivated MAPs no serious losses due to insect and mite pests are noted and the use of control measures is considered unnecessary (Catizone et al. 1986; Pollini 1998; Joy et al. 2001a, b), experimental data on production loss caused by different population levels of each pest species are in many cases lacking. Hence, specific information about pest monitoring methods, economic thresholds and effectiveness of control, both natural and adopted by growers, must be derived from other crops, in which the same pests are present.

In spite of this fragmented information, more than 700 insect and mite species associated to about 80 MAPs, both cultivated and wild, are reported (Table 11.2). Most of them are polyphagous or oligophagous, and their economic importance is quite variable, depending on the host plant, geographical region and cropping conditions. The most harmful species are included in the following orders and families: Coleoptera (Chrysomelidae, Curculionidae, Elateridae), Diptera (Agromyzidae, Cecidomyidae), Hemiptera (Aphididae), Lepidoptera (Noctuidae, Geometridae, Tortricidae). Besides insects, several species of mites and nematodes are reported as injurious to MAPs.

4.1 Roots and Bulbs

Main insect pests of MAPs roots and bulbs are included in Hemiptera Lachnidae, Diptera Heleomyzidae and Anthomyidae, Lepidoptera Noctuidae, Hepialidae and Cossidae, but the most injurious are probably the Coleoptera of the family Elateridae (click beetles), like some species of *Agriotis* damaging roots of *Cichorium* spp. or bulbs of garlic. Larvae of Elateridae, also known as wireworm, penetrate into the roots or bulbs, boring them and thus facilitating the infection by microbial pathogens, that speed up the plant death. Adult monitoring may be carried out using pheromone traps, even if the specific pheromone is commercially available only for a limited number of species and the correlation between catches and the risk of infestation is not clearly assessed. Larvae can be monitored extracting them from soil samples or using traps based on the presence of wheat seeds that produce CO_2, attractive to the wireworms. Chopped potatoes placed in the soil can be also used as baits to look for the presence/absence of the insects.

Another group of serious pests of roots in MAPs is represented by nematodes. The most frequently reported genera are *Meloidogyne* and *Platylenchus* (Catizone et al. 1986; Joy et al. 2001a, b). The first one, whose species are also called root-knot nematodes, affects the regular development of roots deforming the normal root cells, and making the roots distorted by multiple galls. Plant parasitism due to *Plathylenchus* species is less evident, because of the absence of galls, and consists in the destruction of root cells by the nematodes living inside roots. Up to 30,000–40,000 living nematodes may be extracted from 100 g of infested roots.

Root pests may severely damage MAPs, especially those cultivated for roots, bulb or tuber production, as infestations may dramatically reduce quantitative and qualitative productions. In many cases infestations may result in wilting, stunt and consequently death of the plant. As first symptoms of the presence of root parasites are not specific, early detection is not easy, so that identification of the pest is often too late to allow effective control measures. Moreover, the control of these pests is sometimes difficult to achieve, due to their wide host range and ability to survive for prolonged time in the soil, making them able to attack the same crop in its subsequent cultural cycles. In order to limit the occurrence of soil pests infestations on cultivated MAPs, certified planting material and nematode-resistant cultivars, if available, should be used. In field, preventive strategies such as removal of alternative hosts of nematodes, trap crops for wireworms, crop rotation and intercropping with non-host plants should be adopted to reduce the probability of infestation of susceptible crops in subsequent productive cycles. In decision-making about crop rotation, great attention has to be paid in choosing the plant species to be cultivated in sequence, avoiding to use those susceptible to the same pest. As a general rule, a 2-year minimum interval between susceptible crops is required to control a serious nematodes outbreak. For controlling soil-borne pathogens and pests, especially soil nematodes, good results may also be obtained alternating crop rotation with green manuring with biocide crops, like *Raphanus sativus* or *Brassica juncea* (Furlan et al. 2004; Lazzeri et al. 2009). The use of biocide crops, also termed biofumigation, is expanding in recent years as an alternative to chemicals, especially against nematodes (Curto et al. 2005). Among the biocide plants, sudangrass and sorghum contain a chemical, dhurrin, degrading into hydrogen cyanide, which is a powerful nematicide (Luna 1993; Forge et al. 1995; Wider and Abawi 2000), while *Tagetes* species produce thiophenes, polyacetylenic compounds with strong biocide activity, thus making these plants very useful for suppressing nematode populations in the soil (Marotti et al. 2012). Moreover, applications of essential oils has proven useful to reduce soil concentration of wireworms and nematodes (Ntalli and Caboni 2012a, b; Andrés et al. 2012; Barsics et al. 2013).

Table 11.2 Association among insect and mite taxa and MAPs families

Order and family	Apia-ceae (7)	Astera-ceae (22)	Canna-biaceae (1)	Hyperi-caceae (1)	Irida-ceae (3)	Lamia-ceae (15)	Laura-ceae (1)	Lilia-ceae (7)
Acari (2)								
Eriophyidae (10)		4				4	1	
Tetranychidae (2)	1	2	1			1		
Coleoptera (11)								
Chrysomelidae (60)	2	27	1	4		38		2
Curculionidae (51)	6	44	2		1	2		
Elateridae (7)		7						2
Other Families (15)		7				3	2	
Diptera (9)								
Agromyzidae (36)	1	44	2			7		1
Anthomyiidae (8)		5		1				2
Cecidomyiidae (29)	13	10	1	6		4		
Psilidae (1	6	2						
Tephritidae (17)	2	20						
Other Families (7)		4	2			1		2
Hemiptera (21)								
Aleyrodidae (4)		2				1	1	
Aphididae (74)	14	67	1	1	4	13		
Cercopidae (3)		1		2		2		
Cicadellidae (15)		7	1	1		13		
Coccidae (10)		1				1	7	3
Diaspididae (13)						3	10	6
Lachnidae (5)		8						
Miridae (16)	4	11	4			1		
Other Families (24)	4	10		1		5	4	3
Hymenoptera (4)								
Eurytomidae (2)	7							
Tenthredinidae (13)		1		4		7		
Other Families (2)	1	1						
Lepidoptera (27)								
Arctiidae (11)		10	3			1		
Coleophoridae (6)		6				1		
Gelechiidae (13)		4		2		7		
Geometridae (63)	1	51	2	7		14		
Hepialidae (3)		3	2			1		
Lycaenidae (5)		1				3		
Noctuidae (104)	4	67	15	5		12		
Nymphalidae (8)		4	4					
Oecophoridae (11)	8	4		1				
Papilionidae (1)	6							
Pterophoridae (4)		2				3		
Pyralidae (16)	2	7	2			6		
Sphingidae (3)			2					1
Tortricidae (30)	2	24	1	4		6	2	3
Other Families (19)	1	2	2	3	1	4		3
Thysanoptera (3)								
Thripidae (11)	**1**	**6**	**1**			**2**		
Other Families (3)		3						
Total (incl. Collembola and Orthoptera not listed above)	87	479	499	42	6	168	27	29

In bracket the number of families/species belonging to each order/family (Data from Catizone Dorkeld (2006–2012), Fauna Europaea (2012), Biological Records Centre (2013)

Malva-ceae (9)	Papaver-aceae (1)	Papilio-naceae (1)	Plantag-inaceae (2)	Polygo-naceae (2)	Scrophul-ariaceae (2)	Solan-aceae (3)	Valeria-naceae (1)	Verben-aceae (1)	Total (79)
3							3		13
2	1					1			9
9			8	2	2	8			103
22	2	3	3	2					87
									9
	2				2		1		17
2	2	1	1		1	1	3	1	67
						3			11
	1		1				3		39
									8
									22
									9
							1		5
18	3	1	7	6	5	2	6		148
							1		6
4			1		3				30
									12
1									20
									8
					1				21
				1			2		30
									7
				2		1	1		16
									2
			6						20
									7
4									17
2			4		1		3		85
									6
		1							5
9			22		10	3	3		150
3			2		2		1		16
									13
									6
									5
1			1				1		20
						2			5
1			3						46
3			1			1			21
1									**11**
1									4
86	11	6	60	13	27	22	27	1	1,140

et al. (1986), Joy et al. (2001a, b), Pollini (1998), Basso (2009), Barbagallo et al. (2011), Migeon and

4.2 Leaves, Buds and Stems

All the aerial part of MAPs plants (stem, leaves, flowers, fruits and seeds) may be infested by a large number of insects and mites, although most of them are considered as minor pests. Mites attacking leaves of MAPs belong mainly to Tetranychidae, also known as spider mites due to the protective silk webs that they produce on attacked leaves surface. Among spider mites, the most injurious species is *Tetranychus urticae* Koch, that is extremely polyphagous and is known to attack several hundred of different plant species. Spider mites infestations may cause yellowing leaves and result in plant death. Biological control of *T. urticae* is usually achieved by predaceous mites of the family Phytoseiidae naturally occurring in the field or released on the crop (Tsolakis and Ragusa 2004). In particular, the Phytoseiidae *Phytoseiulus persimilis* Athias-Henriot is commonly reared in commercial factories producing biological control agents and successfully used to limit *T. urticae* on many different crop species, both in field and greenhouse (Bostanian et al. 2003; Opit et al. 2004). Moreover, interesting results have been obtained testing essential oils or plant extracts for their acaricide action (Ragusa Di Chiara et al. 2007; Tsolakis and Ragusa 2008). Among the other mites damaging MAPs, Eriophydae are tiny, microscopic mites which live as plant parasites and often establish specific relationships with their host plants, particularly the gall inducing species.

Among the insects, more than 70 species of Hemiptera Aphididae have been recorded on almost 70 % of the MAPs considered in the present review (74 Aphididae species on 53 plant species, Table 11.2). Aphids are often considered the most destructive pests, due to their biological traits like an high reproductive rate, based also on a short life cycle, the ability to spread over great distances, and, in many species, reproductive strategy alternating sexual and asexual generations. Their infestations involve mainly leaves and young buds, but flowers and all tender tissue may be attacked. Infested plants exhibit a variety of symptoms, such as spotted or yellowing leaves, curled leaves, wilting, decreasing of growth rates, low yields and finally death. Moreover, the presence of honeydew may encourage the growth of fungi on plant surface, negatively affecting photosynthesis and gas exchanges. Many of the aphid species recorded on MAPs are known to be oligophagous, and have been recorded from one to five different MAPs species. The two species *Aphis fabae* s.l. and *Myzus persicae* (Sulzer) are widely distributed and highly polyphagous, having been recorded from hundreds of plant species across many families. They are considered among the most injurious pests of many field crops, and *M. persicae* is known to infest also several cultivated trees, such as citrus, plum, peach (Blackman and Eastop 2000). But the most important damage is represented by the transmission of disease-causing organisms, like plant viruses, to their host plants (Catizone et al. 1986; Basso 2009). Actually, both of them are involved in the transmission of over 40 (*A. fabae*) and 180 (*M. persicae*) plant viruses. Aphid populations are usually limited by many natural enemies, among them predators like adults and larvae of Coleoptera Coccinellidae (Sagar and

Kumar 1996) and Neuroptera Chrysopidae, larvae of Diptera Syrphidae, parasitic wasps such as many Hymenoptera Braconidae. When natural control is not enough, removal of heavily infested plants and the use of mineral oils and botanical insecticides can effectively limit aphids' infestations. Different treatment timing must be adopted when plant virus transmission by aphids occurs in the crop, as a severe damage can be caused even by a low population levels of the insects.

Leaves of cultivated and wild MAPs are usually attacked also by many Lepidoptera and Coleoptera, that cause variable injury levels, from surface damage to leaf epidermis to production of mines or holes and more or less severe cutting of the leaf blade. In spite of the large number of species associated to MAPs (nearly 300 Lepidoptera and 130 Coleoptera, see Table 11.2), the occurrence of serious damages is occasionally recorded. The Lepidoptera family of Noctuidae is the most frequently reported. As natural control of Coleoptera and Lepidoptera is often inadequate, treatments may be needed, preferably using botanical insecticides. Moreover, to control those Lepidoptera whose larvae live on leaves or buds surfaces, the insect pathogen *Bacillus thuringiensis* var. *kurstaki* can be effectively used (Pank 1993). It is a Gram-positive, soil dwelling bacterium producing spores and crystalline proteins that are used as insecticides. *B. thuringiensis* is considered as a cornerstone of modern agriculture, because of its selectivity, the absence of detectable phytotoxic effects, scarce or absent effect on humans, pollinators, and most beneficial insects. On the other hand, the action of *B. thuringiensis* based products is limited by low temperatures, the easy removal by rain, the brief period of action, which is due, in part, to sunlight inactivation of the microorganism (Cantwell and Franklin 1966; Frye et al. 1973; Raun et al. 1966; Pasqualini 1994). As *B. thuringiensis* insecticides must be ingested by the target insect, their effectiveness can be improved using some additives like sticky or wetting products, to guarantee a good distribution on plants, and feed stimulating substances (sugar), thus leading the ingestion of a larger amount of toxins and spores and consequently a more rapid death of the insect (Navon et al. 1997).

4.3 Flowers and Seeds

Flowers may be attacked by many insects, whose infestations may negatively affect the production of flower heads and seeds. This is the case of some species of *Cucullia* (Lepidoptera Noctuidae) on chamomile, or some Lepidoptera Oecophoridae on fennel and parsley (Catizone et al. 1986; Pollini 1998; Basso 2009). As regards seeds pests, Hymenoptera Eurytomidae can be found damaging seeds of some MAPs, like *Systole albipennis* Walker on fennel (Patel et al. 1986). Infestations starts in the field, and their incidence may depend on management practices (Kashyap et al. 1994), but the insects may be found also on stored seeds (Mittal and Butani 1995).

4.4 Field Pest Management

In the future, an increase in pests in MAPs, however, may be expected to be a direct consequence of the growing rate of cultivation (Gabler 2002), and it is possible to foresee that a spread of MAPs monocultures over wider areas will result in a major concern about pest control. As a general rule, pest monitoring should be adopted, both by traps and direct monitoring in the field, in order to work out the economic threshold level. Moreover, the effectiveness of natural enemies should be assessed, as in many cases MAPs are cultivated in restricted and marginal areas, frequently close to a semi-natural or natural context, leading to suppose that a more effective natural control by predators and parasitoids usually occurs in comparison with specialized crops cultivated on larger areas. Experiments aimed at evaluating products useful for essential oil crop protection should certainly be performed, taking into account also botanical insecticides (Regnault-Roger 1997; Isman 2000; Gahukar 2012) and mineral products like clays (Lo Verde et al. 2011a, b), because of the specific orientation of markets towards the high "naturality" content of such products that implies special care in their field management (Carrubba and Catalano 2009). Furthermore, the repellant action of many essential oil crops on insect pests has been tested with promising results, suggesting the use of MAPs as intercrop species (IIRR 1993).

Finally, as in several MAPs pollination is carried out by honeybees and wild bees, particular attention must be paid in choosing the products to be applied and timing of treatments, to avoid negative effects on helpful fauna, including both pollinators and natural enemies.

5 Conclusive Remarks

Information on weeds, pests and diseases affecting MAPs in their typical cultivation areas represents an important fund of experience also in the new environments, in which these plants are currently spreading. Many methods may be suggested for their management, both direct and indirect, involving agronomic, manual, mechanical, biological and chemical strategies. Each of them shows pros and cons, and research about this topic is far from complete. The introduction of chemical products for plant protection has certainly brought an advantage as concerns the easy and cheap management of fields, but many environmental and health issues are of concern. It is widely acknowledged that in cultivation of MAPs, chemical products for weed, diseases and pests control should be kept to a minimum, and applied only when no alternative measures are available (WHO 2003). It should be taken into account that the introduction of these molecules inside the environment may exert significant effects on environmental balances, and that, additionally, sometimes they may have some effect also on extracts chemical composition. When control measures becomes necessary, integrated management should be preferred, limiting the use of chemicals to a minimum, both in terms of number of treatments and of dosage, and selecting highly effective, low-toxicity and low-residue products.

Today, although most of MAP's cultivation is still mainly concentrated in some world regions, herbs and their derivatives are used throughout the world, also due to human population migratory flow and to the globalization process, that in the last decades caused a spread of regional gastronomies in many countries. Therefore, the choice of pest management strategies must take into account the regulatory requirements that apply both for the grower and the end-user countries. As a consequence, the number of active substances that can be used should be limited even if they are allowed in production countries. Actually, the presence of harmful residuals, above all when herbs are addressed to direct human consumption (as medicinal or flavoring items), is another important concern related to chemical management in MAPs. Hence, the minimum interval between the last treatment and harvest time should be observed, according to the recommendations from the manufacturer and in compliance with regional and/or national regulations on maximum residue limits (Kosalec et al. 2009; WHO 2003).

In recent years, however, non-polluting agrotechnology has been rapidly developing, and organic farming of MAPs is gaining an increasing interest in the perspective of environmental protection, sustainable management of resources, and achievement of good quality drugs. In organic cultivation chemicals cannot be used in the form of fertilizers, herbicides and pesticides, hence plants management becomes labor intensive. This stands mainly for weed-control, whereas many insects, mites, nematodes and pathogenic microorganisms may be controlled through biological methods and the use of plant-derived products. Although MAPs protection against harmful organisms is considered as a part of the general agricultural context, MAPs and their products still play a symbolic role in the way of thinking about health and well-being. Hence, a further increase of researches on the biology and sustainable management of weeds, diseases and pests is expected to occur in the near future.

References

Agrios GN (1997) Plant pathology, 4th edn. Academic, San Diego, p 618

Akhtar Y, Isman MB, Niehaus LA, Lee CH, Lee HS (2012) Antifeedant and toxic effects of naturally occurring and synthetic quinones to the cabbage looper, *Trichoplusia ni*. Crop Prot 31:8–14. doi:10.1016/j.cropro.2011.09.009

Álvarez LA, Pérez-Sierra A, Armengol J, García-Jiménez J (2007) Characterization of *Phytophthora nicotianae* isolates causing collar and root rot of lavender and rosemary in Spain. J Plant Pathol 89(2):261–264

Andrés MF, González-Coloma A, Sanz J, Burillo J, Sainz P (2012) Nematicidal activity of essential oils: a review. Phytochem Rev. doi:10.1007/s11101-012-9263-3

Anzalone A, Cirujeda A, Aibar J, Pardo G, Zaragoza C (2010) Effect of biodegradable mulch materials on weed control in processing tomatoes. Weed Technol 3(24):369–377. doi:10.1614/WT-09-020.1

Arnon I (1992) Agriculture in drylands, principles and practices. Elsevier, Amsterdam, p 975

Ascard J (1995) Thermal weed control by flaming: biological and technical aspects. Dissertation, Swedish University of Agricultural Science

Barbagallo S, Binazzi A, Pennacchio F, Pollini A (2011) An annotated checklist of aphids surveyed in the Italian regions of Tuscany and Emilia Romagna. Redia 44:59–96

Barberi P (2002) Weed management in organic agriculture: are we addressing the right issues? Weed Res 2(42):177–193. doi:10.1046/j.1365-3180.2002.00277.x

Barsics F, Haubruge E, Verheggen FJ (2013) Wireworms' management: an overview of the existing methods, with particular regards to *Agriotes* spp (Coleoptera: Elateridae). Insects 4:117–152. doi:10.3390/insects4010117

Basso F (2009) Piante officinali aromatiche e medicinali. Aspetti bioagronomici, aromatici e fitoterapeutici. Pitagora Editrice, Bologna, p 381 (in Italian)

Bellardi MG, Benni A, Bruni R, Bianchi A (2006) A virus disease affecting *Salvia officinalis* L. "Maxifolia" and its effects on essential oil production and composition. Acta Horticult 723:381–386

Benelli G, Flamini G, Canale A, Cioni PL, Conti B (2012) Toxicity of some essential oil formulations against the Mediterranean fruit fly *Ceratitis capitata* (Wiedemann) (Diptera Tephritidae). Crop Prot 42:223–229. doi:10.1016/j.cropro.2012.05.024

Bertetti D, Pensa P, Tinivella F, Gullino ML, Garibaldi A (2010) *Golovynomyces biocellatus* nuovo agente del mal bianco riscontrato su *Mentha spicata* L. coltivata in Italia. Centro di competenza per l'innovazione in campo agro-ambientale (AGROINNOVA), Università degli Studi di Torino, Grugliasco, pp 36–38 (in Italian)

Bertetti D, Martini P, Repetto L, Gullino ML, Garibaldi A (2012) *Golovinomyces biocellatus*, nuovo agente di mal bianco su *Origanum vulgare* "Compactum" coltivato in Italia. Centro di competenza per l'innovazione in campo agro-ambientale (AGROINNOVA), Università degli Studi di Torino, Grugliasco, pp 23–25 (in Italian)

Biological Records Centre Website (2013), Database of Insects and their Food Plants. http://www.brc.ac.uk/dbif/homepage.aspx. Accessed Jan 2013

Blackman RL, Eastop VF (2000) Aphids on the world's crops: an identification and information guide, 2nd edn. Wiley, Chichester, pp 1–466

Blackman GE, Templeman WG, Halliday DJ (1951) Herbicides and selective phytotoxicity. Annu Rev Plant Physiol 6(2):199–230

Bokor P (2011) Diseases and pests of lemon balm (*Melissa officinalis*). Acta fytotechnica et zootechnica Special Number, pp 42–45

Bond W, Grundy AC (2001) Non-chemical weed management in organic farming systems. Weed Res 5(41):383–405. doi:10.1046/j.1365-3180.2001.00246.x

Bond W, Turner RJ, Grundy AC (2003) A review of non-chemical weed management. HDRA, the Organic Organisation, Ryton Organic Gardens, Coventry, p 81

Bostanian NJ, Trudeau M, Lasnier J (2003) Management of the two-spotted spider mite, *Tetranychus urticae* (Acari: Tetranychidae) in eggplant fields. Phytoprotection 84:1–8

Boyd NS, Brennan EB, Fennimore SA (2006) Stale seedbed techniques for organic vegetable production. Weed Technol 4(20):1052–1057. doi:10.1614/WT-05-109.1

Cabrera MG, Vobis G, Alvarez RE (2010) Powdery mildew on *Salvia officinalis* in Corrientes Argentina. Mycosphere 1(4):289–291

Cantwell GE, Franklin BA (1966) Inactivation by irradiation of spores of *Bacillus thuringiensis* var *thuringiensis*. J Invertebr Pathol 8:256–258

Carrubba A, Catalano C (2009) Essential oil crops for sustainable agriculture – a review. In: Lichtfouse E (ed) Climate change, intercropping, pest control and beneficial microorganisms. Springer, Dijon, pp 137–188. doi:10.1007/978-90-481-2716-0_8

Carrubba A, la Torre R (2005) Prove di pacciamatura organica su Origano (*Origanum heracleoticum* L.). In: Proc. XXXVI Convegno SIA, Foggia, 20–22 Sept 2005. pp 274–275 (In Italian)

Carrubba A, Militello M (2013) Nonchemical weeding of medicinal and aromatic plants. Agron Sustain Dev 33(3):551–561. doi:101007/s13593-012-0122-9

Carrubba A, la Torre R, Matranga A (2002) Effect of the choice of different row arrangements on the bio-agronomical behaviour of *Origanum heracleoticum*. Acta Horticult 576:247–252

Carrubba A, la Torre R, Saiano F, Aiello P (2008) Sustainable production of fennel and dill by intercropping. Agron Sustain Dev 28:1–10. doi:10.1051/agro:2007040

Carrubba A, Calabrese I, Ascolillo V (2009) Non-chemical weeds management in two Mediterranean culinary herbs. Acta Horticult 826:51–57

Carrubba A, Catalano C, Militello M, Pasquale A, Strano T, Ruberto G (2010) Variabilità dell'olio essenziale di finocchio (*Foeniculum vulgare* Mill.) per effetto di trattamenti agronomici. In: Sarli G, Alvino A, Cervelli C (eds) Proc IV Convegno Nazionale Piante Mediterranee – Le potenzialità del territorio e dell'ambiente, Marina di Nova Siri (MT), 7–10 Oct 2009 pp 661–666 (in Italian)

Catizone P, Marotti M, Toderi G, Tétényi P (1986) Coltivazione delle piante medicinali e aromatiche. Patròn Editore, Bologna, p 399 (in Italian)

Cavoski I, Al Chami Z, Bouzebboudja F, Sasanelli N, Simeone V, Mondelli D, Miano T, Sarais G, Ntalli NG, Caboni P (2012) *Melia azedarach* controls *Meloidogyne incognita* and triggers plant defense mechanisms on cucumber. Crop Prot 35:85–90. doi:10.1016/j.cropro.2012.01.011

Chaudhary GR (2000a) Weed management in coriander (*Coriandrum sativum*). Indian J Agric Sci 9(70):603–605

Chaudhary GR (2000b) Weed-population dynamics and fennel (*Foeniculum vulgare*) growth as influenced by integrated weed management. Indian J Agron 2(45):421–428

Chicouene D (2007) Mechanical destruction of weeds. A review. Agron Sustain Dev 1(27):19–27. doi:10.1051/agro:2006012

Curto G, Dallavalle E, Lazzeri L (2005) Life cycle duration of *Meloidogyne incognita* and host status of Brassicaceae and Capparaceae selected for glucosinate content. Nematology 7:203–212

De La Fuente EB, Gil A, Lenardis AE, Pereira ML, Suárez SA, Ghersa CM, Grass MY (2003) Response of winter crops differing in grain yield and essential oil production to some agronomic practices and environmental gradient in the Rolling Pampa, Argentina. Agric Ecosyst Environ 99:159–169. doi:10.1016/S0167-8809(03)00131-2

Demarco MF, Sarruggieri H, Lopez MA (1999) Good agricultural practices for the organic production of medicinal plants. Acta Horticult 502:21–27

Dung JKS, Schroeder BK, Johnson DA (2010) Evaluation of *Verticillium* wilt resistance in *Mentha arvensis* and *M longifolia* genotypes. Plant Dis 94(10):1255–1260

Duppong LM, Delate K, Liebman M, Horton R, Romero F, Kraus G, Petrich J, Chowdbury PK (2004) The effect of natural mulches on crop performance, weed suppression and biochemical constituents of Catnip and St. John's Wort. Crop Sci 44:861–869. doi:10.2135/cropsci2004.8610

Edwards J, Taylor PA, Parbery DG, Halloran GM (1999) Peppermint rust in Victoria: the incidence-severity relationship and its implication for the development of an action threshold. Aust J Agr Res 51:91–95

Evenhuis A (1998) On ecology and control of *Mycocentrospora acerina* in caraway (*Carum carvi*). Dissertation, Landbouw Universitei Wageningen, p 198

Faraone N, De Cristofaro A, Maltese M, Vitagliano S, Caleca V (2012) First data on the repellent activity of essential oils of *Citrus limon* towards medfly (Ceratitis capitata). N Mediterr 11(4):31–34

Farooqi AA, Sreeramu BS (2004) Cultivation of medicinal and aromatic crops. Universities Press Private limited Hyderabad, p 656

Fauna Europaea (2012) Fauna Europaea version 2.5. Web service available online at http://www.faunaeur.org. Accessed 8 Feb 2013

Fontana E, Hoeberechts J, Nicola S (2006) Effect of mulching in medicinal and aromatic plants in organic farm guest houses. Acta Horticult 723:405–410

Forge TA, Ingham RE, Kaufman D (1995) Winter cover crops for managing root-lesion nematodes affecting small fruit crops in the Pacific Northwest. Pacific Northwest Sustainable Agriculture, March, p 3

Frużyńska-Jóźwiak D, Andrzejak R (2007) The incidence of diseases and pathogenic fungi on selected medicinal and spice plants in the area of Poznań. Phytopathol Pol 46:47–51

Frye RD, Scholl CG, Scholz EW, Funke BR (1973) Effect of weather on a microbial insecticide. J Invertebr Pathol 22:50–54

Furlan L, Bonetto C, Patalano G, Lazzeri L (2004) Potential of biocidal meals to control wireworm populations. In: Proceedings of the first international symposium on "Biofumigation: a possible alternative to methyl bromide?" Firenze, 31 Mar – 1 Apr 2004. Agroindustria 3:313–316.

Gabler J (2002) Breeding for resistance to biotic and abiotic factors in Medicinal and Aromatic plants: general situation and current results in annual Caraway (*Carum carvi* L. var. annuum). In: Johnson CB, Franz C (eds) Breeding research on aromatic and medicinal plants. The Haworth Herbal Press, Binghamton, pp 1–11

Gahukar RT (2012) Evaluation of plant-derived products against insect pests and diseases of medicinal plants: a review. Crop Prot 42:202–209. doi:10.1016/j.cropro.2012.07.026

Galambosi B, Szebeni-Galambosi Z (1992) The use of black plastic mulch and ridges in the production of herbicide free herbs. Acta Horticult 306:353–356

Garibaldi A, Gullino ML, Minuto G (1997) Diseases of basil and their management. Plant Dis 81(2):124–132

Gil A, De La Fuente E, Lenardis A, Ghersa C, Van Baren HC, Di Leo Lira P, Suarez S, López Pereira M (1998) Yield and composition of Coriander (*Coriandrum sativum* L.) essential oils related to soil environment and weed competition. In: Proc annual conference new crops and new uses: biodiversity and sustainability, Phoenix, 8–11 Nov 1998

Giorgi A, Mandrini S, Bona S (2006) Produzione di assenzio in Valle Camonica. Proceedings III national meeting. *Piante Mediterranee*, Bari, 27 Sept–1 Oct, p 120 (In Italian)

Glawe DA, Pelter GQ, du Toit LJ (2005) First report of powdery mildew of carrot and parsley caused by *Erysiphe heraclei* in Washington State. Plant Health Prog. doi:10.1094/PHP-2005-0114-01-HN

Hall JC, Van Eerd LL, Miller SD, Owen MDK, Prather TS, Shaner DL, Singh M, Vaughn KC, Weller SC (2000) Future research directions for weed science. Weed Technol 3(14):647–658

Hartley MJ (1993) Herbicide tolerance and weed control in culinary herbs. In: Proceedings of 46th New Zealand plant protection conference, Christchurch, 10–12 Aug 1993, pp 35–39

IIRR (1993) International institute of rural reconstruction. The bio-intensive approach to small scale household food production, Silang, p 180

Isman MB (2000) Plant essential oils for pests and disease management. Crop Prot 19:603–608. doi:10.1016/S0261-2194(00)00079-X

James TK, Rahman A, Douglas JA (1991) Control of weeds in five herb crops. In: Proc 44th New Zealand weed and pest control conference, Ruakura Agricultural Centre, New Zealand, pp 116–120

Joshi RD, Dubey LN (1972) Observations on a virus disease of *Salvia spendens* occurring in Gorakhpur. Phyton 14:309–311

Joy PP, Thomas J, Mathew S, Jose G, Joseph J (2001a) Aromatic plants. In: Bose TK, Kabir J, Das P, Joy PP (eds) Tropical horticulture, vol 2. Naya Prokash, Calcutta, pp 633–733

Joy PP, Thomas J, Mathew S, Skaria BP (2001b) Medicinal plants. In: Bose TK, Kabir J, Das P, Joy PP (eds) Tropical horticulture, vol 2. Naya Prokash, Calcutta, pp 449–632

Karamanos AJ (2000) The cultivation of Sage. In: Kintzios SE (ed) Cultivation and breeding; sage. The genus salvia. OPA (Overseas Publishers Association), Amsterdam, pp 93–108

Kashyap RK, Chaudhary OP, Thakral KK (1994) Influence of management practices on the incidence of *Systole albipennis* Walker in fennel (*Foeniculum vulgare* Mill) seeds. JOSAC 3(2):124–129

Kassai-Jáger E, Kiss L (2010) First report of Powdery mildew on lemon balm (*Melissa officinalis*) caused by *Golovinomyces biocellatus* in Hungary. Plant Dis 94(9):1169

Kegge W, Pierik R (2010) Biogenic volatile organic compounds and plant competition. Trends Plant Sci 3(15):126–132. doi:10.1016/j.tplants.2009.11.007

Koike ST, Subbarao K, Roelfs A, Hennen J, Tjosvold S (1998) Rust disease of oregano and sweet marjoram in California. Plant Dis N/A. http://www.arsusdagov/research/publications/publicationshtm?seq_no_115=99476. Accessed 6 Dec 2012

Kosalec I, Cvek J, Tomić S (2009) Contaminants of medicinal herbs and herbal products. Arh Hig Rada Toksikol 4(60):485–501. doi:10.2478/10004-1254-60-2009-2005

Kothari SK, Singh JP, Singh K (1989) Chemical weed control in Bulgarian coriander (*Coriandrum sativum* L.). Int J Pest Manag 35:2–5. doi:10.1080/09670878909371309

Kothari SK, Singh CP, Singh K (2002) Weed control in rose-scented geranium (*Pelargonium* spp). Pest Manag Sci 58:1254–1258. doi:10.1002/ps.592

Kristiansen PE (2003) Sustainable weed management in organic herb and vegetable production. Ph.D. dissertation, University of New England, Armidale

Lazzeri L, Curto G, Dallavalle E, D'Avino L, Malaguti L, Santi R, Patalano G (2009) Nematicidal efficacy of biofumigation by defatted Brassicaceae meal for control of *Meloidogyne incognita* (Kofoid et White) Chitw. on zucchini crop. J Sustain Agric 33:349–358

Liberato JR, Cunnington JH (2007) Powdery mildew on *Mentha* in Australia. Australas Plant Dis Notes 2:83–86

Lo Verde G, Caleca V, Lo Verde V (2011a) The use of kaolin to control Ceratitis capitata in organic citrus groves. Bull Insectol 64(1):127–134. ISSN 1721-8861

Lo Verde G, Rizzo R, Barraco G, Lombardo A (2011b) Effects of Kaolin on Ophelimus maskelli (Hymenoptera: Eulophidae) in laboratory and nursery experiments. J Econ Entomol 104(2):180–187. doi:10.1603/EC10164

Lövenstein H, Lantinga EA, Rabbinge R, Van Keulen H (1993) Principles of production ecology. TPE-WAU, Wageningen, p 118

Luna J (1993) Crop rotation and cover crops suppress nematodes in potatoes. Pacific Northwest Sustainable Agriculture, March, p 45

Machowicz-Stefaniak Z, Zalewska E, Król E (2008) Biotic effect of caraway phyllosphere fungi on the pathogenic fungus *Septoria carvi* Syd. Herba Pol 54(3):70–80

Madia M, Gaetán S (2005) Outbreak of powdery mildew on common sage in Argentina. Plant Dis 89:911

Marotti I, Marotti M, Piccaglia R, Nastri A, Grandi S, Dinelli G (2012) Thiophene occurrence in different *Tagetes* species: agricultural biomasses as sources of biocidal substances. J Sci Food Agric 90(7):1210–1217

Martini A (1996) Prototipo per il pirodiserbo delle colture officinali. Proceedings International Meeting. *Coltivazione e miglioramento delle piante officinali*, Trento, 2–3 June 1994, pp 663–666 (In Italian)

Martini P, Cacciola SO, Savona S, Bozzano G (2006) Root and basal stem rot caused by *Phytophthora nicotianae* on Thyme in Liguria. Acta Horticult 723:477–480

Martini P, Odasso M, Rapetti S, Repetto L, De Rino E, Gullone C (2009) Alterazioni di origine batterica su ranuncolo, salvia e rosmarino. Protezione delle colture 2:109–110 (in Italian)

Migeon A, Dorkeld F (2006–2012) Spider Mites Web: a comprehensive database for the Tetranychidae. http://www.montpellierinrafr/CBGP/spmweb. Accessed 30 Jan 2013

Miresmailli S, Bradbury R, Isman MB (2006) Comparative toxicity of *Rosmarinus officinalis* L essential oil and blends of its major constituents against *Tetranychus urticae* Koch (Acari: Tetranychidae) on two different host plants. Pest Manag Sci 62:366–371. doi:10.1002/ps.1157

Mitchell RB, Aberneth RJ (1993) Tolerance of clary sage, coriander and araway to herbicides applied pre- and post-emergence. In: Proceedings of 46th New Zealand plant protection conference, Christchurch, 10–12 Aug 1993, pp 24–29

Mittal VP, Butani PG (1995) Chemical control of seed midge (*Systole albipennis* Walker) infesting stored fennel. Gujarat Agric Univ Res J 21(1):189–192

Navon A, Keren S, Levski S, Grinstein A, Riven Y (1997) Granular feeding baits based on *Bacillus thuringiensis* products for the control of lepidopterous pests. Phytoparasitica 25(1):101–110

Ntalli NG, Caboni P (2012a) Botanical nematicides: a review. J Agric Food Chem 60(40):9929–9940. doi:10.1021/jf303107j

Ntalli NG, Caboni P (2012b) Botanical nematicides in the Mediterranean Basin. Phytochem Rev. doi:10.1007/s11101-012-9254-4

OEPP/EPPO (2007) Phytotoxicity assessment. EPPO Bull/Bull OEPP 37:4–10

Opit GP, Nechols JR, Margolies DC (2004) Biological control of twospotted spider mites, *Tetranychus urticae* Koch (Acari: Tetranychidae), using *Phytoseiulus persimilis* Athias-Henriot (Acari: Phytoseidae) on ivy geranium: assessment of predator release ratios. Biol Control 29(3):445–452

Pank F (1992) The influence of chemical weed control on quality characters of medicinal and aromatic plants. Acta Horticult 306:145–154

Pank F (1993) Methods of contemporary large scale cultivation of medicinal and aromatic plants. Acta Horticult 331:89–108

Parbery DG (1996) Trophism and the ecology of fungi associated with plants. Biol Rev 71:473–527

Pasqualini E (1994) Use of *Bacillus thuringiensis* in Italy: current status. In: Salama HS, Morris ON, Rached E (eds) The biopesticide *Bacillus thuringiensis* and its applications in developing countries. Proceedings of the International workshop Cairo, 4–6 Nov 1993, pp 53–57

Patel RC, Yadav DN, Dodia JF, Patel AR (1986) On the occurrence of *Systole albipennis* Walker (Hymenoptera: Eurytomidae) infesting fennel in Gujarat. Gujarat Agric Univ Res J 12(1):51–52

Pillonel N, Fischer S, Baroffio C (2012) Cause del deperimento della camomilla romana. Rev Suisse Vitic Arboric Hortic 44(4):234–241

Pollini A (1998) Manuale di entomologia applicata. Edagricole, Bologna, p 1462 (in Italian)

Ragusa Di Chiara S, Tsolakis H, Ragusa E, Alonzo G, Saiano F (2007) Effects of some botanical pesticides on *Tetranychus urticae* Koch (Acariformes, Tetranychidae) and its predator *Cydnodromus californicus* (McGregor) (Parasitiformes, Phytoseiidae) in laboratory trials. In: Acarology XI Merida, 8–13 Sept 2002, Societad Latinoamericana de Acarologia 11:347–354. ISBN/ISSN: 978-970-32-4451-5

Raun ES, Sutter GR, Revelo MA (1966) Ecological factors affecting the pathogenicity of *Bacillus thuringiensis* var *thuringiensis* to the European corn borer and fall armyworm. J Invertebr Pathol 8:365–375

Regnault-Roger C (1997) The potential of botanical essential oils for insect pest control. Integr Pest Manag Rev 2:25–34

Reuveni R, Dudai N, Putiesky E, Elmer WH, Wick RL (1997) Evaluation and identification of basil germplasm for resistance to *Fusarium oxysporum* fsp *basilicam*. Plant Dis 81:1077–1081

Rodeva R, Gabler J (2004) First report of *Phomopsis diachenii* in Bulgaria. Mycol Balc 1:153–157

Sagar P, Kumar N (1996) Natural enemies of *Hyadaphis coriandri* and feeding rate of *Coccinella septempunctata* on it in the Punjab. Int Pest Control 38(1):26–27

Salamone A, Scarito G, Camerata Scovazzo G, Fascella G (2009) Control of powdery mildew in cut roses using natural products in the greenhouse. Floric Ornam Biotechnol 3(Special Issue 1): 121–125, Global Science Books

Schippmann U, Leaman DJ, Cunningham AB (2002) Impact of cultivation and gathering of medicinal plants on biodiversity: global trends and issues. In: FAO (ed) Biodiversity and the ecosystem approach in agriculture, forestry and fisheries. Satellite event on the occasion of the ninth regular session of the commission on genetic resources for food and agriculture, 12–13 Oct 2002, Inter-departmental working group on biological diversity for food and agriculture, Rome, p 21

Simon JE, Chadwick AF, Craker LE (1984) Herbs: an indexed bibliography 1971–1980. The scientific literature on selected herbs, and aromatic and medicinal plants of the temperate zone. Archon Books, Hamden

Singhania SP (2001) Agrotechnology of some essential oil yielding plants of commercial importance in Assam. In: Sahoo S, Ramesh DB, Rao YR, Debata BK, Misra VN (eds) Conservation and utilization of medicinal plants. Allied Publishers, New Delhi, pp 149–158

Sivesind EC, Leblanc ML, Cloutier DC, Seguin P, Stewart KA (2009) Weed response to flame weeding at different developmental stages. Weed Technol 3(23):438–443. doi:10.1614/WT-08-155.1

Smith R, Lanini WT, Gaskell M, Mitchell J, Koike ST, Fouche C (2000) Weed management for organic crops. University of California, Division of Agriculture and Natural Resources, Publication 7250

Tóth Š, Danilovič M (2004) The rationalisation of selected medicinal herbs pesticidal control. In: Proceedings of the 3rd conference on medicinal and aromatic plants of Southeast European countries, 5–8 Sept 2004, Nitra, pp 29–30

Tsolakis H, Ragusa E (2004) Osservazioni su un caso di controllo biologico naturale di *Tetranychus urticae* Koch (Acariformes Tetranychidae) su fragola in serra a conduzione biologica. In: Proceedings of the XIX Italian National Congress of Entomology, Catania 10–15 June 2002, pp 1377–1381

Tsolakis H, Ragusa S (2008) Effects of a mixture of vegetable and essential oils and fatty acid potassium salts on *Tetranychus urticae* and *Phytoseiulus persimilis*. Ecotoxicol Environ Saf 70: 276–282. ISSN: 0147-6513

Týr Š, Vereš T (2011) Weed infestation of *Silybum marianum* (L) Gaertn canopies in the years 2008–2010. Acta fytotechnica et zootechnica Special number Nitra, Slovaca Universitas Agriculturae Nitriae, pp 46–48

Tzanetakis IE, Mackey IC, Martin RR (2005) Tulip virus X(TVX) associated with lemon balm *Melissa officinalis* variegation: first report of TVX in the USA. Plant Pathol 54:562

WHO (2003) WHO guidelines on good agricultural and collection practices (GACP) for medicinal plants. World Health Organization, Geneva, p 72

Wichura A, Braun U, Weber RWS, Hildebrands A (2012) *Golovinomyces orontii* and other powdery mildews on *Rosmarinus officinalis*. Plant Pathol Quar 2(2):162–166

Wider TL, Abawi GS (2000) Mechanism of suppression of *Meloidogyne hapla* and its damage by a green manure of Sudan grass. Plant Dis 84:562–568

Zalewska E (2010) Pathogenicity of *Colletotrichum dematium* (Fr) Grove to caraway *Carum carvi* L. Acta Agrobot 63(1):137–147

Zheljazkov VD, Topalov V (1992) Effect of mechanical and chemical weed control on the growth, development and productivity of Mentha growing for planting material. In: Proceedings of the 23rd international symposium on essential oils, SAC, Auchincruive, Ayr, Scotland, 9–12 Sept 1992

Zheljazkov V, Yankov B, Topalov V (1996) Effect of mechanical and chemical weed control on the growth, development and productivity of *Mentha piperita* and *M arvensis* var *piperascens* grown for planting material. J Essent Oil Res 8:171–176

Zheljazkov V, Zhalnov I, Nedkov NK (2006) Herbicides for weed control in Blessed Thistle (*Silybum marianum*). Weed Technol 4(20):1030–1034

Zimowska B (2008) Fungi threatening the cultivation of sage (*Salvia officinalis* L) in south-eastern Poland. Herba Pol 54(1):15–24

Zimowska B (2011) Conidiogenesis of *Phoma strasseri* the fungus responsible for black stem and rhizomes rot in peppermint (*Mentha piperita*). Acta Sci Pol-Hortorum Cultus 10:171–178

Zumelzú G, Darré C, Novo RJ, Bracamonte RE (2001) Preemergent control of annual weeds in oregano (*Origanum vulgare* L). Acta Horticult 502:181–185

Chapter 12
Introduction to the Precision Medicinal Plant Production

Miklós Neményi

Abstract Precision site specific plant production technologies are becoming more and more widespread in countries with developed agriculture. It is not surprising that in the field of medicinal crop production the need of applying precision methods arising, too. The use of this new technique and technology can only be successful if it serves sustainable production and is used for the purpose of ecological conservation. Interestingly enough, this latter area is more advanced in the field of annual and perennial forest herbs as well as in description of interplant competition than in up-to-date (precision) plant production technologies. A number of new methods of how to use remote sensing in ecological research can be learned from medicinal herbs sciences. The chapter describes the possibilities of use of precision crop production technologies in the cultivation and hand harvesting of medicinal plants.

Keywords GIS • GPS • Remote sensing • Ecophysiological models • Sustainability and diversity • Site specific plant management

1 Introduction

The definition of precision plant production from the engineering and IT aspect is fundamentally not focused on defining the word "precision", but rather on concentrating on the collection and management of data based on site-specific i.e. positioned means. A given technology can be very precise; however it cannot be called part of precision plant production if the various circumstances (soil properties, topological differences, earlier treatments, etc.) within spatially diverse treatment units (management zones) are not treated differently.

Accurate data collection and treatment concerning technical aspects differ from conventional plant production in precision plant production, since in precision plant production the technological changes have to be not only spatially located but also

M. Neményi (✉)
Faculty of Agriculture and Food Science, University of West Hungary, Vár, 2, Mosonmagyaróvár, Hungary
e-mail: nemenyim@mtk.nyme.hu

Á. Máthé (ed.), *Medicinal and Aromatic Plants of the World*, Medicinal and Aromatic Plants of the World 1, DOI 10.1007/978-94-017-9810-5_12

maintain the level of accuracy of detection and treatment in accordance with site specific plans.

However, as the economic aspects cannot be neglected, the required accuracy has to be examined carefully in order to reach the goal of precision plant production. The experiments proved that the biological systems are able to tolerate specific inaccuracies; moreover they can correct the inaccuracies.

From the technological aspect nearly everything could be achieved concerning accuracy, but at the same time increasing accuracy involves rising operational costs.

Inside the field variability regarding fertility conditions have been known for a long time, the knowledge of the older generation having been passed down to the next generation. Nevertheless precision plant production has opened up new horizons for both the promotion of environmental friendly, sustainable technologies and the establishment of a harmonic connection between natural and agro-ecological systems.

Precision plant production technologies provide the possibility for linking up the various production levels (from the individual plant through field, land, region to the global level), since the basic information is very precise (Barbaro et al. 2011; Kovács et al. 2014). At the same time the new technology improves the economic conditions while decreasing the environmental impact (Németh et al. 2007).

Conventional plant production treated the whole field as one homogeneous unit. The experiences showed that in many cases even 1 ha of a given field is too large to treat as one homogeneous management zone.

One of the most difficult tasks is the allocation of management zones within the field in which primarily soil physical, chemical and topological properties can be considered as a homogeneous unit. Fuzzy logic and neural networks (artificial intelligence) are used to solve such tasks. (Mike – Hegedűs F 2006). However, the tendency is to move from the management zones level to the individual crop level. The problem is that these parcels have only quasi homogeneous characteristics, and consequently we take the average values of different parameters into consideration. The solution is the on-the-go detection technologies, since in this way data acquisition is cost effective and large databases can be collected, therefore based on the data analysis the size of the homogeneous treatment units can be defined.

Based on the characteristics of the management zones the required treatments (e.g. amount of fertilizers, etc.) can be defined with the help of decision support models in such a way that the potential productivity is maximized, and at the same time the requirement of sustainability remains.

Precision crop production technology has not come into general use so far, although techniques and IT (Information Technology) would be available for application in this field of medicinal plant production as well.

At the same time regarding the pharmaceutical industry it would be extremely important to apply the knowledge of the variables in a given field. Therefore the need for a specific compound or compound group in the plants should be met, not neglecting the environmental and quality requirements and producing the maximum possible yield.

Fig. 12.1 Using microcopter for site specific crop management

It is a common fact that in practice site specific plant production technology is not able to reduce, for instance, the applied amount of crop protection agents significantly, although in the case of medicinal plant production it would be a significant step forward if it was possible to apply the agent within a very short time period after the infection or pests appear on the plant.

It can be stated that the required high standard of practical sustainable agriculture in medicinal plant protection has not been realized yet. For instance the resolution of the pictures provided by drone technology (UAV, Unmanned Aerial Vehicle technology, e.g. Microcopter) is good enough to detect relatively small objects from 40 to 50 m height it is possible to detect a 2–3 cm size object (Fig. 12.1). Therefore we are already close to being able to detect the insects by appearance immediately. Due to the quick response the applied amount of chemicals can be significantly decreased as well as potential loss arising from the quality of the produced plant.

One of the basic elements of precision technology, namely GIS (Geographical Information Systems) is widely applied in the monitoring of wild animals, in the case of investigation of range of their spreading or the TSA (Time Series Analysis) of their appearance. The tendency seems to be the opposite to the traditional plant monitoring: in the one case the investigation starts at the local (individual) level and moves towards the regional level, in the other case the investigation starts from the regional level and moves towards the local level, down to the level of each individual field or even smaller units.

2 Sustainability and Diversity

One of the possible definitions of sustainability can be found in the Brundtland Report (Our common future, 1987): “Humanity has the ability to make development sustainable to ensure that it meets the needs of the present without compromising the ability of future generations to meet their own needs.”

This was the most important contribution to the thinking on sustainability. To the social, political, economic, ecological and other aspects a totally new one has been added: namely, the emotional constraint of ethics. But from the agrotechnological and social points of view the definition above should be supplemented with the expectation of maximum utilization of arable land's fertility potential. It is important to note that hunger kills more people every year than AIDS, malaria and TB put together (FAO). At the same time the demand for naturally degradable raw materials for industry and the medicinal plants has also been increasing year by year.

The main pillars of the definition of sustainability in the natural environment are:

The maintenance of first and foremost ecosystem structures, diversity, functions, productivity (biomass production) over time despite the external stress being renewed steadily.

Morowitz (1968) in his book entitled Energy Flow in biology citing Bridgeman wrote: "It springs to eye that the tendency of living organisms is to organize their surroundings, that is, to produce 'order' where formerly there was disorder." Why do we not have the right to do the same?

Why don't we have the right to protect our culture and therefore to improve its life conditions in order to get more yield. The answer is given by Margulis (1998). In her book „Symbiotic Planet" she quotes from James E. Lovelock: "No organism feeds on its own waste....One organism's waste is another's food. Failing to distinguish anyone's food from someone else's waste, the Gaian system recycles matter on the global level...The sum of planetary life, Gaia, displays a physiology that we recognize as environmental regulation."

Main characteristics of sustainable agro-ecology are:

- CO_2 neutral production technologies;
- nutrient replacement and plant production without environmental pollution;
- avoidance of ground and super-terrestrial water nitrification, eutrophication and erosion;
- prevention of soil and air pollution;
- keeping the gradients (above all the diversity gradient) between the natural and agro ecosystems in the long term.

Consequently, the meanings of the terms "sustainable agricultural production" and "natural regulations" are very closely related. However, the two terms will be realised in different ways. The solution of the following tasks needs a dynamic approach, because the ecology is a dynamic system.

Nowadays there is no question that the use of precision crop production technologies is inevitable.

There is no question that present agricultural practice causes soil erosion and harmfully affects genetic and species diversity. Arable land and manipulated forests as well as other human management areas cover two thirds of the total land, at the same time the protected areas cover only 8 % of the surface. The remaining area is urbanized or wilderness.

Since the beginning of the last century, due to intensive farming practices 75 % of the genetic diversity has disappeared from the arable lands. If we would like to sustain biodiversity in the environment of arable land, we have to reverse the loss of global biodiversity.

On the other hand the most probably occurring climate change cannot be neglected, which will change agricultural technology and practice fundamentally. The decision makers at state level as well as the farmers have to take into account the indicators generating diversity increase, as well as technology development. Increase of diversity must be the task of the local communities (Saunier and Meganck 1995).

Fortunately the situation is not everywhere as critical as in the so called developed countries, therefore intensive research is carried out in order to survey the current situation, monitoring the changes to make the adequate intervention count. This is especially true for the medicinal plants. There are some very good examples for this:

In South Uganda 187 various medicinal plants were investigated from 163 different genera and 53 families. Different parts of these plants are used for medical preparation: leaves, shoots, roots, bark, twigs, sap, bulbs, flowers, seeds, internodes, and fruits. These plants were either cultivated in gardens or were collected from the wilderness. The author states that it has to be ensured that these medicinal plants are continuously available as raw materials (Ssegawa 2007). This means that instead of collecting the medicinal plant from the wilderness they have to be produced in controlled circumstances.

Quantitative analysis was carried out in Tekai Tembeling Forest Reserve (rain forest in Malaysia) with 6,788 individual trees and other plants, which meant 331 species, 179 genera and 87 families. The authors have come to the conclusion that as the forest is very rich and diverse in medicinal species, conservation assessment has to be started in order to increase the diversity of the medicinal species living in the area (Eswani et al. 2010).

Other investigations of plant diversity were carried out in the Manali Wildlife Sanctuary of North Western Himalayas, in order to estimate the potential of bio sources, identify the species, preferably the native or endemic and threatened medicinal plants. All together 270 different species have been investigated, which were 84 families and 197 genera. The most medicinal plant has been found between 2,000 and 2,800 m above sea level. As the altitude increased, the number of species decreased. In total, there were 62 native species and 98 endemic. The authors suggest a systematic monitoring in order to investigate the changes in the population as well as in the changes of habitat of the endangered medicinal plants (Rana Man and Samant 2011).

The above mentioned examples clearly show that for successful monitoring the most up to date monitoring devices have to be to solve the problem of conserving the diversity of medicinal plants.

3 GIS (Geographical Information System)

GIS (Geographical Information System) is basically a system for spatial information management, which helps to make informed and intelligent decision making. It is important to emphasize that well trained staff is required for data analysis for reliable information collection from the large databases. On the other hand for data process and analysis various mathematical methods are built into the GIS software (Fourie 2009). GIS from the users' point of view is a multi-level digital mapping system which is able to manipulate data stored at various layers, therefore required data can be collected and relations between the different layers can be investigated by mathematical methods (Fig. 12.2). Input data can be collected from various sources: satellite, airborne or UAV based image collection is widely applied for data collection, moreover close-to-surface (1–5 m height) images and hand collected soil samples are all important sources for GIS input.

GIS enables the integration of data sources into one processing software system. It has to be emphasized that GIS is a multi-dimensional system, which also integrates a special dimension, namely time (TSA, Time Series Analysis) if monitored data is imported into the software. The up-to-date GIS software is able to carry out sophisticated calculations, for instance erosion prediction in a small agricultural field or a larger region. GIS can also be a useful tool for seed and fertilizer production companies as well as for the farm chemical industry or crop insurance companies. GIS software is widely used in the governmental sector as well as at the research institutes, primarily universities. The fertilizer application level or other chemicals application in the different regions or counties can be evaluated. Risk analysis can also be carried out concerning damage to wildlife or crop stress.

For models applied in the ecosystem or agro-ecosystem GIS databases and data sources are required. Application of a spatial data analysis system can also be important for model validation. ESRI (Wyland 2008) established a program called Common Land Unit Program in 1997, which enables us to monitor special plants such as medical plants. One of the possibilities of the data processing is if we want to collect data regularly (monitoring) about a given plant ESRI, South Africa Fourie 2009). Based on the satellite images the outlines of the investigated fields were digitized, and randomly 45 by 45 m areas were designated where data for validation was collected. Subsequent data from all the fields were collected by means of aerial imagery in order to identify the various plant species. This process is followed by statistical analysis.

In case of medical plant monitoring growth rate characteristics of Viper's bugloss (Echium amoenum Fisch. et Mey.) were investigated in the Eshkevarat area located in the northern mountains of Iran, in order to investigate the environmental impact on the extension and preservation of the plant. It was also investigated how this plant influences the economic welfare of the rural society. Data were processed by means of GIS (Eslami and Kaviani 2011). The article clearly presents

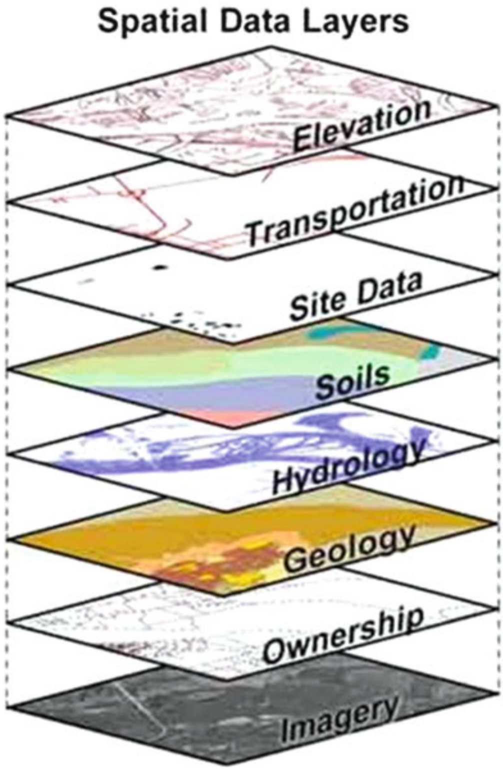


Fig. 12.2 Concept of GIS map layering. Greene R. P.: a strategy for integrating GIS (Source: http://www.niu.edu/ceet/strategicalliance/GIS.htm)

how those natural indicators can be connected to economic parameters by means of the application of GIS.

GIS was applied in the evaluation of the production potential of German Chamomile medicinal plant (Pirbalouti et al. 2012) in the north of Khuzestan province (Iran). Relevant factors were evaluated such as environment-components, soil chemical and physical characteristics (pH, EC: electrical conductivity and organic matter), climate factors (temperature and precipitation) and topography (DEM) at different spatial and temporal resolutions. Data evaluation was carried out by ILWIS (version academic 3.0) GIS software, where 4 categories were concluded. The results of maps in this study identified that 0 %, 1.5 % (15868.35 ha), 32.7 % (345,930 ha) and 65.8 % (696091.6 ha) of land have currently highly suitable (S1), moderately suitable (S2) and marginally suitable (S3) and not suitable (N) for chamomile crop production in north of Khuzestan, respectively. Further information on this subject (GIS) can be collected from the articles: Brase (2006) and Neményi et al. (2003).

4 GPS (Global Positioning System)

More and more accurate positioning of the collected data is required, if GIS is applied for plant production, environmental or ecological data processing. However data collection (detection and sensoring) and application processes (fertilizer of chemical applications, seeding, etc.) require increasing accuracy of positioning. Nowadays on-the-go technology is widespread. This technology means there is a very short time (2–3 s) between the detection and application. During this time calculation of the required dose has to be carried out and application has to be started with very high accuracy.

The first and most advanced satellite based positioning system is called NAVSTAR GPS or GPS system for short. The most important feature of the system is based on the signals sent from various satellites orbiting around the Earth. The GPS receiver locates its position based on time and distance calculations. For the NAVSTAR system the satellites are moving in six orbits, in each orbit there is a minimum of six satellites, therefore in all cases 5 to 11 satellites are visible for the users. As the older satellites have been working for a longer time than expected, there are now 31 functioning satellites sending the positioning signals towards the receivers (Fig. 12.3).

There are other GNSS (Global Navigation Satellite Systems) services: The GLONASS is commanded by Russia, the number of orbits is 3 and there are 8 satellites planned for each orbit. Both systems are operated by the military; however some open signals are available for civilian users as well. The most recent GNSS called Galileo differs from the earlier mentioned ones in a very important way, namely it was planned to be operated by civilian bodies. This system is also planned to have 3 orbits with 10 satellites in each orbit and is expected to be available for the users in 2019.

In satellite technology control segments (ground antennas) are required in order to monitor the accuracy of the positioning. There are several ground antennas for the NAVSTAR GPS system, the most important one is at Colorado Springs, which is the Master Control Station (MCS) for the whole system (Fig. 12.4). The most important task of the ground control station system is to synchronize the atomic clocks on board the satellites to within a few nanoseconds of each other, and to adjust the ephemeris of each satellite's internal orbital model.

In order to have a correct position at least four satellites have to be visible for the users. The positioning is based on measuring distance and time. During positioning we know our distance from various satellites. From each satellite the distance can be only on a surface of a sphere, however if two spheres intersect we can narrow our position into a circle. If we measure our distance from a third satellite, we know that our location is one of two points where the three spheres intersect. Theoretically the fourth sphere is the surface of the Earth itself, however it is more accurate to calculate the distance from the fourth satellite, therefore one of the two remaining points will be designated. The atomic clocks on the satellites are very accurate (1 sec difference is expected in each 300,000–3,000,000 Year). The clock in the receiver is much more inaccurate, therefore the more satellites are visible the more precise the accuracy is going to be.

Fig. 12.3 NAVSTAR GPS constellation, 31 satellites in 6 orbital planes (Source: http://www.ngs.noaa.gov/CORS/)

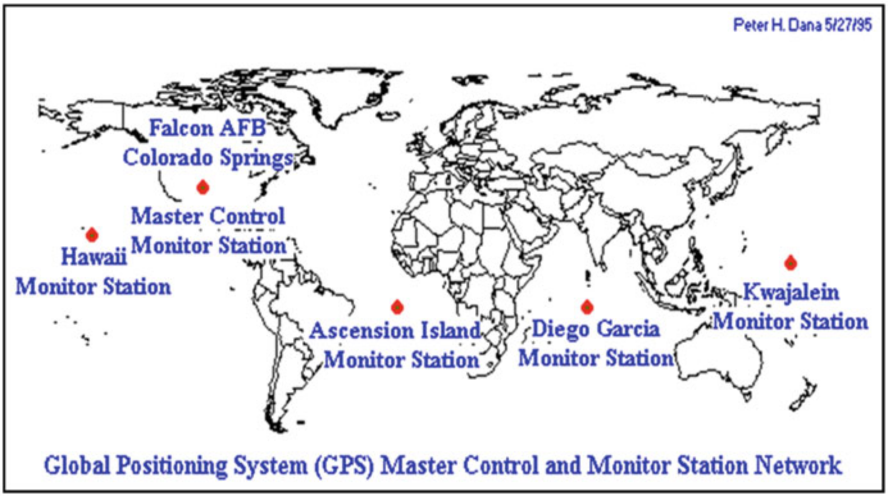


Fig. 12.4 Global Positioning System (GPS) Master Control and Monitor Station Network

Accuracy is measured by two different methods. The so called real time measurement is when the detection of the phenomena and the required action happen at the same time (or within very short time frame). The accuracy of the satellite positioning without any correction is 5–15 m. This accuracy can be increased by various technologies. The geostationary satellites (EGNOS in Europe, Fig. 12.5) or ground based technologies (different services are available regionally or locally at the country level) provide 2–5 m accuracy for positioning. In ideal cases the accuracy can be as good as submeter, however these systems cannot guarantee

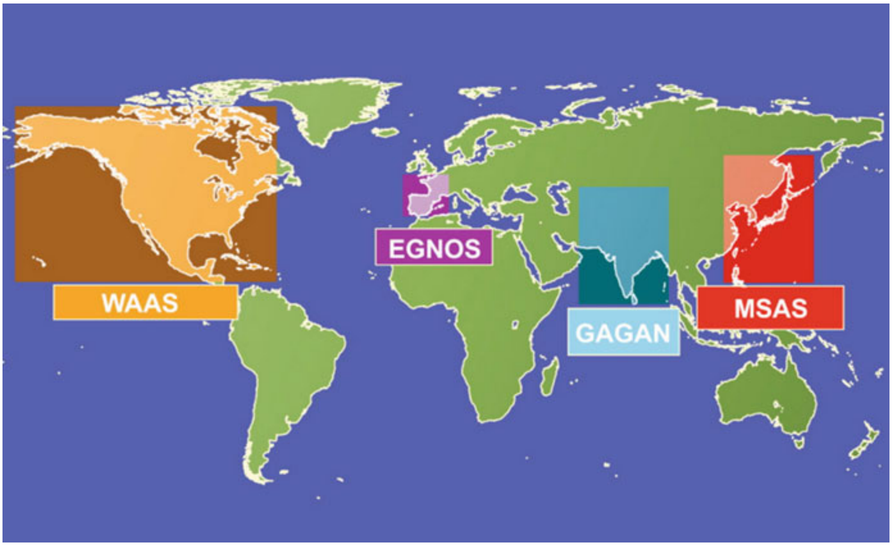


Fig. 12.5 Satellite-Based Augmentation Systems: SBAS (European Space Agency)

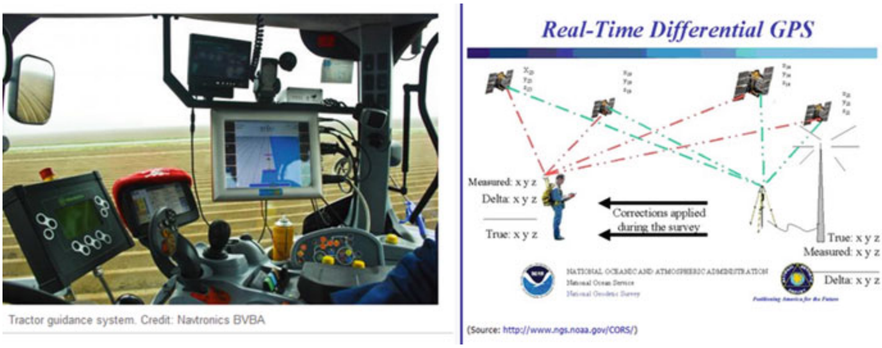


Fig. 12.6 RTK tractor guidance system (Smart Farming May 16, 2013)

this level of accuracy at all. The guaranteed level of accuracy (around 2 m) can be good enough in the case of data collection for yield of fertilizer application; however the need for a more accurate positioning arises in other operations such as seeding or auto steering.

The characteristics of precision agriculture are changing from being only site-specific to very accurate as well. Applying special technologies, such as RTK (Real Time Kinematic) techniques with someone's own base and rover station or using the most accurate positioning service sub-inch (below ±2.5 cm) precision is available (Fig. 12.6). This means that for seeding, mechanical plant cultivation or chemical plant protection a programmed route can be followed by means of auto steering. One of the greatest advantages of this technology is that the tracks for the power-machines can be varied, therefore soil compaction can be avoided. It is also important to emphasize that the increase of accuracy in precision technology also provides economic and environmental benefits.

5 Remote Sensing

As mentioned earlier GIS provides useful information where the source data is precise enough for the data process. The earlier mentioned ESRI methods, where plant species are monitored from an airplane are going to be overtaken by other remote sensing detecting technologies, which will significantly reduce the number of checking. If one thinks about the conservation of diversity, the problem is well described by Damayanti et al. 2011. Until 2001 in Indonesia only 0.05 % of medicinal plants had been identified or data available on them. The following fundamental problems occurred: 1. The number of locals who would be able to recognize the plants are limited as they do not understand the essential differences in the medicinal plants concerning diversity, 2. The number of books and guides are limited, there are not enough taxonomists in these institutes who would be scientifically trained to carry out this work. It is also a crucial problem that this work is time and labour consuming. The authors encourage the younger generation to study and specialize more in this field. They also argue there would be a need for more experts in the research institutes. Scientists in computer and information technologies have to learn how taxonomists identify various medicinal plants. It is clear from the above mentioned example that this problem exists in most tropical countries, and the solution for this could be remote sensing.

Remote sensing is based on the physical phenomena of spectral reflectance and thermal emittance.

It is a non-destructive monitoring technology for providing spatial and temporal parameters that influence the biological processes. In plant production the remote sensing technologies supply site specific information for crop management, i.e. this is a fundamental technological part of precision crop production.

By applying remote sensing technologies a yield prediction can be carried out. This technology is also a basic data source for weed monitoring and in-time detection of diseases and pests. Nutrient, pest management and irrigation scheduling can be applied. One of the great advantages of remote sensing technology is that it is possible to monitor the surroundings of the investigated field as well. By doing so we fulfil the requirements of sustainability. The database provided by remote sensing is necessary for physiological modelling creation of plant growth. Moreover it contributes to the accuracy increase of such models. (This will be discussed in more detail later.)

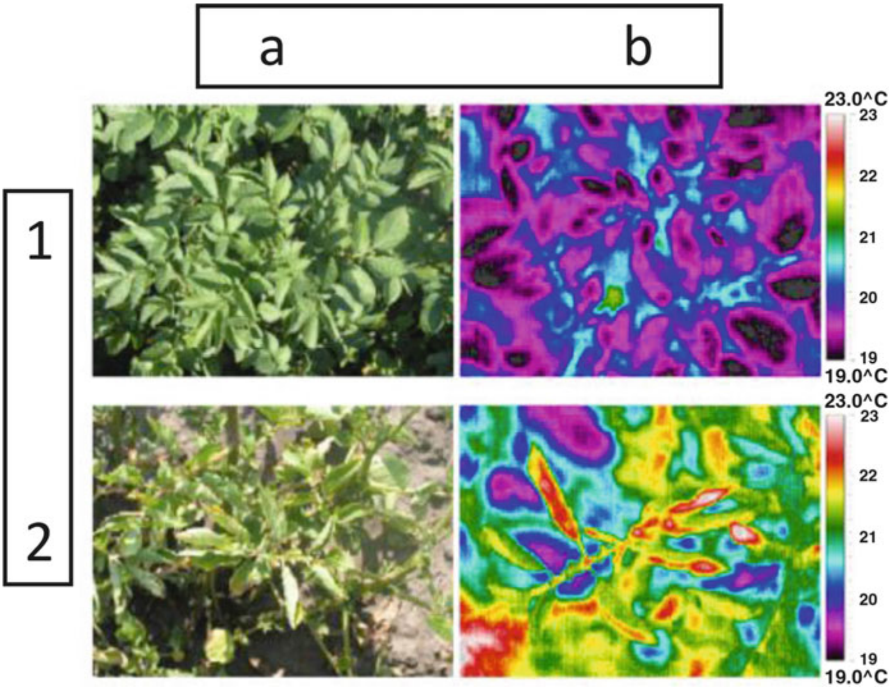


Fig. 12.7 CCD (**a**) and infrared image (**b**) of healthy (1) and virus infected (2) potato plants (Mesterházi 2004)

The main point of the detection of spectral reflectance characteristics of leaves method is that reflectance depends on the state of the leaves in natural light. Green leaf area shows low reflectance in the visible range of the spectrum, whereas in a stressed situation, for instance due to low level of alimentary substances, high reflectance is typical in NIR (Near Infrared Reflectance) range. This is the basic concept of applying vegetation indices. One of the most common vegetation indices is NDVI (Normalized Differential Vegetation Index) which is calculated as (NDVI = NIR-RED/NIR + RED) or RVI (Ratio Vegetation Index) which is calculated as (RVI = NIR/RED). Both indices are applied for crop canopy analysis. All materials are emitting energy in the thermal infrared range (8–14 μ) on Earth. This phenomenon is able to detect physical changes in various fields, as evaporation highly influences the temperature of the surface. The leaves or the stalk of a diseased plant evaporate less than the healthy ones, therefore temperature differences appear (Fig. 12.7). The situation is the same in distinguishing weeds from soil or various plant cultures (Pinter et al. 2003; Mesterházi 2004).

In remote sensing there are two different approaches: multispectral and hyperspectral imaging. Multispectral images (mainly applied in satellite technology) divide the visible range into 3–6 bands. Multispectral imaging systems are

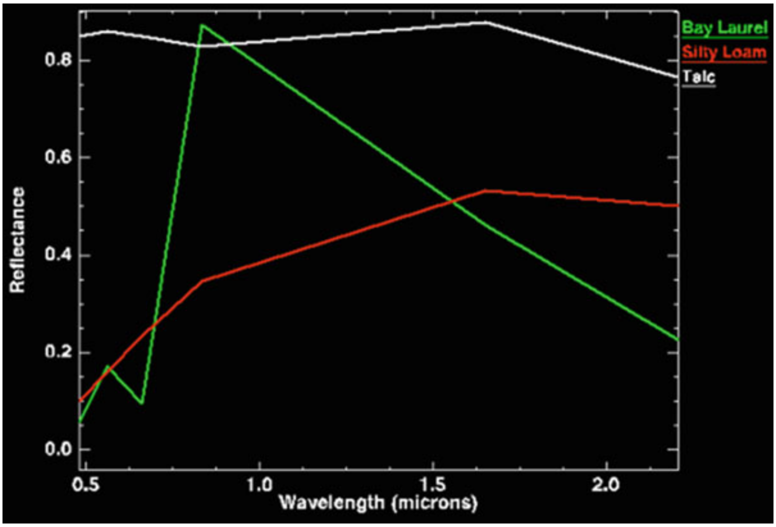


Fig. 12.8 Reflectance spectra of a green bay laurel leaf, the mineral talc, and a silty loam soil provided by multispectral *Landsat 7 ETM* sensor (Shippert 2003)

able to differentiate between various vegetation and soil types, clear and turbid water and selected man-made objects (Fig. 12.8).

Hyperspectral technology on the other hand divides the reflected electromagnetic spectrum into very narrow bands (of even only one nm band), being able to provide a continuous spectra in the visible, NIR, mid-infrared and thermal infrared portions (Govender et al. 2007; Shippert 2003) (Fig. 12.9).

As the use of drugs is increasing dramatically; it is essential to have precise information about the Cannabis plant to increase prevention. Azaria et al. (2009) applied hyperspectral imaging technology for recognizing cannabis in laboratory circumstances, oblique view (25–80 m) and airborne (AISA EAGLE 400–1,000 m) respectively. Collected data was evaluated by means of PCA (Principal Component Analysis). It has been concluded that bands in 530–550 and 670–680 nm (leaf and canopy) are the best to recognize Cannabis.

6 Plant Disease Detection

6.1 Weed and Plant Disease Detection

Spectral Camera HS can also be applied for detecting two different weeds in wheat and chickpea. As Fig. 12.10 shows, differences in 400 and 1,000 μm range appear, weeds can be differentiated both from one another as well as from the cultivated plants (Shapira et al. 2010).

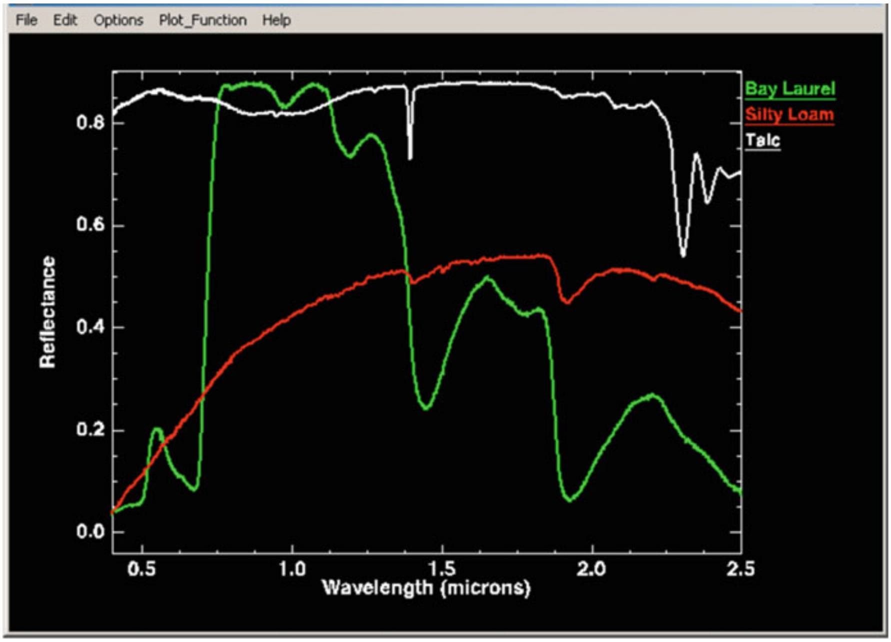
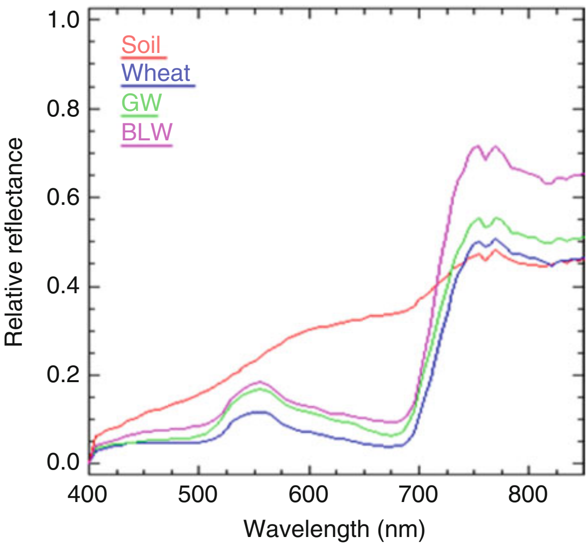


Fig. 12.9 Hyperspectral reflectance spectra measured by laboratory spectrometers for three materials: a green bay laurel leaf, the mineral talc, and a silty loam soil (Shippert 2003) (The *arrows* show the rapid changes in the spectra)

Fig. 12.10 Relative reflectance of wheat or chickpea grass weeds (GW), broad leaf weeds (BLW) vs wavelength (Shapira et al. 2010)



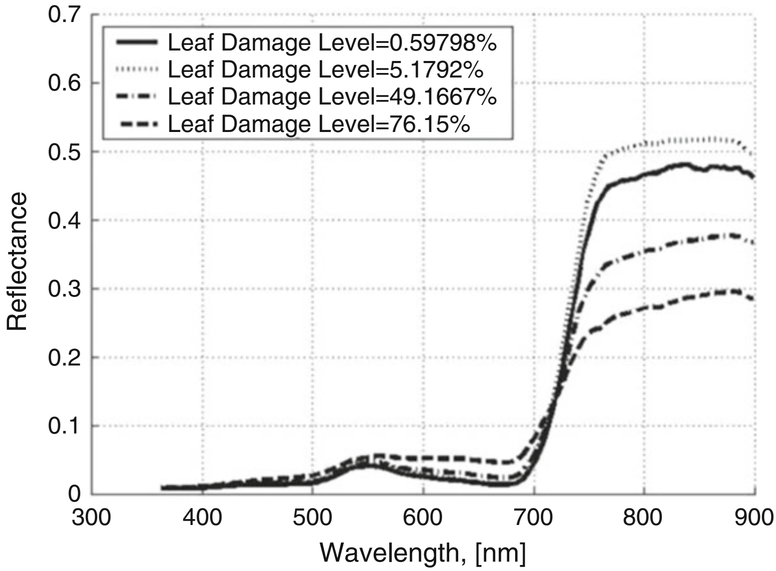


Fig. 12.11 Spectra response of a plant in different damage levels (Sgouros 2008)

In the event that more than one plant appears in a given field requiring identification machine vision technology has to be applied. Detailed information on methodology and practical application of computer vision is available in the work of Andersen (2002).

Content-based Image Retrieval (CBIR) system was used for description of shape and texture features. Fourier, generic Fourier and Gabor-Zernike descriptors the last one provided the highest retrieval efficiency (92 %) (Basavaraj et al. 2012).

Plant disease detection can be carried out by hyperspectral imaging or identification of differences in colour or shape of spots by computer vision based method. It is clearly visible in the figure that different diseases are separable in the NIR range (Fig. 12.11, Sgouros 2008).

7 Ecophysiological Models

The ecophysiological models can be used in two areas. One of them is the description of the increase of the populations in nature, the effect of the factors on growth with special regard to climatic factors. Using this method yield can be predicted. The model is used for the description of growth of different annual and perennial forest herbs as well as description of interplant competition (Popović and Lindquist 2010).

The Intercom model takes the following parameters into account: “geographical latitude, standard daily weather data (incident solar radiation, minimum and maximum temperature, rainfall, average wind speed, and vapour pressure, soil physical properties (field capacity) and numerous parameter values that describe the morphological and physiological characteristics of the species. Under conditions of adequate soil water supply, the water balance component of the model can be removed.”

The other possible application of the ecophysiological models is plant production. Primarily it is used for yield prediction, and the models are developed for this. Until now models for medicinal plant production do not exist. These models, parallel to the earlier mentioned ones, consider the technological elements such as soil cultivation and technological features of the earlier cultivated plants in the investigated field. The novel aspect in these models is that they are applied in site specific plant production, which means yield prediction is carried out site specifically (Nyéki et al. 2013).

8 Technical Environment of Precision Plant Production

Where the soil physical morphological and technological data is available artificial intelligence (fuzzy logic) is applied in order to decide the size and the location of the management zones. As mentioned earlier, within these zones the parameters are considered to be similar.

In order to carry out reliable yield prediction, knowledge about previous yield data is necessary. In practice yield and moisture monitoring devices are built into combine harvesters (Fig. 12.12).

Since the accuracy of grain yield and moisture content measurements is very important research continues (Csiba et al. 2013). Earlier the soil sample collection was very costly and scattering was high, therefore averaging made the data unreliable. A new technological development, namely on-the-go data collection makes data collection possible regarding numerous physical and chemical properties of the soil (Mouazen et al. 2013; Mesterházi 2004; Neményi et al. 2006; Nagy et al. 2013). Using these databases as well as using the data available from the climatic conditions monitoring, fertilisation replenishment plans are relatively easy to give to a specific management zone. It has to be mentioned that fertilization decision support models are continuously being developed (Nyéki et al. 2013). The up-to-date variable rate spreaders apply the nutrient components according to the fertilization plan in the case of solid, liquid or organic forms. Nowadays precision agriculture has enough data for variable rate planting and there are several seeding machines that are able to do this work. In site specific applications variable rate irrigation is also the focus of research which is in close connection with climate change (Kovács et al. 2014).

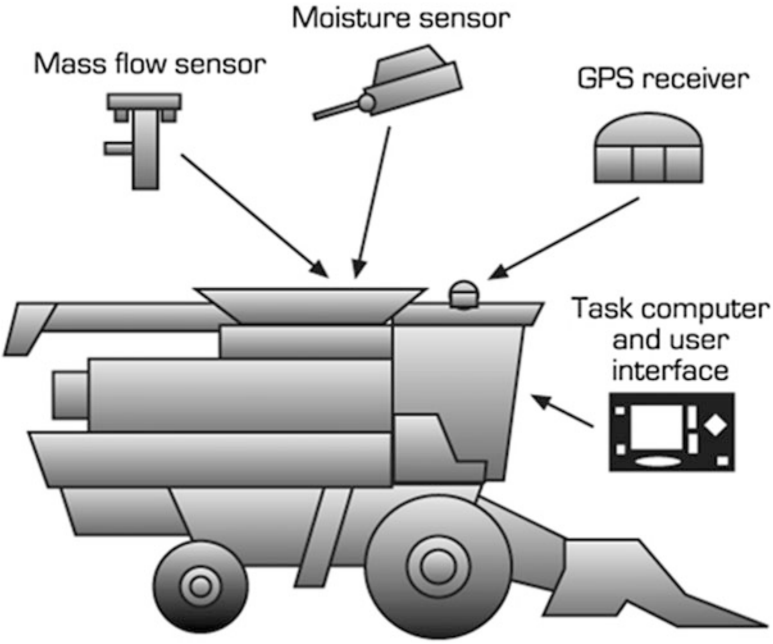


Fig. 12.12 Components of yield and grain moisture content mapping system in combine harvester (Grisso et al. 2009)

Precision plant production technologies in its full technological applications integrate precision weed control and pest management as well as site specific protection against diseases.

Precision management technologies can be carried out based on two different practical solutions: 1, by monitoring, when weed mapping and data collection is based on remote sensing technologies which can be UAV or ground based; in this case weed data is integrated into a program which later helps in GPS positioned site specific application; 2, on-the-go solution, when detection and application are carried out in nearly real time.

The various herbicides have traditionally been mixed with water in a tank. This is not an environmentally friendly method because the calculation of the amount of chemicals required for a field is very difficult and thus a given zone of field can be overstressed by double- spraying the rest. In the last decade map-based direct injection spraying has gained ground. In this system the first step is the identification and classification of weeds, the second step is weed mapping and choosing the appropriate chemicals, and finally filling them into tanks of sprayers. The map-based direct injection sprayer sprinkles only the adequate amount of chemical on the plot covered by weeds. Consequently, the up-to-date plant protection machines are able to protect the crops against various weeds with different chemicals. This technology enables significant reduction in the use of chemicals.

The latest state-of-the-art thermal micro-spray system developed by the researchers of Davis University (Zhang et al. 2012) can revolutionize nature-friendly weed control.

The hyperspectral machine vision system distinguishes weeds from plants in the row. It enables an intra-row weed management using food grade oil heated to approximately 160–180 °C. This procedure does not use polluting chemicals.

Another modern trend in the medicinal herbs production is the use of mechanical weed control, generally to avoid the residues of herbicides in medicinal plants. Using computer vision (visible and Near-infrared Reflectance) is the most suitable way of this form of weed control that leads the fight against weeds, not only in the row, but also among the individual plants, too.

The author is convinced that biologists, biosystems engineers, GIS scientists, chemists, physicists, plant-breeders, biotechnologists and last but not least agricultural engineers using their collective experience and knowledge can utilize the potential of a given field in an environmentally friendly and sustainable way. These thoughts are valid for precision medicinal plant production as well.

References

Andersen HJ (2002) Outdoor computer vision and weed control. Ph. D. Dissertation. Aalborg University

Azaria I, Goldshelger N, Ben-Dor E, Bar-Hamburger R (2009) Detection of cannabis plants by hyper-spectral remote sensing means. In: Proceedings of 6th EARsel SIG IS workshop (Imaging Spectroscopy) Tel –Aviv

Barbaro M, Rocca M, Danuso F (2011) A methodology for evaluating land suitability for medicinal plants at a regional level. Ital J Agron 6(4):e34

Basavaraj SA, Suvarna SN, Govardhan A (2012) Suitability of shape and texture features in retrieval of medicinal plants' images in Indian context. Int J Mach Learn Comput 2(6):848–854

Brase TA (2006) Precision agriculture. Thomson Delmar Learning, Clifton Park

Csiba M, Kovács AJ, Virág I, Neményi M (2013) The most common errors of capacitance grain moisture sensors: effect of volume change during harvest. Precision Agric 14:215–223

Damayanti EK, Hikmat A, Zuhud EAM (2011) Indonesian tropical medicinal plants diversity: problems and challenges in identification. International workshop, linking biodiversity and computer vision technology to enhance sustainable utilisation of Indonesian Tropical Medicinal Plants, Bogor, Augustus 11

Eslami A, Kaviani B (2011) The use of geographical information system (GIS) for evaluation in precision agriculture (in Hungarian). Ph.D. Thesis, University of West Hungary, Mosonmagyaróvár

Eswani N, Kudus KA, Nazre M, Noor Awang AG, Ali M (2010) Medicinal plant diversity and vegetation analysis of logged over hill forest of Tekai Tembeling forest reserve, Jerantut, Pahang. J Agric Sci 2(3):189–210

Fourie A (2009) Better crop estimates in South Africa. Winter issue of ArcUser magazine

Grisso R, Alley M, McClellan P (2009) Precision farming tools: yield monitor. Virginia Cooperative Extension. Publication: 442–502

Govender M, Chetty K, Bulcock H (2007) A review of hyperspectral remote sensing and its application in vegetation and water resource studies, Water vol 33 No 2

Kovács AJ, Nyéki A, Milics G, Neményi M (2014) Climate change and sustainable precision crop production with regard to maize (zea mays l.). In: Proceeding of international conference on precision agriculture. Sacramento, 20–23 July 2014

Margulis L (1998) The symbiotic planet. Weidenfeld and Nicolson, London, p 146

Mesterházi PÁ (2004) Development of measurement technique for GPS-aided Plant production. Ph.D. Thesis, University of West Hungary, Mosonmagyaróvár

Mike-Hegedűs F (2006) Applying fuzzy logic and neural networks to database evaluation in precision agriculture. Ph.D. Theses. University of West Hungary, Mosonmagyaróvár

Morowitz HJ (1968) Energy flow in biology. Academic Press, New York, p 179

Mouazen AM, Alhwaimel SA, Kuang B, Waine TW (2013) Fusion of data from multiple soil sensors for the delineation for water holding capacity zones. In: Stafford JV (ed) Precision agriculture '13. Wageningen Academic Publishers, Leida, 7–11 July 2013, pp 745–751

Nagy V, Milics G, Smuk N, Kovács AJ, Balla I, Jolánkai M, Deákvári J, Szalay KD, Fenyvesi L, Štekauerová V, Wilhelm Z, Rajkai K, Németh T, Neményi M (2013) Continuous field soil moisture content mapping by means of apparent electrical conductivity (ECa) measurement. Hydrol Hydromech 61(4):305–312

Neményi M, Mesterházi Á, Zs P, Zs S (2003) The role of GIS and GPS in precision farming. Comput Electron Agric 40:45–55

Neményi M, Mesterházi Á, Milics G (2006) An application of tillage force mapping as a cropping management tool. Biosyst Eng 94:351–357

Németh T, Neményi M, Harnos ZS (2007) Methodology of precision agriculture (In Hungarian). JATE Press, Szeged

Nyéki A, Milics G, Kovács AJ, Neményi M (2013) Improving yield advisory models for precision agriculture with special regards to soil compaction in maize production. In: Stafford JV (ed) Precision agriculture '13. Wageningen Academic Publishers, Leida, 7–11 July 2013, pp 443–451

Pinter PJ, Hatfield JL, Schepers JS, Barners EM, Moran MS, Daughtry CST, Upchurch DR (2003) Remote sensing for crop management. Photogramm Eng Remote Sens 69(6):647–664

Pirbalouti AG, Bahrami M, Golparvar AR, Abdollah K (2012) GIS based land suitability assessment for German chamomile production. Bulg J Agric Sci 17(1): 93–98. Agricultural Academy

Popović ZS, Lindquist JL (2010) Evaluation of the intercom model for predicting growth of forest herbs. Arch Biol Sci Belgrade 62(1):175–183

Rana Man S, Samant SS (2011) Diversity, indigenous uses and conservation status of medical plants in Manali wildlife sanctuary, North western Himalaya. Indian J Tradit Knowl 10(3):439–459

Saunier RE, Meganck RA (1995) Conservation of biodiversity and the new regional planning. Department of Regional Development and Environment Executive Secretariat for Economic and Social Affairs General Secretariat, Organization of American States

Sgouros A (2008) Hyperspectral imaging and spectral classification algorithms in plant pathology. Ph.D. Thesis, Technical University of Crete

Shapira U, Herrman I, Karnieli A, Bonfil J D (2010) Weeds detection by ground-level hyperspectral data. ISPRS Archive Vol. XXXVIII, Part 4-8-2-W9, Core Spatia. Databases–updating, maintenance, and services-from theory to practice. Haifa, 2010

Shippert P (2003) Introduction to hyperspectral image analysis. Online J Space Commun 3:1–13

Ssegawa P (2007) Medicinal plants diversity, uses and ecological factors that influence their distribution in the Sango Bay area, Southern Uganda. Ph.D. Thesis, University Makerere the extension of *Echium amoenum* Fisch. et Mey., a medicinal plant, in the Northern part of Iran. J Med Plants Res 5(10):2107–2109

Wyland J (2008) Agribusiness grows with crop-specific maps. Sept issue of ArcWatch magazine

Zhang Y, Slaughter DC, Staab ES, Giles DK, Downey D (2012) Automated weed control in organic row crops using hyperspectral species identification and thermal micro-dosing. Crop Protection 41:96–1005

Chapter 13
Medicinal Plants to Control Diseases and Pests

Vera Sergeeva

Abstract The control of pests and diseases continues to attract interest and requires a high level of attention. Pest control is fundamental to sustainable crop production in terms of both quality and quantity. Environmental protection of ecosystems and biodiversity is now also factored into the equation. Modern pest management utilizes a variety of methods to prevent crop loss and maintain yield quality. Increasingly popular among them is the use of plant extracts and essential oils, known as a natural source of antimicrobials. Plant oils have antibacterial, antifungal, antiviral, antiparasitic, antifeedant, antitermitic and antinematicidal properties; thus these biologically active natural products from essential oils and plant extracts have the potential to replace synthetic pesticides in the management of fungal rotting in fruits and vegetables, suppression of soil-borne plant diseases, and the control of pests in stored product and in the field. The discovery of natural products with low mammalian and environmental toxicity for integrated pest management (IPM) will ensure a safe and reliable food supply into the future.

Keywords Extracts • Essential oils • Phytochemicals • Biopesticides • Pest control • Commercialization

1 Introduction

The United Nations Food and Agricultural Organization (FAO) estimates the annual loss of food crops to pests and diseases as 20–25 % of potential world yield (Dubey et al. 2008). Despite impact given to agriculture the experts believe that about 20–60 % of our produce is lost every year in post-harvest stages during transit and storage (Babu et al. 2003). An estimated one third of global agricultural production valued at several billion dollars is destroyed annually by over 20,000 species of pests in field and storage (Mariapackiam and Ignacimuthu 2008). Plant

V. Sergeeva (✉)
School of Science and Health, University of Western Sydney, Locked Bag 1797,
2751 Penrith, NSW, Australia
e-mail: sergeeva@tpg.com.au

Á. Máthé (ed.), *Medicinal and Aromatic Plants of the World*, Medicinal
and Aromatic Plants of the World 1, DOI 10.1007/978-94-017-9810-5_13

protection therefore plays an extremely important role in increasing the production of agricultural crops for our growing populations.

A number of methods are used for pest management. In recent years medicinal plants have been widely studied, as the bioactive compounds they contain are known to inhibit or retard the growth of microorganisms. The current increasing demand for healthy food and sustainable agriculture has seen a parallel increase in interest in the use of oils and medicinal plant extracts to replace conventional chemical pesticides in control pests and diseases. Bioactive compounds of plants are generally extracted in the aqueous form, using ethanol or other organic solvents, or by steam when present in the essential oils (Khoddami et al. 2013).

The ability of plants to produce various secondary metabolites may lead to a significant reduction of pest population dynamics via new and efficient plant-extract based botanical pesticides, providing natural protection against agricultural pests (Isman 2006; Pino et al. 2013). A group of compounds classified as allomones show a broad spectrum of activity against plant pathogenic fungi and pest insects, ranging from insecticidal, antifeedant, repellent, oviposition deterrent, growth regulatory and antivector activities (Dubey 2011; Ebadollahi 2013). The effectiveness of these substances has resulted in the development of new botanical pesticides safe for both the environment and human health (Koul and Walia 2009; Ebadollahi 2013).

2 Historical Background

The benefits of essential oils have been recognised for thousands of years. Their use in anointing rituals and healing the sick is described in the New Testament of the Bible, and as early as 2500 BC herbs and oils were used to protect seeds and stored grain from insect attack. The first botanical insecticide dates back to the seventeenth century, when it was shown that nicotine from tobacco leaves killed plum beetles (Raja 2014).

Having observed that some plants protect themselves better than others, humans also developed the use of plants as pesticides. Historically, botanicals were used before other kinds of pesticides. They are mentioned in Hieroglyph, Chinese, Greek and Roman antiquity and also in India, where the use of the neem tree (*Azadirachta indica* Juss.; Meliaceae) was reported in the Veda, a body of manuscripts written in archaic Sanskrit dated at least 4,000 years ago (Philogène et al. 2005; Koul and Walia 2009). It is thus difficult to assess exactly where and when plants or plant extracts were systematically used in plant protection or, more generally, in agriculture. In the eighteenth century, some publications dealt with plant-based formulations to control insect pests (Shepard 1951). At the end of the nineteenth century, methods including the use of toxic plants or minerals, oils, tars, sulfocalcic sprays, boiling water and so forth were commonly put into practice (Philogène et al. 1984). Integration of empirical and scientific observations led to the development of plant extracts.

The first botanicals and allelochemicals to be used as pesticides came from easily available products. Pest insects were targeted more than pathogens because they could be easily identified. In last years published numerous papers: 61 articles in 1980 and more than 1,250 articles in 1980–2014; several recent books and chapters have reviewed biopesticides of plant origin (Koul and Dhaliwal 2001; Regnault-Roger et al. 2005; Giordano and Costs 2013; Isman and Grieneisen 2014).

Before WWII four main groups of compounds were commonly used: nicotine and alkaloids from tobacco plant *Nicotiana tabacum*, rotenone and rotenoids from *Lonchocarpus sp*., pyrethrum and pyrethrins from *Chrysanthemum cinerariifolium*, and vegetable oils. The use of these substances, because of their toxicity to nontargeted species (nicotine) or the instability of molecules (pyrethrum), decreased with the commercialization of chemically synthesized insecticides developed during the war (organochlorides, organo phosphates, and carbamates), which were easier to handle and less expensive. This situation continued until the 1960s (Alburo and Olofson 1987).

The negative effects on non-target species and environmental risks resulting from the massive use of chemical pesticides created renewed interest for botanicals, however, and, while substantially greater effort was devoted to the development of new synthesized pesticides, research on biopesticides of plant origin was actively pursued throughout the second half of the twentieth century (Isman 2008; Ntalli and Menkissoglu-Spiroudi 2011). This is well illustrated by the development of pyrethrinoids, synthesized molecules derived from pyrethrum, and the neem tree.

Overall, however, modern use of botanical pesticides has been marginal compared with other control methods. That said, improvement in the understanding of plant allelochemical mechanisms of activity offer new prospects for their use in crop protection, as has the examination of the reasons behind their limited use. (Isman 2006; Dubey 2011).

3 Plants: Factories of Natural Chemicals

Botanicals are a rich source of organic chemicals on earth. The plant kingdom is, in effect, a natural laboratory in which a great number of chemicals are biosynthesized. Many plants have developed natural, biochemical mechanisms to defend themselves from weed competition and animal, insect and fungal attacks. Some contain components toxic to pathogens, so discourage feeding by insects and other herbivores; others provide protection or immunity from diseases caused by some pathogens; yet others help plants compete for resources by discouraging competition among different plant species (Dubey 2011; Gurjar et al. 2012). By studying the diverse chemistry of various plant species, scientists have discovered many useful compounds that can be used as biopesticides (Koul and Dhaliwal 2001). Major classes of plant compounds important for plant chemical interactions include: Nitrogen containing compounds, i.e., alkaloids, glucosinolates; Phenolics, i.e., simple phenols, flavonoid; Terpenoids, i.e., monoterpenes, sesquiterpenes, limonoids (Jeyasankar et al. 2014; Miresmailli and Isman 2014).

Chemical pesticides are known to pollute the environment, soil and water, as well as causing deleterious effects on human health and the biosphere. In the past, synthetic pesticides have played a major role in crop protection programs and have immensely benefited mankind. Nevertheless, their indiscriminate use has resulted in the development of resistance, resurgence and outbreak of new pests; toxicity to non-target organisms (poisoning of farm workers and consumers; destruction of fish, birds, and other wildlife); and hazardous environmental effects (groundwater contamination; disruption of natural biological control and pollination), endangering the sustainability of ecosystems and threatening human and environmental health (Jeyasankar and Jesudasan 2005). Recognition of these hazardous effects has resulted in a challenge to current pest management practices.

Hence there is a need to search for an environmentally safe and economically viable strategy for the control of diseases and to reduce the dependence on synthetic agrochemicals, which in turn has led to a recent increased interest towards the use of plant products as novel chemotherapeutants in plant protection (Isman 2006; Gurjar et al. 2012). In particular, research has targeted biodegradable compounds with selective toxicity which, when extracted from the plant and applied to infested crops, provide efficient protection. Positive results in the control of pests and fungal pathogens have been obtained using essential oils, compounds produced by many species of plants as secondary metabolites. The popularity of botanical solutions is subsequently increasing and plant products are now being used globally as “green” pesticides (Ignacimuthu 2004; Dubey et al. 2008).

For example, increased resistance to conventional chemicals has created a need to identify new effective insecticidal compounds, while also taking into account the impact of control methods on native flora and fauna. This has stimulated studies of alternative controls in naturally occurring insecticides derived from plants, and bioactive compounds from many medicinal and aromatic species (Isman 2006).

Many substances with repellent and/or antifeedant effects are found in the allomone group Dubey 2011). Since these effects are closely related only to insect behavior, plant extracts that contain these substances are selective and thus friendly to non-target organisms. This is of great importance for the development of new botanical insecticides which are safe for the environment (Pavela 2013).

The move toward ‘green’ chemistry processes and the continuing need for new crop protection tools with novel modes of action makes discovery and commercialization of natural products as green pesticides an attractive and profitable pursuit that is commanding attention (Isman and Machial 2006; Koul et al. 2008).

4 Mode of Action

Mode of action refers to the specific biochemical interaction through which a pesticide produces its effect. Usually, the mode of action includes the specific enzyme, protein, or biological step affected. While most other classifications are the pests controlled, physical characteristics, or chemical composition, mode of action specifically refers to the biological process which the pesticide interrupts (Khambay et al. 2003; Bloomquist et al. 2008). Knowing the mode of action is integral for scientists to improve the quality and sustainability of a product. Plants offer an alternative source of insect-control agents because they contain a range of bioactive chemicals, many of which are selective and have little or no harmful effect on non-target organisms and the environment (Isman 2006). One plant species may possess substances with a wide range of activities; for example, extracts from the neem tree *A. indica* are antifeedant, antioviposition, repellent and growth-regulating (El-Wakeil 2013).

5 Biological Activities of Plant Extracts and Essential Oils and Their Constituents

5.1 Antifeedant and Repellent

Plant chemicals (botanicals) with antifeedant properties against economically impacting insect pests have been studied in many countries, due to their enormous importance in the field of insect pest management. The concept of insect antifeedants gained strength in the 1970s–1980s with demonstration of the potent feeding deterrent effect of azadirachtin and neem seed extracts on a large number of pest species (El-Wakeil 2013). Antifeedants are the naturally-occurring chemicals in certain plants which play a major role in the unsuitability of non-host plants as food for insects due to adverse physical effects (Immaraju 1998; Jeyasankar et al. 2014).

Chemical isolation of these active ingredients is important not only for understanding the ecological aspects of insect pest's relationship, but also for their potential in pest control. Antifeedant chemicals may be defined as being either repellent without making direct contact to insect, or suppressant/deterrent from feeding once contact has been made with insects (Dubey 2011; Jeyasankar et al. 2014).

Ecologically, antifeedants play a significant role since they never kill the target insects pests directly but allow them to remain available to their natural enemies (predator and parasites), thus helping to safeguard the natural ecological balance. Further, monophagous, oligophagous and polyphagous insects die due to the application of antifeedants on their food plants, via starvation (Isman 2006; Jeyasankar et al. 2014).

No commercial products based purely on antifeedant effectiveness are presently known (Dubey 2011). Nevertheless, some products containing primary substances with both insecticidal and an antifeedant and antioviposition effect are currently being used. Botanical pesticides that have come on market over the past 20 years are those based on the terpenoid azadirachtin, a limonoid found in seeds of the Indian neem tree *A. indica*, which has been used traditionally to control pests and diseases (Saxena 1989).

The study evaluated the activity of ethyl acetate and ethanol extracts from the fruit and seeds of *Cabralea canjerana* (Meliaceae) on South American fruit fly *Anastrepha fraterculus*. Limonoids and triterpenes were detected in fruit and seed extracts, and the results highlighted toxic and deterrent properties (antifeedant and antioviposition) of extracts from *C. canjerana* to which *A. fraterculus* is vulnerable. The different modes of action of the extracts of fruits and seeds of *C. canjerana* on *A. fraterculus*, particularly the antifeedant activity and oviposition deterrence indicate their possible use in the integrated pest management of this fruit fly (Magrini et al. 2014). Also ethyl acetate extract of seeds of *Achyranthes aspera* showed higher insecticidal and growing inhibition activities against *Henosepilachna vigintioctopunctata* (Jeyasankar et al. 2014).

Research has also indicated that pesticides based on Myrtaceae essential oils could be used in a variety of ways to control a large number of insect pests (Ebadollahi 2013). As well, Adel et al. (2010) have several studies on physiological, biochemical and histopathological effects of Artemisia monosperma against the cotton leaf worm, Spodoptera *littoralis* in Egypt. Sammour et al. (2011) had comparative studies on the efficacy of neemix and basil oil formulations on the cowpea aphid *Aphis craccivora* in laboratory conditions in Egypt.

Botanical insecticides based on the repellent effect of essential oils are particularly promising when used in closed areas, where the fumigation effect can be used – e.g. in granaries and households for protection against storage pests (Isman 2006). Research shows that biochemical pest control is promising, however in practice one problem encountered is the short persistence of the effects (Koul and Dhaliwal 2001).

5.2 *Fumigant*

One of the most valued properties of essential oils is their fumigant activity against insects, which enables control of pests in storage without direct application to the insect. In this context, essential oils have received much attention as potentially useful bioactive control compounds, showing broad spectrum activity against insects but low mammalian toxicity, while also degrading rapidly in the environment (Bakkali et al. 2008; Koul et al. 2008; Rajendran and Sriranjini 2008).

Many plant essential oils have fumigant action, such as those of *Aremisia sp.*, *Anethum sowa*, *Curcuma long* and *Lippia alba*. Common essential oils with bioactivity, either as an insecticide or repellent, are clove, thyme, mint, lemongrass, cinnamon, rosemary and oregano oils (Isman and Machial 2006).

Results indicate that *Eucalyptus dives* and *Leptospermum petersonii* essential oils merit further investigation for their potential as fumigants against early instars and eggs of Queensland fruit fly (Hidayat et al. 2013). The essential oil of *Blumea balsamifera* was found to possess strong fumigant toxicity to the maize weevil, *Sitophilus zeamais* (Chu et al. 2013). The exact mode of action of these oils as fumigants is unknown but the oils mainly act in the vapor phase via the respiratory system. There are no established natural fumigants in use against pests attacking grains, dry stored food and other agricultural products (Tripathi et al. 2009; Hidayat et al. 2013).

5.3 Antifungal

Plant diseases, particularly fungal infections, contribute significantly to agricultural crop losses globally. Work carried out with plant extracts and essential oils has indicated that these have potential in the control of phytopathogenic fungi. Considering the complexity of these compounds, it must be admitted that there are various actional mechanisms, many of which are still unknown. The mechanisms may be due to direct fungitoxic action, inhibiting mycelial growth and spore germination, or to activisation of the plant's defense mechanisms, such as indication of phytoalexins and proteins related to resistance, indicating the presence of compounds with elicitor characteristics (Stangarlin et al. 1999). However, there are only limited data available on the antifungal activity of essential oils against plant fungal pathogens.

The cutting edge of research into these compounds and their mode of action focuses on the control of phytopathogenic fungi. The effect on mycelial growth of fungi has been seen for compounds from various medicinal plants. A number of scientific investigations have highlighted the importance and the contribution of many plant families i.e. Asteraceae, Liliaceae, Apocynaceae, Solanaceae, Caesalpinaceae, Rutaceae, Piperaceae, Sapotaceae, etc., used as medicinal plants (Sheetal and Singh 2008).

Cinnamon oil exhibited strong inhibitory effects on *Botrytis cinerea*. It completely suppressed the mycelial growth of fungus (Sirirat et al. 2009). Management of anthracnose caused by *Colletotrichum spp*. is an important issue for the tropical fruit industry because of the resulting financial losses. Essential oil extracted from lemon grass *Cymbopogon spp*. has proven a viable method for controlling post harvest anthracnose of mango fruit (Salomone et al. 2008). Agapanthus, carica, syzygium and allium extracts were active on *Colletotrichum spp*. in vitro and also reduced anthracnose disease of bean and cowpea, thus are potential seed treatments in anthracnose disease control (Masangwa et al. 2013). The study results showed the potential of some plant essential oils, i.e. basil oil (*Ocimum basilicum*), orange oil (*Citrus sinensis*), lemon oil (*Citrus Medica limonum*) and mustard oil (*Brassica juncea*), to reduce postharvest losses induced by *Colletotrichum gloeosporioides* in mango fruits (Abd-AllA and Haggag 2013). The cow urine-based plant extract of four plants *Anacardium occidentale, Pimenta dioica, Alpinia galangal* and *Anisomeles indica* is promising in the control of anthracnose disease in chilli (Prashith Kekuda et al. 2014).

5.4 Antiviral

In vitro studies have been carried out into the antiviral activities of essential oils against the tobacco mosaic virus (TMV). Results indicate that essential oils isolated from artemisia and lemongrass, and the individual compound citronellal, have the potential to be used as an effective alternative for the treatment of tobacco plants infected with tobacco mosaic virus (TMV) under greenhouse conditions (Lu M et al. 2013). The anti-viral protein (AVP) extracts from Great bougainvillea (*Bougainvillea spectabilis*) and Chilean Mesquite (*Prosopis chilinesis*) were found to be effective in reducing the sunflower necrosis virus (SFNV) infection both in cowpea and sunflower plants (Lavanya et al. 2009).

5.5 Antinematicidal

Recent research has shown that specific plants' 'phytochemical make-up' or plant extracts produce a significant reduction in the nematode population or control them (Abbasi et al. 2008; Okeniyi et al. 2010). Further studies need to be conducted in the field to as certain the nematicidal ability of the extracts in the soil. The development of new nematicides is a difficult task and the field has not yet reached maturity: efforts have largely consisted of basic, descriptive research. The mode of action of most nematicidal phytochemicals is largely unknown. Commercial nematicides based on plant essential oils have not yet appeared in the market (Andres et al. 2012).

5.6 Antitermitic

A recent study revealed the antitermitic activity of some Pakistani medicinal plants traditionally used in local remedies. These plant seed ethanolic extracts have the potential to develop new and safe products for control of *Odontotermes obesus*, a naturally occurring termite. The screening results suggest that Fennel *(Foeniculum vulgare),* Syrian Rue/African Rue *(Peganum harmala),* Mint *(Mentha spp.) and Basil (Ocimum basilicum)* have promising capability in termite control. There is now need to conduct field studies on their use as a commercial antitermitic agent

(Abbas et al. 2013). Endod (*Phytolacca dodecandra)* leaf extracts also showed a 100 % mortality effect on both soldier and worker termites observed after 48 h. These results were significantly different than those of neem (*A. indica)* leaf water extract, but need further investigation against termites *Microterms spp*. in field condition (Shiberu et al. 2013).

5.7 Herbicides

Weeds have a greater negative impact on crop yields than any other agricultural pests (Oerke 2006). Many recently discovered allelochemicals have potential for development as natural product herbicides (Duke and Lydon 1987; Ercisli et al. 2005; Brown 2006). Manuka essential oil contains natural beta-triketones, which target the same plant enzyme as some commercial synthetic herbicides. With this component, small amounts of manuka oil can be combined with a commercial organic herbicide of lemongrass essential oil to achieve greater results (Dayan et al. 2011).

6 Commercial Products and Uses

Botanical insecticides presently play only a minor role in insect pest management and crop protection, predominantly due to increasingly stringent regulatory requirements in many jurisdictions. In spite of the wide-spread recognition of insecticidal properties in plants, only a handful of pest control products directly obtained from plants are in use because the commercialization of new botanicals is hindered by a number of issues (Isman and Machial 2006). Botanicals used as insecticides presently constitute only 1 % of the world insecticide market (Rozman et al. 2007).

6.1 Plant-Based Products Currently on the Market

Plant essential oils are produced commercially from several botanical sources, many of which are members of the mint family (*Lamiaceae*). The oils are generally composed of complex mixtures of monoterpenes, biogenetically related phenols, and sesquiterpenes. Examples include 1,8-cineole, the major constituent of oils from rosemary (*Rosmarinus officinale*) and eucalyptus (*Eucalyptus globus*); eugenol from clove oil (*Syzygium aromaticum*); thymol from garden thyme (*Thymus vulgaris*); and menthol from various species of mint (*Mentha* species) (Koul et el. 2008).

These results have spurred the development of essential oil-based insecticides, fungicides, and herbicides for agricultural and industrial applications and for the consumer market, using rosemary oil, clove oil, and thyme oil as active ingredients. Interest in these products has been considerable, particularly for control of greenhouse pests (Isman and Grieneisen 2014).

Among the traditional botanicals, those registered for use in the USA are pyrethrum, neem, rotenone, sabadilla, ryania and nicotine. Several azadirachtin-based insecticides are sold in the USA and a number of essential oils are exempt from registration. In Canada only pyrethrum, rotenone, nicotine are registered for use there. Mexico allows the products registered in the USA. In Europe, pyrethrum, neem and nicotine are allowed, however, since 2008 rotenone is no longer allowed in the European Union. In fact, neem has still to make headway in these countries. In Asia, India leads in the use of botanicals, with a number of products registered under provisional legistration. According to Isman (2006) neem-based products are in abundance in addition to pyrethrum, rotenone, nicotine and essential oils. However, neem is yet to be approved in Australia, New Zealand and the Philippines. In Latin America, Brazil leads in registered products based on pyrethrum, rotenone, neem, garlic and nicotine. Throughout Latin America, plant oils and extracts are produced by cottage industry. Data on regulated products for most African countries is not known. Apparently, only pyrethrum is approved for use in South Africa (Koul and Walia 2009).

China has carried out research into botanical pesticides research since the 1930s and 17 botanical pesticides have now been registered, including: *insecticides* – azadirachtin, rotenon, perethrins, vertrine, nicotine, Celastrus angulatus, camphor (d-camphor), eucalyptol; *fungicides* – eugenol, carvacrol, ethylicin, Da Huang Su Jia Ni; *insecticide/fungicide* – matrine and cnidiadin (Shuyou 2013; Pan et al. 2013).

A mixture of essential oils trademarked *hexahydroxyl* (EcoPCO EcoSMART Technologies, Franklin, Tennessee) and based on distinct combinations of different plant essential oils that significantly enhance the activity of these oils against insect was formulated. (Isman and Akhtar 2007; Koul and Walia 2009).

Recently several organic herbicide products have appeared on the market. Lanini (2010) these include *GreenMatch* (55 % d-limonene), *Matratec* (50 % clove oil), *WeedZap* (45 % clove oil + 45 % cinnamon oil) and *GreenMatch EX* (50 % lemongrass oil).

6.2 Barriers to Commercialization of Botanicals

Plant oils themselves, or products based on plant oils, are mostly nontoxic to mammals, birds, and fish (Isman 2006). However, as broad-spectrum pesticides, both pollinators and natural enemies are vulnerable to poisoning by products based on essential oils. Owing to their volatility, essential oils have limited persistence under field conditions: therefore, although natural enemies are susceptible via direct contact, predators and parasitoids re-invading a treated crop one or more days after treatment are unlikely to be poisoned by residue contact with pesticides (Ismail et al. 2004; El-Sebai et al. 2005).

Factors affecting the use of botanical pesticides include: ongoing raw material availability; standardization of botanical extracts containing a complex mixture of active constituents; solvent types; rapid degradation of the extract in storage after preparation; adequate biomass to justify extraction; feasibility of extraction near the harvest site; registration; market opportunities for botanical pesticides and the need for appropriate weather conditions during usage. Success of botanical pesticides in the field will therefore largely depend on finding solutions to these issues (Tripathi et al. 2009; Isman 2006; Dubey 2011).

7 Future Research for Improved Efficacy of Botanical Pesticides

There are around 800 plant species from all over the world which have been found to exhibit biocidal activity, and some plant products have been recommended for the control of insect-pest and diseases of various agricultural, horticultural, fruit and other economic crops (Tripathi and Tripathi 1999). As an example, neem and its products have been tested for their pesticidal properties against more than 300 species of insects belonging to different orders throughout the world (Tripathi 2000).

All characteristics indicate that the use of antifeedant and repellent substances for protection has great potential. By studying plant organisms that protect themselves against the pest attack, we can obtain pesticide-active substances which enable us to control this attack in an ecological way (Koul et al. 2008). There is a need to develop new plant protection products for many reasons: the growing demand for food, environmental issues, increasingly stringent ecotoxicological and toxicological requirements, and the continuous development of pest resistance to available pesticides. Plants produce a unique set of secondary metabolites that may play an important role in a sustainable pest management, as new products directly or as novel chemical frameworks for synthesis (Isman 2006; Pino et al. 2013). They are also important for identifying original modes of action (Koul and Dhaliwal 2001). For a successful research and development process leading to a commercial product, a wide range of criteria (especially biological, environmental, toxicological, regulatory, and commercial) must be satisfied (Pino et al. 2013). Among the major challenges to be faced by the candidates (new or known phytochemicals) to reach the market are the sustainable use of raw materials (availability and accessibility), standardization of chemically complex extracts, and regulatory requirements and approval (Miresmailli and Isman 2014).

References

Abbas M, Shahid M, Iqbal M, Anjum F, Sharif S, Ahmed S, Pirzada T (2013) Antitermitic activity and phytochemical analysis of fifteen medicinal plant seeds. J Med Plants Res 7(22):1608–1617

Abbasi WM, Ahmed N, Zaki N, Shaukat SS (2008) Effect of *Barleria acanthoides* Vahl. On root-knot nematode infection and growth of infected okra and brinjal plants. Pak J Bot 40416(5):2193–2198

Abd-AllA MA, Haggag WM (2013) Use of some plant essential oils as post-harvest botanical fungicides in the management of anthracnose disease of mango fruits (*Mangi feraindica* L.) caused by *Colletotrichum gloeosporioides* (penz). Int J Agric For 3(1):1–6. doi:10.5923/j.ijaf.20130301.01

Adel MM, El-Hawary FM, Abdel-Aziz NF, Sammour EA (2010) Some physiological, biochemical and histopathological effects of Artemisia monosperma against the cotton leafworm, *Spodoptera littoralis*. Arch Phytopathol Plant Prot 43:1098–1110

Alburo R, Olofson H (1987) Agricultural history and the use of botanical insecticides in Argao, Cebu. Philipp Quar Cul Soc 15:151–172

Andres M, Gonzalez-Coloma S, Sanz J, Burillo J, Sain P (2012) Nematicidal activity of essential oils: a review. Phytochem Rev 11:371–390

Babu A, Hern A, Dorn S (2003) Sources of semiochemicals mediating host finding in *Callosobruchus chinensis* (Coleoptera: Bruchidae). Bull Entomol Res 93:187–192

Bakkali F, Averbeck S, Averbeck D, Idaomar M (2008) Biological effects of essential oils – a review. Food Chem Toxicol 46(2):446–475

Bloomquist JR, Boina DR, Chow E, Carlier PR, Reina M, Gonzalez- Coloma A (2008) Mode of action of the plant-derived silphinenes on insect and mammalian GABAA receptor/chloride channel com plex. Pestici Biochem Physiol 91:17–23

Brown C (2006) Allelopathic plants: nature's weed killers. Earth friendly gardening. https://earthfriendlygardening.wordpress.com/2006/11/30/allelopathic-plants-natures-weedkillers/

Chu SS, Du SS, Liu ZL (2013) Fumigant compounds from the essential oil of Chinese *Blumea balsamifera* leaves against the Maize Weevil (*Sitophilus zeamais*). J Chem 7, ID 289874. doi:10.1155/2013/289874

Dayan FE, Howell J, Marais JP, Ferreira D, Koivunen M (2011) Manuka oil, a natural herbicide with preemergence activity. Weed Sci 59(4):464–469

Dubey NK (2011) In: Dubey NK (ed) Natural products in plant pest management. CABI, Wallingford/Oxfordshire/Cambridge, MA, p 293

Dubey NK, Srivastava B, Kumar A (2008) Current status of plant products as botanical pesticides in storage pest management. J Biopesticides 1(2):182–186

Duke SO, Lydon J (1987) Herbisides from natural compounds herbicides from natural compounds. Weed Technol 1(2):122–128

Ebadollahi A (2013) Essential oils isolated from myrtaceae family as natural insecticides. Annual Rev Res Biol 3(3):148–175

El-Sebai TN, El-Wakeil NE, Abdallah SA (2005) Efficacy of certain mineral, natural oils and insecticides against the Whitefly, Bemisia tabaci on cucumber plants and their side effects on the associated predators. Bull Entomol Soc Egypt, Econ Ser 31:229–241

El-Wakeil NE (2013) Botanical pesticides and their mode of action. Gesunde Pflanzen 65:125–149. doi:10.1007/s10343-013-0308-3

Ercisli S, Esitken A, Turkkal C, Orhan E (2005) The allelopathic effects of juglone and walnut leaf extracts on yield, growth, chemical and PNE compositions of strawberry cv. Fern Plant Soil Environ 51(6):283–287

Giordano A, Costs A (2013) Book: plant extracts: role in agriculture, health effects and medical applications. Nova, Hauppauge, p 393

Gurjar MS, Ali S, Akhtar M, Singh KS (2012) Efficacy of plant extracts in plant disease management. Agric Sci 3(3):425–433. doi:10.4236/as.2012.33050

Hidayat Y, Heather N, Hassan E (2013) Repellency and oviposition deterrences effects of plant essential and vegetable oils against female Queensland fruit fly *Bactrocera tryoni* (Froggatt) (Diptera:Tephritidae). Aust J Entomol 52:379–386

Ignacimuthu S (2004) Insect pest management with green pesticides. J Sci Ind Res 63:694–696

Immaraju JA (1998) The commercial use of azadirachtin and its integration into viable pest control programmes. Pestic Sci 54:285–289

Ismail AI, Abd El-Salam AME, Soliman MMM (2004) Field evaluation of plant derivative and chemical compounds and their mixtures against Ceroplastes floridensis Com. on orange trees. Egypt J Biol Pest Control 14:175–179

Isman MB (2006) Botanical insecticides, deterrents, and repellents in modern agriculture and an increasingly regulated world. Annu Rev Entomol 51:45–66. doi:10.1146/annurev.ento.51.110104.151146

Isman MB (2008) Botanical insecticides: for richer, for poorer. Pest Manag Sci 64:8–11

Isman MB, Akhtar Y (2007) Plant natural products as source for developing environmentally acceptable insecticides. In: Shaaya I, Nauen R, Horowitz A (eds) Insecticides design using advanced technologies. Springer, Berlin, Heidelberg, pp 235–248

Isman M, Grieneisen ML (2014) Botanical insecticide research: many publications, limited useful data. Sci Direct 19(3):140–145. doi:10.1016/j.tplants.2013.11.005

Isman MB, Machial CM (2006) Pesticides based on plant essential oils: from traditional practice to commercialization. In: Rai M, Carpinella MC (eds) Naturally occurring bioactive compounds. Elsevier, Amsterdam, pp 29–44

Jeyasankar A, Jesudasan RWA (2005) Insecticidal properties of novel botanicals against a few lepidopteran pests. Pestology 29(10):42–44

Jeyasankar A, Premalatha S, Elumalai K (2014) Antifeedant and insecticidal activities of selected plant extracts against Epilachna beetle, Henosepilachna vigintioctopunctata (Coleoptera: Coccinellidae). Adv Entomology 2(1):1–6. doi:10.4236/ae.2014.21003

Khambay BP, Batty D, Jewess PJ, Bateman GL, Hollomon DW (2003) Mode of action and pesticidal activity of the natural product dun nione and of some analogues. Pest Manag Sci 59:174–182

Khoddami A, Wilkes M, Roberts T (2013) Techniques for analysis of plant phenolic compounds. Molecules 18: 2328–2375. doi:10.3390/molecules18022328

Koul O, Dhaliwal GS (2001) Advances in biopesticide research, vol I-Phytochemical biopesticides. Harwood Academic Publishers gmbh, Amsterdam, 223 pp

Koul O, Walia S (2009) Comparing impacts of plant extracts and pure allelochemicals and implications for pest control. CAB Rev: Perspect Agric, Vet Sci, Nutr Nat Resour 4(049):1–30

Koul O, Walia S, Dhaliwal GS (2008) Essential oils as green pesticides: potential and constraints. Biopesticides Int 4:63–84

Lanini WT (2010) Organic herbicides—do they work? Dept plant sciences, UC Davis, CA. UC Davis Field Day, UCNFA News, p 11–12

Lavanya N, Saravanakumar D, Rajendran L, Ramiah M, Raguchander T, Samiyappan R (2009) Management of sunflower necrosis virus through anti-viral substances. Arch Phytopathology Plant Pro tection 42:265–276. doi:10.1080/03235400601037149

Lu M, Han Z, Xu Y, Yao L (2013) In vitro and in vivo anti-tobacco mosaic virus activities of essential oils and individual compounds. J Microbiol Biotechnol 23(6):771–778

Magrini FE, Specht A, Gaio J, Girelli CP, Migues I, Heinzen H, Sartori VC, Cesio V (2014) Viability of Cabralea canjerana extracts to control the South American fruit fly, *Anastrepha fraterculus*. J Insect Sci 14:47

Mariapackiam S, Ignacimuthu S (2008) Larvicidal and histopathological effects of the oil formulations on *Spodoptera litura*. In: Ignacimuthu S, Jayaraj S (eds) Recent trends in insect pest management. Elite Publishing House, New Delhi, pp 231–235

Masangwa JIG, Aveling AS, Kritzinger Q (2013) Screening of plant extracts for antifungal activities against Colletotrichum species of common bean (*Phaseolus vulgaris* L.) and cowpea (*Vigna unguiculata* (L.) Walp). J Agric Sci 151:482–491. doi:10.1017/S0021859612000524

Miresmailli S, Isman MB (2014) Botanical insecticides inspired by plant–herbivore chemical interactions. Trends Plant Sci 19(1):29–35
Ntalli NG, Menkissoglu-Spiroudi U (2011) Pesticides of botanical origin: a promising tool in plant protection. In: Margarita Stoytcheva (ed.) Pesticides – Formulations, Effects, Fate. Ch. 1, InTech, p 3–24
Oerke EC (2006) Crop losses to pests. J Agric Sci 144:31–43
Okeniyi MO, Fademi OA, Orisajo SB, Adio SO, Otunoye AH, Adekunle OV (2010) Effect of botanical extract on root-knot nematode (Meloidogyneincognita) infection and growth of cocoa seedlings. J Appl Biosci 36:2346–2352
Pan R, Feng A, Lan K (2013) China crop protection monthly report, Guangzhou CCM Information Science & Technology Co., Ltd., Issue 02, p 23
Pavela R (2013) New possibilities of using the Antifeedant and repellent effects of plant extracts in the protection of plants and human health (p 219–240) Book: Adriana Giordano, Alfieri Costs plant extracts: role in agriculture, health effects and medical applications, Nova Science Publishers, Inc (US), 393 pp
Philogène BJR, Arnason JT, Towers GHN, Abramowski Z, Campos F, Champagne D, McLachlan D (1984) Berberine: a naturally occur ring phototoxic alkaloid. J Chem Ecol 10:115–123
Philogène BJR, Regnault-Roger C, Vincent C (2005) Botanicals: yesterday's and today's promises. In: Regnault-Roger C, Philogène BJR, Vincent C (eds) Biopesticides of plant origin. Lavoisier Publishing, Paris, pp 1–15
Pino O, Sánchez Y, Rojas MM (2013) Plant secondary metabolites as an alternative in pest management. I: background, research approaches and trends Rev. Protección Veg. 28 (2) La Habana mayo-ago. ISSN 1010–2752
Prashith Kekuda TR, Vivek MN, Manasa M, Yashoda K, Noor Nawaz AS, Raghavendra HL (2014) Antifungal effect of cow urine extracts of selected plants against Colletotrichum capsici isolated from anthracnose of chilli. Intl J Agri Crop Sci 7(3):142–146
Raja N (2014) Botanicals: sources for eco-friendly biopesticides. J Biofertil Biopestici 5:1. doi:10.4172/2155-6202.1000e122
Rajendran S, Sriranjini V (2008) Plant products as fumigants for stored-product insect control. J Stored Prod Res 44(2):126–135
Regnault-Roger C, Phylogeny BJR, Vincent C (2005) Botanicals: yesterday's and today's promise. In: Regnault RC, Philogene BJR, Vincent C (eds) Biopesticides of plant origin. Lavoisier Publishing, Secaucus, pp 1–15
Rozman V, Kalinovic I, Korunic Z (2007) Toxicity of naturally occurring compounds of Lamiaceae and Lauraceae to three stored-product insects. J Stored Prod Res 43:349–355
Salomone A, Scaritto G, Sacco A, Cabras G, Angioni A (2008) Inhibitory effects of the main com pounds of oregano essential oil against some pathogenic fungi. Modern fungicide and Antifungal compounds V. Deutsche Phytomedizinische Gesellschaft 44:345–360
Sammour EA, El-Hawary FM, Abdel-Aziz NF (2011) Comparative study on the efficacy of neemix and basil oil formulations on the cowpea aphid Aphis craccivora Koch. Arch Phytopathol Plant Prot 44:655–670
Saxena RC (1989) Insecticides from neem. In: Arnason JT et al (eds) Insecticides of plant origin. American Chemical Society, Washington, DC, pp 110–135
Sheetal V, Singh SP (2008) Current and future status of herbal medicines. Vet World 1(11):347–350
Shepard H (1951) The chemistry and action of insecticides. McGraw Hill, New York, p 504
Shiberu T, Ashagre H, Negeri M (2013) Laboratory evaluation of different botanicals for the control of termite, Microterms spp (Isoptera: Termitidae) 2(3):1–3. doi:10.4172/scientificreports696
Shuyou H (2013) Biopesticide development in China AGROW World crop protection news Informa UK Ltd, p 15–16
Sirirat S, Wimolpun R, Sanit S (2009) Antifungal activity of essential oils derived from some medicinal plants against grey mould (*Botrytis cinerea*). Asian J Food Ag Ind 36:229–233

Stangarlin JR, Schwan-Estrada KRF, Cruz MES, Nozaki MH (1999) Plantas medicinais e controle alternativo de fitopatógenos. Biotecnologia Ciência & Desenvolvimento, Brasília 2(11): 16–21
Tripathi YC (2000) Potential of neem constituents in pest management. Everyman's sci 35(2):78–84
Tripathi YC, Tripathi G (1999) Modern trends in environmental pollution and ecoplanning, first edition edited by Arvind Kumar, 01/1999: chapter chapter 4: importance of phytopesticides in environmental protection. ABD Publishers, Jaipur, pp 82–96. ISBN 81-95-771-01-26
Tripathi AK, Upadhyay S, Bhuiyan M, Bhattacharya PR (2009) A review on prospects of essential oils as biopesticide in insect-pest management. J Pharmacogn Phytother 1(5):052–063

Chapter 14
Quality Assurance of Medicinal and Aromatic Plants- Good Agricultural and Collection Practices (GAP & GCP)

A.K.S. Rawat and S.K. Tewari

Abstract There has been increasing interest of use of herbal products globally and this has led to the spurt in the use of plant based medicines across the world. This has created opportunities for developing countries like India with traditional knowledge base to develop globally acceptable newer herbal drugs/neutraceuticals and convert their rich bioresources and associated traditional knowledge for economic wealth and thereby bring prosperity to the nation. However, the Indian herbal drug industries generally face the problem of adulteration and substitution, which may be deliberate or some time unintentional. Such adulteration and substitution lead to poor quality and batch to batch inconsistency. Medicinal plant materials are supplied through collection from wild populations and also from organized cultivation. Under the overall context of quality assurance of herbal medicines, W.H.O. has developed the Guidelines on Good Agricultural and Collection Practices (GACP) for medicinal plants, providing general technical guidance on obtaining medicinal plant materials of good quality for the sustainable production of herbal products classified as medicines. It is extremely important to establish the reference samples and to determine the quality parameters of the medicinal plants and adulterant/substitute by undertaking extensive and intensive study of the traditional treatise of the classical medicines or traditional practices, combined with the modern scientific knowledge and methods.

Keywords Herbal drugs • Quality control • Drug standardization • GAP • GCP

1 Introduction

There has been great interest in the use of herbal medicines/products for the health care, cosmaceuticals and nutraceutical purposes across the world. The global herbal market is about US$ 90 billion which is growing at the rate of 10–15 % annually and is expected to cross five trillion US$ by the year 2050 Anonymous (2000). The

A.K.S. Rawat (✉) • S.K. Tewari (✉)
CSIR-National Botanical Research Institute, Lucknow, Uttar Pradesh, India
e-mail: pharmacognosy1@rediffmail.com; pharmacognosy1@rediffmail.com

Á. Máthé (ed.), *Medicinal and Aromatic Plants of the World*, Medicinal and Aromatic Plants of the World 1, DOI 10.1007/978-94-017-9810-5_14

latest export statistics of India's Ayurveda, Yoga & Naturopathy, Unani, Siddha and Homoeopathy (AYUSH) and herbal products have revealed that India stands as second leading exporter in the world accounting for Rs. 1.32 billion for the year 2010–2011 Subrat et al. (2002). As per the latest statistics available from Pharmexcil, China is the top country leading in the world in the export of herbals with $1329.72 million followed by India with an export figure of $790.56 million for the year 2010. The major commodities or products of export under AYUSH include medicaments of Ayurvedic, homoeopathic and bio-chemical systems. The herbal exports too have seen a considerable increase of Rs. 367.6 million from Rs. 5707.6 million in year 2009–2010 to Rs. 6025.7 million in the year 2010–2011. The major herbal exports of India include *Psyllium* husk, *senna* leaves and pods, and various other herbs and herbal products. Among all, the *Psyllium* husk (*Isabgol* husk) contributes a major chunk of herbal export, accounting to Rs. 4250.5 million (Anonymous 2013).

The traditional medicine in India functions through two streams i.e. the folk stream and the classical organized stream that includes the Ayurveda, Unani, Siddha, etc. Around 8,000 wild plants are used in these systems. Although the global market of herbal drugs is growing at a fast pace, the Indian share needs to improve constantly to meet this pace. The major reasons for this lag is the lack of proper quality and safety of herbal drugs despite having in-depth knowledge in traditional herbal medicines. There are opportunities in twenty-first century for developing countries like India with traditional knowledge base to develop globally acceptable newer herbal drugs/neutraceuticals and convert their rich bioresources and associated traditional knowledge for economic wealth and thereby bring prosperity to the nation.

Indian herbal drug industries generally face the problem of adulteration and substitution. It is observed that in herbal markets of the country, sometimes not only various species of particular genus but entirely different taxa are being sold under the same vernacular name. For example in the name of '*Talispatra*', an important Ayurvedic drug, different leaves of *Taxus wallichiana, Abies spectabilis* and *Rhododendran anthopogan* are being sold in Dehradun, Kolkata and Amritsar markets, respectively. Similarly, in the name of '*Chirayata'*, different *Swertia* species and *Andrographis paniculata* are also being sold in various crude drug markets. It is also observed that in the name of '*Daruharidra*', '*Pittapapara*', '*Vidarikand*', '*Pashanbhed*' and '*Ashok*', two or more different plant species are being sold in the markets. The means of such adulteration and substitution may be deliberate or some time unintentional. Such adulteration and substitution lead to poor quality and batch to batch inconsistency.

1.1 Reasons for Substitution and Adulteration

The extinction of many species due to deforestation, increasing demand with decreasing resource base and incorrect identification of many plants have resulted in adulteration and substitution of raw drugs. The concept of substitution has prevailed since historical times, and in Ayurveda we can find this in the treatise of *Bhavaprakasha* and *Yogaratnakara*. However, the concepts of substitution given by our preceptors was totally different than the present day prevailing trend of adulteration and substitution. Adulteration is a practice of substituting the original crude drug partially or fully with other substances which are either free from or inferior in therapeutic and chemical properties or addition of low grade or spoiled drugs or entirely different drug similar to that of original drug substituted with an intention of enhancement of profits. Adulteration may also be defined as mixing or substituting the original drug material with other spurious, inferior, defective, spoiled, useless other parts of same or different plant or harmful substances or drug which do not confirm with the official standards. A drug shall be deemed to be adulterated if it consists, in whole or in part, of any filthy, putrid or decomposed substance (Om et al. 2013). For substitution of the drug, several reasons are prevalent:

- Non-availability of the drug: Substitution for *Ashtavarga Dravyas* (group of eight crude drugs).
- Uncertain identity of the drug: For the herb *Lakshmana*, different species such as *Arlia quinquefolia, Ipomea sepiaria* etc. are considered.
- Cost of the drug: *Kumkuma* being costly herb is substituted by *Kusumbha*.
- Geographical distribution of the drug: *Rasna* (*Pluchea lanceolata*) is used in Northern India while in southern parts, *Alpinia galanga* is considered as the source.
- The adverse reaction of the drug: *Vasa* is a well known *Rakta-Pittahara* (cures bleeding disorder) drug, but due to its abortifacient activity its utility in pregnant women is limited, instead drugs such as *Laksha*, *Ashoka* etc. are substituted.

1.2 Types of Substitution

A drug to be considered as a substitute needs to fulfill certain criteria. These are evident from following cases of substitution:

- Similarity in Rasa-panchakas: *Bharangi* and *Kantakari*.
- Exhibit similar therapeutic effects: *Ativisha* and *Musta*.
- In a formulation, the *pradhana dravya* i.e., the major ingredient should never be substituted. For example, while preparing *Bharangyaadi Guda,* do not substitute *Bharangi* with any other drug.
- Substitution with totally different drug: Here we can consider two types of *Gokshura – Tribulus terrestris* (Zygophylaceae) and *Pedalium murex* (Pedaliaceae). *T. terrestris* has the chemical constituents like chlorogenin, diosgenin, rutin, rhamnose and alkaloids while *P. murex* has sitosterol, ursolic acid, vanilin, flavonoids and alkaloids.

- Substitution of different species: Here we can consider *Bharangi* (*Clerodendron indicum*) and *Kantakari (Solanum xanthocarpam*). *Bharangi* has *tikta rasa* and l*aghu, ruksha guna* and has *kapha* and *vatahara* properties, while *Kantakari* has *katu vipaka* and *ushna virya*. Both *C. indicum* and *S. xanthocarpam* have shown anti-hisaminic activity and both are commonly employed in the diseases related to the respiratory system, which are commonly associated with release of histamines and other autacoids. Both the species are proved for nephroprotective, lothotriptic, diuretic and hepatoprotective activities.
- Substitution of the species belonging to same family: The example of *Datura metal* and *Datura stramonium* can be considered here. Their utility has been proved as bronchodialatory and inhibitor of secretion of mucous membrane. The alkaloid present in both the species are well proven bronchodilators and also inhibit the secretion of mucous membrane of the respiratory tract. Thus as far as the diseases of the respiratory tract are concerned, both *D. metal* and *D. stramonium* are beneficial.
- Context specific substitution: The *Amalaki (Embelica officinalis)* and *Bhallataka (Semicarpus anacardium)* can be considered as example. The *amalaki* has *laghu guna* and *lavana vargitha pancharasa*, *madhura vipaka*, *sheeta virya* and *tridoshahara* property. It has chemical constituents such as vitamin C, phyllembin, linolic acid, indole acetic acid, ellagic acid, salts etc. while *bhallataka* has *laghu, teekshna, snigdha guna, katu, tikta, kashaya rasa, mardhura vipaka, ushna virya* and *kapha vatahara* properties due to biflavonoids, anacardic acid, nicotinic acid, riboflavin, thiamine and essential oils. Research profile of *E. officinalis* shows anti-oxident, hepato-protective, microbial, hypoglycemic, hypolipidemic actions. The research profile of *Semecarpus* shows anti-tumour, hypotensive, anti–cytotoxic and anticancerous properties etc. Both *Amalaki* and *Bhallataka* are *Rasayana* drugs. *Amalaki* is commonly employed as *Kamya Rasayana* and *Bhallataka* as *Nimittika Rasyana*. In current practice, the *Rasayana* formulations are being employed as an adjuvant therapy in chronic as well as malignant diseases. *Amalaki* can be employed as *Rasayana* in chronic debilitating diseases like bronchial asthma, diabetes etc., while *bhallataka* would be better choice in malignant conditions, both in solid tumors and in leukaemia.

1.3 Types of Adulterants

Drugs are generally adulterated or substituted with substandard, inferior or artificial drugs.

1.3.1 Substitution with Substandard Commercial Varieties

Adulterants resemble the original crude drug morphologically, chemically, therapeutically but are sub standard in nature and cheaper in cost. This is the most common type of adulteration.

1.3.2 Substitution with Superficially Similar Inferior Drugs

Inferior drugs may or may not have any chemical or therapeutic value. They resemble only morphologically, so due to its resemblance they are used as adulterants.

1.3.3 Substitution with Artificially Manufactured Substance

The drug is adulterated with the substance which has been prepared artificially. The artificially manufactured substance resembles the original drug. This method is followed for the costlier drugs.

1.3.4 Substitution with Exhausted Drug

The same drug is admixed but that drug is devoid of medicinally active substance as it has been extracted already. Mainly volatile oil containing drugs like clove, coriander, fennel, caraway are adulterated by this method. As it is devoid of colour and taste due to extraction, natural colour and taste is manipulated with additives.

1.3.5 Substitution with Synthetic Chemicals to Enhance Natural Character

Synthetic chemicals are used to enhance natural character of the exhausted drug. E.g. citral is added to citrus oils like lemon and orange oils.

1.3.6 Presence of Vegetative Matter of Same Plant

Some miniature plants growing along with the medicinal plants are added due to their colour, odour, and constituents.

1.3.7 Harmful Adulterants

Some are harmful materials as the adulterant, are collected from market waste materials and admixed with the drug. It is done for the liquid drugs.

1.3.8 Adulteration of Powders

The drugs which are in the form of powders are frequently adulterated. For example dextrin is added in ipecacuanha, exhausted ginger in ginger, red sanders wood in capsicum powder and powdered bark adulterated with brick powder.

The adulteration and substitution are generally of two types; undeliberate or unintentional and deliberate adulteration, which can be illustrated by following examples:

Undeliberate/Unintentional Substitution or adulteration due to confusion of common vernacular name of herbal drugs, as described below.

Vernacular name	Plant species
Punarnava	*Boerhaavia diffusa, Trianthema portulacastrum*
Brahmi	*Bacopa monnieri, Centella asiatica*
Shankhpushpi	*Evolvulus alsinoides, Convolvulus microphyllus*
Talishpatra	*Taxus wallichiana, Abies spectabilis, Rhododendron* spp.
Ratanjot	*Arnebia benthamii, Arnebia nobilis, Arnebia ecuroma, Onosma hispidium*

Deliberate Adulteration Gross substitution by different plant materials is done deliberately. Sometimes in place of the genuine crude, substituted products which have similar appearance to the genuine are mixed. The examples are cited below.

Drug	Genuine	Adulterate/substitute
Ashoka	*Saraca indica*	*Polyalthea longifolia, Shorea robusta*
Kurchi	*Holarrhena antidysentrica*	*Wrightia tinctoria*
Senna	*Cassia acutifolia/Cassia angustifolia*	*Cassia auriculata*

1.3.9 Lack of Confidence in the Quality of Drug in Traditional Medicine Is Hindering Us from Capitalizing These Systems at Global Level

It is well documented that the quantity and nature of secondary metabolites in medicinal plants is influenced by growth, season, edaphic and environmental factors. Nema et al. (2013) reported high concentration of alenin, barbolin and isobarbolin in basal leaves as compared to the leaves from apex and middle in case

Fig. 14.1 Three pillars of herbal drug development

of *Aloe vera*. Similarly rhizome of *Rheum emodi* collected in summer has maximum anthraquinone which is responsible for biological activity as compared to rhizome collected in winter Nishioka (1991). The change in quantity and nature of secondary metabolites also result in variation in therapeutic value.

Therefore, it is extremely important to establish the reference samples and to determine the quality parameters of the medicinal plants by undertaking extensive and intensive studies of the traditional treatises of the classical medicines or traditional practices, combined with the modern scientific knowledge and methods and using the latest analytical and computational tools such as HPLC, GC, HPTLC, etc. (Fig. 14.1).

1.4 Need of Quality Control and Initiatives by Government of India

The entire process of development of herbal drugs/products, based on traditional knowledge needs proper taxonomically identified quality raw material and scientific validation of claims. Further, to get constant supply of quality raw material, whether collected from wild or cultivated and procured from market, one has to follow, Good Collection Practices (GCP), Good Agriculture Practices (GAP), Good Ethical Practices (GEP), Good Procurement Practices (GPP), and Good Storage Practices (GSP).

The Indian government has taken a number of initiatives including the preparation of the Ayurvedic Pharmacopoeia of India (AYUSH). Under this, Dept. of AYUSH has published six volumes containing 519 single herb monographs (Anonymous 1999–2012) and 101 classical ayurvedic formulations (Anonymous 2008). Further, Indian Council of Medical Research (ICMR) has also prepared monographs of individual plants in "Quality Standards of Indian Medicinal Plants", a compendium containing 377 monographs in eleven volumes (Anonymous 2003–2013). Indian Pharmacopoeia Commission (IPC) initiated a programme to supply botanical reference standards (BRS) and chemical reference standards (CRS) to herbal drug industries to maintain quality of their products. Realizing the problem of adulteration and substitution in herbal drugs, CSIR-National Botanical Research Institute, Lucknow has been engaged for the last two decades in developing parameters for quality control of crude drug and standardization of polyherbal formulations. Under the programme over 150 crude single herbal drugs and about 20 polyherbal formulations have been standardized. Some of the important raw drugs evaluated are *'Amraharidra', 'Ashoka', 'Bhuimala', 'Chiriata', 'Daruharidra', 'Kali Musli', 'Kalmegh', 'Pitpapra', 'Safed musli', 'Salam panja', 'Satawar', 'Gokharu', 'Resha khatmi', 'Talispatra', 'Vidarikand'*, etc.

2 Standardization of Herbal Products

New technologies are constantly being developed to isolate and identify the components responsible for the activity of medicinal plants. But these technologies should consider and possibly use the fact that the biological activity of plant extracts often results from additive or synergistic effects of its components. Another possibility is the qualitative and quantitative variations in the content of bioactive phytochemicals, which are currently considered major detriments in its use as a medicine. Different stresses, locations, climates, microenvironments and physical and chemical stimuli, often called elicitors, qualitatively and quantitatively alter the content of bioactive secondary metabolites. Enzymatic pathways leading to the synthesis of these phytochemicals are highly inducible (Eder and Cosio 1994). This is particularly true for phytochemicals that are well documented for their pharmacological activity, such as alkaloids (Facchini and De Luca 1994), phenylpropanoids (Dixon and Paiva 1995) and terpenoids (Trapp and Croteau 2001) whose levels often increase by two to three orders of magnitude following stress or elicitation (Darvill and Albersheim 1984). Thus, elicitation-induced, reproducible increases in bioactive molecules, which might otherwise be undetected in screens, should significantly improve reliability and efficiency of plant extracts in drug discovery while at the same time preserving wild species and their habitats. Standardization, optimization and full control of growing conditions should guarantee a cost-effective and quality-controlled production of many plant-derived compounds. This kind of standardization and quality control of the plant based drugs will improve efficacy of these drugs and promote its usage.

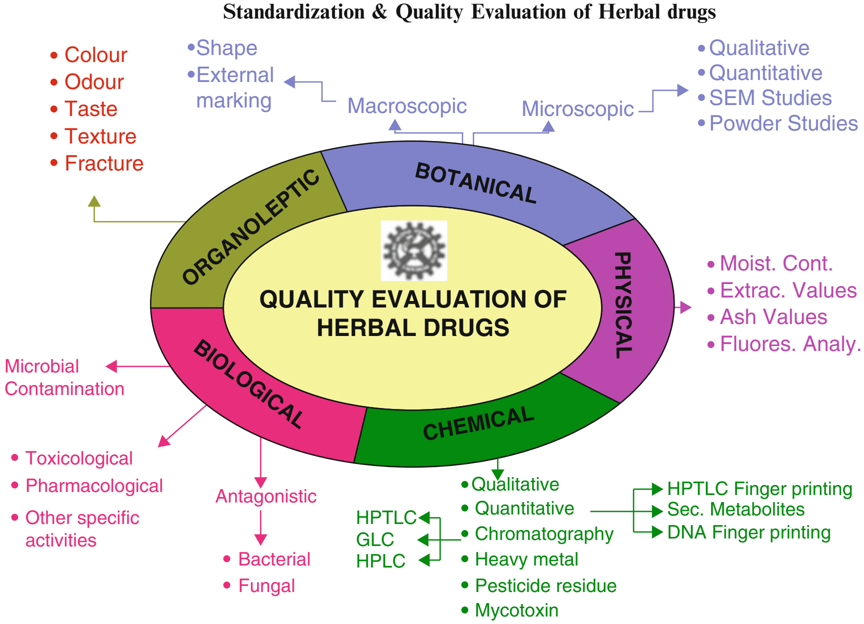


Fig. 14.2 Quality and standardization of herbal drugs/formulations

Most of the herbal drugs produced currently in the developing countries lack proper quality specification and standards and therefore facing difficulties for keeping consistency in quality in batch to batch products. Most of these drugs do not have well defined and characterized composition. The three pillars of ideal herbal drug and their rational use are quality, safety and efficacy (Fig. 14.2). The traditional medicines used to be an individual based treatment regime wherein the traditional physicians used handpicked plant materials to prepare drugs/formulations to treat their patients.

A well-experienced traditional physician in the past used to have specific knowledge and special ability to collect the right plants having the therapeutically useful agents from certain specific habitats. These experienced medicinal plant collectors had intimate knowledge of plant species and could identify therapeutically effective plant from a population of a species. With such unique expertise they were able to maintain certain level of standards in the therapeutic quality of the herbal drugs. There had been a decline or almost extinction of such experienced plant collectors by the turn of twentieth century itself due to a variety of reasons. One of the reasons was the transformation of traditional medicine from the individualized system to a commercial manufacturing system. This transformation resulted in great deterioration in the whole procedure and process of traditional medicine. Indeed, quality of the drugs became the greatest casualty in this transformation. Over 80 % of the raw material required for traditional medicines/herbal medicines used to be collected from wild resources. The increase in demand of medicinal plants for the commercial herbal medicine sector led to the indiscriminate and unscientific collection without any consideration for the quality of the material collected. The increase in commercial demand for medicinal plants from wild resources has also encouraged many to use adulterants or spurious materials, which further complicated the quality standards of plant based drugs and pharmaceuticals (Pushpangadan 1999).

It is now well known that the therapeutic activity of a medicinal plant is due to the presence of certain biologically active chemical constituents, which are either primary or secondary metabolites. The expression of many of these compounds particularly those of the secondary metabolite category are controlled and conditioned by a variety of factors such as its genetic predisposition, habitat of the plant agro climatic conditions, season and also the stage of growth and development of the plant etc. The traditional Indian system of medicines like *Ayurveda, Siddha, Unani* and *Amchi* etc. provided specific instructions for collection by indicating location/ edaphic conditions, habitat, seasonal and even the stage of the plant growth and developmental stage. Scientific investigations now provide ample evidence to the fact that there is a flux of change in the presence of very many of these chemical constituents, particularly those of the secondary metabolites, in such varied conditions described above. Therefore, it is extremely important to establish the reference samples and to determine the quality parameters of the medicinal plants by undertaking extensive and intensive study of the traditional treatise of the classical medicines or traditional practices, combined with the modern scientific knowledge and methods and using the latest analytical and computational tools. These tools have been used successfully in quality assurance of raw drugs/formulations. Some of our experiences are shared in the following points:

2.1 Pharmacognostic Evaluation of **Curcuma caesia** *Roxb. Rhizome*

Curcuma caesia Roxb. (Zingiberaceae) is commonly known as ‘black turmeric’. In India, it grows in West Bengal, Madhya Pradesh, Orissa, Bihar, North-East and Uttar Pradesh and is widely used by ethnic communities for various ailments. Rhizomes of the plant are used for sprains and bruises and are also employed in cosmetics. In West Bengal it finds an important place in traditional system of medicine and is also used as a substitute for turmeric in fresh stage. Detailed pharmacognostical evaluation of the rhizome sample was done to develop pharmacopoeial standards of this species which are shown in Fig. 14.3 (Plate 1 and 2) (Verma et al. 2010).

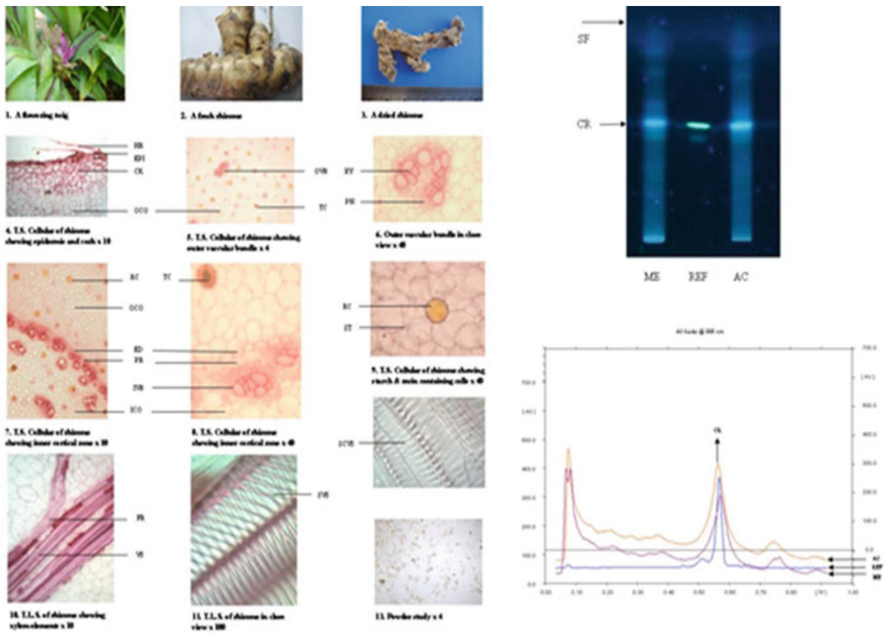


Fig. 14.3 Plate 1- Macro and Microscope characters of rhizome of *Curcuma cassia*. *OCO* Outer cortex, *ICO* inner cortex, *EPI* Epidermis, *CK* Cork cells, *OVB* Outer vascular bundle, *IVB* inner Vascular bundle, *FR* fiber, *ST* Starch, *VS*, Vessel, *XY* Xylem, *PM* Phloem, *ED* Endodermis, *PR* pericycle, *HR* hair, *TC* tannin collecting cell, *RC* Resin containnng cell, *SVS* Spiral vessel and *SCVS* scaleform vessel. **Plate 2** HPTLC profile of *Curcuma cassia* rhizome and reference sample (Uokr UV. 366 mm). *SF* Solvent froth, *CR* Curcumin, *REF* Reference sample, *AC* Acetate extract and *ME* Methanol extract

2.2 *Comparative Pharmacognostic Evaluation of Three Species of* Swertia *L*

Swertia spp. (Gentianaceae) commonly known as *Chiraita* in herbal drug markets of India is used to protect liver and a wide range of diseases. The official species, *Swertia chirayita* (Roxb.) Karsten is known for its potent activity against malaria, liver-disorder, fever, diabetes and also as appetite stimulant. However, several other species of *Swertia* viz. *S. alata* (Royle ex D. Don) Clarke and *S. paniculata* Wall. are being used as substitutes /adulterants for *S. chiraita* in India, Japan, China, Pakistan and other Asian Countries. The detailed morphological and anatomical studies revealed that there are marked differences in morphological characters such as shape of leaves (elliptic or ovate -lanceolate in *S. chirayita*, linear or linear-lanceolate in *S. paniculata* and ovate -oblong in *S. alata*) and stem (narrowly winged in *S. alata*, while cylindrical to obtusely four-angled in *S. chirayita* and *S. paniculata*). However, the main distinguishing features among these species are: flowers pentamerous in *S. paniculata* and tetramerous in *S. chirayita* and *S. alata,*

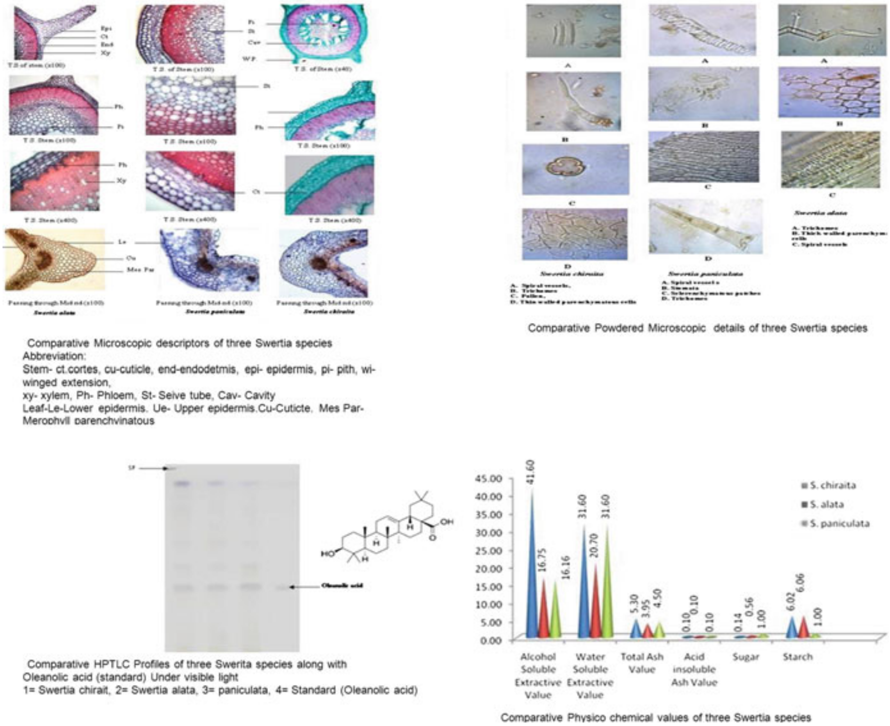


Fig. 14.4 Comparative pharmacognostical parameters (microscopy and phytochemical) of three species of *Swertia*

and each petal in *S. chirayita* have two glands, whereas in other two species there is single gland on each petal (Fig. 14.4). There are some minor variations in the microscopic characters as calcium oxalate crystals are absent in *S. alata*. No appearance of cavity in the pith region of stem in *S. alata* and *S. paniculata* while cavities present in premedullary region of *S. chirayita*. Besides, there is also variation in physicochemical parameters and oleanolic acid concentration among these species (Bisht et al. 2011).

2.3 *Phytochemical Evaluation of* Decalepis hamiltonii

Decalepis hamiltonii Wight & Arn. (Asclepiadaceae) is widely used in the traditional systems of medicine for many ailments. Roots are claimed to be useful as anti-inflammatory and antipyretic, antiulcer, antioxidant, hepatoprotective, neuroprotective, anxiolytic, antifungal and antibacterial. Tuberous roots are used as a cooling agent and blood-purifier. In our market survey it was observed that the plant is sold as a substitute of *Hemidesmus indicus*.

HPTLC, physico-chemical, fluorescence analysis and phytochemical evaluation were used to establish the authenticity of *Decalepis hamiltonii* root. The moisture content (18.23 %), total ash (8.08 %) and acid insoluble ash (0.77 %) are considered to be an important and useful parameter for detecting the presence of moisture and inorganic substances like silicate ion. Similarly the alcohol-soluble extractives (15.00 %) and water-soluble extractives (30.25 %) are indicators of the total solvent soluble components, sugars (0.16 %), starch (1.20 %) and tannins (4.64 %) shows quantity of different metabolites in this plant. Successive solvent extractions are carried through soxhlet apparatus and are indicators of the total soluble component at different polarity viz. hexane, chloroform, acetone, ethanol and water respectively. In HPTLC analysis composition of mobile phase was optimized by testing different solvent systems of varying polarity and the best results were obtained by using Toluene: Ethyl acetate (90:10 v/v) for Vanillin (Rf-0.42 $\pm$ 0.02). The R_f value of vanillin in the sample track was found to be 0.42 $\pm$ 0.02 while vanillin content was found to be 0.094 % w/w (Srivastava et al. 2011).

2.4 Comparative Botanical and Phytochemical Evaluation of Medicinally Important Stem Bark of Ficus *Species*

Pharmacognostical evaluation viz. botanical study, physicochemical parameters and HPTLC analysis and antioxidant studies to make a comparison among stem barks of four *Ficus* species viz. *F. carica, F. religiosa, F. glomerata* and *F. retusa*. Determinations of various physicochemical constants were carried out according to the methods provided in Ayurvedic Pharmacopoeia of India (API). The macro and microscopical character of these *Ficus* species showed moderate variation. Tannin content was found to be maximum in *F. religiosa* (6.36 %) and minimum in *F. carica* (0.14 %). This result was supported by microscopical studies which showed the presence of numerous dark brown cell contents in case of *F. religiosa*. Total phenolic content was also found to be maximum in *F. religiosa* (20.57 %) and minimum in *F. carica* (3.04 %). Sugar content was found to be maximum in *F. religiosa* (2.04 %) and minimum in *Ficus glomerata* (1.36 %). Starch content was found to be maximum in *F. carica* (6.44 %) and minimum in *F. glomerata* (1.49 %) (Rawat et al. 2012). HPTLC analysis showed the presence of β-sitosterol and lupeol in the ethanolic extract of bark of all the four *Ficus* species (Fig. 14.5). The R_f values of β-sitosterol and lupeol was found to be 0.46 and 0.62 respectively using toluene: ethyl acetate (80: 20 v/v) which is clearly visualized in HPTLC chromatogram and in densitometric chromatogram. Concentration of β-sitosterol was found maximum in *F. carica* (0.131 %) and minimum in *F. glomerata* (0.041 %) and conc. of lupeol was found to be maximum in *F. retusa* (0.069 %) and minimum in *F. religiosa* (0.020 %).

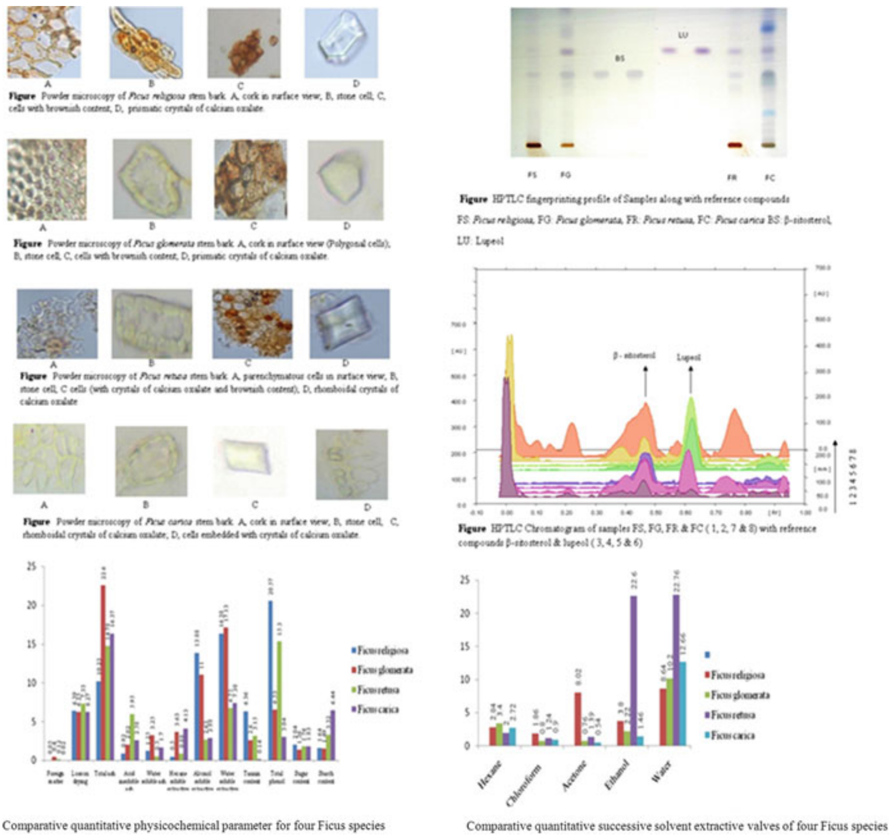


Fig. 14.5 Comparative pharmacognostical parameters (microscopy and phytochemical) of four species of *Ficus*

3 The Complexity of Herbal Products

Herbal products may contain a single herb or combinations of several different herbs believed to have complementary and/or synergistic effects. Some herbal products, including many traditional medicine formulations, also include animal products and minerals. Herbal products are sold as either raw plants or extracts of portions of the plant. Extraction involves boiling or percolating the herb in water, alcohol, or other solvents to release biologically active constituents of the plant. These liquid extracts may then be heated or dried to create more concentrated liquids, pastes, or powders. Both the raw herb and the extract contain complicated mixtures of organic chemicals, which may include fatty acids, sterols, alkaloids, flavonoids, glycosides, saponins, tannins, and terpenes. It is often difficult to determine which component, if any, of the herb has biological activity in humans. In addition, the processing of herbs, such as heating or boiling, may alter the pharmacological activity of the organic constituents. Similarly, a host of environmental factors, including soil, altitude, seasonal variation in temperature, atmospheric humidity, length of daylight, rainfall pattern, shade, dew, and frost conditions, may affect the levels of components in any given batch of an herb.

Chemical variations in germplasms of several plant species are well documented. Different chemotypes of *Ocimum, Hedychium, Thymus, Acorus, Piper, Valariana,* etc. exist in nature and their different compounds resulted in variation in pharmacological activities and quality parameters. Few such case studies are summarized in the following sections:

3.1 *Chemotypic Variations in* **Acorus calamus**

In case of *Acorus calamus,* our studies showed that 37 accessions collected from different phyto-geographical zones of India had remarkable variation in the essential oil content of rhizome. It was maximum in NBA-10 (9.5 % d/w) collected from North-West Himalayan zone (1,097 m altitude and rainfall 13–428 mm, high altitude clay soil) while it was minimum (2.3 % d/w) in NBA-16 collected from Lucknow, thus showing role of ecological conditions in variation of metabolites (Fig. 14.6).

3.2 *Comparative Antioxidant Activity and Quantification of Protodioscin and Prototribestin in Fruits of* **Tribulus terrestris** *Collected from Different Phyto-Geographical Zones of India*

Tribulus terrestris is a valuable herb known for its medicinal and dietary application in the traditional medicines of India and all over the world. Various chemical constituents of *Tribulus terrestris* have been found to possess various medicinal properties. Protodioscin and prototribestin are steroidal saponin compound(s) most notably in the *Tribulus* families. A simple, rapid, cost-effective and accurate high performance thin layer chromatographic method has been developed for quantification of protodioscin and prototribestin in the fruit of *T. terrestris* (Rawat et al. 2013). The collected accessions were successfully analyzed and depending on origin, a significant difference in the concentration of the protodioscin and prototribestin was observed. Separation and quantification was achieved by HPTLC using mobile phase of n butanol: glacial acetic acid: water (8.0:0.6:2.0 *v/v/v*) on precoated silica gel 60 F_{254} aluminum plates with 45 min TLC chamber saturation and densitometric determination was carried out after derivatization with anisaldehyde-sulphuric acid reagent in absorption-reflectance mode. Among the analysed accessions, NBT-06 (arid zone, Rajasthan) was found to contain maximum prototribestin (0.636 %) while protodioscin was maximum (0.317 %) in

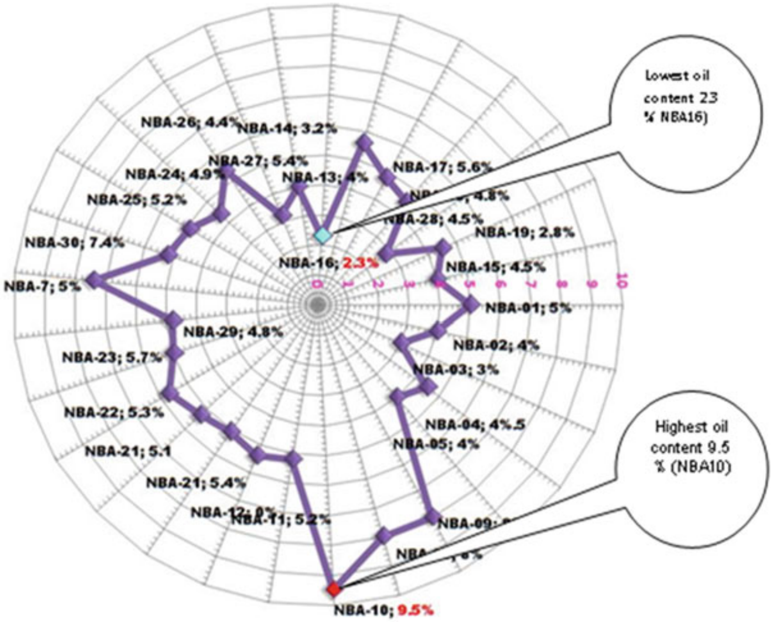
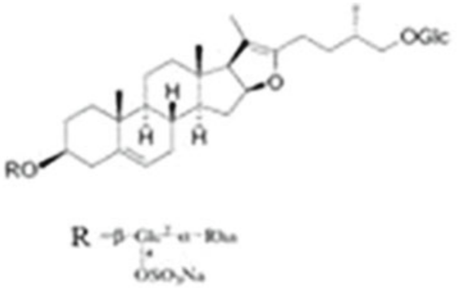


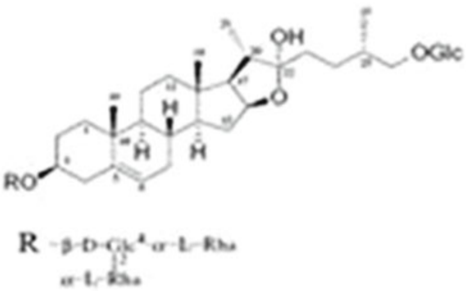
Fig. 14.6 Essential oil content (%) in rhizomes of *Acorus calamus* accessions collected from different phytogeographical zones of India

NBT-03 (Eastern Ghats, Tamilnadu). Prototribestin was not detected in NBT-03 (Tamilnadu), NBT-05 (U.P.), NBT-08 (U.P.) & NBT-34 (M.P.) while protodioscin was not detected in NBT-01 (Gujarat) and NBT-31 (U.P.) through HPTLC. The highest antioxidant activity was observed in Pali (Rajasthan) sample of arid zone (Fig. 14.7).

The demand of botanicals for producing standardized and quality herbal products is on rise and to promote their export, it is essential to maintain the quality of herbs used for the preparation of these products. Therefore, identity of plant species, variety/chemotypes, time of harvest, drying, storage, packing are some of the important steps for producing quality herbal medicine having batch to batch consistency and desirable therapeutic effects. Quality assurance can be achieved by using pharmacognostical parameters, HPTLC, HPLC, GC and other modern tools (Singh et al. 2007; Tiwari et al. 2012). These steps are important for keeping raw herbs devoid of foreign matter and devoid of pesticides and heavy metal as per WHO limits. Some essential steps to ensure quality of herbal product include compliance with GMP, preparation of standard formulations, preparation of SOP's, strict adherence to standard protocols and quality of raw materials etc.



Prototribestin (PT)



Protodioscin

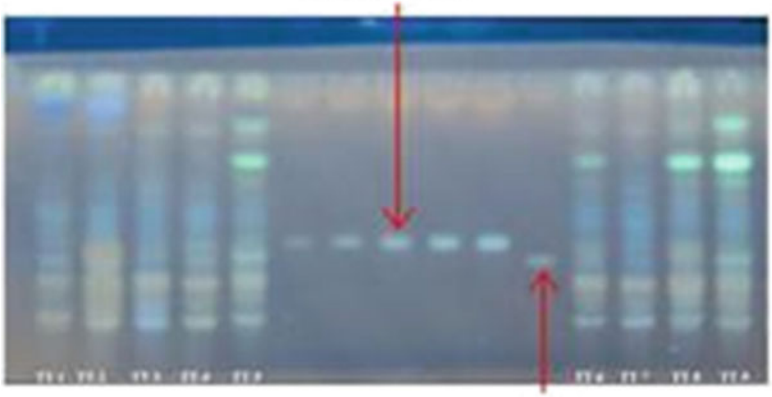


Fig. 14.7 HPTLC profile of *Tribulus terristris* accessions with Protodiaosin (*PD*) Standard (After derivitization with vallanilin-sulphuric acid under UV366)

4 Good Agricultural Practices for Medicinal Plants

This section presents general guidelines on good agricultural practices for medicinal plants. It describes general principles and provides technical details for the cultivation of medicinal plants. It also describes quality control measures, where applicable.

4.1 Selection of Medicinal Plants

Where applicable, the species or botanical variety selected for cultivation should be the same as that specified in the national pharmacopoeia or recommended by other authoritative national documents of the end-user's country. In the absence of such national documents, the selection of species or botanical varieties specified in the pharmacopoeia or other authoritative documents of other countries should be considered. In the case of newly introduced medicinal plants, the species or botanical variety selected for cultivation should be identified and documented as the source material used or described in traditional medicine of the original country.

4.2 Botanical Identity

The botanical identity – scientific name (genus, species, subspecies/variety, author, and family) – of each medicinal plant under cultivation should be verified and recorded. If available, the local and English common names should also be recorded. Other relevant information, such as the cultivar name, ecotype, chemotype or phenotype, may also be provided, as appropriate. For commercially available cultivars, the name of the cultivar and of the supplier should be provided. In the case of landraces collected, propagated, disseminated and grown in a specific region, records should be kept of the locally named line, including the origin of the source seeds, plants or propagation materials.

4.3 Specimens

In the case of the first registration in a producer's country of a medicinal plant or where reasonable doubt exists as to the identity of a botanical species, a voucher botanical specimen should be submitted to a regional or national herbarium for identification. Where possible, a genetic pattern should be compared to that of an authentic specimen. Documentation of the botanical identity should be included in the registration file.

4.4 Seeds and Other Propagation Materials

Seeds and other propagation materials should be specified, and suppliers of seeds and other propagation materials should provide all necessary information relating to the identity, quality and performance of their products, as well as their breeding history, where possible. The propagation or planting materials should be of the appropriate quality and be as free as possible from contamination and diseases in order to promote healthy plant growth. Planting material should preferably be resistant or tolerant to biotic or abiotic factors. Seeds and other propagation materials used for organic production should be certified as being organically derived. The quality of propagation material – including any genetically modified germplasm – should comply with regional and/or national regulations and be appropriately labelled and documented, as required. Care should be taken to exclude extraneous species, botanical varieties and strains of medicinal plants during the entire production process. Counterfeit, substandard and adulterated propagation materials must be avoided.

4.5 Cultivation

Cultivation of medicinal plants requires intensive care and management. The conditions and duration of cultivation required vary depending on the quality of medicinal plant materials required. If no scientific published or documented cultivation data are available, traditional methods of cultivation should be followed, where feasible. Otherwise a method should be developed through research. The principles of good plant husbandry, including appropriate rotation of plants selected according to environmental suitability, should be followed, and tillage should be adapted to plant growth and other requirements. Conservation Agriculture (CA) techniques should be followed where appropriate, especially in the build-up of organic matter and conservation of soil humidity. CA also includes "no-tillage" systems and aims to conserve, improve and make more efficient use of natural resources through integrated management of available soil, water and biological resources combined with external inputs. It contributes to environmental conservation as well as to enhanced and sustained agricultural production. It can also be referred to as resource-efficient/resource- effective agriculture.

4.5.1 Site Selection

Medicinal plant materials derived from the same species can show significant differences in quality when cultivated at different sites, owing to the influence of soil, climate and other factors. These differences may relate to physical appearance or to variations in their constituents, the biosynthesis of which may be affected by extrinsic environmental conditions, including ecological and geographical variables, and should be taken into consideration. Risks of contamination as a result of pollution of the soil, air or water by hazardous chemicals should be avoided. The impact of past land uses on the cultivation site, including the planting of previous crops and any applications of plant protection products, should be evaluated.

4.5.2 Ecological Environment and Social Impact

The cultivation of medicinal plants may affect the ecological balance and, in particular, the genetic diversity of the flora and fauna in surrounding habitats. The quality and growth of medicinal plants can also be affected by other plants, other living organisms and by human activities. The introduction of non-indigenous medicinal plant species into cultivation may have a detrimental impact on the biological and ecological balance of the region. The ecological impact of cultivation activities should be monitored over time, where practical. The social impact of cultivation on local communities should be examined to ensure that negative impacts on local livelihood are avoided. In terms of local income- earning opportunities, small-scale cultivation is often preferable to large-scale production, in particular if small-scale farmers are organized to market their products jointly. If large-scale medicinal plant cultivation is or has been established, care should be taken that local communities benefit directly from, for example, fair wages, equal employment opportunities and capital reinvestment.

4.5.3 Climate

Climatic conditions, for example, length of day, rainfall (water supply) and field temperature, significantly influence the physical, chemical and biological qualities of medicinal plants. The duration of sunlight, average rainfall, average temperature, including daytime and night-time temperature differences, also influence the physiological and biochemical activities of plants, and prior knowledge should be considered.

4.5.4 Soil

The soil should contain appropriate amounts of nutrients, organic matter and other elements to ensure optimal medicinal plant growth and quality. Optimal soil conditions, including soil type, drainage, moisture retention, fertility and pH, will be dictated by the selected medicinal plant species and/or target medicinal plant part. The use of fertilizers is often indispensable in order to obtain large yields of medicinal plants. It is, however, necessary to ensure that correct types and quantities of fertilizers are used through agricultural research. In practice, organic and chemical fertilizers are used. Human excreta must not be used as a fertilizer owing to the potential presence of infectious microorganisms or parasites. Animal manure should be thoroughly composted to meet safe sanitary standards of acceptable microbial limits and destroyed by the germination capacity of weeds. Any applications of animal manure should be documented. Chemical fertilizers that have been approved by the countries of cultivation and consumption should be used. All fertilizing agents should be applied sparingly and in accordance with the needs of the particular medicinal plant species and supporting capacity of the soil. Fertilizers should be applied in such a manner as to minimize leaching.

Growers should implement practices that contribute to soil conservation and minimize erosion, for example, through the creation of streamside buffer zones and the planting of cover crops and “green manure” (crops grown to be ploughed in), such as alfalfa.

4.5.5 Irrigation and Drainage

Irrigation and drainage should be controlled and carried out in accordance with the needs of the individual medicinal plant species during its various stages of growth. Water used for irrigation purposes should comply with local, regional and/or national quality standards. Care should be exercised to ensure that the plants under cultivation are neither over- nor under-watered. In the choice of irrigation, as a general rule, the health impact of the different types of irrigation (various forms of surface, sub-surface or overhead irrigation), particularly on the risks of increased vector-borne disease transmission, must be taken into account.

4.5.6 Plant Maintenance and Protection

The growth and development characteristics of individual medicinal plants, as well as the plant part destined for medicinal use, should guide field management practices. The timely application of measures such as topping, bud nipping, pruning and shading may be used to control the growth and development of the plant, thereby improving the quality and quantity of the medicinal plant material being produced.

Any agrochemicals used to promote the growth of or to protect medicinal plants should be kept to a minimum, and applied only when no alternative measures are available. Integrated pest management should be followed where appropriate. When necessary, only approved pesticides and herbicides should be applied at the minimum effective level, in accordance with the labelling and/or package insert instructions of the individual product and the regulatory requirements that apply for the grower and the end-user countries. Only qualified staff using approved equipment should carry out pesticide and herbicide applications. All applications should be documented. The minimum interval between such treatments and harvest should be consistent with the labelling and/or package insert instructions of the plant protection product, and such treatments should be carried out in consultation and with the by agreement of the buyer of the medicinal plants or medicinal plant materials. Growers and producers should comply with maximum pesticide and herbicide residue limits, as stipulated by local, regional and/or national regulatory authorities of both the growers' and the end-users' countries and/or regions. International agreements such as the International Plant Protection Convention and *Codex Alimentarius* should also be consulted on pesticide use and residues.

4.6 Harvest

Medicinal plants should be harvested during the optimal season or time period to ensure the production of medicinal plant materials and finished herbal products of the best possible quality. The time of harvest depends on the plant part to be used. Detailed information concerning the appropriate timing of harvest is often available in national pharmacopoeias, published standards, official monographs and major reference books. However, it is well known that the concentration of biologically active constituents varies with the stage of plant growth and development. This also applies to non-targeted toxic or poisonous indigenous plant ingredients. The best time for harvest (quality peak season/time of day) should be determined according to the quality and quantity of biologically active constituents rather than the total vegetative yield of the targeted medicinal plant parts. During harvest, care should be taken to ensure that no foreign matter, weeds or toxic plants are mixed with the harvested medicinal plant materials.

Medicinal plants should be harvested under the best possible conditions, avoiding dew, rain or exceptionally high humidity. If harvesting occurs in wet conditions, the harvested material should be transported immediately to an indoor drying facility to expedite drying so as to prevent any possible deleterious effects due to increased moisture levels, which promote microbial fermentation and mould. Cutting devices, harvesters, and other machines should be kept clean and adjusted to reduce damage and contamination from soil and other materials. They should be stored in an uncontaminated, dry place or facility free from insects, rodents, birds and other pests, and inaccessible to livestock and domestic animals.

Contact with soil should be avoided to the extent possible so as to minimize the microbial load of harvested medicinal plant materials. Where necessary, large drop cloths, preferably made of clean muslin, may be used as an interface between the harvested plants and the soil. If the underground parts (such as the roots) are used, any adhering soil should be removed from the medicinal plant materials as soon as they are harvested. The harvested raw medicinal plant materials should be transported promptly in clean, dry conditions. They may be placed in clean baskets, dry sacks, trailers, hoppers or other well-aerated containers and carried to a central point for transport to the processing facility. All containers used at harvest should be kept clean and free from contamination by previously harvested medicinal plants and other foreign matter. If plastic containers are used, particular attention should be paid to any possible retention of moisture that could lead to the growth of mould.

When containers are not in use, they should be kept in dry conditions, in an area that is protected from insects, rodents, birds and other pests, and inaccessible to livestock and domestic animals.

Any mechanical damage or compacting of the raw medicinal plant materials, as a consequence, for example, of overfilling or stacking of sacks or bags, that may result in composting or otherwise diminish quality should be avoided. Decomposed medicinal plant materials should be identified and discarded during harvest, post-harvest inspections and processing, in order to avoid microbial contamination and loss of product quality.

5 Personnel

Growers and producers should have adequate knowledge of the medicinal plant concerned. This should include botanical identification, cultivation characteristics and environmental requirements (soil type, soil pH, fertility, plant spacing and light requirements), as well as the means of harvest and storage. All personnel (including field workers) involved in the propagation, cultivation, harvest and post-harvest processing stages of medicinal plant production should maintain appropriate personal hygiene and should have received training regarding their hygiene responsibilities. Only properly trained personnel, wearing appropriate protective clothing (such as overalls, gloves, helmet, goggles, face mask), should apply agrochemicals. Growers and producers should receive instruction on all issues relevant to the protection of the environment, conservation of medicinal plant species, and proper agricultural stewardship.

6 Good Collection Practices for Medicinal Plants

This section describes the general strategies and basic methods for small and large scale collection of fresh medicinal plant materials. Collection practices should ensure the long-term survival of wild populations and their associated habitats. Management plans for collection should provide a framework for setting sustainable harvest levels and describe appropriate collection practices that are suitable for each medicinal plant species and plant part used (roots, leaves, fruits, etc.). Collection of medicinal plants raises a number of complex environmental and social issues that must be addressed locally on a case-by-case basis. It is acknowledged that these issues vary widely from region to region and cannot be fully covered by these guidelines. More guidance can be found in the WHO/IUCN/WWF Guidelines on the Conservation of Medicinal Plants to deal comprehensively with the sustainable use and conservation of medicinal plants.

6.1 Permission to Collect

In some countries, collection permits and other documents from government authorities and landowners must be obtained prior to collecting any plants from the wild. Sufficient time for the processing and issuance of these permits must be allocated at the planning stage. National legislation, such as national "red" lists, should be consulted and respected. For medicinal plant materials intended for export from the country of collection, export permits, phytosanitary certificates, Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) permit(s) (for export and import), CITES certificates (for re-export), and other permits must be obtained, when required.

6.2 Technical Planning

Prior to initiating a collection expedition, the geographical distribution and population density of the target medicinal plant species should be determined. Distance from home base and quality of the target plant(s) available are factors to be considered. When the collection sites have been identified, local and/or national collection permits should be obtained, as indicated in Sect. 4.1. Essential information on the target species (taxonomy, distribution, phenology, genetic diversity, reproductive biology and ethnobotany) should be obtained. Data about environmental conditions, including topography, geology, soil, climate and vegetation at the prospective collecting site(s), should be collated and presented in a collection management plan.

Research on the morphology of the target medicinal plant species and variability of its populations should be carried out in order to develop a "search image" for the species. Copies of photographs and other illustrations of the target medicinal plant (s) from books and herbarium specimens, and ethnographical information (common or local names) of the target species and plant parts are useful field instruments, especially for untrained workers. Botanical keys and other taxonomic identification aids are useful at collection sites where either related species, or unrelated species of similar morphological characteristics, may be found. Rapid, safe and dependable transportation to carry personnel, equipment, supplies and collected medicinal plant materials should be arranged in advance.

A collection team familiar with good collecting techniques, transport, and handling of equipment and medicinal plant materials, including cleaning, drying and storage, should be assembled. Training of personnel should be conducted regularly. The responsibilities of all those involved in collection should be clearly set out in a written document. All stakeholders, in particular, manufacturers, traders and government, are accountable for the conservation and management of the targeted medicinal plant species. The social impact of field collection on local communities should be examined and the ecological impact of field collection activities should be monitored over time. The stability of the natural habitat(s) and the maintenance of sustainable populations of the target species in the collection area(s) must be ensured.

6.3 Selection of Medicinal Plants for Collection

Where applicable, the species or botanical variety selected for collection should be the same as that specified in the national pharmacopoeia or recommended by other authoritative national documents of the end-user's country, as the source for the herbal medicines concerned. In the absence of such national documents, the selection of species or botanical varieties specified in the pharmacopoeia or other authoritative documents of other countries should be considered. In the case of newly introduced medicinal plants, the species or botanical variety selected for collection should be identified and documented as the source material used or described in traditional medicine in original countries.

Collectors of medicinal plants and producers of medicinal plant materials and herbal medicines should prepare botanical specimens for submission to regional or national herbaria for authentication. The voucher specimens should be retained for a sufficient period of time, and should be preserved under proper conditions. The name of the botanist or other experts who provided the botanical identification or authentication should be recorded. If the medicinal plant is not well known to the community, then documentation of the botanical identity should be recorded and maintained.

6.4 Collection

Collection practices should ensure the long-term survival of wild populations and their associated habitats. The population density of the target species at the collection site(s) should be determined and species that are rare or scarce should not be collected. To encourage the regeneration of source medicinal plant materials, a sound demographic structure of the population has to be ensured. Management plans should refer to the species and the plant parts (roots, leaves, fruits, etc.) to be collected and should specify collection levels and collection practices. It is incumbent on the government or environmental authority to ensure that buyers of collected plant material do not place the collected species at risk. Medicinal plant materials should be collected during the appropriate season or time period to ensure the best possible quality of both source materials and finished products. It is well known that the quantitative concentration of biologically active constituents varies with the stage of plant growth and development. This also applies to non-targeted toxic or poisonous indigenous plant ingredients. The best time for collection (quality peak season or time of day) should be determined according to the quality and quantity of biologically active constituents rather than the total vegetative yield of the targeted medicinal plant parts.

Only ecologically non-destructive systems of collection should be employed. These will vary widely from species to species. For example, when collecting roots of trees and bushes, the main roots should not be cut or dug up, and severing the taproot of trees and bushes should be avoided. Only some of the lateral roots should be located and collected. When collecting species whose bark is the primary material to be used, the tree should not be girdled or completely stripped of its bark; longitudinal strips of bark along one side of the tree should be cut and collected. Medicinal plants should not be collected in or near areas where high levels of pesticides or other possible contaminants are used or found, such as roadsides, drainage ditches, mine tailings, garbage dumps and industrial facilities which may produce toxic emissions. In addition, the collection of medicinal plants in and around active pastures, including riverbanks downstream from pastures, should be avoided in order to avoid microbial contamination from animal waste. In the course of collection, efforts should be made to remove parts of the plant that are not required and foreign matter, in particular toxic weeds. Decomposed medicinal plant materials should be discarded.

In general, the collected raw medicinal plant materials should not come into direct contact with the soil. If underground parts (such as the roots) are used, any adhering soil should be removed from the plants as soon as they are collected. Collected material should be placed in clean baskets, mesh bags, other well aerated containers or drop cloths that are free from foreign matter, including plant remnants from previous collecting activities. After collection, the raw medicinal plant materials may be subjected to appropriate preliminary processing, including elimination of undesirable materials and contaminants, washing (to remove excess soil), sorting and cutting. The collected medicinal plant materials should be protected from insects, rodents, birds and other pests, and from livestock and domestic animals.

If the collection site is located some distance from processing facilities, it may be necessary to air or sun-dry the raw medicinal plant materials prior to transport. If more than one medicinal plant part is to be collected, the different plant species or plant materials should be gathered separately and transported in separate containers. Cross-contamination should be avoided at all times. Collecting implements, such as machetes, shears, saws and mechanical tools, should be kept clean and maintained in proper condition. Those parts that come into direct contact with the collected medicinal plant materials should be free from excess oil and other contamination.

6.5 Personnel

Local experts responsible for the field collection should have formal or informal practical education and training in plant sciences and have practical experience in fieldwork. They should be responsible for training any collectors who lack sufficient technical knowledge to perform the various tasks involved in the plant collection process. They are also responsible for the supervision of workers and the full documentation of the work performed. Field personnel should have adequate botanical training, and be able to recognize medicinal plants by their common names and, ideally, by their scientific (Latin) names. Local experts should serve as knowledgeable links between non-local people and local communities and collectors. All collectors and local workers involved in the collection operation should have sufficient knowledge of the species targeted for collection and be able to distinguish target species from botanically related and/or morphologically similar species. Collectors should also receive instructions on all issues relevant to the protection of the environment and the conservation of plant species, as well as the social benefits of sustainable collection of medicinal plants.

The collection team should take measures to ensure the welfare and safety of staff and local communities during all stages of medicinal plant sourcing and trade. All personnel must be protected from toxic and dermatitis-causing plants, poisonous animals and disease-carrying insects. Appropriate protective clothing, including gloves, should be worn when necessary.

7 Common Technical Aspects of Good Agricultural Practices for Medicinal Plants and Good Collection Practices for Medicinal Plants

Medicinal plants should be harvested in sustainable manner. ‘Sustainability’ is a principle that has been used for centuries in agriculture, forestry and in the management of natural resources and has been simply described as a system that meets the needs of the present without compromising the ability of future generations to meet their own needs. The important components of sustainable harvesting include timing of harvesting, material to be harvested, harvesting techniques, harvesting equipment and storage etc. These practices are common to the medicinal plant produce, obtained from collection as well as cultivation.

7.1 Inspection and Sorting

Raw medicinal plant materials should be inspected and sorted prior to primary processing. The inspection may include:

- Visual inspection for cross-contamination by untargeted medicinal plants and/or plant parts;
- Visual inspection for foreign matter;
- organoleptic evaluation, such as: appearance, damage, size, colour, odour, and possibly taste.

7.2 Primary Processing

Appropriate measures of primary processing are dependent on the individual materials. These processes should be carried out in conformity with national and/or regional quality standards, regulations and norms. In some cases, purchasers may request that specific protocols are followed. These protocols should also comply with national and/or regional regulatory requirements that apply in the producer and the purchaser countries. As far as possible, standard operating procedures should be followed. If modifications are made, they should be justified by adequate test data demonstrating that the quality of the medicinal plant material is not diminished.

Harvested or collected raw medicinal plant materials should be promptly unloaded and unpacked upon arrival at the processing facility. Prior to processing, the medicinal plant materials should be protected from rain, moisture and any other conditions that might cause deterioration. Medicinal plant materials should be exposed to direct sunlight only where there is a specific need for this mode of drying. Medicinal plant materials that are to be used in the fresh state should be harvested/collected and delivered as quickly as possible to the processing facility in order to prevent microbial fermentation and thermal degradation. The materials may be stored under refrigeration, in jars, in sandboxes, or using enzymatic and other appropriate conservation measures immediately following harvest/collection and during transit to the end-user. The use of preservatives should be avoided. If used, they should conform to national and/or regional regulations for growers/collectors and end-users.

Medicinal plant materials that are to be employed fresh should be stored under refrigeration, in jars, in sandboxes, or using enzymatic or other appropriate conservation measures, and transported to the end-user in the most expeditious manner possible. The use of preservatives should be avoided. If used, this should be documented and they should conform to national and/or regional regulatory requirements in both the source country and the end-user country. All medicinal plant materials should be inspected during the primary-processing stages of production, and any substandard products or foreign matter should be eliminated mechanically or by hand. For example, dried medicinal plant materials should be inspected, sieved or winnowed to remove discoloured, mouldy or damaged materials, as well as soil, stones and other foreign matter. Mechanical devices such as sieves should be regularly cleaned and maintained. All processed medicinal plant materials should be protected from contamination and decomposition as well as from insects, rodents, birds and other pests, and from livestock and domestic animals.

7.3 Drying

When medicinal plant materials are prepared for use in dry form, the moisture content of the material should be kept as low as possible in order to reduce damage from mould and other microbial infestation. Information on the appropriate moisture content for particular medicinal plant materials may be available from pharmacopoeias or other authoritative monographs. Medicinal plants can be dried in a number of ways: in the open air (shaded from direct sunlight); placed in thin layers on drying frames, wire-screened rooms or buildings; by direct sunlight, if appropriate; in drying ovens/rooms and solar dryers; by indirect fire; baking; lyophilization; microwave; or infrared devices. When possible, temperature and humidity should be controlled to avoid damage to the active chemical constituents. The method and temperature used for drying may have a considerable impact on the quality of the resulting medicinal plant materials. For example, shade drying is preferred to maintain or minimize loss of colour of leaves and flowers; and lower temperatures should be employed in the case of medicinal plant materials containing volatile substances. The drying conditions should be recorded.

In the case of natural drying in the open air, medicinal plant materials should be spread out in thin layers on drying frames and stirred or turned frequently. In order to secure adequate air circulation, the drying frames should be located at a sufficient height above the ground. Efforts should be made to achieve uniform drying of medicinal plant materials and so avoid mould formation. Drying medicinal plant material directly on bare ground should be avoided. If a concrete or cement surface is used, medicinal plant materials should be laid on a tarpaulin or other appropriate cloth or sheeting. Insects, rodents, birds and other pests, and livestock and domestic animals should be kept away from drying sites.

For indoor drying, the duration of drying, drying temperature, humidity and other conditions should be determined on the basis of the plant part concerned (root, leaf, stem, bark, flower, etc.) and any volatile natural constituents, such as essential oils. If possible, the source of heat for direct drying (fire) should be limited to butane, propane or natural gas, and temperatures should be kept below 60 °C. If other sources of fire are used, contact between those materials, smoke and medicinal plant material should be avoided.

7.4 Specific Processing

Some medicinal plant materials require specific processing to: improve the purity of the plant part being employed; reduce drying time; prevent damage from mould, other microorganisms and insects; detoxify indigenous toxic ingredients; and enhance therapeutic efficacy. Common specific processing practices include pre-selection, peeling the skins of roots and rhizomes, boiling in water, steaming, soaking, pickling, distillation, fumigation, roasting, natural fermentation, treatment with lime and chopping. Processing procedures involving the formation of certain shapes, bundling and special drying may also have an impact on the quality of the medicinal plant materials.

Antimicrobial treatments of medicinal plant materials (raw or processed) by various methods, including irradiation, must be declared and the materials must be labelled as required. Only suitably trained staff using approved equipment should carry out such applications, and they should be conducted in accordance with standard operating procedures and national and/or regional regulations in both the grower/collector country and the end-user country. Maximum residue limits, as stipulated by national and/or regional authorities, should be respected.

References

Anonymous (1999–2012) The ayurvedic pharmacopoeia of India. Part-I (Monographs) vol III, 1st edn. Government of India, Department of Indian Systems of Medicine and Homoeopathy, New Delhi

Anonymous (2000) Medicinal plants: the global view, international development research centre (IDRC). New Delhi Research Programme

Anonymous (2003–2013) Quality standards of Indian medicinal plants, vol VIII. Indian Council of Medical Research, New Delhi

Anonymous (2008) The ayurvedic pharmacopoeia of India. Part-II (formulations), vol II, 1st edn. Ayush Department, New Delhi

Anonymous (2013) Ayurveda, vol 2, No 3. Association of manufacturers of ayurvedic medicines, Ghaziabad

Bisht D, Gupta M, Srivastava S, Datt B, Rawat AKS (2011) Comparative pharmacognostic evaluation of three species of S*wertia* L. (Gentianaceae). Pharmacognosy J 3(19):7–12

Darvill AG, Albersheim P (1984) Phytoalexins and their elicitors—a defense against microbial infection in plants. Annu Rev Plant Physiol 35:243–275

Dixon RA, Paiva NL (1995) Stress-induced phenylpropanoid metabolism. Plant Cell 7(7):1085–1097

Eder J, Cosio EG (1994) Elicitors of plant defense responses. Int Rev Cytol 148:1–36

Facchini PJ, De Luca V (1994) Differential and tissue-specific expression of a gene family for tyrosine/dopa decarboxylase in opium poppy. J Biol Chem 269:26684–26690

Nema J, Shrivastava SK, Mitra NG (2013) Chemical composition of *Aloe ferox* under stresses of soil pH and desiccation. Int J Chem 03:44–48

Nishioka I (1991) Biological activities and the active components of rhubarb. Int J Orient med 16:193–212

Om P, Jyoti, Kumar A, Kumar P, Mann NK (2013) Adulteration and substitution in Indian medicinal plants: an overview. J Med Plants Stud 1(4):127–132

Pushpangadan P (1999) Medicinal plants industrial possibilities for India in 21st Century. Nature Watch 1(1):14–18

Rawat AKS, Tiwari SS, Srivastava A, Srivastava S (2012) Comparative pharmacognostic and phytochemical standardization of stem bark of four medicinally important *Ficus* species and their antioxidant studies. *Asian J Tropical Biomed* 2:S1–6

Rawat AKS, Srivastava A, Tiwari SS, Srivastava S (2013) Quantification of protodioscin and prototribestin in fruits of *Tribulus terrestris* L. collected from different phyto-geographical zones of India. J Liq Chromatogr Relat Technol 36(13):1810–1821

Singh DP, Srivastava SK, Govindarajan R, Rawat AKS (2007) Determination of Bergenin and Gallic acid in different *Bergenia* species by high performance liquid chromatography. Acta Chromatographia 19:246–252

Srivastava S, Tiwari SS, Rawat AKS (2011) Phytochemical standardization of *Decalepis hamiltonii* Wight & Arn. Root Nat Acad Sci Letter 34(1&2):41–47

Subrat N, Iyer M, Prasad R (2002) The ayurvedic medicine industry: current status and sustainability. Published by Ecotech Services (India) Pvt. Ltd, 63

Tiwari SS, Pandey MM, Srivastava S, Rawat AKS (2012) Simultaneous determination of Picrosides (Picroside-I and Picroside-II) in *Picrorhiza kurroa* and its substitute *Picrorhiza scrophulariiflora* through high performance thin layer chromatography. Biomed Chromatogr 26(1):61–68

Trapp S, Croteau R (2001) Defensive resin biosynthesis in conifers. Annu Rev Plant Physiol Plant Mol Biol 52:689–724

Verma D, Srivastava S, Singh V, Rawat AKS (2010) Pharmacognostic evaluation of *Curcuma caesia* Roxb. Rhizome Nat Prod Sci 16(2):107–110

Chapter 15
In Vitro Micropropagation of Medicinal and Aromatic Plants

Ákos Máthé, Fathi Hassan, and Ahmad Abdul Kader

Abstract Modern biotechnological methods like *in vitro* micropropagation technique hold tremendous potential for the production of high-quality plant-based medicine. They also allow to achieve the large scale multiplication of disease-free plants, faster cloning and the conservation of desired genotypes, in a very short span of time. Via genetic transformation techniques, the modification of both genetic information of MAPs and the regulation of genes responsible for the production of valuable biologically active substances has also become possible in either higher amounts or with better properties.

Micropropagation protocols are worked out for many plant species cultured *in vitro* to provide macro- and micro- mineral nutrients, vitamins, source of carbohydrates, appropriate environmental conditions (light intensity, photoperiod and temperature) and plant growth regulators required to obtain high regeneration rates. As such they are expected to facilitate commercially feasible micropropagation. Well-defined cell culture methods have also been developed to produce pharmacologically important secondary metabolites. Genetic engineering is applied to produce transgenic medicinal plants and metabolites.

This chapter offers a brief insight into the present status of the biotechnology of MAPs. Special emphasis has been placed on the *in vitro* micropropagation and rapid clonal multiplication of selected elite genotypes, the regulation of organogenesis and somatic embryogenesis, the exploitation of advantages in somaclonal variation and genetic engineering techniques for both crop improvement and *in vitro* germplasm conservation. The production of flavor and volatile constituents in tissue cultures will also be briefly discussed, similarly to the application of farther biotechnological approaches.

Á. Máthé (✉)
Faculty of Agriculture and Food Science, University of West Hungary, Budapest, Hungary
e-mail: akos.mathe@upcmail.hu

F. Hassan
Department of Plant Biotechnology, Institute of Plant Genetics, Leibniz University of Hannover, Herrenhauser street 2, Hannover 30419, Germany

A. Abdul Kader
Biotechnology Department, General Commission for Scientific Agricultural Research (GCSAR), Damascus 12573, Syria

Á. Máthé (ed.), *Medicinal and Aromatic Plants of the World*, Medicinal and Aromatic Plants of the World 1, DOI 10.1007/978-94-017-9810-5_15

Keywords *In vitro* propagation • Biotechnology • Medicinal plants • Metabolites • Micropropagation • Transgenics • Genetic transformation

Abbreviations

BA	6-benzylaminopurine
B5	Gamborg et al. (1976) medium
2,4-D	2,4-dichlorophenoxy acetic acid
IAA	indole-3-acetic acid
IBA	indole-3-butyric acid
MS	Murashige and Skoog (1962) medium
N6	Chu et al. (1975) medium
NAA	naphthalene-1- acetic acid
BA	6-benzylaminopurine
Kn	kinetin
TDZ	Thidiazuron
2ip	2-isopentenyladenine
GA3	Gibberellic acid
MAPs	medicinal and aromatic plants
WPM	Woody Plant Medium (McCown and Lloyd 1981)

1 Introduction

Micropropagation or Plant tissue culture is defined as aseptic asexual plant propagation on a defined culture medium, in culture vessels, under controlled conditions of light and temperature. It is the science and art of growing isolated plant cells, tissues, organs or whole plants on semi-solid or in liquid synthetic nutrient media, under aseptic conditions. The name micropropagation refers to the miniature shoots/plantlets initially produced (in culture vessels) in this mode of plant propagation. Micropropagation allows to capture maximum genetic gain from the genetic variability of natural populations. When appropriate selection criteria and methods are adopted, micropropagation of economically important plants can be feasible (Kane et al. 2008; Ganguli 2009; Kumar and Loh 2012).

The interest in *in vitro* mass propagation of Medicinal and Aromatic Plants (MAPs) has distinctly increased for various reasons like:

- Clonal multiplication of high yielding elite genotypes to generate good quality planting material with higher rate of multiplication.
- Rapid multiplication of plants which do not set seeds in a particular climate or those whose seeds have low germination capacity, where, environment can be controlled or altered to meet specific needs of the plant.

- Availability of plants all year round (independently of regional or seasonal variations)
- Resistance of plants to pests, diseases and herbicides.
- Production of uniform clones from highly heterozygous plants.
- *In vitro* selection for resistance to biotic and abiotic stresses and production of plants with modified genotypes to avoid a danger of monoculture, i.e. genetic erosion.
- Conservation of genetic resources of species and endangered plants which are facing extinction and *in vitro* clonal multiplication and dissemination of elite clones and *ex- situ* conservation of endangered medicinal plants.
- Identification and production of clones with desired characteristics and exploiting somaclonal variation and genetic engineering techniques for crop improvement.
- Identification of fast growing medicinal trees for production of medicines.
- Safe exchange of germplasm
- Production of secondary metabolites (flavor, volatile constituents, etc.) in tissue culture.
- Preservation of genetic materials by cryopreservation

Due to the vast scope of its application some experts are refer to tissue culture research as a "botanical laser" (Chawla 2006; Ganguli 2009; Trigiano and Gray 2011; Kumar and Loh 2012).

Tissue culture protocols have been developed for a wide range of MAPs. These include both endangered rare and threatened plant species. Additionally, biotechnology with its apparently unlimited potentials, offers alternatives to conventional crop improvement, large scale multiplication of disease-free plants, faster cloning and conservation of plant genotypes, in a relatively short span of time. It also opens up possibilities to modify and regulate their genetic information with the aim of producing valuable natural substances, in either higher amounts or with better properties (Rout et al. 2000a, b; Schippmann et al. 2002; Ganguli 2009; Rath and Puhan 2009; Yaadwinder 2010; Tasheva and Kosturkova 2012).

This chapter offers a brief insight into the present status of biotechnology applications related to micropropagation, rapid clonal multiplication, and improvement of MAPs.

2 Micropropagation of Medicinal and Aromatic Plants

In conventional open field cultivation, many of medicinal species show a low rate of fruit-set and seed germination or have long periods of growth that result in extremely slow multiplication rates. The productivity of many MAPs is low due to the lack of high yielding, biotic stress tolerant cultivars and the absence of disease–free planting materials of elite genotypes (Gantait et al. 2011). Similarly, there is a lack of sufficient planting materials and commercial propagation techniques. As a result, there is a pressure on the wild populations with pharmaceutical uses. Consequently, there is an urgency to preserve such wild populations for future uses by adopting improved and efficient mass propagation techniques.

The conventional methods are frequently time consuming, less efficient and sometimes even unsuccessful. As a contrast, *in vitro* propagation techniques allow to achieve large scale multiplication of disease free plants in short time around the year, under aseptic conditions (Rout et al. 2000a, b; Rath and Puhan 2009; Lakshimi and Reddy 2009; Yaadwinder 2010). Micropropagation methods have been elaborated for many medicinal plants including some **threatened species** including: *Adhatoda beddomei, Aegle marmelos, Aristolochia spp., Artemisia spp., Azadirachta indica,* ***Acorus calamus, Boswellia serrata****, Catharanthus roseus, Centella asiatica,* ***Cammigonum polygonoides, Caralluma spp., Ceropegia bulbosa, C. fantastica****,* ***Chlorophytum borivilianum, Commiphora wightii, Coptis teeta,*** *Coleus froskohlii, Dioscorea spp., Glycorrihiza glabra, Gymnema sylvestre, Immondsia chinensis, Mentha spp.,* ***Nepenthes khasiana,*** *Panax spp., Papaver somniferum, Phyllanthus spp., Plantago zeylanica,* ***Podophyllum hexandrum,*** *Podophyllum spp.,* ***Rhodiola rosea, Spilanthes acmella, Tecomella undulat****, Terminalia arjuna, Tribulus terrestris, Tylophora indica, Vitex negundo, Withania somnifera* (Chandore et al. 2010; Singh et al. 2011; Neha 2011; Tasheva and Kosturkova 2012; Yadav et al. 2012).

Biotechnological tools are important in order to select, multiply and conserve the important genotypes of MAPs. Micropropagation is used for the rapid multiplication of stock plant materials i.e. to produce large numbers of progenies under sterile conditions. It covers all types of aseptic plant cultures: Seed culture, Embryo culture, Organ culture, Callus culture, Cell culture, Protoplast culture and Anther culture. Micropropagation has advantages and superiority over conventional propagation approaches because of high multiplication rate and disease-free plants.

Efficient regeneration systems are prerequisites for the genetic transformation of plants. Additionally, by creating new and/or in part, semi-natural products, the new innovative discipline of metabolics will clearly open the door to explore thousands of plants and their constituents (Trigiano and Gray 2011; Gantait et al. 2011).

Plant regeneration can follow either the organogenic or embryogenic pathways and is dependent on the manipulation of both inorganic and organic constituents in the nutrient media, according to the type of explants and the species.

Four basic methods are used to propagate plants *in vitro*:

- enhanced axillary shoot proliferation
- node culture
- *de novo* formation of adventitious shoots through shoot organogenesis
- non-zygotic (somatic) embryogenesis

In vitro propagation techniques are based on axillary branching, adventitious shoot formation through organogenesis and somatic embryogenesis (Jwala and Shekhar 2013).

In vitro plant tissue culture – one of the key tools in plant biotechnology – exploits the totipotency nature of plant cells. Medicinal and aromatic plants industry has applied immensely *in vitro* propagation approaches for the large scale multiplication of elite superior varieties. As a result, hundreds of plant tissue culture laboratories have been established worldwide (Gantait et al. 2011).

2.1 In Vitro *Micropropagation of MAPs Through Organogenesis*

2.1.1 Propagation by Meristem and Meristem Tip-Culture

Culture of the apical meristematic dome alone, from either terminal or lateral buds, for the purpose of pathogen elimination. The meristem tip comprises the apical meristem plus one or two subtending leaf primordia. A meristematic tissue, in most plants, consists of undifferentiated cells (meristematic cells) and is found in zones of the plant where growth can take place. The meristematic cells give rise to various organs of the plant, and maintain plant growth. The essence of meristem-tip culture is the excision of the organized shoot apex from a selected donor plant for subsequent *in vitro* culturing. The excised meristem tip is typically small (often <1 mm in length) and is removed by sterile dissection under the microscope. The explants comprise the apical dome and a limited number of the youngest leaf primordia, and excludes any differentiated provascular or vascular tissues. A major advantage of such explants is that they have a potential to exclude (eliminate) pathogenic organisms that may have been present in the donor plant from the *in vitro* culture. A second advantage is the genetic stability inherent in this technique. Shoot development, directly from the meristems, avoids callus formation and adventitious organogenesis thus ensuring that genetic instability and/or somaclonal variation are minimized. If cases where no virus elimination is needed, the less demanding related technique of shoot–tip culture can be used (Jwala and Shekhar 2013).

2.1.2 Propagation by Shoot and Node Culture

Propagation from preexisting meristems through shoot tip and node culture is the most reliable and widely used method for the micropropagation of a large number of species including MAPs. Both methods rely on the stimulation of axillary shoot growth from lateral buds following disruption of apical dominance of the shoot apex. Shoot tip culture refers to the *in vitro* propagation by repeated enhanced formation of axillary shoots from shoot tips or lateral buds cultured on media supplemented with cytokinins. The axillary shoots produced are either subdivided into shoot tips and nodal segments that serve as secondary explants for further proliferation or are treated as microcuttings for subsequent rooting. **Node culture**, a simplified form of shoot culture is another method for production from preexisting meristems. Propagation from axillary shoots has proved to be a reliable method for the micropropagation of a large number of MAPs. Currently, the most frequently used micropropagation method for commercial production utilizes enhanced axillary shoot proliferation from cultured meristems. Regeneration of shoots from meristem tips is often used to produce initial explants for large scale micropropagation. (Kane 2011, in: Trigiano and Gray 2011; Jwala and Shekhar 2013). Saha et al. (2010) established a reliable plantlet regeneration protocol using nodal explants for large-scale production of ***Ocimum kilimandscharicum*** and for long term germplasm storage *in vitro* for conservation. They also assessed the genetic fidelity of the micropropagated plants. Recently, Dangi et al. (2014) reported a successful protocol for micropropagation of the important medicinal tree ***Terminalia bellerica*** from nodal explants.

Micropropagation via organogenesis has been obtained in many medicinal species (Khalid et al. 2005; Jabeen et al. 2007; Vadodaria et al. 2007; Jan et al. 2010; Kapaia et al. 2010; Kranthi et al. 2011; Swaroopa et al. 2011;. . ..etc. Hence, more and more medicinal species are now micropropagated via *in vitro* culture techniques. Numerous factors are reported to influence the success of *in vitro* propagation of medicinal plants. Some important reviews are the following: Rout et al. 2000b; Nalawade et al. 2003; Ozel et al. 2006; Rajeswari and Paliwal 2006; Chaturvedi et al. 2007; Neelofar et al. 2007; Rath and Puhan 2009, Sasikumar et al. 2009, Sharma et al. 2010, Bawra et al. 2010, Karaguzel et al. 2012; Sayanika et al. 2012; Amin et al. 2013). As a rule, these reviews cover a wide range of MAPs.

Plant regeneration from shoot meristems has yielded encouraging results in medicinal plants such as: ***Acalypha wilkesiana*** (Sharma et al. 2007), ***Acorus calamus*** (Neha 2011), ***Aegle marmelos*** (Arumugam et al. 2003); ***Aloe vera*** syn barbadensis Mill. (Baksha et al. 2005; Ujjwala 2007), ***Amomum krervanh*** (Wondyifraw and Surawit 2004), ***Artemisia judaica*** L. (Pan et al. 2003), ***Asparagus adscendens*** Roxb. (Mehta and Subramanian 2005); ***Astragalus cicer*** (Basalma et al. 2008a), ***Bixa orellana*** (De Paiva et al. 2003), ***Calophyllum apetalum*** (Nair and Seeni 2003), ***Carthamus tinctorius*** L. (Basalma et al. 2008b), ***Centella asiatica*** L. (Tiwari et al. 2000), ***Capsicum frutescens*** (Sudha and Ravishankar 2003), ***Carthamus tinctorius*** L. (Basalma et al. 2008b), ***Catharanthus roseus*** (Debnath et al. 2006), ***Chlorophytum borivilianum*** (Haque et al. 2009; Rizvi and Kukreja 2014), ***Citrullus colocynthis*** (Meena et al. 2010), ***Clerodendrum inerme*** (Srinath et al. 2009), ***Cordia verbenacea*** L. (Lameira and Pinto 2006), ***Curculigo orchioides*** Gaertn. (Bhavisha and Jasrai 2003), ***Echinops spinosissimus*** Turra (Pan et al. 2003), ***Elettaria cardamomum*** (Nadganda et al. 1983; Bajaj et al. 1993), ***Eleutherococcus koreanum*** (Park et al. 2005), ***Garcinia indica*** (Malik et al. 2005), ***Ginkgo biloba*** (Tommasi and Scaramuzzi 2004), ***Gloriosa superb*** (Arumugam and Gopinath 2012), ***Gynura procumbens*** (Lour.) Merr. (Chan et al. 2009); ***Hoslundia opposita*** Vahl. (Prakash and Van Staden 2007), ***Hypericum perforatum*** L. (Danova et al. 2012; Savio et al. 2012), ***Labisia pumila*** (Hartinie and Jualang 2007), ***Leptadenia reticulate*** (Kalidass et al. 2008), ***Mollugo nudicaulis*** (Nagesh and Shanthamma 2011), ***Ornithogalum ulophyllum*** (Ozel et al. 2008), ***Ocimum gratissimum*** L (Gopi et al. 2006), ***Peganum harmala*** (El-Tarras et al. 2012); ***Phyllanthus urinaria*** (Kalidass and Mohan 2009), ***Picrorhiza Kurroa*** Royle ex

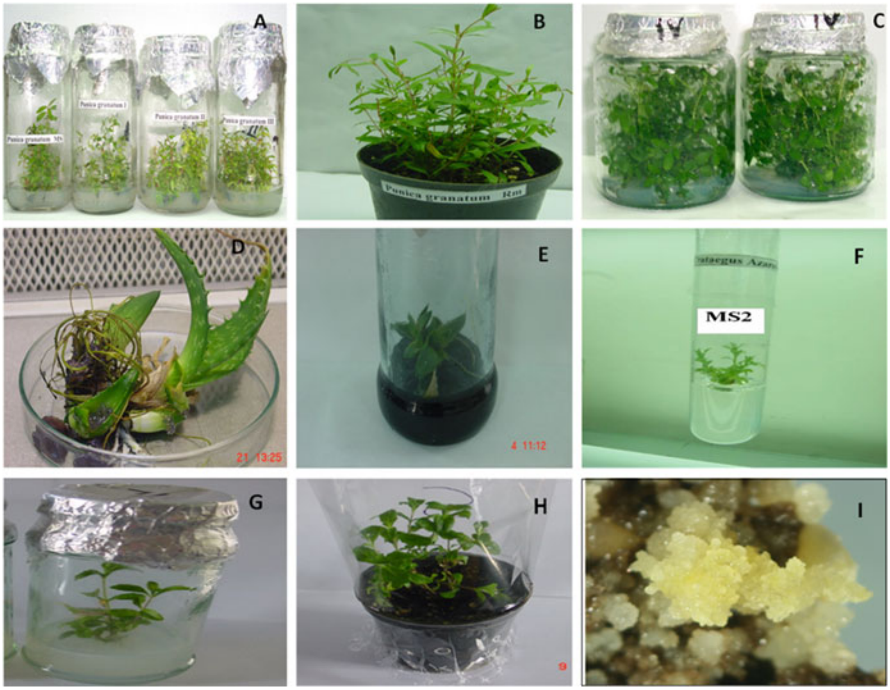


Fig. 15.1 *In vitro* propagation of different medicinal plants. (Source: personal work of last author). (**a**) Shoot induction and multiplication in *Punica granatum*. (**b**) Acclimatized *in vitro* micropropagated plantlets of *Punica granatum*. (**c**) Shoot induction and multiplication in *Myrtus communis* L. (**d**) Shoot induction and multiplication in *Aloe vera*. (**e**) Root induction in *Aloe vera* (**f**) Shoot induction and multiplication in *Crategus azarolus*. (**g**) Shoot induction and multiplication in *Stevia rebundiana*. (**h**) Root induction and acclimatization of *Stevia rebundiana*. (**i**) Embryogenic callus in *Vitis vinifera* (Alzubi, personal com.)

Benth. (Jan et al. 2010), ***Plantago lanceolata*** (Khawar et al. 2005), ***Pluchea lanceolata*** (Arya et al. 2008), ***Salvia species*** (Mederos-Molina 2006), ***Stevia rebaudiana*** Bert (Das et al. 2011), ***Taxus chinensis*** (Wang et al. 2004), ***Thapsia garganica*** (Makunga et al. 2003); ***Urgenia indica*** (Mohan et al. 2005); ***Urginea maritime*** (Aasim et al. 2008), ***Vitis thunbergii*** Sieb. Et Zucc. (Lu 2005); ***Vitex agnus-castus*** (Balaraju et al. 2008), etc. Figure 15.1 illustrates the different stages of micropropagation of some selected medicinal species.

A good example of such studies is on ***Rhodiola rosea*** that is an endangered medicinal species with a limited distribution. It has an outstanding importance for the pharmaceutical industry, in the prevention and cure of cancer, heart and nervous system diseases etc. Tasheva and Kosturkova (2012) presented research on initiation of *in vitro* cultures in *Rhodiola rosea* and some other *Rhodiola* species, with variable achievements in induction of organogenic and callus cultures, regeneration and micropropagation. Many farther research results have been reported on other species (Dimitrov et al. 2003; Gogu et al. 2008; Bozhilova et al. 2008; Tasheva and Kosturkova 2011).

Besides medicinal plants, a few aromatic plants with medicinal value have also been micropropagated to a reasonable extent. Selected examples include a clonal propagation method of ***Crataegus monogyna*** Jacq. (Lindm.) reported by Wawrosch et al. 2007. Nikam et al. (2008) established a protocol for *in vitro* micropropagation of ***Ceropegia hirsuta*** through improvement, conservation and micropropagation of ***Ceropegia intermedia*** from nodal explants. Chandore et al. (2010) developed a novel *in vitro* protocol for multiplication and restoration of ***C. fantastica*** in Western Ghats. Chavan et al. (2011) evaluated factors effecting *in vitro* propagation of ***Ceropegia attenuata*** and reported high frequency of shoot induction achieved on MS medium. Krishnareddy et al. (2011) reported an efficient protocol for multiple shoot induction in ***Ceropegia juncea.*** Protocols for clonal multiplication of many economically important Zangiberaceous species like *Amomum subulatum* (large cardamom), *Curcuma aromatica* (kasturi turmeric), *Curcuma domestica* and *Curcuma zedoaria* were also develpoed (Prakash et al. 2004, in: Gantait et al. 2011).

2.1.3 *In Vitro* Micropropagation of MAPs via Callus Culture

Callus culture is the culture of a differentiated tissue from explants allowed to dedifferentiate *in vitro* and so-called callus tissue is produced. This callus is induced to form organs. Organs produced include shoots, roots, geophytic structures (i.e., bulbs, tubers) and flowers. Adventitious shoots and roots are the most common forms of adventitious organ formation (Trigiano and Gray 2011; Jwala and Shekhar 2013). There are numerous reports describing the regeneration of various medicinal plants via callus culture. Regeneration of ginger plantlets through callus phase has been reported from leaf, vegetative bud, ovary, anther explants and anther callus from diploid and tetraploid ginger (Nirmal Babu et al. 1997). Organogenesis and plantlet formation were achieved from the callus cultures of tumeric (Salvi et al. 2001). *In vitro* differentiation of shoot buds directly from explants of medicinal plants or via callus cultures has been demonstrated. Manjkhola et al. (2005) reported organogenesis and somatic embryogenesis in ***Arnebia euchroma*** (Royle) Johnston (*Boraginaceae*) callus cultures from leaf explant on MS medium supplemented with 2.5 μM IBA and 2.5 μM BAP with 72 % plantlets survived under nursery conditions. Further, somatic embryos were encapsulated for use as synthetic seeds. Nikam and Savanth (2007) focused on the callus cultures, micropropagation and domestication of ***Ceropegia sahyadrica***. They had some proposals to improve this plant via genetic engineering in the field concerned with tubers just like potatoes etc. Kondamudi et al. (2010) induced an excellent development of a callus which had the ability for organogenesis and morphogenesis for the endangered taxon ***Ceropegia pusilla***. Several further reports are available on the regeneration of various medicinal plants via callus culture (Patra et al. 1998; Basu and Chand 1998; Dhar and Joshi 2005; Sharma 2005; Nurazah et al. 2009).

Recently, Pan et al. (2013) reported callus induction and multiple shoot formation in six endangered medicinal plants and subsequent media optimization for their potential commercial applications. These are: *Rauwolfia serpentina* (Sarpagandha), *Coleus barbatus*, *Tylophora indica* (Indian ipecac), *Acorus calamus* (Sweet flag), *Ornithogalum* (Star of Bethlehem) and *Cycas revoluta* (Sago palm). In *Ornithogalum*, multiple *in vitro* bulbs were formed. In case of *Cycas revoluta* (leaf explant), swelling was observed at 5 mg/l of 2,4-D in MS media. They concluded further exploitation of plants produced for probable micropropagation and plant regeneration on a commercial scale. *In vitro* bulbs were observed in the media supplemented with 5 mg/l BAP in *Ornithogalum* (bulbs germinated on further subculture; all media were B5 based). Apical buds were observed in the media supplemented with 0.1 mg/l BAP in *Coleus barbatus* (buds germinated in subsequent subcultures; all media were MS based). Nodal explants of *Acorus calomus* were supplemented with MS + BAP (0.5 mg/l) and IAA (1 mg/l) on MS media where three apical buds were initiated and also germinated on further subculture (Pan et al. 2013).

2.2 **In Vitro** ***Micropropagation of MAPs Through Somatic Embryogenesis***

Somatic embryogenesis (direct and indirect) is the developmental process by which somatic cells undergo restructuring through the embryogenic pathway to generate embryogenic cells. Subsequently, these cells go through a series of morphological and biochemical changes that result in the formation of a somatic embryo and ultimately, the generation of a new plants:

- direct somatic embryogenesis, where somatic embryos differentiate from the explants without an intervening embryogenic callus phase,
- indirect somatic embryogenesis, where somatic embryos differentiate indirectly after an embryogenic callus phase.

Plant regeneration via somatic embryogenesis from single cells, that can be induced to produce an embryo and then a complete plant, has been demonstrated in numerous medicinal plant species. Efficient development and germination of somatic embryos are prerequisites for commercial plantlet production (Trigiano and Gray 2011).

The embryogenic pathway, as opposed to the organogenic pathway, can be a more efficient and productive system for large–scale propagation. Plant regeneration via somatic embryogenesis is the *in vitro* process used to reduce multiplication time. It potentially offers an efficient system for mass clonal propagation and regeneration of plants from genetic transformation and somatic hybridization experiments. The method has the potential to produce large numbers of plantlets in a relatively short period of time. Since the embryo contains both a root and an apical shoot meristem, the rooting stage required in conventional *in vitro* bud or shoot propagation method is obviated. Somatic embryos are small and can be adequately handled in scaled-up procedures. They are amenable to storing and separation by image analysis, dispensing by automated systems and can be encapsulated and either stored or planted directly with the aid of mechanized systems. Furthermore, manipulation of somatic embryos to function as synthetic seeds would allow clonal germplasm to be stored efficiently in seed repositories if viability can be maintained for adequate lengths of time (Trigiano and Gray 2011).

Somatic embryogenesis has been reported in many different medicinal species like: ***Dendrocalamus strictus*** (Rao et al. 1985), ***Cephaelis ipecacuanha*** (Rout et al. 2000a), ***Bambusa eduli*** (Lin et al. 2004). Das et al. (1999) reported that the somatic embryos of ***Typhonium trilobatum*** were germinated on MS medium supplemented with 0.01 mg/l NAA and 2 % (w/v) sucrose after 2 weeks of culture. Pandit et al. (2008) gave a comprehensive protocol for the micro-tuberization for threatened ***Ceropegia*** species. They proposed a novel phenomenon of microtuber proliferation. Nikam et al. (2008) established a protocol for *in vitro* micropropagation of ***Ceropegia hirsute***. Successful plant regeneration has been achieved in ***Holostemma ada-kodien*** (Martin 2003b), ***Aloe vera*** by somatic embryogenesis (Garro-Monge et al. 2008). Murthy et al. (2010a; b) developed a reproducible protocol based on somatic embryogenesis for the propagation of the endangered species. ***Ceropegia spiralis*** produced plants in shorter period. They also tested the scope of domesticating this species, as it has beautiful flowers and medicinal properties as well.

2.3 Protoplast Culture of Medicinal and Aromatic Plants

Plant protoplasts are defined as cells that have had their rigid cellulose cell wall removed without damaging the external cell membrane that surrounds the nucleus and cytoplasm. The use of protoplasts not only enables mass production of plants, but it can also be a rich source of genetic diversity and somaclonal variation. Protoplast cultures have proven particularly useful for increasing the production of alkaloids and other natural products in plants. Plants have been regenerated from isolated protoplasts of a number of medicinal plants and somatic hybrids (Bajaj 1988, Rout et al. 2000; Aziz et al. 2006). Cell lines with high shikonin contents were obtained from protoplast-derived callus of fennel (Miura and Tabata 1986), ***Asparagus*** (Elmer et al. 1989), ***Lithospermum erythrorhizon*** (Kumar 1992). In addition, the fusion between protoplasts of ***Hyoscyamus muticus*** × ***Nicotiana tabacum*** resulted in somatic hybrid plants that showed high growth rates (Jia et al. 1983). Regeneration of plants from protoplasts of different medicinal plants has been reported. Chen et al. (1997a,b) standardized the protocol on protoplast isolation from shoot tip cultures of ***Asparagus***. Subsequently, they achieved plant regeneration from protoplasts. About 80 % of the protoplasts showing cell division developed into colonies of at least 25–30 cells, of which 25 % initiated somatic embryos. Successful plant regeneration was achieved by protoplast culture in ***Artemisia judaica*** and ***Echinops spinosissimus*** (Pan et al. 2003). Jian-Feng et al. (2009) reported on the Protoplast isolation and plant regeneration from leaves of ***Rhodiola sachalinesis***.

2.4 Anther-Culture of Medicinal and Aromatic Plants

Anther-culture is the process of using anthers to culture haploid plantlets. Haploids may be produced from male (androgenesis) or female (gynogenesis) gametophytes. *In vitro* androgenesis involves culture of anthers or isolated microspores. Gynogenesis may be induced in unfertilized ovule or ovary culture. Success of hapolid cultures depends on genotype, media and culture conditions. Haploids of many MAPs have been produced *in vitro*. Induction frequencies of almost 100 % and a yield of more than one thousand plantlets or calli have occurred under optimal conditions, from one anther from ***Datura innoxia*** (Trigiano and Gray 2011).

Anther-culture techniques were effective for the production of haploid plants of several species (Bajaj 1983a,b; Wesolowska and Skrzypczak 1985; Alatorre et al. 1998, Rout et al. 2000). Perez-Bermudez et al. (1985) reported the induction of somatic embryogenesis and plant regeneration from cultured anthers of ***Digitalis obscura***. Incubation in the darkness significantly increased the callus proliferation and embryo development. They also achieved shoot-bud regeneration from callus, on a medium containing 1.0 mg/l BA and 2.0 mg/l 2,4-D. Gautam et al. (1993) reported the induction of callus from anthers of ***Azadirachta indica*** on Nitchís (N6) medium containing 10 μM IAA, 1 μM BA.

2.5 **In Vitro** *Rooting of MAPs*

Prolific rooting of *in vitro*-cultured micro-shoots is critical for the successful establishment of these shoots, both in the greenhouse and field. *In vitro* induction of roots from growing shoots has been achieved in standard media containing auxin and in media in the absence of auxin, depending on plant genotype in different medicinal plants (Rout et al. 2000b; Martin 2003c, Rajneesh and Shyamal 2009, Sharafi et al. 2013a). There is marked variation in the rooting potential of different plant species, and systematic trials are often needed to define the conditions required for root induction. Moderate to high concentrations of all cytokinins inhibit rooting (Rout et al. 2000b). Faria and Illg (1995) obtained 100 % rooting in the excised shoots of ***Zingiber spectabile*** in liquid or Gelrite-gelled medium containing 5 μM IAA or NAA. In ***Aloe barbadensis***, MS medium containing 0.5 mg L^{-1} NAA showed 95 % rooting with healthy and thick roots, however increasing the NAA concentrations lowered the quality and number of roots

(Baksha et al. 2005) and MS medium containing 1 mg L^{-1} IAA was more effective for rooting than medium without any growth hormones (Ujjwala 2007). In ***Aloe vera***, IAA showed very poor effects, at all concentrations, whereas MS medium containing 0.2 mg L^{-1} of NAA produced most number of roots as compared to 0.2 mg L^{-1} IBA (Ahmed et al. 2007). Husain et al. (2007) employed a two step procedure for rooting of ***Pterocarpus marsupium*** by first giving pulse treatment with IBA (200 μM) for 4 days followed by subsequent transfer to semi-solid half strength MS medium containing IBA (0.2 μM) + phenolic acids.

2.6 *Acclimatization and Field Establishment of* In Vitro *Micropropagated MAPs*

Acclimatization is a critical phase of micropropagation (Jwala and Shekhar 2013). Jha and Sen (1985) reported that prior to transfer to soil, all of the rooted plantlets of ***Bowiea volubilis*** were maintained for 4–6 weeks in MS salts with 0.5 % sucrose and incubated at 24–30 °C for 4 weeks for hardening. After 4 weeks, the plantlets were transferred to soil and showed 80 % survival. Satheesh and Bhavanandan (1988) reported that when micropropagated plants of ***Plumbago rosea*** were transferred to pots containing a 1:1 soil and sand mixture under greenhouse conditions, about 60 % of the plants survived. A high survival (96 %) was recorded when plantlets of ***Pinellia ternata*** were transplanted into a 1:2:1 mixture of vermiculite: loam soil: peat moss (Tsay et al. 1989). Jha and Jha (1989) noted the highest survival of ***Cephaelis ipecacuanha*** when the plants were maintained for a 4-week period in liquid MS medium and then transferred to greenhouse conditions. Saxena et al. (1997) reported that rooted plantlets of ***Psoralea corylifolia*** were successfully transferred to a 1:1 mixture of soil and sand. About 95 % of the regenerated plants survived in the greenhouse. Salvi et al. (2002) reported on micropropagation and field evaluation of micropropagated plants of turmeric.

3 Conservation Through Low Temperature Storage and Cryopreservation of Medicinal and Aromatic Plants

Germplasm can be stored *in vitro* in variety of forms including isolated protoplasts, cells from suspension, callus cultures, meristem tips, somatic embryos, shoot tips and propagules at various stages of development. Based on culture growth *in vitro* conservation can be classified into two groups:

– Slow growth cultures in cold storage.
– Cryopreservation at (−196 °C). (Jwala and Shekhar 2013).

Medium- and long-term *in vitro* low temperature storage and cryopreservation have been used successfully for conservation of different medicinal plants to store a range of tissue types, including meristems, anthers/pollens, embryos, calli, shoot tips and even protoplasts (Bekheet 2000; Ahuja et al. 2002; Baek et al. 2003; Martin and Pradeep 2003; Rao 2004; Sarasan et al. 2006; Rathore and Singh 2013). Cryopreservation is long-term conservation method in liquid nitrogen (−196 °C) in which cell division and metabolic and biochemical processes are arrested. Since whole plants can regenerate even from frozen cultures, cryopresevation provides an opportunity for conservation of endangered medicinal plants. For example, low temperature storage has been reported to be effective for cell cultures of medicinal and alkaloid-producing plants such as *Rauvolfia serpentine, Digitalis lanata, Atropa belladonna, Hyoscyamus* spp. When plants are regenerated and no abnormality is seen, either in fertility or in alkaloid content, the materials can be stored using cryopreservation methods (Bajaj 1988). However, the system will depend on the availability of liquid nitrogen based methods (Tripathi and Tripathi 2003).

4 *In Vitro* Development of Encapsulated Seeds of MAPs

The artificial seed technology is an exciting and rapidly growing domain of research in plant cell and tissue culture. Encapsulated seeds are produced either by encasing somatic embryos in a protective coating or by desiccating somatic embryos with or without coating (Rout et al. 2000a). Protocols of synthetic seed technology for successful micropropagation, storage and transport of plants have been defined for several crops (Rout et al. 2000a; Nagananda et al. 2011). Seeds, somatic embryos, shoot-tips, and pollens can be encapsulated in different concentrations of sodium alginate for both storage and micropropagation. Somatic embryos of celery (***Apium graveolens***) immobilized in alginate were successfully grown into whole plants (Redenbaugh et al. 1986). Martin (2003a) accomplished clonal propagation and encapsulation of *in vitro* formed bulbs of ***Ipsea malabarica*** using rhizome and its reintroduction to the natural habitat. Half strength MS medium supplemented with 6.97 μM Kn induced four shoots per explant within 50 days. Transfer of the isolated shoots increased rate of shoot multiplication to more than ten shoots. In subsequent culturing they developed bulbs. *In vitro* bulbs were encapsulated and 100 % conversion to plantlets using growth regulator-free ½ strength MS or 6.97 μM Kn. supplemented medium was observed (Martin 2003a).

5 *In Vitro* Production of Secondary Metabolites of MAPs

5.1 Production of Secondary Metabolites of MAPs from Cell Suspension/Liquid/ and Large-Scale Cultures in Bioreactors/Bioprocessing

Secondary metabolites can be used as pharmaceuticals, agrochemicals, aromatics and food additives. Plant derived compounds include many terpenes, polyphenols, cardenolides, steroids, alkaloids and glycosides. Totipotency of plant cells ensures that they possess the entire genetic characteristics of the plant, thus making it possible to synthesize these compounds *in vitro*. Tissue culture offers an effective and potential alternative metabolite production because the amount of secondary metabolites produced in tissue cultures can occasionally be even higher than in parent plants.

The main advantages associated with *in vitro* plant systems include the manipulation of environmental conditions, rapid production and the use of simpler and cheaper downstream processing schemes for product recovery from culture medium. The capability to cultivate callus cells and organs in liquid media has also made an important contribution to modern plant biotechnology with respect to the production of commercially valuable compounds. Callus cells obtained from the transgenic plants can be grown in simple, chemically defined liquid media to establish transgenic cell suspension cultures for recombinant protein production (Jwala and Shekhar 2013).

Bioactive compounds extracted from plants are widely used. Studies for obtaining secondary plant metabolites such as active compounds for the production of pharmaceutical and cosmetics, hormones, enzymes, proteins, antigens, food additives and natural pesticides from harvest of the cultured cell and callus cultures have been carried out on an increasing scale.

Biotechnological culturing of plant cells and tissues involve two major methodologies, i.e.: cell culture and clonal-propagation techniques.

Cell culture studies being with callus initiation for the purpose of determining the medium that best adapts for cultivation. Callus lines are then generally screened for their productivity, so that the best performing lines can be transferred to cell suspensions. Elicitation is usually one of the most successful approach used to increase the production of secondary metabolites in cell suspensions. This is the final step, in bioreactor studies, leading to a possible commercial production of secondary metabolites. Bioreactors can be regarded as biological factories producing high-quality metabolic products.

Growth in medicinal plant tissue cultures can be scaled-up using so called continuous culture systems, such as bioreactors that could allow the automated high-level isolation of secondary metabolites.

While the second method of plant cell culture is based on the clonal propagation of a plant on large-scale regeneration from cells belonging to an explant which is potentially very useful. Liquid media have been used for plant cells and somatic embryos, and organ cultures in both agitated flasks or various types of bioreactors. Bioreactors have also been used for the culturing of hairy roots, mainly as a system for secondary metabolite production.

Cell culture techniques, however, are limited to only a handful of applications such as production of shikonin from ***Lithospermum erythrohizon***, berberine from ***Coptis japonica*** and ginseosides from ***Panax ginseng***.

Suspension culture liquid media allow a close contact with the plant tissue and thus stimulate and facilitate the uptake of nutrients and plant growth regulators, leading to better shoot and root development. Continuous shaking promotes a reduced expression of apical dominance that generally leads to induction and proliferation of numerous axillary buds. The growth and multiplication rate of shoots is enhanced by forced aeration, since continuous shaking of medium provides ample oxygen supply to the tissue which ultimately leads to their faster growth (Bajaj 1989; Jwala and Shekhar 2013).

Bioreactors are usually described in a biochemical context as self contained, sterile environments which depend on liquid nutrient or liquid/air inflow and out flow systems, designed for intensive culture and affording maximal opportunity for monitoring and control over micro environmental conditions (agitation, aeration, temperature, dissolved oxygen pH etc.). Since bioreactors provide a rapid and efficient plant propagation system for many important plant species, this method seems promising and is utilized to avoid intensive manual handling in large scale production at industrial level. They utilize liquid media. Automation of micropropagation in bioreactors has been advanced by several authors as a possible way of reducing cost of micropropagation (Mehrotra et al. 2007).

The large scale production of plants using bioreactors employing liquid medium can be achieved through induction of somatic embryogenesis, as in ***Digitalis lanata*** (Greidziak et al. 1990), or by using shoot meristem multiplication technique as in ***Digitalis purpurea*** (Hagimori et al. 1984) and in ***Stevia rebundiana*** (Akita et al. 1994). Some other important medicinal plants propagated in bioreactors include: *Atropa belladonna*, *Asparagus officinalis*, *Artemisa annua*, *Acanthopanax koreanum*, *Chlorophytum borivilianum, Digitalis lanata and* Brahmi (Mehrotra et al. 2007; Praveen 2009; Haque et al. 2009; Rizvi and Kukreja 2014).

In suspension cultures of ***Catharanthus roseus***, MS medium was reported to be best for producing serpentine. 2,4-D was reported to cease metabolite production (DiCosmo and Misawa 1995). Production of Rosavin was reported as a product of glycosylation by ***Rhodiola rosea*** (roseroot) cell cultures (Wu et al. 2003). In ***Catharanthus roseus***, auxins promoted callus induction. Auxin removal and cytokinins alone were found to increase secondary metabolites production (DiCosmo and Misawa 1995). Ginseng root tissue cultures in a 20 Tons bioreactor produced 500 mg/L/day of the saponins that is considered as a very good yield (Charlwood and Charlwood 1991). Bey combining 2.5 mg l^{-1} IBA and 0.1 mg l^{-1} kinetin, the production of ginsenoside saponins in cell cultures of ***Panax quinquefolium***, was even higher than in adult plants (Zhong et al. 1996). Pande et al. (2002) reported that the yield of lepidine from ***Lepidium sativum*** Linn. depends upon the source and type of explants. The production of ginsenosides in hairy root cultures of American Ginseng, ***Panax quinquefolium*** L. was reported recently (Kochan et al. 2013). Wu et al. (2003) described high salidroside yields in the suspension culture of ***Rhodiola sachalinensis***. Thymol was extracted from callus cultures of ***Nigella sativa*** (Al-Ani 2008).

Advances in plant tissue culture have enabled commercial scale production of plant metabolites (Furmanowa et al. 1999; Matkowski. 2000; Verpoorte et al. 2002; Casado et al. 2002; Wu et al. 2003; Oksman-Caldentey and Inzé 2004, Zhou and Wu 2006, Shilpa et al. 2010). Bioreactor systems have been applied for embryogenic and organogenic cultures of several plant species (Levin et al. 1988; Preil et al. 1988). Much progress has been achieved in the optimization of these systems for the production and extraction of valuable medicinal plant ingredients such as ginsenosides and shikonin. Roots cultivated in bioreactors have been found to release medicinally active compounds, including the anticancer drug isolated from various ***Taxus*** species, into the liquid media of the bioreactor which may then be continuously extracted for pharmaceutical preparations (Murch et al. 2000; Debnath 2009).

The effects of salicylic acid were characterized on different explants of St. John's wort, ***Hypericum perforatum*** L. (cells, calli and shoots) cultured *in vitro*. The production of both hypericin and pseudohypericin has doubled in elicited cell suspension cultures. Furthermore, phenyl-propanoids that are among the most frequently observed metabolites affected upon treatment of *in vitro* culture material with elicitors, were produced and the enzymatic activities of phenylalanine ammonia lyase and of chalcone isomerase were stimulated upon elicitation (Gadzovska et al. 2013). Different other studies on *in vitro* techniques approaches of ***H. perforatum*** L. have been also reported (Murch et al. 2000; Kirakosyan et al. 2000; Walker et al. 2002; Pasqua et al. 2003; Karppinen et al. 2007; Kornfeld et al. 2007; Liu et al. 2007a, b; Xu et al. 2008; Karakas et al. 2009; Danova et al. 2010; Filippini et al. 2010; Germ et al. 2010; Karppinen 2010; Coste et al. 2011; Danova et al. 2012; Savio et al. 2012; Gadzovska et al. 2013).

5.2 Production of Secondary Metabolites of MAPs by Organ Cultures

Plant regeneration can be obtained in two morphogenic pathways: **organogenesis** (the formation of unipolar organs) and **somatic embryogenesis** (the production of bipolar structures, somatic embryos with a root and a shoot meristem). When plant somatic cells are isolated and cultured under *in vitro* conditions, they are capable of expressing their totipotency. The injured cells in the outer layers of isolated explants emit ethylene that induces de-differentiation. Cell division can occur in an unorganized pattern with the formation of meristematic centers directly in the explant tissues. Meristematic centers form directly on the explants, in some plants, and can develop into either shoots, roots or somatic embryos. Organ cultures can be used for both secondary metabolite production and plant propagation.

Shoot-Cultures There are few examples in the special literature that focus on secondary metabolite production from shoot cultures, e.g.: *Spathiphyllum cannifolium* and *Stevia rebaudiana.* Organ-cultures of *Lavandula officinalis* were cultivated in 5 L bubble column bioreactor to obtain rosmarinic acid. Moreover, shoot culture of *Ananas comosus* was performed in a 10 L airlift bioreactor and organ culture of *Hypericum perforatum* in 2 L stirred tank bioreactor to produce hypericin (Jwala and Shekhar 2013).

Somatic Embryogenesis (the production of bipolar structures, somatic embryos with a root and a shoot meristem) is also an alternative method for the production of certain metabolites. In most cases, the somatic embryos can be cryopreserved which makes it possible to establish gene banks.

Production of rosmarinic acid was enhanced by increasing sucrose levels in the culture media (Misawa, 1985). Instead of sucrose using glucose (in MS medium) as a source of carbon was also found to enhance production of podophyllotoxin in cell cultures of *Podophyllum hexandrum* and the cultures grown in dark produced better friable calli and higher podophyllotoxin than the ones grown in light. Agitating speeds for cultured cells is reported to influence cell viability and product synthesis. Also, it was found that *in vitro* grown cells showed much more damage at 200 rpm and more viable cells were found between speeds 125–150 rpm (Chattopadhyay et al. 2002).

In vitro techniques have been widely explored for rapid and efficient production of ginseng *Panax vietnamensis* biomass and ginsenosides. Cell and adventitious root cultures of *P. vietnamensis* have been established which opens the way to commercial applications. Various physiological and biochemical parameters affecting the biomass production and ginsenoside accumulation have been investigated. These parameters are affected by various phytohormones, sucrose and activated charcoal (AC) which result in influencing shoot regeneration and proliferation from callus, as well as adventitious and secondary root formation. The saponin analysis of calli and roots show the presence of ginsenoside-Rg1, majonoside-R2, and ginsenoside-Rb1. These results indicated that *P. vietnamensis* biomass has a great potential to produce saponin as a new source for the pharmaceutical and cosmetic industry (Nhut et al. 2013).

6 Biotransformation versus *In Vitro* Production of Secondary Metabolites in Medicinal and Aromatic Plants

This technology, also called **biofarming**, is used to produce secondary metabolites *in vitro*. It is the process of chemical conversion or alteration of one compound to another using cultured cells or enzymatic action (Chasseaud and Hawkins 2006). Plants can synthesize some rare enzymes that can synthesize further important compounds. Chemical synthesis of these compounds is sometimes unfeasible. Different types of cell cultures (suspension and hairy root) are used to transform natural or synthetic aromatic compounds, steroids, alkaloids, coumarins, terpenoids, lignans and many other compounds through biotransformation. The biotransformation process includes reactions such as reduction, oxidation, hydroxylation, acetylation, esterification, glucosylation, isomerization, methylation, demethylation, epoxidation and many more (Pras et al. 1995; Uden et al. 1995; Rout et al. 2000a; b; Giri et al. 2001; Rao and Ravishankar 2002; Rao et al. 2002; Sharafi et al. 2013b).

Some of famous examples on pharmaceutical products obtained by biotransformation are: producing Rhaziminine from ***Rhazya stricta*** (Saeed et al. 1993)***, Podophyllum hexandrum*** for Podophyllotoxin (Uden et al. 1995); ***Mucuna prureins*** for DOPA (3,4-dihydroxyphenylalanine) (Pras et al. 1995); ***Catharanthus roseus*** for Viincrisitne (DiCosmo and Misawa 1995), ***Eucalyptus perriniana*** for Taxol derivatives (Hamada et al. 1996), Codeine (Wilhelm and Zenk 1997), ***Spirulina platensis*** for Morphine (Rao et al. 1999), ***Capsicum frutescens*** for Vinallin, Capsaicin (Rao and Ravishankar 1999) and ***Capsicum frutescens*** for Digoxin and purpureaglycoside (Rao et al. 2002). Studies on biotransformation in ***Peganum harmala*** and conversion of tryptamine to serotonin by callus and cell suspension cultures of *Peganum harmala* (Berlin 1999). Krajewska-Patan et al. (2002) reported on biotransformation and the influence of elicitation on the tissue cultures of rose root (***Rhodiola rosea***). György et al. (2004) and György (2006) reported on enhancing the production of cinnamyl glycosides in compact callus aggregate cultures of ***Rhodiola rosea*** by biotransformation of cinnamyl alcohol. He produced Glycoside from *Rhodiola rosea in vitro* cultures. Thymol was extracted from callus cultures of ***Nigella sativa*** (Al-Ani 2008).

7 Genetic Transformation of MAPs/Genetic Engineering Studies

Agrobacterium transformation provides a method for the routine genetic transformation of several important medicinal species. *A. tumefaciens*-mediated transformation systems are well established for *Taxus* (yew), *Echinacea, Scrophularia* (figwort), *Digitalis* (foxglove), *Thalictrum* (meadow-rues) and *Artemisia* (Siahsar et al. 2011). Genetic transformation with bacterial vector systems has been widely used with medicinal plants including *Artemisia annua*, *Taxus sp*., *Papaver somniferum*, *Ginkgo biloba* and *Camptotheca acuminate* and species from Family *Solanaceae*.

Increasing the production of pharmacologically active natural products represents one of the main targets for the genetic manipulation of medicinal plants. The use of genetically modified plant cell cultures such as hairy root cultures for Solanaceae species or for *Artemisia annua* offers a rational approach to allow the over-expression of genes encoding biosynthetic enzymes and to overcome the rate-limiting steps in biosynthesis.

Top ten ranked medicinal plants most commonly used in the United States and Europe are: *Hypericum perforatum, Panax ginseng, Allium sativum, Echinacea purpurea, Ginkgo biloba*, *Saba serrulata*, *Tanacetum parthenium, Valeriana officinalis, Ephedra distachya*.

It should be noted that not only plants are of great interest for the pharmaceutical industry, but also defined natural products. 11 % of the 252 drugs considered to be basic and essential by the WHO are isolated and used directly from plant sources. Remarkably, approximately 40 % of pharmaceutical lead-compounds for the synthetic drug industry, are derived from natural sources (Siahsar et al. 2011; Trigiano and Gray 2011; Jwala and Shekhar 2013)..

The question also arises as to whether there is a need for the use of biotechnology and gene technology with medicinal and aromatic plants? The answer is definitely: yes, since biotechnology could be applied as a method for enhancing the formation and accumulation of desirable natural products (Trigiano and Gray 2011; Jwala and Shekhar 2013).

Micropropagation, cell and hairy root cultures as well as gene technologies are all important techniques for plant propagation, however these are mostly used to improve the production and yield of desired natural products. Two well described examples of this are artimisinin and paclitaxel, both of which are available in plants, albeit in only small quantities.

As a consequence, not only are both natural products expensive, but *Taxus* species are endangered due to unsustainable cutting and collection. In an attempt to overcome these problems for both drugs, intensive research is being carried out worldwide, including combinational biosynthesis, or improved bioprocessing in bioreactors for both artimisinin and paclitaxel.

The application of biotechnological techniques to MAPs has received considerable interest, especially when the final product is defined, purified and natural. The manipulation of MAPs is well known and accepted both by scientists and consumers if the pathways and product yield can be optimized to create precursors for semi-syntheses (e.g., baccatin-III to paclitaxel), food components (e.g. vitamins), pesticide resistance, and cellular storage conditions, as shown for *Mentha* x *piperita* with enhanced resistance against fungal attacks and abiotic stress (Trigiano and Gray 2011; Jwala and Shekhar 2013).

Julsing et al. (2007) discussed the prospects and limitations of genetic engineering of medicinal plants. Genetic transformation has been reported for various species. Naina et al. (1989) reported the successful regeneration of transgenic neem plants (***Azadirachta indica***) using *Agrobacterium tumefaciens* containing a recombinant derivative of the plasmid pTi A6. Hairy root lines of ***Datura metel*** were established following infection of aseptic stem segments with *Agrobacterium rhizogenes* strain A4 and cultured in hormone-free B5 solid medium. The growth and production of hyoscyamine and scopolamine (mg/g dry wt.) of these root cultures was encouraged by using B5 liquid medium with half-strength salts (Giri et al. 1997). The genetic transformation of ***Atropa belladona*** has been reported using *Agrobacterium tumefaciens*, with an improved alkaloid composition (Yun et al. 1992; Cucu et al. 2002). *Agrobacterium* mediated transformation of *Echinacea purpurea* has also been demonstrated using leaf explants (Koroch et al. 2002).

Genetic transformation could be used as a powerful tool for enhancing the productivity of **novel secondary metabolites** with a limited yield. Hairy root cultures, of *Agrobacterium rhizogenes*, have been found to be suitable for the production of secondary metabolites because of their stability and high productivity in hormone-free culture conditions. A number of plant species including medicinal plants have already been successfully transformed with *A. rhizogenes*. The hairy root culture system of ***Artemisia annua*** L. was established by infection with *A. rhizogenes*. The optimum artemisine concentration was 4.8 mg/L (Cai et al. 1995). Giri et al. induced the development of hairy roots in ***Aconitum heterophyllum*** using *A. rhizogenes* (Giri et al. 1997). Pradel et al. (1997) developed a system for producing transformed plants from root explants of **Digitalis lanata**. They evaluated different wild strains of *A. rhizogenes* for the productions of secondary products (cardenolides, anthraquinones, and flavonoids) obtained from hairy roots and transgenic plants. They reported higher amounts of anthraquinones and flavonoids in the transformed hairy roots than in untransformed roots. An efficient protocol for the development of transgenic opium poppy (***Papaver somniferum*** L.) and California poppy (***Eschscholzia californica*** Cham.) root cultures using *A. rhizogenes* is reported (Park and Facchini 2000). Bonhomme et al. (2000) has reported the tropane alkaloid production by hairy roots of ***Atropa belladonna*** obtained after transformation with *A. rhizogenes*. Argolo et al. (2000) reported the regulation of solasodine production by *A. rhizogenes*-transformed roots of *Solanum aviculare*. Souret et al. (2002) have demonstrated that the transformed roots of ***Artemisia annua*** are superior to whole plants in terms of yield of the sesquiterpene artemisinin. Shi and Kintzios have reported the genetic transformation of ***Pueraria phaseoloides*** with *A. rhizogenes* and puerarin production in hairy roots. The content of puerarin, in hairy roots, reached a level of 1.2 mg/g dry weight and was 1.067 times higher than the content in the roots of untransformed plants. Thus, these transformed hairy roots have great potential as a commercially viable source of secondary metabolites (Shi and Kintzios 2003).

Several farther studies have been reported on genetic engineering of different medicinal plants. Due to the richness of scientific literature on the topic, only a few selected references have been reviewed here.

8 Conclusions

Biotechnology, and within that micropropagation of medicinal plants seem to offer an unlimited and untapped source of chemical compounds with high drug potentials.

Protocols have been developed in laboratories across the world for the clonal multiplication of hundreds of plant species including also endangered species. Large-scale plant tissue cultures are likely to offer attractive alternatives to the traditional plantation methods with the advantage that they offer a controlled supply of biochemicals independently of plant availability.

The primary importance of *in vitro* propagation of rare, critically endangered, and vulnerable species is to generate a large number of planting material from one single explant, without destroying the mother plant. Thus, these can contribute to restore natural habitats and conserve biodiversity. The significance of efficient *in vitro* protocols is to obtain maximum number of plantlets with proper rooting and subsequent acclimatization in the field, in a minimum period of time.

Plant tissue culture has already significantly contributed to agricultural biotechnology including medicinal plants. Large scale micropropagation applications assist to produce large numbers of plants with comparable genotypes (clones). Plant tissue culture is also a key tool in recovering transgenic plants. Besides genetic transformation for crop improvement, its applications include: selection of variants/mutants (somaclonal variation/ mutation), cryopreservation, di-haploid production and embryo rescue, and production of useful chemicals (e.g. taxol). Liquid cultures have been used for detecting trace elements and determining their toxicities to crop plants (Kopittke et al. 2010). Micropropagation is an important technology also because many secondary plant metabolites cannot be synthesized chemically. Plant tissue culture could enable the rapid multiplication and sustainable use of medicinal plants for future generations. Micropropagation also holds a potential as a tool for the production of high quality plant-based metabolites (and medicines). Pharmacologically active compounds from plants represent one of the largest and most diverse groups of plant secondary metabolites. Advances in tissue culture, combined with improvements in genetic engineering, specifically transformation technologies, has opened up new perspectives for the high volume production of pharmaceuticals, neutraceuticals, and other beneficial substances.

In the present study, *in vitro*-propagation of MAPs, their significance and the wide scope of investigations on micropropagation and mass cloning of MAPs have been highlighted.

References

Aasim M, Khawar KM, Ozcan S (2008). *In vitro* regeneration of red squill *Urginea maritime* (L.) Baker. using thidiazuron biotechnology & biotechnological equipment 22(4): 925–928

Ahmed S, Kabir AH, Ahmed MB, Razvy MA, Ganesan S (2007) Development of rapid micropropagation method of *Aloe vera* L. Seed Sci J 24:121–128

Ahuja A, Sambyal M, Koul S (2002) *In vitro* propagation and conservation of *Atropa acuminate* Royle ex Lind- an indigenous threatened medicinal plant. J Plant Biochem and Biotech 11:121–124

Akita M, Shigeoka T, Koizumi Y, Kawamura M (1994) Mass propagation of shoots of *Stevia rebundiana* using a large-scalebioreactor. Plant Cell Rep 13:180–183

Al-Ani NK (2008) Thymol Production from Callus culture of *Nigella sativa* L. Plant Tissue Cult Biotech 18(2):181–185

Alatorre MFS, Borys MW, Salazar A (1998) Pollen germination *in vitro* of Mexican *Crataegus spp*. Acta Societais Botanicorum Polonie 67(1):45–49

Amin S, Kaloo ZA, Singh S, Altaf T (2013) Micropropagation of medicinally important plant species of family Asteraceae – a review. Int J Recent Sci Res 4(8):1296–1303

Argolo AC, Charlwood BV, Pletsch M (2000) The regulation of solasodine production by *Agrobacterium rhizogenes*-transformed roots of *Solanum aviculare*. Planta Med 66:448–451

Arumugam A, Gopinath K (2012) *In vitro* micropropagation using corm bud explants: an endangered medicinal plant of *Gloriosa superb*L. Asian J Biotech 4(3):120–128

Arumugam S, Rao AS, Rao MV (2003) *In vitro* propagation of *Aegle marmelos* (L.) Corr., a medicinal tree. In: Jain SM, Ishii K (eds) Micropropagation of woody trees and fruits, vol 75. Kluwer, Dordrechet, pp 269–315

Arya D, Patni V, Kant U (2008) *In vitro* propagation and quercetin quantification in callus cultures of Rasna (*Pluchea lanceolata* Oliver & Hiern.). Indian J Biotechnol 7:383–387

Aziz ZA, Davey MR, Lowe KC, Power JB (2006). Isolation and culture of protoplasts from the medicinal plant *Centella asiatica*. Rev Bras Pl Med. Botucatu 8: 105–109

Baek HJ, Kim HH, Cho EG, Chae YA, Engelmann F (2003) Importance of explant size and origin and of preconditioning treatments for cryopreservation of garlic shoot apices by vitrification. Cryo Lett 24:381–388

Bajaj YPS (1983a) Haploid protoplasts. In: Giles KI (ed) Plant protoplasts. Academic, London/New York, pp 113–40

Bajaj YPS (1983b) *In vitro* production of haploids. In: Evans DA, Sharp WR, Ammirato PV, Yamada Y (eds) Handbook of plant cell culture, vol 1. Macmillan, New York, pp 228–287

Bajaj YPS (1988) Regeneration of plants from frozen (21968C) protoplasts of *Atropa belladonna*, *Datura innoxia* and *Nicotiana tabacum*. Ind J Exp Biol 26:289–292

Bajaj YPS (ed) (1989) Biotechnology in agriculture and forestry 7. Medicinal and aromatic plants 2. XIX, 545 S. ISBN: 3-540-19196-8/ 0-387-19196-8

Bajaj YPS, Reghunath BR, Gopalakrishnan PK (1993) *Elettaria cardamomum* Maton (cardamom): aromatic compounds, *in vitro* culture studies and clonal propagation. In: Bajaj YPS (ed) Biotechnology in agriculture and forestry, medicinal and aromatic plants IV. Springer, Berlin, pp 132–147

Baksha R, Jahan MAA, Khatum R, Munshi JL (2005) Micropropagation of *Aloe barbadensis* Mill. through *In vitro* Culture of Shoot tip Explants. Plant Tissue Cult Biotechnol 15:121–126

Balaraju K, Agastian P, Preetamraj JP, Arokiyaraj S, Ignacimuthu S (2008) Micropropagation of *Vitex agnus-castus*, (*Verbenaceae*)—a valuable medicinal plant. In vitro Cell Dev Biol – Plant 44(5):436–441

Basalma D, Uranbey S, Gurlek D, Ozcan S (2008a) TDZ-induced plant regeneration in *Astragalus cicer* L. Afr J Biotechnol 7:955–959

Basalma D, Uranbey S, Mirci S, Kolsarici O (2008b) TDZ x IBA induced shoot regeneration from cotyledonary leaves and *in vitro* multiplication in Safflower (*Carthamus tinctorius* L.). Afr J Biotechnol 7:960–966

Basu P, Chand S (1998) Tropane alkaloids from callus cultures, differentiated roots and shoots of *Hyoscyamus muticus* L. Plant Biochem Biotech 7:39–42

Bawra B, Dixit M, Chauhan NS, Dixit VK, Saraf DK (2010) *Leptadenia reticulata* a rasayana herbs: a review. Asian J Plant Sci 9:314–319

Bekheet SA (2000) *In vitro* conservation of *Asparagus officinalis*. Biologia Plantarum 43:179–183

Berlin J (1999) Genetic transformation of *Peganum harmala*. In: Bajaj YPS (ed) Biotechnology in agriculture and forestry. Transgenic medicinal plants. Springer, Berlin

Bhavisha BW, Jasrai YT (2003) Micropropagation of an Endangered Medicinal Plant: *Curculigo orchioides* Gaertn. Plant tissue Cult 13:13–19

Bonhomme V, Laurain-Mattar D, Lacoux J, Fliniaux M, Jacquin-Dubreuil A (2000) Tropane alkaloid production by hairy roots of *Atropa belladonna* obtained after transformation with *Agrobacterium rhizogenes* 15834 and *Agrobacterium tumefaciens* containing *rol* A, B, C genes only. J Biotechnol 81:151–158

Bozhilova M, Evstatieva L, Tasheva K (2008) Salidroside content in *in vitro* propagated *Rhodiola rosea* L. In: Proceedings of the 5th Conference on medicinal and aromatic plants of Southeast European Countries (CMAPSEEC'08), Brno, Czech Republic

Cai G, Li G, Ye H, Li G (1995) Hairy root culture of *Artemisia annua* L. by Ri plasmid transformation and biosynthesis of artemisinin. Chin J Biotechnol 11:227–235

Casado JP, Navarro MC, Utrilla MP, Martinez A, Jimenez J (2002) Micropropagation of *Santolina canescens* Lagasca and *in vitro* volatiles production by shoot explants. Plant Cell Tiss Org 69:147–153

Chan LK, Lim SY, Pan LP (2009) Micropropagation of *Gynura procumbens* (Lour.) Merr. an important medicinal plant. J Med Plants Res 3(3): 105–111. http://www.academicjournals.org/JMPR, ISSN 1996-0875© 2009 Academic Journals

Chandore AN, Nimbalkar MS, Gurav RV, Bapat VA, Yadav SR (2010) A protocol for multiplication and restoration of *Ceropegia fantastica* Sedgw.: a critically endangered plant species. Curr Sci 99:1593–1596

Charlwood BV, Charlwood KA (1991) Terpenoid production in plant cell cultures. In: Harbourne JB, Tomas-Barberan FA (eds) Ecological chemistry and biochemistry of plant terpenoids. Clarendon, Oxford, pp 95–132

Chasseaud LF, Hawkins DR (2006) Biotransformation of drugs. Encyclopedia of Pharmaceutical Technology: 3rd edn. pp 310–324

Chattopadhyay S, Srivastava AK, Bhojwani SS, Bisaria VS (2002) Production of podophyllotoxin by plant cell cultures of Podophyllum hexandrum in bioreactor. J Biosci Bioeng 93:215–220

Chaturvedi HC, Madhu J, Kidwai NR (2007) Cloning of medicinal plants through tissue culture. a review. Indian J Exp Biol 45:937–948

Chavan JJ, Mansingraj SN, Avinash AA, Sharad SK et al (2011) Micropropagation and *in vitro* flowering of endemic and endangered plant *Ceropegia attenuata* Hook. J Plant Biochem Biotechnol 20:276–282

Chawla HS (2006) Introduction to plant biotechnology. Rastogi Publications, Meerut

Chen GY, Conner AJ, Christey MC, Fautrier AG, Field RJ (1997a) Protoplast isolation from shoots of *Asparagus* cultures. Int J Plant Sci 158:537–542

Chen GY, Conner AJ, Christey MC, Fautrier RJ (1997b) Field Culture and regeneration of protoplasts from shoots of *Asparagus* culture. Int J Plant Sci 158:543–555

Chu CC, Wang CC, Sun CS, Hsu KC, Yin KC, Chu CY, Bi FY (1975) Establishment of an efficient medium for anther culture of rice through comparative experiments on the nitrogen sources. Sci Sin 18:659–668

Coste A, Vlase L, Halmagyi A, Deliu C, Coldea G (2011) Effects of plant growth regulators and elicitors on production of secondary metabolites in shoot cultures of *Hypericum hirsutum* and *Hypericum maculatum*. Plant Cell Tiss Org Cult 106:279–288

Cucu N, Negoianu-Tenea G, Gavrila L (2002) Genetically modified medicinal plants. II. Transfer and expression of a marker kanamycine resistance gene in *Atropa belladonna* plants. Rom Biotechnol Lett 7:869–874

Dangi B, Khurana-Kaul V, Kothari SL, Kachhwaha S (2014) Micropropagation of Terminalia bellerica from nodal explants of mature tree and assessment of genetic fidelity using ISSR and RAPD markers. Physiol MolBiol Plants 20(4):509–516

Danova K, Čellárová E, Macková A, Daxnerová Z, Kapchina-Toteva V (2010) *In vitro* culture of *Hypericum rumeliacum* Boiss. and production of phenolics and flavonoids. In Vitro Cell Dev Biol Plant 46:422–429
Danova K, Nikolova-Damianova B, Denev R, Dimitrov D (2012) Influence of vitamins on polyphenolic content, morphological development, and stress response in shoot cultures of *Hypericum spp*. Plant Cell Tiss Organ Cult 110(3):383–393
Das P, Palai SK, Patra A, Samantaray S, Rout GR (1999) *In vitro* somatic embryogenesis in *Typhonium trilobatum* Schott. Plant Growth Regul 27:195–199
Das A, Gantait S, Mandal N (2011) Micropropagation of an elite medicinal plant: *Stevia rebaudiana* Bert. Inter J Agri Res 6(1):40–48
De Paiva N, Da Mota TR, Otoni WC (2003) Direct organogenesis from hypocotyl-derived explants of annatto (*Bixa orellana*). Plant Cell Tiss Organ Cult 75:159–167
Debnath SC (2009) Zeatin and TDZ-induced shoot proliferation and use of bioreactor in clonal propagation of medicinal herb, roseroot (*Rhodiola rosea* L). J Plant Biochem Biotechnol 18(2):245–248
Debnath M, Malik CP, Bisen PS (2006) Micropropagation: a tool for the production of high quality plant-based medicines. Current Pharmaceutical Biotech 7:33–49
Dhar U, Joshi M (2005) Efficient plant regeneration protocol through callus for *Saussurea obvallata* (DC.) Edgew. (*Asteraceae*): effect of explant type, age and plant growth regulators. Plant Cell Rep 24:195–200
DiCosmo F, Misawa M (1995) Plant cell and tissue culture: Alternatives for metabolite production. Biotechnol Adv 13:425–453
Dimitrov B, Tasheva K, Zagorska N, Evstatieva L (2003) *In vitro* cultivation of *Rhodiola rosea* L. Genet Breed 32(1–2):3–6
Elmer WH, Ball T, Volokita M, Stephens CT, Sink KC (1989) Plant regeneration from callus derived protoplasts of *Asparagus*. J Am Soc Hortic Sci 114:1019–1024
El-Tarras A, El-Awady AM, Attia OA, El Dessoky DS (2012) *In vitro* multiplication of the important medicinal plant, harmal (*Rhazya stricta* Decne). J Med Plants Res 6(19):3586–3590
Faria RT, Illg RD (1995) Micropropagation of *Zingiber spectabile* Griff. Sci Hortic 62:135–137
Filippini R, Piovan A, Borsarini A, Caniato R (2010) Study of dynamic accumulation of secondary metabolites in three subspecies of *Hypericum perforatum*. Fitoterapia 81:115–119
Furmanowa M, Hartwich M, Alfermann AW, Ko´zminski W, Olejnik M (1999) Rosavin as a product of glycosylation by *Rhodiola rosea* (roseroot) cell cultures. Plant Cell Tiss Org Cult 56(2):105–110
Gadzovska S, Maury S, Delaunay A, Spasenoski M, Hagège D, Courtois D, Joseph C (2013) The influence of salicylic acid elicitation of shoots, callus, and cell suspension cultures on production of naphtodianthrones and phenylpropanoids in *Hypericum perforatum* L. Plant Cell, Tissue Organ Cult (PCTOC) 113(1):25–39
Gamborg OL, Murashige T, Thorpe TA, Vasil IK (1976) Plant tissue culture media. In Vitro 12:473–478
Ganguli A (2009) Applied biotechnology and plant genetics. Oxford Book company, Delhi, p 320. ISBN 978-81-89473-48-8
Gantait S, Mandal N, Nandy S (2011) Advances in micropropagation of selected aromatic plants: a review on vanilla and strawberry. Am J Biochem Mol Biol 1(1):1–19
Garro-Monge G, Gatica-Arias AM, Valdez-Melara M (2008) Somatic embryogenesis, Plant regeneration and Acemannan detection in Aloe (Aloe barbadensis MILL.). Agronomia Costarricense 32(2):41–52
Gautam VK, Nanda K, Gupta SC (1993) Development of shoots and roots in anther-derived callus of *Azadirachta indica* A. Juss. a medicinal tree. Plant Cell Tiss Org Cult 34:13–18
Germ M, Stibilj V, Kreft S, Gaberščik A, Kreft I (2010) Flavonoid, tannin and hypericin concentrations in the leaves of St. John's wort (*Hypericum perforatum* L.) are affected by UV-B radiation levels. Food Chem 122:471–474

Giri A, Banerjee S, Ahuja PS, Giri CC (1997) Production of hairy roots in *Aconitum heterophyllum* wall. using *Agrobacterium rhizogenes*. In vitro Cell Dev Biol Plant 33(4):293–306

Giri A, Dhingra V, Giri CC, Singh A, Owen PW, Lakshami NM (2001) Biotransformations using plant cells, organ cultures and enzyme systems: current trends and future prospects. Biotechnol Adv 19:175–199

Gogu G, Hartan M, Nicuta D, Maftei D (2008) "Preliminary data regarding *Rhodiola rosea* L. *in vitro*," in Proceedings of the scientific papers of the 5th Conference on Medicinal and Aromatic Plants of Southeast European Countries (CMAPSEEC'08), Brno, Czech Republic, p 1–6

Gopi C, Nataraja SY, Ponmurugan P (2006) *In vitro* multiplication of *Ocimum gratissimum* L. through direct regeneration. Afr J Biotechnol 5(9):723–726

Greidziak N, Diettrich B, Luckner M (1990) Batch cultures of somatic embryos of *Digitalis lanata* in gas lift fermenters, development and cardenolide accumulation. Plant Med 6:175–178

György Z (2006) Glycoside production by *in vitro Rhodiola rosea* cultures, Ph.D. thesis, Acta Universitatis Ouluensis C Tehnica 244, Oulu, Finland

György Z, Tolonen A, Pakonen M, Neubauer P, Hohtola A (2004) Enhancing the production of cinnamyl glycosides in compact callus aggregate cultures of *Rhodiola rosea* by biotransformation of cinnamyl alcohol. Plant Sci 166(1):229–236

Hagimori MT, Matsi T, Mikami Y (1984) Jar fermentor culture of shoot forming cultures of *Digitalis purpurea* using a revised media. Agric Biol Chem 48:965–970

Hamada H, Sanada K, Furuya T, Kawabe S, Jaziri M (1996) Biotransformation of Paclitaxel by cell suspension cultures of *Eucalyptus Perriniana*. Nat Prod Res 9:47–52

Haque R, Saha S, Bera T (2009) Micropropagation of an important medicinal plant *Chlorophytum borivilianum*. Int J Ph Sci 1(1):153–163

Hartinie M, Jualang GA (2007) *In vitro* germination and plantlet establishment of *Labisia pumila* (Bl.) F. Vill. Sci Hortic 115:91–97

Husain MK, Anis M, Shahzad A (2007) *In vitro* propagation of Indian Kino (*Pterocarpus marsupium* Roxb.) using thidiazuron. In Vitro Cell Dev Biol Plant 43:59–64

Jabeen N, Shawl AS, Dar GH, Jan A, Sultan P (2007) Micropropagation of *Inula racemosa* Hook. f.A valuable medicinal plant. Int J Bot 3(3):296–301

Jan A, George T, Shawl SA, Neelofar J, Kozgar IM (2010) Improved micropropagation protocol of an Endangered medicinal plant- *Picrorhiza Kurroa* Royle ex Benth. promptly through Auxin treatments. Chiang Mai J Sci 37(2):304–313

Jha S, Jha TB (1989) Micropropagation of *Cephaelis ipecacuanha* Rich. Plant Cell Rep 8:437–439

Jha S, Sen S (1985) Regeneration and rapid multiplication of *Bowiea volubilis* Harv. in tissue culture. Plant Cell Rep 4:12–14

Jia J, Potrykus I, Lazal GB, Saul M (1983) *Hyoscyamus-Nicotiana* fusion hybrid selected via auxotroph concentration and verified by species specific DNA hybridization. In: Protoplasts, Poster Proceeding, Biekhauser, Basel/Boston/Stuttgart, pp 110–1

Jian-Feng L, Yun-Qing C, Zhi-Wen C (2009) Protoplast isolation and plant regeneration from leaves of *Rhodiola sachalinesis*. Chinese Traditional and Herbal Drugs 7: 2010–2014 (Chinese)

Julsing KM, Quax WJ, Kayser O (2007) The engineering of medicinal plants: prospects and limitations of medicinal plant biotechnology. In: Kayser O, Quax WJ (eds) Medicinal plant biotechnology: from basic research to industrial applications, Wiley, p 1–8

Jwala A, Shekhar KA (2013) Plant tissue culture: modern techniques and practices. Encyclopedia of plant tissue culture, vol 1–5, 1st edn. Campus Books International, New Delhi

Kalidass C, Mohan VR (2009) *In vitro* rapid clonal propagation of *Phyllanthus urinaria* Linn. (*Euphorbiaceae*)- a medicinal plant. Researcher 1(4):56–61

Kalidass C, Manickam VS, Glory M (2008) *In vitro* studies on *Leptadenia reticulate* (Retz.) Wight & Arn. (*Asclepiadaceae*). Indian J Multidisciplinary Res 4(2):221–225

Kane ME, Kauth Ph, Stewart S (2008) Micropropagation. In: Beyl CA, Trigiano RN (eds.) Plant propagation Concepts and laboratory exercises. 462 pp CRC Press/Taylor & Francis, Boca Raton, p 319–332

Kapaia VY, Kapoora P, Raoa IU (2010) *In vitro* propagation for conservation of rare and threatened plants of India –a review. Inter J Biol Technol 1(2):1–14

Karaguzel O, Kaya A, Biner B, Aydinsakir K (2012) In vitro propagation of native Ornithogalum species in West Mediterrenean region of Turkey. Afr J Agric Res 7(17):2669–2673

Karakas O, Toker Z, Tilkat E, Ozen HC, Onay A (2009) Effects of different concentrations of benzylaminopurine on shoot regeneration and hypericin content in *Hypericum triquetrifolium* Turra. Nat Prod Res 23:1459–1465

Karppinen K (2010) Biosynthesis of hypericins and hyperforins in *Hypericum perforatum* L (St. John's wort)—precursors and genes involved. Acta Universitatis Ouluensis, A Scientiae Rerum Naturalium 564, p 74

Karppinen K, Hokkanen J, Tolonen A, Mattila S, Hohtola A (2007) Biosynthesis of hyperforin and adhyperforin from amino acid precursors in shoot cultures of *Hypericum perforatum*. Phytochemistry 68:1038–1045

Khalid MK, Cigdem AO, Semiha B, Sebhattin O, Orhan A (2005) Efficient shoot regeneration in Syrian Rue (*Peganum harmala* L.) Under *in vitro* Conditions. Int J Agric Biol 5:790–793

Khawar KM, Sarihan EO, Sevimay CS et al (2005) Adventitious shoot regeneration and micropropagation of *Plantago lanceolata* L. Periodicum Biologorum 107(1):113–116

Kirakosyan A, Hayashi H, Inoue K, Charchoglyan A, Vardapetyan H (2000) Stimulation of the production of hypericins by mannan in *Hypericum perforatum* shoot cultures. Phytochemistry 53:345–348

Kochan E, Wasiela M, Sienkiewicz M (2013) The production of ginsenosides in hairy root cultures of American Ginseng, *Panax quinquefolium* L. and their antimicrobial activity. In vitro Cell Dev Biol – Plant 49(1):24–29

Kondamudi R, Vijayalakshmi V, Murthy KSR (2010) Induction of morphogenetic callus and multiple shoot regeneration in *Ceropegia pusilla* Wight and Arn. Biotechnology 9:141–148

Kopittke PM, Blamey FC, Asher CJ, Menzies NW (2010) Trace metal phytotoxicity in solution culture: a review. J Exp Bot 1–10, doi:10.1093/jxb/erp385

Kornfeld A, Kaufman PB, Lu CR, Gibson DM, Bolling SF et al (2007) The production of hypericins in two selected *Hypericum perforatum* shoot cultures is related to differences in black gland structure. Plant Physiol Biochem 45:24–32

Koroch AR, Kapteyn J, Juliani HR, Simon JE (2002) *In vitro* regeneration and *Agrobacterium* transformation of *Echinecea purpuria* leaf explants. Trends New Crop New News, 2002

Krajewska-Patan A, Mscisz A, Kedzia B, Lutomski J (2002) The influence of elicitation on the tissue cultures of rose root (*Rhodiola rosea*). Herba Polonica 48(2):77–81

Kranthi G, Pavan U, Mahende A, Srinivasa RK, Venugopal RK, Kiranmayee K, Sadanandam A (2011) Micropropagation of a valuable ethnomedicinal plant *STREBLUS ASPER* LOUR. J Phytology 3(2):18–23

Krishnareddy PV, Karuppusamy S, Pullaiah T (2011) *In vitro* propagation of *Ceropegia juncea* Roxb. Afr J Plant Sci 3:345–357

Kumar A (1992) Somatic embryogenesis and high frequency plantlet regeneration in callus cultures of *Thevetia peruviana*. Plant Cell Tiss Org Cult 51:47–50

Kumar PP, Loh CS (2012) Plant tissue culture for biotechnology. In: Altman A, Hasegawa PM (eds) Plant biotechnology and agriculture for the 21st century. Academic, Elsevier, pp 131–138

Lakshimi BJ, Reddy KJ (2009) *In vitro* propagation of some medicinal plants as a biotechnological tool for conservation – a review. In: Trevedi, PC (ed) Biotechnology: tissue culture to proteomics. Aavishkar publishers and distributors. Pointed Publishers, India, p 115–127 ISBN: 978-81-7132-5771

Lameira OA, Pinto JEBP (2006) *In vitro* propagation of *Cordia verbenacea* L. (*Boraginaceae*) Rev. Bras. Pl. Med., Botucatu 8:102–104

Levin R, Gaba V, Tal B, Hirsch S, De Nola D, Vasil K (1988) Automated plant tissue culture for mass propagation. Biotechnology 6:1035–1040

Lin C-S, Lin C-C, Chang W-C (2004) Effect of thidiazuron on vegetative tissue-derived somatic embryogenesis and flowering of bamboo *Bambusa edulis*. Pl Cell Tiss Org Cult 76:75–82

Liu XN, Zhang XQ, Sun JS (2007a) Effects of cytokinins and elicitors on the production of hypericins and hyperforin metabolites in *Hypericum sampsonii* and *Hypericum perforatum*. Plant Growth Regul 53:207–214

Liu XN, Zhang XQ, Zhang SX, Sun JS (2007b) Regulation of metabolite production by precursors and elicitors in liquid cultures of *Hypericum perforatum*. Plant Cell Tiss Organ Cult 91:1–7

Lu MC (2005) Micropropagation of *Vitis thunbergii* Sieb. et Zucc., a medicinal herb, through high-frequency shoot tip culture. Sci Hortic 107:64–69

Makunga NP, Jager AK, Van SJ (2003) Micropropagation of *Thapsia garganica*—a medicinal plant. Plant Cell Rep 21:967–973

Malik SK, Chaudhury R, Kalia RK (2005) Rapid *in vitro* multiplication and conservation of *Garcinia indica*: a tropical medicinal tree species. Sci Hortic 106:539–553

Manjkhola S, Dhar U, Joshi M (2005) Organogenesis, embryogenesis and synthetic seed production in *Arnebia euchroma* – a critically endangered medicinal plant of the Himalaya. In Vitro Cell Dev Biol Plant 41:244–248

Martin KP (2003a) Clonal propagation encapsulation and re-introduction of *Ipsea malabarica* (Reich b.f.) JD Hook., an endangered orchid. *In vitro* Cell Dev Biol Plant 39: 322–326

Martin KP (2003b) Plant regeneration through somatic embryogenesis on *Holostemma ada-kodien*, a rare medicinal plant. Pl Cell Tiss Org Cult 72:79–82

Martin KP (2003c) Rapid *in vitro* multiplication and *ex vitro* rooting of *Rotula aquatica* Lour., a rare rhoeophytic woody medicinal plant. Pl Cell Rep 21:415–420

Martin KP, Pradeep AK (2003) Simple strategy for the *in vitro* conservation of *Ipsea malabarica*, an endemic and endangered orchid of the Western Ghats of Kerala. India Pl Cell Tiss Org Cult 74:197–200

Matkowski A (2000) Plant *in vitro* culture for the production of antioxidants – a review. Biotechnol Adv 26:548–560

McCown BH, Lloyd G (1981) Woody plant medium (WPM) – a mineral nutrient formulation for microculture of woody plant species. HortScience 16:453–453

Mederos-Molina S (2006) Micropropagation of *Salvia broussonetii* Benth. – a medicinal plant species. Plant Tissue Cult & Biotech 16(1):19–23

Meena MC, Meena R, Patni V (2010) High frequency plant regeneration from shoot tip explants of *Citrullus colocynthis* (Linn.) Schrad. An important medicinal herb. Afr Biotechnol 9(31):5037–5041

Mehrotra S, Goel MK, Kukreja AK, Mishra BN (2007) Efficiency of liquid culture systems over conventional micropropagation: a progress towards commercialization. Afr J Biotechnol 6(13): 1484–1492 http://www.academicjournals.org/AJB

Mehta SR, Subramanian RB (2005) Direct *In vitro* Propagation of *Asparagus adscendens* Roxb. Plant Tissue Cult 15:25–32

Misawa M (1985) Production of useful plant metabolites (ed. Fiechter A). Adv Biochem Eng Biotechnol. Springer, Berlin, pp 59–88

Miura Y, Tabata M (1986) Direct somatic embryogenesis from protoplasts of *Foeniculum vulgare* Miller. Plant Cell Rep 5:310–313

Mohan JSS, Patel S, Sharma U (2005) *In vitro* clonal propagation of *Urgenia indica* (Roxb.) Kunth. Prajna. J Pure Appl Sci 13:21–28

Murashige T, Skoog F (1962) A revised medium for the rapid growth and bioassays with tobacco tissue cultures. Physiol Pl 15:473–479

Murch SJ, Krishna Raj S, Saxena PK (2000) Tryptophan is a precursor for melatonin and serotonin biosynthesis in *in-vitro* regenerated St. John's wort (*Hypericum perforatum* L. cv. Anthos) plants. Plant Cell Rep 19:698–704

Murthy KSR, Kondamudi R, Vijayalakshmi V (2010a) Micropropagation of an endangered medicinal plant *Ceropegia spiralis* Wight. J Agr Tech 6:179–191
Murthy KSR, Kondamudi R, Pullaiah T (2010b) High frequency somatic embryogenesis in *Ceropegia spiralis* Wight – An endemic and endangered medicinal plant. Indian J Biotechnol 9:414–418
Nadganda RS, Mascarenhas AP, Madhusoodanan K (1983) Clonal multiplication of cardamom (*Eletteria cardamomum* Maton) by tissue culture. Plantation Crops 11:60–64
Nagananda GS, Nalini S, Rajath S (2011) Regeneration of encapsulated Protocorm like bodies of medicinally important vulnerable orchid *Flickingeria nodosa* (dalz.) Seidenf. Int J Bot 7:310–313
Nagesh KS, Shanthamma C (2011) Micropropagation and antioxidant activity of *Mollugo nudicaulis* Lam. J Med Plant Res 5(6):895–902
Naina NS, Gupta PK, Mascarenhas AF (1989) Genetic transformation and regeneration of transgenic neem (*Azadirachta indica*) plants using *Agrobacterium tumefaciens*. Curr Sci 58:184–187
Nair LG, Seeni S (2003) *In vitro* multiplication of *Calophyllum apetalum* (*Clusiaceae*), an endemic medicinal tree of the Western Ghats. Plant Cell Tiss Organ Cult 75:169–174
Nalawade SM, Sagare AP, Lee CY, Kao CL, Tsay HS (2003) Studies on tissue culture of Chinese medicinal plant resources in Taiwan and their sustainable utilization. Botanical Bulletin of Academia Sinica 44(2):79–98
Neelofar J, Shawl AS, Dar GH (2007) Micropropagation of *Inula racemosa* Hook. f. a valuable medicinal plant. Int J Bot 3:296–301
Neha Bhagat (2011) Conservation of endangered medicinal plant (*Acorus calamus*) through plant tissue culture. J Pharmacognosy 2(1):21–24. Available online at http://www.bioinfo.in/contents.php?id=70
Nhut DT, Hai NT, Huy NP, Chien HX, Nam NB (2013) New achievement in *Panax vietnamensis* research. doi: 10.1007/978-94-007-5500-0_3. In: Jain, SM Gupta SD (eds) Biotechnology of neglected and underutilized crops. doi:10.1007/978-94-007-5500-0_3, Springer, Netherlands, pp 43–57
Nikam TD, Savanth RS (2007) Callus culture and micropropagation of *Ceropegia sahyadrica* Ans. et Kulk: An edible starchy tuberous rare asclepiad. Indian J Plant Physiol 12:108–114
Nikam TD, Savanth RS, Parage RS (2008) Micropropagation of *Ceropegia hirsuta* Wt. & Arn., a starchy tuberous asclepiad. Indian J Biotechnol 7:129–132
Nirmal Babu K, Ravindran PN, Peter KV (1997) Protocols for micropropagation of spices and aromatic crops. Indian Institute of Spices Research, Calicut, p 35
Nurazah Z, Radzali M, Syahida A, Maziah M (2009) Effects of plant growth regulators on callus induction from *Cananga odorata* flower petal explants. Afr J Biotechnol 8(12):2740–2743
Oksman-Caldentey KM, Inzé D (2004) Plant cell factories in the post-genomic era: new ways to produce designer secondary metabolites. Trends Plant Sci 9:433–440
Ozel CA, Khawar KM, Mirici S, Ozcan S, Arslan O (2006) Factors affecting *in vitro* plant regeneration of the critically endangered Mediterranean knapweed (*Centaurea tchihatcheffii* Fisch et. Mey). Naturwissenschaften 93:511–517
Ozel CA, Khawar KM, Karaman S, Ates MA, Arslan O (2008) Efficient *in vitro* multiplication in *Ornithogalum ulophyllum* Hand Mazz from twin scales. Sci Hortic 116(1):109–112
Pan ZG, Liu CZ, Murch SJ, El-Demerdash M, Saxena PK (2003) Plant regeneration from mesophyll protoplasts of the Egyptian medicinal plants *Artemisia judaica* L. and Echinops spinosissimus Turra. Plant Sci 165:681–687
Pan S, Neeraj A, Srivastava KS, Kishore P, Sarethy IP (2013) Effects of growth regulators on *in vitro* response and multiple shoot induction in some endangered medicinal plants. OA Biotechnology 2(1):3
Pande D, Malik S, Bora M, Srivastava PS (2002) Rapid protocol for *in-vitro* micropropagation of *Lepidium sativum* Linn and enhancement in the yield of lepidine. In-vitro Cell Dev Biol- Plant 38:451–455

Pandit SS, Aneeshkumar N, Naik DD (2008) Towards conservation of threatened *Ceropegia* species endemic to a biodiversity hot spot: *in vitro* micro tuber production and proliferation, a novel strategy. J For Sci 24:79–88
Park SU, Facchini PJ (2000) *Agrobacterium rhizogenes*-mediated transformation of opium poppy, *Papaver somniferum* L., and California poppy, *Eschscholzia californica* cham, root cultures. J Exp Bot 51:1005–1016
Park S-Y, Ahn J-K, Lee W-Y, Murthy HN, Paek K-Y (2005) Mass production of *Eleutherococcus koreanum* plantlets via somatic embryogenesis from root cultures and accumulation of eleutherosides in regenerants. Plant Sci 168:1221–1225
Pasqua G, Avato P, Monacelli B, Santamaria AR, Argentieri MP (2003) Metabolites in cell suspension cultures, calli, and *in vitro* regenerated organs of *Hypericum perforatum* cv. Topas. Plant Sci 165:977–982
Patra A, Rai B, Rout GR, Das P (1998) Successful plant regeneration from callus cultures of *Centella asiatica* (Linn.) Urban. Plant Growth Regul 24:13–16
Perez-Bermudez P, Cornejo MJ, Segura J (1985) Pollen plant formation from anther cultures of *Digitalis obscura*. Plant Cell Tiss Org Cult 5:63–68
Pradel H, Dumkelehmann U, Diettrich B, Luckner M (1997) Hairy root cultures of *Digitalis lanata*. secondary metabolism and plant regeneration. J Plant Physiol 151:209–215
Prakash S, Elongomathavan R, Seshadri S, Kathiravan K, Ignacimuthu S (2004) Efficient regeneration of *Curcuma amada* Roxb. plantlets from rhizome and leaf sheath explants. Plant Cell Tissue Organ Cult 78:159–165
Prakash S, Van Staden J (2007) Micropropagation of Hoslundia opposita Vahl–a valuable medicinal plant. S Afr J Bot 73:60–63
Pras N, Woerdenbag HJ, VanUden W (1995) Bioconversion potential of plant enzymes for the production of pharmaceuticals. Plant Cell Tiss Org Cult 43:117–121
Praveen N (2009) *In vitro* regeneration of Brahmi shoots using semisolid and liquid cultures and quantitative analysis of bacoside A. Acta Physiologiae Plantarum 31(4):723–728
Preil W, Florek P, Wix U, Beck A (1988) Towards mass propagation by use of bioreactors. Acta Horticult 226:99–105
Rajeswari V, Paliwal K (2006) *In vitro* propagation of *Albizia odoratissima* L.F. (Benth.) from cotyledonary node and leaf nodal explants. In Vitro Cell Dev Biol Plant 42:399–404
Rajneesh KA, Shyamal KN (2009) *In vitro* Shoot Cut: A High Frequency Multiplication and Rooting Method in the Bamboo *Dendrocalamus hamiltonii*. Biotechnology 8:259–263
Rao NK (2004) Plant genetic resources: advancing conservation and use through biotechnology. Afr J Biotech 3:136–145
Rao SR, Ravishankar GA (1999) Biotransformation of isoeugenol to Vanilla flavour metabolites and capsaicin in suspended and immobilized cell cultures of *Capsicum frutescens*: study of the influence of beta-cyclodextrin and fungal elicitor. Process Biochem 35:341–348
Rao RS, Ravishankar GA (2002) Plant cell cultures: chemical factories of secondary metabolites. Biotechnol Adv 20(2):101–153
Rao IU, Rao IVR, Narang V (1985) Somatic embryogenesis and regeneration of plants in the bamboo *Dendrocalamus strictus*. Plant Cell Rep 4:191–194
Rao SR, Tripathi U, Ravishankar GA (1999) Biotransformation of codeine to morphine in freely suspended cells and immobilized cultures of *Spirulina platensis*. World J Microbiol Biotechnol 15:465–469
Rao RS, Usha T, Ravishankar GA (2002) Biotransformation of digitoxin to digoxin in cell cultures of Capsicum frutescens in the presence of β-cyclodextrin. Biocatal Biotransform 20:137–143
Rath SP, Puhan P (2009) *In vitro* micropropagation of medicinal plants- a retrospect. In: Trevedi PC (ed) Biotechnology: tissue culture to proteomics. Aavishkar publishers and distributors/ Pointed Publishers, India, pp 91–114
Rathore S, Singh N (2013) *In vitro* conservation of *Bacopa monniere*- an endangered medicinal plant. Global J. of BioScience Biotechnol (G.J.B.B.) 2(2):187–192

Redenbaugh K, Viss P, Slade D, Fujii J (1986) Scale-up Artificial seeds. In: Somers DA, Gengenbach BG, Biesboer DD, Hackett WP, Green CE (eds) Proc VI Int Cong Plant Tissue and Culture, Univ Minnesota, Minneapolis, MN, p 132.

Rizvi MZ, Kukreja AK (2014) Micropropagation of an endangered medicinal herb *Chlorophytum borivilianum* Sant. et Fernand. in bioreactor. Afr J Biotechnol 13(17):1772–1778

Rout GR, Samantaray S, Das P (2000a) *In vitro* somatic embryogenesis from callus cultures of *Cephaelis ipecacuanha* A. Richard. Sci Hortic 86:71–79

Rout GR, Samantary S, Das P (2000b) *In vitro* manipulation and propagation of medicinal plants. Biotechnol Adv 18(2):91–120

Saeed SA, Simjee RU, Farah M, Naheed S, Atta UR (1993) Rhaziminine from Rhazya stricta a dual inhibitor of arachidnoic acid metabolism and plateletactivating factor-nduced platelet aggregation. Planta Med 59:566–568

Saha S, Dey T, Ghosh P (2010) Micropropagation of *Ocimum kilimandschricum* Guerke (*Labiatae*). Acta Biol Cracov Bot 52(2):50–58

Salvi ND, George L, Eapen S (2001) Plant regeneration from the leaf base callus of turmeric and random amplified polymorphic DNA analysis of the regenerated plants. Plant Cell Tiss Org Cult 66:113–119

Salvi ND, George L, Eapen S (2002) Micropropagation and field evaluation of micropropagated plants of turmeric. Plant Cell Tiss Org Cul 68:143–151

Sarasan V, Cripps R, Ramsay MM, Atherton C, McMichen M, Prendergast G, Rowntree JK (2006) Conservation *in vitro* of threatened plants – progress in the past decade. In Vitro Cell Dev Biol Plant 42:206–214

Sasikumar S, Raveendar S, Premkumar A, Ignacimuthu S, Agastian P (2009) Micropropagation of *Baliospermum montanum* (Willd.) Muell. Ara.-A threatened medicinal plant. Indian J Biotechnol 8:223–226

Satheesh KK, Bhavanandan KV (1988) Micropropagation of *Plumbago rosea* Linn. Plant Cell Tiss Org Cult 15:275–278

Savio LEB, Astarita LV, Santarém ER (2012) Secondary metabolism in micropropagated *Hypericum perforatum* L. grown in non-aerated liquid medium. Plant Cell Tiss Organ Cult 108:465–472

Saxena C, Palai SK, Samantaray S, Rout GR, Das P (1997) Plant regeneration from callus cultures of *Psoralea corylifolia* Linn. Pl Growth Regul 22:13–17

Sayanika DW, Bengyella L, Sharma GJ (2012) *In vitro* Seed Germination and Micropropagation of Edible Bamboo *Dendrocalamus giganteus* Munro using Seeds. Biotechnology 11:74–80

Schippmann U, Leaman DJ, Cunningham AB (2002) Impact of cultivation and gathering of medicinal plants on biodiversity: global trends and issues. Inter-Department Working Group on Biology Diversity for Food and Agriculture. FAO, UN, Rome, Italy

Sharafi A, Haleh HS, Amir M, Pejman A, Khadijeh R, Valentine ON (2013a) A reliable and efficient protocol for inducing hairy roots in *Papaver bracteatum*. Plant Cell, Tissue Organ Cult(PCTOC) 113(1):1–9

Sharafi A, Haleh HS, Amir M, Pejman A, Bahman HK, Khadijeh R (2013b) Metabolic engineering of morphinan alkaloids by over-expression of codeinone reductase in transgenic hairy roots of *Papaver bracteatum*, the Iranian poppy. Biotechnol Lett 35(3):445–453

Sharma N (2005) Micropropagation of *Bacopa monneiri* L. Penn – an important medicinal plant. M.Sc. Dissertation. Department of biotechnology and environmental sciences, Thapar Institute of Engineering and Technology, Patiala, India

Sharma A, Meena MC, Patni V, Arora DK (2007) *In vitro* multilplication of *Acalypha wilkesiana* dwarf through nodal segment culture. J Plant Sci Res 23(1–2):79–82

Sharma S, Neelima R, Barkha K, Dipika P, Baljinder K, Sarita A (2010) Conservation of biodiversity of highly important medicinal plants of India through tissue culture technology-a review. agriculture and biology J North America Online: doi: 10.5251/abjna. 2010.1.5.827. 833.© ScienceHuβ, http://www.scihub.org/ABJNA

Shi HP, Kintzios S (2003) Genetic transformation of *Pueraria phaseoloides* with *Agrobacterium rhizogenes* and puerarin production in hairy roots. Plant Cell Rep 21:1103–1107

Shilpa K, Varun K, Lakshmi BS (2010) An alternate method of natural drug production: elciting secondary metabolite production using plant cell culture. J Plant Sci 5:222–247

Siahsar B, Rahimi M, Tavassoli A, Raissi A (2011) Application of biotechnology in production of medicinal plants. American-Eurasian. J Agric Environ Sci 11(3):439–444

Singh HP, Parthasarathy VA, Nirmal Babu K (2011) Advances in horticulture biotechnology-regeneration systems- fruit crops, plantation crops and spices, vol 1. Westville Publishing House, New Delhi, p 484

Souret FF, Weathers PJ, Wobbe KK (2002) The mevalonate-independent pathway is expressed in transformed roots of *Artemisia annua* and regulated by light and culture age. In-vitro Cell Dev Biol Plant 38:581–588

Srinath G, Sridhar V, Renuka R (2009) *In vitro* Propagation of the Medicinal Herb – *Clerodendrum inerme*. Advanced Bio Tech 9(3):21–23

Sudha G, Ravishankar GA (2003) Putrescine facilitated enhancement of capsaicin production in cell suspension cultures of *Capsicum frutescens*. J Plant Physiol 160:339–346

Swaroopa G, Subhash K, Ghansham D (2011) An improved plant regeneration system for high frequency multiplication of *Rubia cordifolia* L.: a rare medicinal plant. Asian J Biotechnol 3:397–405

Tasheva K, Kosturkova G (2011) *Rhodiola rosea* L. *in vitro* plants morphophysiological and cytological characteristics. Rom Biotechnol Lett 16(6):79–85

Tasheva K, Kosturkova G (2012) The role of biotechnology for conservation and biologically active substances production of *Rhodiola rosea* : Endangered medicinal species. The Scientific World Journal, vol. 2012, Article ID 274942, p 13, 2012. doi:10.1100/2012/274942

Tiwari KN, Sharma NS, Tiwari K, Singh BD (2000) Micropropagation of *Centella asiatica* (L.), a valuable medicinal herb. Plant Cell Tiss Org 63(3):179–185

Tommasi F, Scaramuzzi F (2004) *In vitro* propagation of *Ginkgo biloba* by using various bud cultures. Biol Plant 48:297–300

Trigiano RN, Gray DJ (eds) (2011) Plant Tissue culture, Development and Biotechnology. CRC Press, Boca Raton, FL

Tripathi L, Tripathi JN (2003) Role of biotechnology in medicinal plants. Trop J Pharm Res 2(2):243–253

Tsay HS, Gau TG, Chen CC (1989) Rapid clonal propagation of *Pinellia ternata* by tissue culture. Plant Cell Rep 8:450–454

Uden WV, Bouma AS, Baracht JF et al (1995) The production of podophyllotoxin and its 5-methoxy derivative through bioconversion of cyclodextrin-complexed desoxypodophyllotoxin by plant cell cultures. Plant Cell Tiss Org Cult 42:73–79

Ujjwala JS (2007) *In vitro* Regeneration of *Aloe barbadensis*. Biotechnology 6:601–603

Vadodaria HK, Samantaray S, Maiti S (2007) Micropropagation of *Glycyrrhiza glabra* Linn. an important medicinal plant. J Cell Tissue Res 7(1):921–926

Verpoorte R, Contin A, Memelink J (2002) Biotechnology for the production of plant secondary metabolites. Phytochem Rev 1(1):13–25

Walker TS, Bais HP, Vivanco JM (2002) Jasmonic acid-induced hypericin production in cell suspension cultures of *Hypericum perforatum* L. (St. John's wort). Phytochemistry 60:289–293

Wang YD, Yuan YJ, Wu JC (2004) Induction studies of methyl jasmonate and salicylic acid on taxane production in suspension cultures of *Taxus chinensis* var. mairei. Biochem Eng J 19:259–265

Wawrosch C, Prinz S, SoleimanY, Kopp B (2007) Clonal propagation of *Crataegus monogyna* Jacq. (Lindm.). Planta Med, p 73

Wesolowska M, Skrzypczak L (1985) *In vitro* androgenetic cultures of *Hyoscyamus niger,* H. albus and alkaloid content assay. Acta Soc Bot Pol 54:107–113

Wilhelm R, Zenk MH (1997) Biotransformation of thebaine by cell cultures of *Papaver somniferum* and *Mahonia nervosa*. Phytochemistry 46:701–708

Wondyifraw T, Surawit W (2004) Micropropagation of Krawan (*Amomum krervanh* Pierre ex Gagnep). Sci Asia 30:9–150
Wu S, Zu Y, Wu M (2003) High yield production of salidroside in the suspension culture of *Rhodiola sachalinensis*. J Biotechnol 106(1):33–43
Xu MJ, Dong JF, Zhang XB (2008) Signal interaction between nitric oxide and hydrogen peroxide in heat shock-induced hypericin production of *Hypericum perforatum* suspension cells. Sci Chin Ser C Life Sci 51:676–686
Yaadwinder S (2010) *In vitro* micropropagation of medicinal plants by tissue culture. Plymouth Stud Sci 4(1):432–449
Yadav K, Singh N, Verma S (2012) Plant tissue culture: a biotechnological tool for solving the problem of propagation of multipurpose endangered medicinal plants in India. J Agric Technol 8(1):305–318
Yun DJ, Hashimoto T, Yamada Y (1992) Metabolic engineering of medicinal plants: transgenic *Atropa belladonna* with an improved alkaloid composition. Proc Natl Acad Sci U S A 89:11799–11803
Zhong JJ, Bai Y, Wang SJ (1996) Effects of plant growth regulators on cell growth and ginsenoside saponin production by suspension cultures of *Panax quinquefolium*. J Biotechnol 45:227–234
Zhou LG, Wu JY (2006) Development and application of medicinal plant tissue cultures for production of drugs and herbal medicinals in China. Nat Prod Rep 23:789–810

Chapter 16
Herbal Medicinal Preparations in Different Parts of the World

Dezső Csupor

Abstract In the past years the market of medicinal plants and plant-based products have been changed significantly. Globalisation is gaining strength even in medicinal plant industry since the former local medicinal plants are distributed worldwide through the Internet. National rules regarding medicinal plants and herbal medicinal preparations are also developed in a greatly differing way in certain countries. On the one hand, strict regulation of medicinal products results in unreasonable hurdles in, on the other hand, underregulation of certain product categories leads to the existence of products with dubious quality. Processed medicinal plant products are shifting from the category of medicines to the food sectors and according to the strengthening health conscious behaviour novel products categories are invented (functional foods, fortified foods, etc.). The aim of this chapter is to present an overview of the major product categories and of the regulation and market characteristics of certain countries.

Keywords Medicinal plants • Phytotherapeutics • Regulation • Food supplements • Medical devices • Herbal medicines

1 Introduction

In the past years significant changes have taken place in the marketing, distribution and use of medicinal plants. Use and application shows diversification and its volume is increasing rapidly; significant reproportionations in cultivation are already in progress; and new medicinal plant processing centres has been established (primarily in Asia). We may say that globalisation is gaining strength even in this small segment of the healthcare industry. Accordingly to this phenomenon the range of products containing (also) medicinal plants which reach the consumers in industrialised countries has expanded, and this inevitably brought along changes in the legal regulation. Strangely, this implied diversification, liberalisation and stricting, at the same time. Processed products of extremely

D. Csupor (✉)
Department of Pharmacognosy, Szeged University, Eötvös street 6, Szeged, Hungary
e-mail: csupor.dezso@pharmacognosy.hu

Á. Máthé (ed.), *Medicinal and Aromatic Plants of the World*, Medicinal and Aromatic Plants of the World 1, DOI 10.1007/978-94-017-9810-5_16

differing quality, which seem to be medicines and occur in a dumping-like manner impose a serious challenge for both healthcare professionals and consumers. The proliferating, nonetheless inadequately harmonised national and regional provisions of law and implementing regulations causing problems of interpretation and legal vacuum by their mere nature also open the chance for misuse and malfeasance. In recent years malfeasant acts posing a threat to product quality and consumer safety have became frequent in this field (Lord et al. 1999; Nowack 2004; Schoepfer et al. 2007).

The so called "green wave" started in the 1970s is part of a larger and complex process simultaneously taking place in many fields of everyday life and has continually been accelerating during the past 10 or 15 years. We are the witnesses of a dynamic transformation that is unprecedented and unique. Intermediate product groups have occurred to which the already existing regulatory and licensing-authorisation concepts and frameworks cannot be or scarcely be applied. The pace of development motivated by possibilities surpasses deep beyond the chances of legislation, law enforcement, market supervision and consumer protection. Today, national and regional demands requiring the adaptation of the already existing regulatory frameworks to the actual market situation; and particularly the establishment of new regulatory restrictions being better guarantors of consumer safety are manifesting themselves at the same time.

2 Medicinal Plant Use in Different Parts of the World

Pharmaceutical drug consumption of the world is greatly uneven: while a 1/4 part of the Earth's population living in the industrialised countries consume 70–80 % of all the pharmaceutical drugs, the remainder 3/4 part living in the so called third world have access only to 10–20 % of all marketed pharmaceutical products. Concerning access to medicinal plants and products manufactured from them also shows high dividedness, but the ratio is practically the inverse of the above. It is unfortunately known that the majority of the nearly four billion people living in the developing countries have access neither to medical care, nor to the modern pharmaceutical drugs; cannot even afford most of the drugs sold by free retail at local street markets, and the remainder cheap products, which they do can afford, are generally worthless counterfeits. Local healers and traditional preparations of herbal or animal origin embody "pharmaceutical" therapy for these people. The composition of the *materia medica* in these countries is similar to what could be found in Europe in a couple 100 years ago, i.e. the basis of "therapy" is provided by the locally available drugs of herbal (and in a smaller proportion, animal) origin. Unregulatedness is a characteristic of this sector: national level regulation of the local traditional medicine is missing or inadequate. Quality warrants being effective with regards to medicinal plants may only be provided and ensured by pharmacopoeias adopted and/or inherited from earlier eras, by recommendations established upon the aid of the WHO, (World Health Organization 1996, 2000, 2003; World

Health Organization Regional Office for the Western Pacific 1993) and in case of raw materials, to be exported by the quality specifications of the importing party.

In a major part of the world (primarily in the Far East) healthcare provided in these two different levels (modern and folk) is completed by a third level based on professional, secondary and post-secondary training and education: the institutionalised traditional medicine and its *materia medica*. In this part of the world and in a smaller extent in South America, too, a very significant transformation is in progress in these days. This will change not only the terms of local supply and standards, but it also exerts an ever growing impact on Europe and North America. The agricultural cultivation of medicinal plants is rapidly increasing, the processing methods are modernised, and the volume of raw material, semi-finished and finished product export aimed at the developed world is also increasing. According to commercial data, five herbal drugs out of the ten medicinal plants ranking highest (soy, garlic, *Ginseng*, *Ginkgo*, *Cimicifuga*) in terms of commercial turnover are cultivated to a significant extent in the developing world. Nowadays, the majority of the primary industrial processing workload is also carried out in these parts of the world (primarily in China) (Brinckmann 2007). By this means the countries of the Far East are gradually becoming semi-finished and finished product suppliers. According to certain data, nearly 80 % of medicinal plant export aimed at the United States is originated from China, and in a smaller proportion, from India. It can be presumed that this tendency may be similar in Europe, as well. All the above described phenomena have changed the European and American product markets to a considerable extent and raise novel regulatory problems.

Following the example and active initiative provided by China in the first place, the World Health Organization (WHO) has also recognised that the supply for the so called third world may only be provided by rational fortification and development of the locally available resources (the provision of traditional care, those raw materials which are locally available and suitable for drug production, and the local manufacturing industry). Therefore, the WHO announced the Traditional Medicine Strategy, within the frame of which the professional evaluation of the most important medicinal plants used in local medicinal systems of the developing regions has been started in 1986 (World Health Organization 1999–2006). Jointly with the Development Programmes of the UN (UNDP, UNIDO) and based on the already operating models local medicinal plant processing factories and research bases has been established in Asia, Latin America and Africa. These initiatives have also contributed to the fact that in the last two or three decades a series of completely new medicinal plants occurred and gained acceptance into the European pharmacopoeias and medicinal plant usage. Large international research databases show that the intensity and quality level of the previously moderately accessible Chinese, Japanese, Thai and Indian medicinal plant research is developing in an explosively rapid pace. In all probability these processes may show further intensification in the near future and will verify the applicability of further Asian, South American and African medicinal plants. These taxa, their drugs and the concentrates manufactured from them will occur in the European and American markets in an accelerated tempo, and by such these will further increase the pressure exerted on the national agricultural cultivation and processing efforts, as well as will stimulate the regulatory demands.

It is long known that grounds of pharmaceutical drug supply, the main quality requirements and the major pharmaceutical drug application tendencies show considerable similarity in the countries of the developed world, and despite all national peculiarities are converging in nature. This, however, is prevalent to medicinal plants and products manufactured from them only to a limited extent. In those European countries where monastic healing tradition inherited from the Middle Ages (e.g.: Germany, Switzerland, and France) has rooted itself deep in the common knowledge the pharmaceutical industry steadily maintained its interest and support towards medicinal plants, thus healing with medicinal plants (phytotherapy) is also remained alongside the medicine based on the use of "modern" synthetic substances and preparations. As a result of this and with regards to quality issues the product range based on medicinal plants has further developed and completely transformed. Hundreds of preparations based on medicinal plant extracts has emerged to the level of accepted and registered pharmaceutical drugs. With the elimination of marketing barriers and national borderlines these products have occurred in the other European countries as well.

All in all, however, compared to synthetic drugs a significant decline has taken place concerning the entire turnover of medicinal plants and herbal preparations, which yet showed immense cross-country differences. In Germany, which is the largest processor and consumer of medicinal plants, several hundred over-the-counter (OTC) preparations having recognition and acceptance at the level of pharmaceutical drugs and containing medicinal plant extracts are available in pharmacies. The role of this product group is also significant in France, Switzerland and Austria, while their turnover share in other countries is less important.

In the second half of the last century the tide has turned in all intents and purposes concerning the population's interest and preferences towards the preparations used in and for healing measures. This phenomenon originated from the technologically developed countries. In the world of high technology the interest of the population has started to increase towards anything which is natural or closer to nature. From the 1970s, signs of a similar tendency change have also started to occur in the fields of medicine and pharmaceutical drug therapy; and this may be partially responsible for that herbal products continue to gain grounds even at the present time. Strengthening of the influence of the Asian traditional medicinal systems in Europe is practically fashion-like, as it can also be said about the use and application of medicinal plants originating from other continents and being hitherto unknown in Europe. In the American continent and throughout Europe a greater part of the adult population (30–80 %, depending on the pathology intended to be treated) makes use of these trends and procedures, alike the preparations used within the frame of them. In the rank order of procedures and products used phytotherapy is amongst the most frequently used ones (Yeh et al. 2002; Egede et al. 2002; Meyer and Gillatt 2002).

National rules regarding medicinal plants and herbal medicinal preparations are also developed in a greatly differing way in certain countries of Europe, and even in

North America (e.g.: regulations and product authorisation practices of the US and Canada are harshly different). In the integrating Europe and among given continents the differences between the national product authorisation and marketing systems has become to act as serious obstacles towards free flow of products and quality control. This also contributes to a greater extent to the decline and export inaptitude of domestic products of countries concerned.

3 Medicinal Plant, Pharmaceutical Drug or Food?

In response to the increasing interest of the population several major industrial branches has quickly reacted: a part of the large and medium sized pharmaceutical factories searching for new possibilities, newly established medicinal plant processors, and the food industry. In possession of the already accumulated know-how and technology the former appeared on the market with more and more pharmaceutical drug-like processed and ready-packed preparations, while newly entering small and micro companies are occasionally presented themselves with original herb combinations or with the replications of already marketed and proven preparations. Smaller companies can comply only partially or cannot comply at all with the quality and accreditation requirements being obligatory for registered pharmaceutical drugs: *good agricultural practice* (GAP), *good manufacturing practice* (GMP) and *good laboratory practice* (GLP); therefore, these companies manufacture products of lower levels of processing or of lower levels of quality. In certain countries the authorities have introduced several new product categories differing in quality along with the slackening of licensing conditions of the marketing authorisation procedures.

The food industry has also targeted the "back to the nature" consumer attitude as well as aimed to satisfy the strengthening health conscious behaviour by elaborating and supporting of a series of novel concepts. Products that may be classified as *"health food"*, *"dietary supplement"*, more recently as *"pharmaco-nutrition"*, as *"functional food"* for athletes, or as *"fortified food"* have arisen in order to satisfy these demands (however, the legal background of these category descriptions are frequently ungrounded). Observing the increasing vitamin and mineral consumption the manufacturers soon recognised that the previously very limited food supplement product range (the basis of which is provided by nearly two dozen of vitamins and minerals) can be expanded by other endobiotic (e.g.: fatty acids, carnitine, chondroitine sulfate) or purified xenobiotic substances of herbal or animal origin having characteristic physiological effects (e.g.: β-carotene, lycopene, chitosan). From this point it took only one step to "discover" medicinal plants as possible ingredients of these products. In order to resolve the contradiction between the supplementation of the usual diet and the applications in expressly therapeutic purposes, first the American, later the European legislation introduced the possibility for the use of "other substances with nutritional or physiological effects" (European Parliament and of the Council 2002). With this measure the narrow limits implied by vitamins and minerals has at once been extended to ingredients and finished products of nearly infinite numbers, origins and characteristics. As a result of the liberalisation of the rules of legislation and the extension of the range food supplement ingredients the number of products has abruptly increased and several thousand new products has been introduced to the worldwide market.

The major part of herbal ingredients (*botanicals*) is borrowed by the food supplement manufacturing and application from the *materia medica* of the traditional or evidence based medicines. These plant taxa were usually investigated, proved and applied by medicinal research targeting therapeutic purposes, which research activities were performed under therapeutic circumstances and in patients with definitively diagnosed disorders. Restriction of the applicability of food supplements exclusively to the supplementation of the diet ("*The labelling, presentation and advertising must not attribute to food supplements the property of preventing, treating or curing a human disease, or refer to such properties*" (European Parliament and of the Council 2002)). Conceals a fundamental discrepancy, because the knowledge on medicinal plants and their preparations has not been gained through the nourishment of the healthy, but through the healing of the ill.

Although products may form many groups, but regarding the main directions of medicinal plant use and the corresponding legal regulatory manners only two can be differed: the pharmaceutical drug-like and the food-like (of course the legal regulations on cosmetics, fragrance and aroma substances have effect on medicinal plants, as well). In the countries of the European Union the legal harmonisation of both fields can look back upon a past longer than a decade, but it is still far from being actually implemented. However, a more serious problem is that these two major fields of application (drugs, foods) have long left un-harmonized within the perplexed and complex legal harmonization committee system of the Union, thus these have evolved and are still evolving to strikingly diverging directions. Harmonisation of drug-like products is carried out in accordance with the evidence based guiding principles established in Europe, with product authorisation procedures elaborated to the smallest detail, and with the regulation of both the marketing and the utilisation – albeit this path has its own hitches, of course, as it will be shown. For the legal regulation of food-like products (first of all, the food supplements) Europe has adopted to a considerable extent the model of the United States.

The fact the simple drugs and herbal teas were succeeded by the products (of higher processing levels) appearing in drug-like package forms should mean the improvement of quality, so to speak; presumed if processing steps are carried out under controlled and systematic circumstances. However, at present there exists neither a legal stipulation system warranting actual protection during the products' lifespan spreading from the raw materials to the finished products and to the consumers, nor an international infrastructure serving quality control. Efforts towards quality, and by this means towards the protection of the consumer shall be considered as an objective declared explicitly or implicitly in all national (or regional, e.g.: EU) regulations, but at the present this objective is realised neither at the appropriate levels, nor in the adequate manners.

4 International Outlook

In the followings the characteristics of the markets of medicinal plant products and of the regulatory practices of certain countries considered to be important in terms of turnover and regulation of products of medicinal plant origin are briefly described. The characteristics of the market and legislative situation of Germany being predominant in Europe and playing an important role in the scientific establishment of phytotherapy, of the United States of America creating the category of food supplements, of China dominating product manufacturing, and of Canada being exemplary in terms of herbal product regulation are reviewed.

4.1 China

In China, traditional medicinal healing with medicinal plants and herbal preparations has its own, thousand year-old roots. Besides the fact that most of the thousand year-old methods and preparations have remained until nowadays it is beyond all questions that China is the beacon of medicinal herb utilisation performed with the cooperation of the physician and the pharmacist. In the fields of traditional medicinal healing and medicinal herb utilisation it is the impact of China that can be perceived as most influential and powerful.

It is estimated that approximately 6,000 such botanical taxa are known in China, which are also used for healing purposes somewhere in its regions. From this number 1967 medicinal plant drugs were official in the 8th edition of the Pharmacopoeia of the People's Republic of China issued in 2005 (total number of pharmacopoeial articles is 3,214) (China Internet Information 2008). Drugs used for the purposes of traditional healing are corresponding to more than 60 % of all pharmacopoeial articles!

Collective manufacturing value of the several hundred Chinese medicinal plant processor companies was approximately USD 14 billion in 2005, and this value is constantly increasing (International Trade Centre UNCT AD/WTO 2006). The vast number of processors and manufacturers and the fact that cultivation takes place mainly in distant rural areas renders understandable the difficulties faced by the Chinese government and supervisory organs and authorities in the assurance of quality expected in Europe and in North America.

China has long integrated the concept and methods of traditional medicine to its own healthcare system. Regarding this from the Chinese viewpoint it can also be said that conventional Western *materia medica* has been integrated to the Chinese medicine. In China, conventional drugs can only be marketed in pharmacies.

However, pharmacists (and lay consumers too) can purchase medicinal plant drugs in local markets and also in local sales. The pharmacist may use these in particular at the doctor's prescription (or at the desire of the patients) to prepare herbal preparations (Zemin 2001). According to certain data, 95 % of all the Chinese hospitals have a ward of traditional medicine, and 30–50 % of the country's drug consumption is realised in the form of traditional preparations.

In China, the rules of pharmaceutical drug registration and official control perceived in the European sense of the terms have evolved in the last 50 years. For the duties and tasks of pharmaceutical drug registration and official control the State Drug and Food Administration (SFDA) established in 1998, and enlarged and reorganised later on in 2003, is responsible with 339 local and 2,321 regional departments and with a staff over 64,000. In China, herbal (and animal) drugs belonging to the system of Traditional Chinese Medicine (TCM) are considered to have equal status as Western pharmaceutical drugs and are applied according to the practice and terminology of the TCM.

The current traditional medicine of China by no means represents a closed and rigid system, but a healing concept instead, to which medicinal plants and preparations "newly discovered" by research (yet being in use from ancient times in one or in another Chinese province), today's manufacturing and recent therapy, alike the newly realised therapeutic possibilities can be and are being integrated. In this sense it is a system in continuous transformation and development, in which new realisations and products created on the basis of scientific investigations and also complying with novel medicinal demands (e.g.: alcohol and opium addiction, treatment of new viral infections) can find their proper places (Szendrei 2006).

Food supplements and related products (e.g.: health food) have also occurred in China despite the very strong traditional medicinal healing. Duties and tasks of administration, authorisation/acceptance pertaining to these products are falling under the powers of the State Administration of Industry and Commerce; however, supervision is carried out by the SFDA in cooperation with other supervisory bodies. These products may only be advertised upon the previous authorisation of the text of the advertisement concerned and for professionals only (Lehmann and Xu 2001).

According to the latest news, due to the growing number of claims raised in respect of quality and of consumer "accidents" a declining tendency has arisen in the United States' so called "herbal products" market involving medicinal plants. This resulted in intensified market supervision at first, then in the conclusion of an important American-Chinese agreement. Within the frame of this a Chinese-American medicinal plant and product quality controlling centre will be established in Shanghai. The aim of this rather important joint investment is to strictly control the quality of raw materials and finished products exported to the USA, thus reducing consumer risks (Cavaliere 2008). All the above described facts and phenomena properly reflects to the importance attached by the Chinese Government to the sector of medicinal plants.

4.2 United States of America

Concerning the use of medicinal plants as pharmaceutical drugs the United States of America can be regarded as the prototype country to firstly "write it off" and to later side track it to the field of nutrition. In the previous decades the Food and Drug Administration (FDA) classified all products basically into three product categories: pharmaceutical drugs, foods, and cosmetics. Pursuant to the original definitions only pharmaceutical drugs can be used to treat, relieve or heal diseases; while in turn products classified as food – including the food supplements – cannot be used for the same. In order to authorise marketing as pharmaceutical drugs the FDA without any kind of allowance provided for the manufacturers of medicinal plant extracts and products stipulated the same level of documentation which is applied to any other pharmaceutical drug of synthetic or natural origin. Other aspects, e.g.: previous marketing and use (tradition) in the estimation of the efficacy and safety of these products were all disregarded. This resulted in the phenomenon that in the last decades herbal preparations were practically not marketed among pharmaceutical drugs but only among the food product groups intended for special functions (e.g.: health food, fortified food, dietary supplements). With the recognition of the fact that in terms of application possibilities medicinal plants are more than food products, but accordingly to the FDA concept they cannot be taken equal to pharmaceutical drugs, their application and use in food supplements have started as an alternative possibility (as it were a lock-gate). For this purpose a separate regulation was elaborated within the Dietary Supplement, Health and Education Act of 1994; however, it accepted that food supplements may be suitable for the prevention of chronic disorders and for the alleviation of their symptoms (U.S. Food and Drug Administration 1994). The Dietary Supplement, Health and Education Act of 1994 and the Federal Food, Drug and Cosmetic Act of 2004 (U.S. Food and Drug Administration 2012) made an attempt to surmount the apparent discrepancies. Application of medicinal plants and "other substances with physiological effects" in food supplements was allowed by order and the use of "health claims" was officially introduced for the authorised recommendations of these products. Therefore such a situation which can be regarded as somewhat intermediate in nature has arisen in which and in principal the same medicinal plants may be used among the ingredients of pharmaceutical drugs and food supplements, and the allowable uses also show overlapping to more and more higher ratios, approaching those already authorised in the case of pharmaceutical drugs. This preposterous situation is properly characterised by the fact that in the US six out of the ten medicinal plant taxa (*Ginkgo*, *Sabal, Echinacea, Cimicifuga, Silybum, and Hypericum*) ranking highest in terms of commercial turnover can be found among the food supplements, from which taxa several are to be found in Europe among the registrated pharmaceutical drugs (Lindstrom et al. 2013).

The popularity of alternative and complementary healing preparations including herbal preparations has started to increase from as early as the 1980s. The expansion of the food supplement market – following a minor decline – is maintained up to the present: according to the annually reported turnover data the growth was of 33 % from 2000 to 2012, and the aggregated value reached USD 5.6 billion in 2012 (Lindstrom et al. 2013). Authorisation of medicinal plants and other substances as ingredients resulted in product combinations in a number that is now far beyond controllable; according to certain estimates more than 10,000 such products are available in the US market.

The American regulation indirectly contributed to the situation that medicinal plant based preparations are practically not used within the frame of the institutional clinical therapy, but within the complementary and alternative medicines, or for self-medication purposes frequently unknown to the attending physician, and even in such situations where participation of a physician would be necessary. By considering this the US federal government has established the National Centre for Complementary and Alternative Medicine (NCCAM) operating under the National Institute of Health (NIH) and having a significant background network composed of universities and research institutions. This Centre supports many topics within which each procedure and product group, including herbal preparations, are evaluated on a critical basis from the points of view of efficacy and reliability, and carry out professional information activities among the population regarding the proper use of these products (http://nccam.nih.gov/).

"Exclusion" of medicinal plants from the range of registered pharmaceutical drugs and concurrently the increasing demand of the population towards natural products manufactured from medicinal plants, as well as the claims raised in respect of the quality incurring as a consequence of the incomprehensible product marketing, and of the consumer damages that got into the public eye have lead to different corrective measures in the US in the last decade. Monographs of the series entitled "USP dietary supplement herbal monographs" elaborated by the Pharmacopeial Commission of the United States of America aimed to improve the reliability of the products by stipulating higher requirements are drawing the quality of the most important medicinal plants used in food supplements near to the quality of pharmaceutical drugs (Schiff et al. 2006). In recent years a strong campaign has been started to inform the population in a more professional manner about medicinal plants and particularly about alternative therapeutic trends and tendencies.

4.3 Canada

Here, the popularity of alternative and complementary preparations and procedures is also high: a recent study established that more than a two third part (70–75 %) of the adult population consumes such preparations, among which the proportion of those containing herbal ingredients is also significant (Health Canada 2010). In consideration of this a separate authority belonging under the Ministry of Health, called Health Products Directorate was founded in 1999 with the mission of establishing a unified regulation for the diverse range of products not registered as pharmaceutical drugs. Before this date these products could be marketed either as pharmaceutical drugs or as food products, but this system gave rise to too many problems in the licensing procedures and in law enforcement. As a result of a process of several years involving comprehensive national and international consultations the Natural Health Products Regulations having powers over all relevant product groups that contain ingredients of natural origin has taken effect on 01 January 2004 (Natural Health Products Regulations 2003). This ordinance defines in an itemised way all those substances of natural origin that may be used in products of natural origin, and excludes all those that are forbidden to be used (pharmaceutical drugs defined in national pharmacopoeias, substances and materials regulated by other ordinances, e.g.: tobacco, doping substances, narcotic and other psychoactive substances). It also regulates all activities related to these products, as well as the formal requirements of marketing (e.g.: obligations of labelling and information provision). Lasting until 2010, this regulation granted a 6-year extension for the requalification of those food supplements which at the time have already been marketed. During this requalification process nearly one half of the products did not comply with the requirements stipulated to remain in the market (Wojewnik 2010), and the imposed costs prompted many manufacturers to withdraw their products. The indisputably positive feature of the Canadian domestic regulation is that the supervision of all medicinal plant containing products belongs to the powers and scope of duties of one joint authority. In principle this resolution assures that in a diverse range of products spreading from pharmaceutical drugs to food products the regulatory and law enforcement activities may be carried out in accordance with unified policies.

4.4 Europe

Europe is the birthplace not just of the modern phytotherapy but of several other healing concepts (e.g.: anthroposophy, homeopathy) which aside of medicinal plants, herbal products and other therapeutic tools are applied with predilection by physicians and paramedical professionals practicing these. However, due to the differing traditions the approach towards products of herbal origin is remarkably different from country to country. In the 1990s, the situation in the field of medicinal plant marketing was analysed by several economic and scientific studies. Recurrent critical conclusions of these were aimed at the national regulations at the time being difficult to compare. The Regulatory Situation of Herbal medicines – A Worldwide Review published by the World Health Organization in 1998 showed a similar picture (World Health Organization 1998b).

From the beginning of the twentieth century, Germany has occupied a leading position in the establishment of the principles and practice of rational phytotherapy and also exerts major effect on the development of the European medicinal plant market. Discovery of several important medicinal plants (e.g.: *Ginkgo, Cimicifuga, Vitex, Aesculus, Hedera, Valeriana, Echinacea, Silybum*), elucidation of their active agents, and verification of their efficacy are linked with the names of German researchers. Registered pharmaceutical drugs containing medicinal plant extracts and having proven to be effective have occurred in the German market even as early as the 1950s, and Germany was in the vanguard of this process. Accordingly, attempts towards new regulations were also initiated there. The German medicines act published in 1976 stipulated that herbal teas and other products of medicinal plant origin should fundamentally comply with requirements identical to those which are applied to pharmaceutical drugs. This act entrusted to the Bundesgesundheitsamt (BGA) the task of requalification of the several thousand products at the time being marketed in the country. In response to the protestations of the manufacturers and physicians practicing phytotherapy the BGA E-Kommission has been established in 1978 having professional evaluation of several hundred medicinal plants occurring in the products concerned as its main objective (to be carried out through positive and negative monographs), thus promoting the requalification of these products. Based on the work of this Commission, 245 positive and 115 negative monographs were published by the BGA until 1994 (Blumenthal 2000). The establishment of the industrial alliance seated in Bonn and operating at the present, as well, called Kooperation Phytopharmaka served to accelerate this process. Following the German model the European Scientific Cooperative on Phytotherapy (ESCOP) has been founded on the basis of European collaboration in 1989. This initiation laid the main emphasis upon the pharmacological and clinical evaluation and assessment of medicinal plants. The ESCOP is still working and until today it has published 115 medicinal plant monographs (European Scientific Cooperative on Phytotherapy 2013). Further changes in the German market of products of medicinal plant origin have been brought by the medicines-related EU directives in the 2000s, but these have much milder effects than in those member states which have traditions of a lesser extent and a less powerful manufacturing sector.

5 Product Categories

5.1 Herbal Medicines

For a long period the integration of the regulatory system concerning medicinal plants has not incited by any of the member states of the European Union. To renounce their own and properly developed system was not in the interest of those member states which had considerable tradition, and the other member states having less significant medicinal plant industry felt no need for a unified legislative environment. From the 1990s, the advancing enlargement of the EU has increased the actuality of this issue's resolution, since the admission of the new Eastern European member states implied not just the opening of an immense market for the large Western European factories and their more developed products, but also the risk of new competitors, as well, because several new member states (Bulgaria, Poland, Hungary) had considerable grounds in medicinal plant cultivation and processing. The occasionally contradictory legal provisions also threatened to make difficult the free flow of products. This was properly reflected by the how the importance of the "policy on medicinal plants" within the European Medicines Agency (EMA) has changed. At first, in the EMA an informal working group dealt with medicinal plant related issues from 1992, and this was transformed to the so called Ad Hoc Working Group on Herbal Remedies in 1997. Its place was undertaken by the Working Party on Herbal Medicinal Products operating within the Committee for Proprietary Medicinal Products (CPMP). At present, within the EMA a separate body, the Committee on Herbal Medicinal Products (HMPC) is dealing with questions and problems related to medicinal plants. Experts are delegated to the HMPC from all 27 member states of the EU, and from Iceland and Norway, as well (http://www.emea.europa.eu; Silano et al. 2004)

The HMPC has been established in 2004, and the Directive 2004/24/EC has entered into force at the same time had its originally declared principal objective in the integration and harmonisation of the European regulatory practices and in saving the values of the diverse products based on medicinal plants (European Parliament and of the Council 2004). Nonetheless it should also be concluded that it rendered more difficult the alignment of (new) member states having less developed processing industry. Since then, this directive has been implemented into the legislation of all member states. The basic principle articulated in this directive that the countless categories of products used with healing purposes and existing throughout the EU should be integrated has later lead to many conflicts and unexpected (or unforeseen by many) consequences. The two categories established by this directive into which all products already being marketed should be re-qualified were (and still are) the traditional herbal medicines and the herbal medicines.

The most important consequence of this directive is the appearance of a new product category being unified in the whole EU: the category of ***traditional herbal medicinal products***. The traditional herbal medicines are products, which can be used without medical diagnosis and supervision, are not restricted by prescriptions; they can be applied orally, externally or by inhalation, and which have been used in medicine for at least 30 years (not necessarily as licensed pharmaceutical drug-like products!), from which at least 15 years could be verified within the European Economic Area (EEA). If efficacy and relative safety is unambiguous on the basis of the long-existing use and experience, then there is no need to perform preclinical and clinical studies for requalification. Therefore, these products are authorised for marketing within the frame of a "simplified licensing procedure". However, their manufacture should be carried out on the basis of strict requirements (GMP) identical with those applying to all other pharmaceutical drugs.

The varied traditional use of medicinal plants in Europe rendered necessary the establishment of such an integrated "register" that *integrates traditional use* within the EU's territory. This seemingly absurd initiative in fact serves the best interest of both the consumers and the manufacturers. Collection and scientific clarification of data warrants that exclusively those plants and only in those indications and recommended doses may be admitted to this integrated “register” from the use of which no hazard is imposed on the consumers. Pursuant to Directive 2001/83/EC this “register” is created in the form of community herbal monographs accepted by the Committee on Herbal Medicinal Products of the EMA (European Parliament and of the Council 2001). These monographs lay down the indications, posology, undesirable effects and contraindications for each herbal drugs based on the documented traditional use (documents of products marketed for at least 15/30 years).

Although the elaboration of monographs recording the traditional use can be regarded as a progressive step, it also carries many disadvantages in its very nature, since traditional use does not warrant efficacy, only renders it likely – and this is contradictory with the principle of all efforts made towards the realisation of a rational phytotherapy. A delimitation of these monographs is that they have surpassed 100 in number, but the majority of them can only be used for the licensing of mono-component products only, because the articulated posology and indication refers to a given medicinal plant’s defined dose (exceptions are the combination monographs on Thymi herba and Primulae radix, and on Valerianae radix and Lupuli flos). A further problem is the “freeze locking” of the traditional indications and dosages, since within the category there is no possibility to introduce a new dosage manner or to broaden the medical indication.

Licensing and authorisation of (non-traditional) ***herbal medicines*** is restricted and bound to strict requirements (GMP) identical with those applying to all other pharmaceutical drugs. This implies a nearly insurmountable obstacle for new plant taxa and/or products to appear (legally) on the market. No matter high efficacy a plant species may seem to possess the patenting and legal protection procedures are much more difficult and risky than in the case of single pharmaceutical drug molecules. The majority of the large drug manufacturers do not take this risk; therefore it is not of everyday occurrence if a pharmaceutical factory – manufacturing primarily synthetics – has a research department dedicated to medicinal plants. It is similarly unusual that a large manufacturer may provide support for clinical studies carried out with medicinal plants. Because of the patenting difficulties related to plants it is hard to prevent that the obtained results should not be used by another manufacturer in order to support its own product.

This whole situation is also reflected at the regulatory level: monographs on medicinal plants elaborated by the HMPC are recording not just the traditional use but the well-established use, as well. Well-established use may be possible in case of those herbal drugs for which the efficacy is proven in clinical trials. In this case the 15/30 year-long presence on the market should not be applied as requirement.

As a result of long and wearisome debates the monographs on medicinal plants are elaborated on the basis of the available scientific evidence and market data by the Monographs and Lists Working Party (MLWP) operating as a section of the HMPC. The foundation of a monograph is the so called assessment report being several ten pages long and summarising the knowledge on the given herbal drug; and the essence of this is the monograph itself summarising in a couple of pages the traditional and well-established uses. Lack of clinical data results in the rejection of the well-established use in case of the majority of herbal drugs. The monographs and assessment reports elaborated by the MLWP and accepted by the HMPC are thenceforward made available for public discussion during which the interested parties (industry, professional organisations) may express their opinion and propose modifications. Thus, the monographs reflect not only to the compromise made by the MLWP members but to the results of a wider, professional-industrial consultation, as well.

The purpose and advantage of these monographs may be that they render easier the licensing and authorisation procedure, however, they became legally binding and may serve as the basis of a Mutual Recognition Procedure only if they get included to the so called Community List. Only those herbal drugs are entered to this Community List which has been proven harmless during the appropriately long period of use (30 years) and can also comply with other safety requirements. At the present, in this Community List 12 herbal drugs can be found (since 2004!), but no decision has been brought even in the case of one of these, i.e. even for these drugs there is no guarantee for that the member states' authorities will apply identical evaluation during their decision making procedures. It may occur that a member state refuses the authorisation of a product fully complying with a given monograph. The monographs shall only be regarded as benchmarks from which both the manufacturers and the licensing authorities may depart. Under such circumstances and in the majority of cases there is no guarantee for that the same product in the different member states of the Union will be provided with the same evaluation and will be marketed with the same indication or recommendation.

It can be assumed that the classification of herbal medicinal products and traditional herbal medicinal products may be the result of a compromise made by the member states; which, however, is difficult to integrate with the preceding legislation and to harmonise with the interests of the many member states. Only sporadic attempts were made in order to save the special traditional (herbal) product categories of the individual member states: a rare example is to be found in Austria where products (frequently of herbal origin) prepared within the traditional folk medicine and on the basis of individual recipes in pharmacies were admitted to the Intangible Cultural Heritage List of the UNESCO, which ensures the survival of the traditional medicinal practice and of certain products (Austrian Commission for UNESCO 2010a, b).

5.2 Food Supplements

Also as a part of the harmonisation of the legislation of the European Union the Directive 2002/46/EC regulating the marketing of food supplements was published in 2002 (European Parliament and of the Council 2002). This directive would like to integrate and harmonise the provisions of law related to food supplements at the time already been present in vast numbers at the markets of the member states.

The European legislators adopted many elements of the partially conservative and palpably discriminative (e.g.: rendering more difficult the chances of certain European medicinal plants to reach the American market), yet to a certain extent exceedingly liberal (food supplements are surprisingly "varied" regarding their composition) US regulation, but unfortunately they did not follow its positive elements in respect of certain important points.

In Europe, the preceding chaotic relations were replaced by a liberally regulated market where the assurance of the free competition on the market and the free flow of products have been attributed with at least as high importance as professional viewpoints and consumer protection. A significant shift was that food supplementation meant up to this point an almost exclusive supplementation of vitamins and minerals, while from there on food supplements with varied composition and numerous herbal ingredients appeared on the market. According to this new regulation besides vitamins and minerals food supplements may contain "other substances with nutritional and physiological effects". "Other" substances occurring in these products are generally medicinal plant derived materials, and by now it is likely that there are more food supplements of (medicinal) plant origin than those "classic" multivitamin-mineral substance-type products. Manufacturers recognised the market gap that has risen to the surface, and as a result of this in many member states the supply created the previously non-existent demand for a new product group, the herbal food supplements. As of today, many medicinal plants are present on the market in the forms of pharmaceutical drugs and food supplements, in fact, the appearance of these products may be the same. However, quality guarantees stipulated for food supplements are much weaker due to the fact that there is no authorisation obligation for these products. During their manufacture the strict rules and requirements of pharmaceutical drug manufacturing may also be considered as irrelevant. In principle, all products which are marketed in one or another member state may occur on the EU's common market. There are national efforts to restrict this, but these are not uniform, and their efficacy is also questionable due to the limits and weaknesses of supervision, control and sanctioning. One example is that the Belgian, French and Italian authorities have decided to develop a common approach for the evaluation of botanicals in the 'BELFRIT' project. A first step in this initiative is the compilation of a list of plants whose use in food supplements could be possibly based on safety assessment. Nevertheless, it is not a legally binding instrument (Cousyn et al. 2013), and cannot be opposed to legal provisions, including those of the member states involved in the project. Regulatory efforts at national levels also exist, like in the case of Belgium where both the positive and negative lists of (medicinal) plants allowed or forbidden to be used in food supplements were elaborated (Belgian Federal Public Service – Health, Food Chain Safety and Environment 1997), while other member states are attempting to resolve the problem by the unilateral employment of either the negative or the positive lists.

It is the manufacturer who bears all responsibility for the legal appropriateness, safety and efficacy; and preceding the marketing of these products their composition and quality are not verified in official laboratories. The manufacturers realised that it is way easier to enter to the market with a food supplement, alike that there are wider possibilities to increase their turnover. The outcome of this is the phenomena that many pharmaceutical factories perform manufacturing and marketing activities related to food supplements, and that more and more manufacturers initiate the "requalification" of their pharmaceutical drug-like products to food supplements. The rapid success is grounded primarily by the broad range of supply, the advertisement activities being more intensive than in the case of pharmaceutical drugs, and the ever-expanding scale of recommendations. Diversified distribution has its own non-negligible role, and asides the pharmacies it covers several shop types and the Internet, as well. For this reason the character of the products, their appearance, a significant part of their ingredients and their recommendations are gradually nearing those of the OTC drugs, while the regulatory guarantees on the quality are (still) lacking.

Accordingly, the EU regulations have not created complete unity even on the market of food supplements; however, serious scruples are primarily not raised by this fact. Beyond the before mentioned reservations related to the lack of quality assurance the source of a very serious problem is the complete blurring of the categories of medicinal and nutritional plants. Total separation of these two categories would naturally be strained, since overlaps are numerous (e.g. garlic), but it is certainly unjustifiable that plants having expressed healing properties and being non-occurrent in the usual human diet (e.g.: St. John' wort, maidenhair tree) should occur in food supplements. Although in pursuance to the provisions of law it is forbidden to attribute healing property to food supplements, unfortunately this rule is stepped over by distributors from time to time. This is partially understandable, since the same quantity (dose) of the same plant will not lose its effect just because it is marketed as a food supplement and not as a pharmaceutical drug. At the same time, it is also true that the extracts used in food supplements do not always have the quality of pharmaceutical drugs (this is not even a requirement in a strict sense), their dosage do not strictly comply with the therapeutic dosage, thus it is conceivable that substances being responsible for the effects may missing or their concentrations may be (too) low. Therefore, although there is evidence for the efficacy of the ingredients concerned; the occurrence of the expected effect of the marketed and frequently complex products is neither guaranteed, nor verified with human clinical studies of probative value.

The above described contradictory situation resulting in numerous misuse and malfeasant acts was attempted to be terminated by creating the list of the so called health claims. These health claims are not medical indications, nonetheless, refer to the character of the physiological/pharmacological effect – and when regarded through the eyes of a lay consumer or a patient, or even of a healthcare professional they do not differ significantly from the medical indications that we are accustomed to. The acceptance of claims being similar to medical indications and their ever-expanding introduction in the eyes of the physicians and of the consumers render food supplements more and more similar to pharmaceutical drugs; and this controversial regulatory action may serve practically the inverse of the originally declared objective.

The list summarising these health claims and making them universal for the whole EU is created by the collaboration of the EU's member states. At present, the refinement and modification of the claims proposed by the manufacturers and professional organisations, as well as the publication of the final list is in progress. No decision has yet been brought upon the health claims of medicinal plants.

5.3 Medical Devices

The category of medical devices was regulated for the first time by Directive 93/42/EEC (European Parliament and of the Council 1993). In recent years, the manufacturers realised the possibilities being implicit in this directive laying down rather broad limits, and as a result of this situation and aside to the conventional medical devices, e.g. breast implants and hip prostheses, more and more products of medicinal plant origin have appeared within this category. This is permitted by the directive since according to the definition *"medical device means any instrument, apparatus, appliance, software, material or other article (...) which intended (...) to be used for human beings for the purpose of: (...) diagnosis, prevention, monitoring, treatment or alleviation of disease, (...) and which does not achieve its principal intended action in or on the human body by pharmacological, immunological or metabolic means, but which may be assisted in its function by such means"*. In these product definitions the first thing to catch the reader's attention is the wording that is hardly interpretable and supposedly striving to achieve the broadest and most inexact possibilities of interpretation ("*which may be assisted in its function by such means*"). The remarkably significant overlapping with the definitions of pharmaceutical drugs and food supplements is also conspicuous. Medicinal plant containing products are generally preparations to be used externally, but weight loss (anti-obesity) products and medicinal cigarettes can also be found amongst them. This variability of products is rendered possible by the fact that no official licensing but only a notification is stipulated for the marketing and distribution of the majority of these products, and accepted registration in one member state opens the whole EU market for the manufacturer. Despite this situation no uniform and integrated European register on medical devices exists at the present. Although the advantages and possibilities being implicit in this category may be considered as comprehensible and worthy of recognition from the viewpoint of manufacturers, yet it is difficult to reason professionally that medical plant preparations may be transferred from a higher ranking quality category to a category of lesser controlled status, while their medicinal use and application is still kept unchanged.

6 Summary

In the last two or three decades medicinal plant use and application has undergone considerable transformation primarily in the industrially developed parts of the world. The structure of the international market has also transformed: the centres of agricultural cultivation and processing has been relocated to the developing regions, and new distributional structures and channels have appeared. Usage and application have diversified, besides the herbal tea culture and pharmaceutical drug consumption being familiar for the Western world new trends of application having different cultural backgrounds and scientific foundations have become "chic", and medicinal plants have appeared in novel products.

The increased populational interest and the situation awareness of the industry stimulated, moreover influenced, the regulatory work of the legislators. The examples of the countries described show that opposed to the worldwide harmonised regulation on pharmaceutical drugs the national regulatory work exercised on medicinal plants and other natural products has retained its previous national differences. While the fashion-like process is spreading in a wave-like manner from one region and country to another, it is unfortunate that the positive elements found in the studied national regulations are rarely adopted by other countries and regions. Examples of regional-type regulations can only be found in Europe; however, these are established in protracted and slow harmonisation processes frequently characterised by the necessity of disadvantageous compromises. Contrary to that the product marketing background is gradually becoming more and more globalised this mosaic-like regulatory situation is leading to disadvantageous regulatory and law enforcement gaps. Opinions insisting on the necessity of internationally integrated and harmonised regulations are gaining strength with each day.

In these fields of healthcare and therapy we are witnessing positive and negative developments at the same time. The positive element of the new regulations is that the global medicinal plant consumption reaches extraordinary dimensions and is continuously increasing. Important medicinal plants of the different continents have become and are becoming common treasures being available everywhere in the world, and the most valuable ones are gradually integrated to the toolbox of the official (evidence based) medicine, as well. Another positive element is that reassessment of medicinal plants which were previously considered to be unreliable by modern medicine, thus consequently rarely applied is intensively pursued nowadays, and more importantly is granted significant funds in Europe, North America and in all the other continents too. The findings rose to the surface from this process provide the hope for and the promise of clarifying the actual values of herbal therapy and its integration into the toolbox of the conventional medicine.

From the antiquity to the present day human utilisation of plants has had many purposes spanning through history. The two main fields were human nourishment and health protection. This duplex role is enriched today with new dimensions, new possibilities and new problems, as well. The overlaps between the use and application of products are more and more expressed with illogical resolutions (possibility of non-admissible nutritional applications, uncertainties about the doses, quality related problems) and professional concerns. While making admissible compromises the level of guarantees relating to the pharmaceutical drug-like medicinal plant products have neared the other pharmaceutical drugs by regulations brought in the Western world, it can also be observed that this process is the inverse of the former situation in the case of other product groups containing medicinal plants, and by this deduction food supplements should be considered in the first place. The new regulations have neared only the approved range of applications of food supplements to the ones of pharmaceutical drugs, but have not done the same for the statutorily stipulated quality and safety guarantees related to these products. This, in company with the rather impotent law enforcement and market controlling guarantees can be considered as the source of unnecessary consumer risks and in a long-term run may lead to the devaluation of medicinal plants.

If the health and safety of the ill and healthy consumers are regarded as the central objective of the assessments and regulations, then the overall picture is still full of contradiction. A completely preposterous situation has evolved particularly in the territory of the European Union. The reasons of this may be sought partly in the bureaucratic and fragmented legislatory and administrational mechanisms of the Union. A suitable example for this is that professional supervision of herbal medicines and food supplements is the delegated task and duty of two completely independent authorities (the EMA and the European Food Safety Authority, EFSA). A uniform and integrated concept, the appropriate inter-authority communication centred around the ill consumers' uncompromised safety are also seem to be lacking. Positive national resolutions are also existing (e.g.: Canada), however, these were disregarded so far by the European legislation. Advantages of the European Union's regulations targeting integration and harmonisation can still be seen only to a modest extent and mainly in the field product flow liberation. The Union level provisions of law bear the signs of compromises so much so that a part of them is completely unsuitable to provide remedy to the actual problems. Other directives provide such broad limits for the market participants that these can serve consumer interests only inappropriately. The un-harmonized regulation combined with the over emphasized principles of producer responsibility and free flow of products resulted in the phenomenon that throughout Europe the vast majority of products are migrating towards product groups providing lesser guaranty for the consumers (under-regulated categories). At the same time the efforts made towards innovation are significantly decreased.

The market of the European Union is quite open to products flowing in from the outside of Europe. There are no efforts made towards quality control that could be compared with those exercised by the USA in relation with China being its largest exporting partner; and it can also be observed that in the European market the ratio of products complying with the requirements merely in a formal way is continually growing.

Acknowledgement This research was supported by the European Union and the State of Hungary, co-financed by the European Social Fund in the framework of TÁMOP 4.2.4. A/2-11-1-2012-0001 'National Excellence Program'. I owe special thanks to Dr. Botond Lajos Borcsa and Klára Horváth-Boros for their precious help in preparing this manuscript.

References

Austrian Commission for UNESCO (2010a) Intangible cultural heritage in Austria, pharmaceutical specialities. http://nationalagentur.unesco.at/cgi-bin/unesco/element.pl?eid=1&lang=en. Accessed 14 Sept 2013

Austrian Commission for UNESCO (2010b) Intangible cultural heritage in Austria, healing knowledge of Pinzgauer men and women. http://nationalagentur.unesco.at/cgi-bin/unesco/element.pl?eid=5&lang=en. Accessed 14 Sept 2013

Belgian Federal Public Service – Health, Food Chain Safety and Environment (1997) Arrete Royal du 29 AOUT 1997 relatif à la fabrication et au commerce de denrées alimentaires composées ou contenant des plantes. http://www.health.belgium.be/filestore/19077559_FR/20120320%20Consolidated%20version%20KB%2029%20Augustus%201997_2_FR.pdf. Accessed 14 Sept 2013

Blumenthal M (2000) Herbal medicine – expanded commission E monographs. American Botanical Council, Austin, pp 197–200

Brinckmann J (2007) Consumer backlash against Chinese ingredients and products continues. MNS medicinal plant and extracts report

Cavaliere C (2008) Opens new facility in China that will test raw materials for dietary supplements. Herb Gram 77:20–21

China Internet Information Center (2008) II. Drug safety supervision system and legal system. http://www.china.org.cn/government/whitepaper/2008–08/20/content_16282375.htm. Accessed 14 Sept 2013

Cousyn G, Dalfrà S, Scarpa B, Geelen J, Anton R, Serafini M, Delmulle L (2013) Project BELFRIT. Eur Food Feed Law Rev 8(3):187

Egede LE et al (2002) The prevalence and pattern of complementary and alternative medicine use in individuals with diabetes. Diabetes Care 25:324–329

European Medicines Agency (2013) http://www.emea.europa.eu. Accessed 14 Sept 2013

European Parliament and of the Council (1993) Council directive 93/42/EEC of 14 June 1993 concerning medical devices

European Parliament and of the Council (2001) Directive 2001/83/EC of the European Parliament and of the Council of 6 November 2001 on the community code relating to medicinal products for human use

European Parliament and of the Council (2002) Directive 2002/46/EC of the European Parliament and of the Council of 10 June 2002 on the approximation of the laws of the member states relating to food supplements

European Parliament and of the Council (2004) The simplified procedure was introduced by directive 2004/24/EC of the European Parliament and of the Council of 31 March 2004 amending, as regards traditional herbal medicinal products, directive 2001/83/EC on the community code relating to medicinal products for human use

European Scientific Cooperative on Phytotherapy (2013) ESCOP monographs, 2nd edn. http://escop.com/publications

Health Canada (2010) What are natural health products? http://www.hc-sc.gc.ca/dhp-mps/prodnatur/index-eng.php. Accessed 14 Sept 2013

International Trade Centre UNCT AD/WTO (2006) Market news service, medicinal plants and extracts, No 18. http://legacy.intracen.org/mas/pdfs/print/english/medicinal-plants.pdf. Accessed 14 Sept 2013
Lehmann Lee & Xu (2001) The drug administration law of the People's Republic of China. http://www.lehmanlaw.com/resource-centre/laws-and-regulations/pharma/the-drug-administration-law-of-the-peoples-republic-of-china-2001.html. Accessed 14 Sept 2013
Lindstrom A, Ooyen C, Lynch ME, Blumenthal M (2013) Herb supplement sales increase 5.5 % in 2012. Herb Gram 99:60–66
Lord GM et al (1999) Nephropathy caused by Chinese herbs in the UK. Lancet 354:481–483
Meyer YP, Gillatt DA (2002) Alternative medications for benign prostatic hyperplasia available on the Internet: a review of the evidence for their use. BJU Int 90:41–44
National Center for Complementary and Alternative Medicine (2013) http://nccam.nih.gov/. Accessed 14 Sept 2013
Natural Health Products Regulations (2003) Can Gaz Part II 137(13):1532–1607
Nowack R (2004) Nierenversagen durch aristolochiasaurehaltige Heilmittel. Z Phytother 25:67–74
Schiff PL Jr et al (2006) The development of USP botanical dietary supplements monographs, 1995–2005. J Nat Prod 69:464–472
Schoepfer AM et al (2007) Herbal does not mean innocuous: ten cases of severe hepatotoxicity associated with dietary supplements from Herbalife products. J Hepatol 47:521–526
Silano M, De Vincenzi M, De Vincenzi A, Silano V (2004) The new European legislation on traditional herbal medicines: main features and perspectives. Fitoterapia 75:107–116
Szendrei K (2006) The place of herbal medicines in addiction treatment. International Expert Group, London
U.S. Food and Drug Administration (1994) Dietary supplement health and education act of 1994. http://www.fda.gov/RegulatoryInformation/Legislation/FederalFoodDrugandCosmeticActFDCAct/SignificantAmendmentstotheFDCAct/ucm148003.htm. Accessed 14 Sept 2013
U.S. Food and Drug Administration (2012) Federal food drug and cosmetic act. http://www.fda.gov/regulatoryinformation/legislation/federalfooddrugandcosmeticactfdcact/. Accessed 14 Sept 2013
Wojewnik P (2010) The Canadian marketplace and regulations for dietary supplements. http://www.naturalproductsinsider.com/articles/2010/01/canadian-supplement-market-and-regs.aspx. Accessed 14 Sept 2013
World Health Organization (1996) Guidelines for the assessment of herbal medicines. WHO expert committee on specifications for pharmaceutical preparations. Technical report series No 863, Geneva
World Health Organization (1998a) Quality control methods for medicinal plant material, Geneva
World Health Organization (1998b) Regulatory situation of herbal medicines. A Worldwide Review, Geneva
World Health Organization (1999–2006) WHO monographs on selected medicinal plants, vol I–IV, Geneva
World Health Organization (2000) General guidelines for methodologies on research and evaluation traditional medicine, Geneva
World Health Organization (2003) WHO guidelines on good agricultural practices for medicinal plants, Geneva
World Health Organization Regional Office for the Western Pacific (1993) Research guidelines for evaluating the safety and efficacy of herbal medicines, Manila
Yeh GY et al (2002) Complementary and alternative medicine use among patients with diabetes mellitus: results of a national survey. Am J Pub Health 92:1648–1652
Zemin J (2001) Drug Administration Law of the People's Republic of China. http://former.sfda.gov.cn/cmsweb/webportal/W45649037/A48335975.html. Accessed 14 Sept 2013

Chapter 17
Medicinal and Aromatic Plants in Scientific Databases

Tomaz Bartol and Dea Baricevic

Abstract This chapter provides assessment of uses and retrieval of terms related to medicinal and aromatic plants in databases available on different platforms/interfaces: ABI/Inform Global (ProQuest; business, management, finance, trade, markets), Agricola (NAL, American National Agricultural Library), Agris-Agrovoc (FAO, Food and Agriculture Organization of the United Nations), CAB Abstracts/OVID (CABI, CAB International; agriculture), Compendex (Ei/Engineering Index, Elsevier; engineering, technical sciences), FSTA (IFIS, International Food Information System; food/drink sciences, technology, human nutrition), Medline/Ebsco-MeSH (NLM, American National Library of Medicine; health, medical sciences (biomedicine), veterinary medicine), Scopus/SciVerse (Elsevier; citation database), Sociological Abstracts (ProQuest; social sciences, e.g. human-environment interactions, rural sociology, ethnology), Web of Science/Web of Knowledge (Thomson Reuters; citation database). Database features and functionalities are reviewed. The applicable terms (used for indexing, controlled glossaries, descriptors, subjects headings, keywords) are located in respective thesauri, e.g.: drug crops, drug plants, essential oil crops, essential oil plants, herbal drugs, herbal medicine, medicinal plants, phytotherapy, "plants, medicinal". Several additional terms and non-descriptors are identified, e.g. aromatic plants, herbaceous agents, herbal remedies, ethnobotany, herbal products, herbal preparations, plant drugs, herbal therapies. Search utilities, principles and rules are tested, e.g. search syntax (query), operators (Boolean, proximity, context), field codes, truncation (wildcard), phrase search, stemming (lemmatization). Bibliometric (scientometric) analysis is conducted in selective databases in order to tentatively assess the numbers and growth of potentially relevant records.

Keywords Bibliometric analysis • Terms for indexing • MAPs on different platforms/interfaces • Information literacy • Information competencies • Search competencies

T. Bartol (✉) • D. Baricevic
Agronomy Department, Biotechnical Faculty, University of Ljubljana, Ljubljana, Slovenia
e-mail: tomaz.bartol@bf.uni-lj.si

Á. Máthé (ed.), *Medicinal and Aromatic Plants of the World*, Medicinal and Aromatic Plants of the World 1, DOI 10.1007/978-94-017-9810-5_17

1 Introduction

Information on medicinal and aromatic plants (MAPS) can be found in many different databases and scientific information systems which are usually dedicated to specific disciplines. Some large global databases cover the widest possible range of information across all sciences. These information systems are constructed according to specific principles. Basic functionalities are usually similar, such as the use of search syntax and search operators. Some other functions, such as controlled glossaries and specific search commands, however, differ significantly among the systems.

In this chapter we wish to provide some context for the field of MAPS and related concepts (herbal medicine, herbal drugs, ethnobotany, etc.) regarding the coverage of this research area across a variety of information resources and search platforms. End users wishing to employ these systems to full capacity need to gain some insight into the system features in order to conduct information retrieval in a more informed way. Retrieval functions in all databases are enhanced with special tools which offer a possibility for a more focused discovery of relevant content as opposed to the rather generalized and less precise retrieval with web search engines, e.g. Google Scholar.

We present some principal global databases which contain valuable information on this subject. International information systems, most notably in the fields of agriculture or biomedicine have been collecting this information over many decades. Some other highly relevant documents can also be found in other research disciplines providing MAPS-related information in other contexts, for example engineering and technologies, economics, or social sciences. Many other information systems and databases exist. Thus, in order to capture a more general picture we will also include two global citation databases Scopus and Web of Science which complement other more specialist databases.

We will analyze selected database features and database-specific search characteristics. All databases allow field-specific retrieval, and offer other functionalities. Most databases employ specialist glossaries (thesauri) which can be used to enhance retrieval results but the end-users are frequently unaware of these tools. We will identify terms and concepts which best reflect the subject matter in this field. In the end, we will conduct an analysis in all databases to offer some information on the growth trends and possible numbers of relevant records which can be retrieved with selected terms in this subject area. Similar principles will provide a basis for a more balanced comparison. This information may help end-users to improve search strategies and enhance the level of personal information literacy and search competencies.

2 Review of Literature

Studies of databases and information systems and related bibliometric (scientometric/informetric) examinations focus on different topics, for example citation analysis, co-word analysis, or analysis of database coverage with regard to different criteria, such as countries, authors or subject matter. MAPS have sometimes been employed as a central topic of research or, more frequently, part of a wider context, for example complementary and alternative medicine (CAM).

Many specialized information resources exist which cover the subject of medicinal plants, for example NAPRALERT (NAtural PRoducts Alert) or AMED (Allied and Alternative (complementary) Medicine). We will focus, however, on larger international information systems which cover an entire discipline (e.g. agriculture or medicine) or all scientific disciplines such as Scopus and Web of Science (henceforward as WOS). Major databases have been investigated regarding the coverage of this field, most notably Medline (PubMed). Saxton and Owen (2005) described in detail the meaning and uses of Medline's Medical Subject Headings (MeSH) in search strategies related to medicinal plants. García-García et al. (2008) investigated phytotherapy in relation to psychiatry in databases Embase and Medline. Coverage of herbal remedies in PubMed was assessed by Hall and Nazir (2005). CAB Abstracts (CAB) and Medline databases were compared by Aalai et al. (2009). Bartol (2012) evaluated WOS, in addition to CAB and Medline. Medline and several other databases were investigated by Boehm et al. (2010) in a more general context of CAM. The authors tested OVID search platform by combining several relevant search terms related to medicinal plants. WOS (Science Citation Index Expanded) was used in relation to selected aspects (dementia research) in herbal medicine (Kim et al. 2012) and CAM more in general (Fu et al. 2011). The field of spices was investigated in CAB database (Senthilkumaran and Amudhavalli 2007). Ethnobotany-related citation behavior was studied in Scopus (Ramos et al. 2012). The importance of correct search strategies in CAM-related retrieval was emphasized by Crumley (2006). Namely, search principles, and thus search results, differ substantially among different platforms or interfaces (Younger and Boddy 2009). Hence, end-users should adapt search strategies accordingly. However, the existing definitions of this field are subject to discussion, thus search strategies are sometimes not specific enough (Wieland et al. 2011). Also, there exists an increasing gap between clinical (evidence-based) concepts and more traditional approaches in this field (Evans 2008).

Selected species or genera have been thoroughly investigated with regard to information aspects in databases, for example Origanum and Lippia (Bartol and Baricevic 2002), Nigella sativa (Anwar 2005), Embelia ribes (Singh et al. 2008), Punica granatum (Al Qallaf 2009), and Artemisia (Ram 2011).

New developments in the field of information retrieval have recently put emphasis on ontological concepts (ontologies) instead of mere descriptors and structured glossaries (thesauri), thus moving towards more advanced uses of metadata and user-based search strategies. Such semantic approaches allow researchers to use concepts instead of mere keywords (Serra Da Cruz and De Macêdo Vieira 2010). New ontological approaches in this field were also employed by (Mustaffa et al. 2012), and used, for example, on Indian medicinal plants (Karanth and Mahesh 2012; Vadivu and Hopper 2012). Semantic relationships of CAM-related and more specific concepts (e.g. herbal medicine, medicinal plants, medicinal herbs) were investigated by (Iyer and Bungo 2011). New Web 2.0-based applications have now provided new possibilities for unification of herbal information, especially in a multicultural community where names and usages can differ in relation to different cultural backgrounds (Lertnattee et al. 2009).

Chinese, Indian (Ayurvedic), or other traditional (herbal) medicines have also been a frequent subject of such analyses. These topics, however, are marked by additional complexities and were thus not an immediate subject of our work.

3 Overview of Databases and Information Systems

This short review presents some selected major international databases and information systems containing selected records on medicinal plants in different fields of science. Most databases are available for subscription through different search platforms. Access to full-text documents is subject to license agreements.

CAB Abstracts (CABI) CAB International (Centre for Agricultural Bioscience International). Principal database for agriculture and related (biotechnology, food science/nutrition, fisheries, forestry, veterinary sciences, environmental and social sciences). Over seven million records (1973 onwards), 330,000 added in 2012. Strong medicinal plants coverage, e.g. botany, physiology, biochemistry, cultivation, pharmacology, plant constituents, herbal drugs.

Agricola (NAL) American National Agricultural Library (USDA-United States Department of Agriculture). Some 4.5 million agriculture and related records from 1970 onwards, incl. many historic digitized materials. Collecting information from all around the world, more recently specialized in a comprehensive coverage of U.S. agricultural information. Freely available on the WWW.http://agricola.nal.usda.gov/

Agris (FAO) Food and Agriculture Organization of the United Nations and some 150 participating national, regional and international institutions. More than three million agriculture and related records (since 1979), including unique documents (e.g. reports or government publications not accessible through other information systems). Indexed by Google Scholar. Freely available on the WWW.http://agris.fao.org/agris-search/index.do

FSTA (IFIS) International Food Information System. Specialized for food sciences, food/drink technology, human nutrition. One million records (since 1969), 52,000 added in 2012. Many records applicable to medicinal and aromatic plants, esp. context of spices (culinary herbs), essential oils, antioxidants etc.

Sociological Abstracts (CSA) CSA (formerly Cambridge Scientific Abstracts, now part of ProQuest). Social sciences and related (e.g. human-environment interactions, rural sociology, ethnology, incl. traditional or alternative medicine). One million records (since 1952), 55,000 added in 2012.

Compendex (Ei/Elsevier) Engineering Index, now part of Elsevier. Engineering and technical sciences. Fifteen million records (since 1884), 900,000 added in 2012. Several thousand medicinal-plants records (e.g. related to plant extracts, mass spectrometry, chromatography, extraction, analysis).

ABI/INFORM Global (ProQuest) Business, management, finance, legal (taxation), trade, markets (since 1923). Complete ABI/INFORM contains over 40 million records, 2,500,000 added in 2012. Thousands of medicinal-plants-related records. Different document types, e.g. trade journals, newspapers, magazines.

Medline (NLM) American National Library of Medicine. Health/medical sciences (biomedicine), incl. veterinary medicine. 19,000,000 references (since 1946), 950,000 added in 2012. Freely available version *PubMed* includes references from Medline and some additional materials. Strong herbal medicine coverage.

Web of Science (Thomson Reuters) Formerly by ISI (Institute of Scientific Information). Accessible through the Web of Knowledge service. General scientific bibliographic and citation database covers all research areas (e.g. life- and applied sciences and technologies, social sciences, arts and humanities (from 1900)). Some 40,000,000 records; 1,800,000 added in 2012.

Scopus (Elsevier) a.k.a SciVerse Scopus. General bibliographic and citation database covers all research areas. Some 28,000,000 records (from 1996). Millions of older pre-1996 materials being added, with 2,400,000 records added in 2012. Scopus includes data (and subject headings) from some other databases.

4 Database Features and Search Characteristics

4.1 Subject Headings and Thesauri

Most discipline-specific databases include the functionality of a thesaurus (tree-structured glossary of subject headings – descriptors). This tool facilitates retrieval of terms according to a specialist terminology. Thesauri aggregate search terms in hierarchical structures and include synonymous or related terms. They designate terms which will best relate to a particular concept. Information specialists assign the same term (subject heading or descriptor) to a database record irrespective of the natural-language terms used by authors of a document. Designated descriptors, however, differ amongst disciplines and are not completely standardized across databases. For example, the two agricultural databases CAB and Agricola use the term *medicinal plants*. In Agris, however, *drug plants* are used. Medline employs an inverted descriptor *'plants, medicinal'*.

Bellow, we present some applicable descriptors (DE), as used in each particular databases/thesaurus. Non-descriptors (UF – Used For) are synonymous terms which are listed but not used for subject indexing in that particular system. Related terms (RT) are related descriptors and can be used as complementary search terms. We provide only those related terms which are directly associated with the concepts of *medicinal plants* and *herbal medicine*.

Agris: Agrovoc The largest global multilingual thesaurus. Descriptor *drug plants* is used. Additionally, 155 different related descriptors are listed, mostly individual species and genera (from *Achillea millefolium* to *Xanthium*). Some related descriptors are also applicable (e.g. *phytotherapy*). Descriptor *essential oil crops* is not associated with *drug plants* in this thesaurus. *Ethnobotany* is also available, which, again, is not linked to *drug plants*.

DE:drug plants UF:medicinal plants, pharmaceutical plants, medicinal herbs (non-descriptors) RT:phytotherapy . . . (155 terms)
DE:phytotherapy UF:herbal therapy RT: drug plants (only one term)
DE:essential oil crops UF:aromatic crops (essential oils), flavouring crops, essential oil plants, perfume crops RT:(50 terms)

Agricola: Agricultural Thesaurus Also in Spanish. Descriptor *medicinal plants* is used. Only 11 different related terms apply. Species and genera are organized differently. Other relevant descriptors are available, e.g. *drug crops*, *ethnobotany*, *herbal medicines*, and *stimulant plants*. An independent descriptor *essential oil crops* exists but is not linked to *medicinal plants*. The practical distinction between *medicinal plants* and *drug crops* is not very clear.

DE:medicinal plants UF:drug plants, medicinal herbs, pharmaceutical crops (non-descriptors) RT:drug crops, ethnobotany, herbal medicines, stimulant plants . . . (11 terms)
DE:drug crops UF:medicinal crops. RT:medicinal plants, phytochemicals (6 terms)
DE:herbal medicines UF:herbal drugs, herbal supplements. RT:medicinal plants (5 terms)
DE:essential oil crops (UF:aromatic crops, aromatic plants). RT:(2 terms)

CAB Abstracts: CAB Thesaurus Also a multilingual functionality. Descriptor *medicinal plants* is used. As many as 395 different related terms (*Abrus precatorius* to *Ziziphus nummularia*). Related *essential oil plants* also available, linked to *medicinal plants*. *Herbal drugs* are not linked to *medicinal plants* but linked, however, to *aromatherapy* which does not come about in the other two agricultural thesauri. There is no *phytotherapy*. Several narrower terms are available, e.g. *antibacterial plants*, *antifungal plants, antiviral plants*.

DE:medicinal plants UF:drug plants, medicinal crops, medicinal herbs, officinal plants. RT:antibacterial plants, antifungal plants, essential oil plants . . . (395 terms)
DE:herbal drugs UF:herbal medicines. RT:aromatherapy (8 related terms)
DE:essential oil plants UF:essential oil crops, flavouring crops. RT:medicinal plants . . . (100 terms)

ABI/INFORM Complete Thesaurus contains *herbal medicine*. Many documents are derived from other fields and some records supplied with MeSH descriptors.

DE:herbal medicine UF:herbal remedies. RT:flowers & plants; folk medicine; naturopathy, pharmaceuticals

FSTA: FSTA Thesaurus Specialized food-and-nutrition thesaurus. Descriptor *medicinal plants* is available which, however, is not linked to other terms. Specialized descriptors refer to essential-oil-concepts, e.g. *citronella essential oils*, *hop essential oils*, *mint oils*. Several associated descriptors exist, e.g. *essences*, *spices* and *herbs*. Descriptor *herbs* refers to many crops which are in some other systems classified as medicinal: *artemisia*, *chamomile*, *mint* etc.

Medline: MeSH (Medical Subject Headings). Competent use can only be achieved by skilled users, information specialists. Descriptor "*medicinal plants*" is employed as inverted "*plants, medicinal*". *Phytotherapy* and *herbal medicine* also exist. *Herbal medicine:* "the study of medicines derived from botanical sources". *Phytotherapy:* "use of plants or herbs to treat diseases or to alleviate pain".

DE:"plants, medicinal" UF:healing plants, medicinal herbs, pharmaceutical plants. RT:ethnobotany, phytotherapy (8 different related terms)
DE:phytotherapy UF:herb therapy, herbal therapy. RT: ethnobotany (6 terms)
DE:herbal medicine UF:herbalism RT:phytotherapy, "plants, medicinal" (3 terms)

Web of Science Does not operate a specialized thesaurus. However, author (or publisher) keywords from the original documents are included. wos also employs KeyWords Plus which are constructed from additional relevant terms in the references of processed documents and are added by information specialists.

Scopus Includes keywords from original documents as well as descriptors from other systems (MeSH, Compendex, Emtree). Keywords are labeled with regard to the source-abstract. This method of indexing considerably increases retrieval in Scopus. The consistency of this indexing, however, is subject to variations.

Other Databases Compendex and Sociological Abstracts do not contain descriptors dedicated to *herbal medicine*. Sociological Abstracts, however, provide descriptors both on *traditional medicine* and *alternative medicine*. Compendex contains descriptor *plant extracts*, which is also available in other databases.

4.2 Search Principles

4.2.1 Search Syntax and Search Operators

Different types of search techniques can be employed in databases, such as basic and advanced (expert) search modes. Searches can be carried out by selecting search criteria from drop-down lists (combining different criteria in different lines) or constructing complex search syntax using search operators (Boolean) and field codes. Search syntax allows combining many different search criteria in a single query. This is especially helpful if an end user wishes to couple several synonymous terms and different qualifiers.

All databases employ operators OR, AND, NOT. The NOT operator must sometimes be entered as ‘AND NOT’, for example in Scopus. Operators can be entered in upper or lower case. OR usually connects synonymous/associated terms. AND holds precedence over OR so parentheses need to be applied in order to set priorities. Thus, the second and the third example bellow are equal in meaning.

```
(spices OR herbs) AND (“food industry” OR “food processing”)
(medicinal plants OR aromatic plants) AND (protection OR conservation) =
= ((medicinal OR aromatic) AND plants) AND (protection OR conservation)
```

Proximity (context) operators can also be used in some databases. Proximity (near, within) operators locate search terms within a certain distance of each other (if needed). Operator *Nx* in Medline/Ebsco, for example, locates terms appearing within x words of one another irrespective of the order:

```
medicinal N3 plants
antioxida* N4 plants
```

The above syntax will also retrieve sentences such as … *plants* with biotechnological and *medicinal* applications…, … *antioxidant* activities of some medicinal *plants*.

4.2.2 Database Fields

Searches focus on different fields, e.g. document titles, authors, publishers, etc. Some databases offer dozens of different fields. However, the way the field codes are used varies among databases and search platforms. We provide some examples for the construction of search queries in respective databases (and interfaces), based on the fields *title* and *year of publication* (Table 17.1). Field codes (abbreviations) are usually not case sensitive (except for Medline on EBSCO). In some databases (Scopus, WOS, CAB/OVID) it is possible to search in all topical fields together: document title, abstract and subject headings (descriptors). The search command *TS* = in WOS will return all records with any of the search terms in the fields *title, abstract* or *subject headings*. In Scopus, field-code command *TITLE-ABS-KEY* achieves the same purpose. In CAB/OVID no field code is necessary. The absence of field codes implies that the search will be conducted exactly in *titles*, *abstracts* and *subject headings (descriptors)*.

In other databases, however, search must be adjusted to either a specific field or complete bibliographic record. This depends on the search interface as the same databases can be accessed through different platforms. Precision is needed while constructing search syntax. Information systems are sensitive and may not return results as expected. For example, ProQuest search syntax *TI (arnica)* will only return *ti* and *arnica* occurring anywhere in a document, and not the occurrence of arnica in the title of a document. Search syntax exactly as *TI(arnica)* must be employed, without the white space between *TI* and *(arnica)*! The same applies to *YR(2012)*.

There are some other database-specific characteristics. Search command *ALL* in Proquest databases will retrieve search terms anywhere in the bibliographic record. If prefix *ALL* is omitted then the search will also be performed in the full-texts of documents which may only be available through license agreements.

4.2.3 Truncation (Wildcard), Phrase Search

End users wishing to retrieve relevant terms must take into account different versions, e.g. plural, singular or adjective forms. Truncated (asterisk) *morpholog** will retrieve *morphology* as well as *morphological*. However, *plant** retrieves too much noise (*plantaricin, plantation* …). Sometimes, it is preferable to use an OR syntax (*plant OR plants*). Databases also offer a possibility of using a wildcard within a word. This is helpful in the cases of alternate spelling, for example *analy?e* (*analyse, analyzer*). Wildcards replace either any number or exactly one character. This is again database specific. Searches can be restricted to a particular phrase

Table 17.1 Use of field codes in databases

Database	Title	Publication year
Compendex (Ei)	"medicinal plants" WN TI	2012 WN YR
CAB abstracts (OVID)	"medicinal plants".TI	2012.YR
Sociological abstracts (ProQuest)	TI("medicinal plants")	YR(2012)
Medline (EBSCO)	TI "medicinal plants"	DT 2012
ABI/INFORM complete (ProQuest)	TI("medicinal plants")	YR(2012)
Scopus	TITLE ("medicinal plants")	PUBYEAR = 2012
Web of science (WOS)	TI = "medicinal plants"	PY = 2012

Scopus: *TITLE-ABS-KEY("medicinal plants") AND PUBYEAR = 2012*
WOS: *TS = "medicinal plants" AND PY = 2012*
CAB: *"medicinal plants" AND 2012.YR*

(using quotation marks) in order to exclude less relevant documents. However, caution is needed. Search for *"medicinal plants"* fails to retrieve the phrase *"medicinal and aromatic plants"*. Combined *OR* syntax can be used instead:

Scopus: *TITLE-ABS-KEY("medicinal plant*" OR "aromatic plant*")*
WOS: *TS = ("medicinal plant*" OR "aromatic plant*")*

4.2.4 Stemming/Lemmatization

Some databases increasingly employ the utility of stemming or lemmatization whereby the use of singular (or plural) form of a word will retrieve both singular and plural. For example, *herb* retrieves both *herb* and *herbs*. Frequently, both American and British English spelling variants will be retrieved. Search term *center* will thus retrieve *centers* and also *centre*, *centres*. However, *herb* or *morphology* may not retrieve *herbal* or *morphological*. Also, stemming does not operate in the same way in all systems. Truncation can be used instead, for example *morphol**. Sometimes, stemming or truncation does not work with phases (within quotation marks). Stemming (autostemming) can be switched-off in some systems. In some other systems, however, this a default function and cannot be aborted.

5 Bibliometric Analysis

In our main analysis we examined databases CAB (agriculture), Medline (biomedicine), ABI/Inform (economics), and both citation databases Scopus and WOS (Fig. 17.1). Separately, we also assessed FSTA (food sciences), Compendex (engineering) and Sociological Abstracts (social sciences) (Fig. 17.2). Based on descriptors and non-descriptors referring to *medicinal plants* and *herbal medicine* in all databases under study, we first identified the most frequently occurring synonymous concepts in Scopus which includes descriptors from several different thesauri. We selected all terms that returned at least 500 records (in the complete database), such as *essential oil plants* (650) and *herbal therapy* (700). The most frequently occurring term was *medicinal plants* (73,000) followed by *"plants, medicinal"* (56,000) and *herbaceous agent* (31,000). Bellow, we outline search query based on Boolean *OR*, which was then used in other databases:

> *Search query:* ("medicinal plant*" OR "plants, medicinal" OR "herbaceous agent*" OR phytotherapy OR "herbal medicine*" OR "medicinal herb*" OR ethnobotany OR "herbal drug*" OR "herbal remed*" OR "herbal product*" OR "herbal preparation*" OR "aromatic plant*" OR "plant drug*" OR "drug plant*" OR "herbal therap*" OR "essential oil plant*")

We ran the above search query on the principles of truncated terms (*) in order to retrieve both singular and plural forms. We searched in all topical fields: document title, abstract and subject headings (descriptors). In some databases, these topical fields can be searched with a single field code, for example *TS = phytotherapy* in WOS. In some other databases, we had to combine the three topical fields with the Boolean OR, for example in ABI/Inform: *(TI(phytotherapy) OR AB(phytotherapy) OR SU(phytotherapy))*. In running this search in Compendex, however, we had to take into account the database rule whereby truncation cannot be used within a phrase. Instead of searching for the truncated *"medicinal plant*"* we had to employ the query *("medicinal plant" OR "medicinal plants")*. Many more terms exist, for example *herbal medication*, *medical plants*, *aromatic herbs,* occurring rarely and have thus not been included in further analysis.

Both highly occurring descriptors *"plants, medicinal"* and *herbaceous agent* are derived from thesauri MeSH (Medline) and Emtree (Embase), respectively, so they are database-specific and will not retrieve relevant records in other information systems. In database FSTA we also included the term *herbs*. Namely, this term is a designated descriptor in this database. By interpreting the results in Figs. 17.1 and 17.2 it is necessary to take into consideration that only those records were retrieved which had been supplied with the terms in our search query.

Other more general terms, for example *herbs*, also return many relevant records, especially in ABI/Inform. This database also includes non-scientific publications. Such documents will very frequently use the term *herbs* for many different concepts related to medicinal and aromatic plants as well as spices. However, we have not employed this term in our analysis. It is too unspecific and returns many unrelated records (e.g. *perennial herbs*, *herb biomass*). Similarly, we have not systematically included spices, culinary herbs, and alike. These "multipurpose" terms frequently cover very general culinary aspects and are linked to such items as parsley, capers,

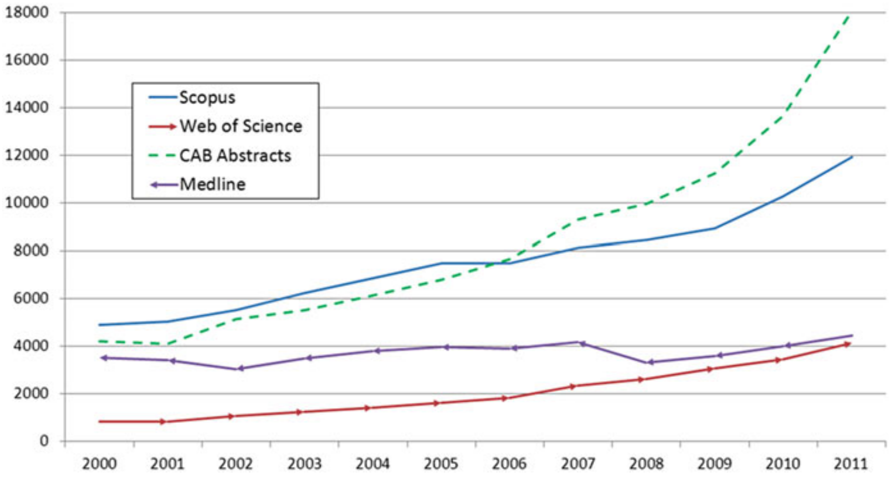


Fig. 17.1 Growth of records related to medicinal plants (herbal medicine) in databases scopus, web of science, CAB abstracts and medline during 2000–2011

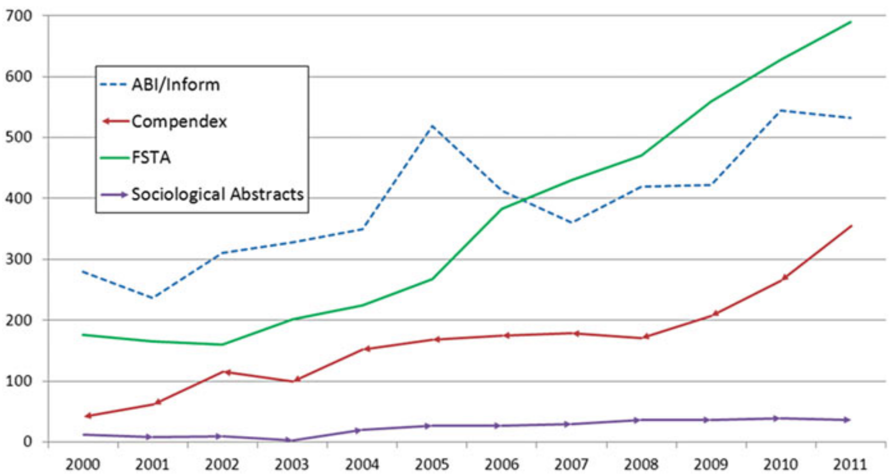


Fig. 17.2 Growth of records related to medicinal plants (herbal medicine) in databases ABI/Inform, compendex, fsta and sociological abstracts during 2000–2011

black pepper or paprika. The inclusion of such common uses would overshadow the more specific medicinal entities.

We have nevertheless compared the databases on similar basis, using the same combination of terms. We can observe constant growth, as expected, with some exceptions. In ABI/Inform there is a peak, in 2005, which is then repeated only in 2010. But this is a specific database which will yield many more records with the use of more common terms. Also, the number of records in Medline is somewhat steady. Scopus returns three times more records than WOS even though the difference in the overall database size is much smaller. This enhanced retrieval in Scopus can possibly be attributed to a more systematic inclusion of subject headings derived from different thesauri. This is an advantage of information retrieval in specialty-based databases as opposed to less subject-controlled information systems (also Web search engines). CAB returns the highest number of medicinal-plants-related records among all databases under study.

Again, these numbers can be attributed to a very systematic indexing of records in this database with the descriptor *medicinal plants* and related descriptors. But here it needs to be emphasized that many more relevant records exist, in all databases. Many records do not contain any broader terms related to this topic and will only be retrieved with more precise terms referring to specific species and genera.

6 Discussion and Conclusions

Information related to medicinal plants, represented by many different concepts, is scattered among many information resources presenting end users with an indomitable task of obtaining comprehensive information. International databases have been assembling such information on a more controlled basis for many decades which offers efficient tools for a more systematic retrieval. Search functionalities usually follow similar principles, however, there exist substantial differences which may hamper successful discovery of knowledge if these utilities are not used correctly.

An important feature of disciplinary databases are controlled glossaries (thesauri) which facilitate retrieval of terms which, however, are not standardized across databases. Many information systems are moving towards the implementation of ontologies which can offset many such problems, but end-users still need to be aware of the possible variants. For example, the term *medicinal plants* is synonymous with *drug plants* or *medicinal herbs*. It is closely related to *essential oil plants* and associated with *herbal medicine* or *herbal remedies*. Broadly, it relates to *natural healing* or *alternative medicine* which, however, can also refer to non-herbal-medicine-related topics, such as acupuncture or yoga. Some other related terms such as *herbs*, *spices*, *culinary plants* are very general and return too many less relevant documents, and should preferably be used in association with additional topical qualifiers, for example *markets, trade*, *plant extracts, antioxidants, pharmacognosy* etc.

End-users usually employ their own preferred natural-language terms and may forget that many other applicable terms exist which are used as designated descriptors. Also, many records do not contain any appropriate broader terms and can only be retrieved with more precise descriptors referring to specific species. Results of uninformed search strategies may thus retrieve too few or too many records. For example, free-text search with the phrase "*medicinal plants*" in Medline will not retrieve records containing the heading *"plants, medicinal"*. Thus, such phrases should be used with caution. Context or proximity operators can sometimes be used instead.

Automatic lemmatization or stemming (auto-stemming) may not retrieve adjective forms. It is sometimes preferable to use truncation. The truncated *morpholog** will thus retrieve *morphology* as well as *morphological*. However, such truncation may not work within phrases.

Using pre-arranged search syntax may be very helpful if end-users wish to repeat the command-search in different information systems and different fields. Search (Boolean) operators have the same value in all information systems, taking into account a precise application of parentheses (brackets). Field codes are platform-specific but should be used with care. End-users should not rely too strongly on 'smart' search techniques. It is always advisable to first check expected search results in each database. Help-pages in each particular information system should also be checked.

Our review has addressed some principal bibliographic databases in different disciplines. Many other relevant information resources on herbal medicine exist and should also be consulted. Information literate end-users should thus strive to build an advanced level of personal search competencies if they wish to use these resources to full potential.

References

Aalai E, Gleghorn C, Webb A, Glover SW (2009) Accessing public health information: a preliminary comparison of CABI's GLOBAL HEALTH database and MEDLINE. Health Info Libr J 26:56–62

Al Qallaf CL (2009) A bibliometric analysis of the *Punica grantum* L. literature. Malays J Libr Inf Sci 14:83–103

Anwar MA (2005) Nigella sativa: a bibliometric study of the literature on Habbat al-Barakah. Malays J Libr Inf Sci 10:1–18

Bartol T (2012) Assessment of indexing trends with specific and general terms for herbal medicine. Health Info Libr J 29:285–295

Bartol T, Baricevic D (2002) Bibliometric analysis of agricultural and biomedical bibliographic databases with regard to medicinal plants genera Origanum and Lippia in the period 1981–1998. Oregano: the genera Origanum and Lippia. Taylor & Francis, London\New York, pp 245–267

Boehm K, Raak C, Vollmar HC, Ostermann T (2010) An overview of 45 published database resources for complementary and alternative medicine. Health Info Libr J 27:93–105

Crumley ET (2006) Exploring the roles of librarians and health care professionals involved with complementary and alternative medicine. J Med Libr Assoc 94:81–89

Evans S (2008) Changing the knowledge base in western herbal medicine. Soc Sci Med 67:2098–2106

Fu J-Y, Zhang X, Zhao Y-H et al (2011) Bibliometric analysis of complementary and alternative medicine research over three decades. Scientometrics 88:617–626

García-García P, López-Muñoz F, Rubio G et al (2008) Phytotherapy and psychiatry: bibliometric study of the scientific literature from the last 20 years. Phytomedicine 15:566–576

Hall M, Nazir N (2005) Quantification of the scientific research in the United States about popular herbal remedies referenced on PubMed. Altern Ther Health Med 11:34–37

Iyer H, Bungo L (2011) An examination of semantic relationships between professionally assigned metadata and user-generated tags for popular literature in complementary and alternative medicine. Inf Res 16:paper 482

Karanth P, Mahesh K (2012) Integrating knowledge base retrieval with web search using semantic roles. Lecture notes in engineering and computer science. In: Proceedings of the international multi conference of engineers and computer scientists, Hong Kong, March 2012. Springer, Heidelberg, pp 344–349

Kim B-Y, Kang JS, Han J-S, Jeon WK (2012) Mapping the dementia research area at the micro-level using co-terms analysis and positioning for traditional herbal medicine. Chin J Integr Med 20:706–711

Lertnattee V, Robkob K, Sornlertlamvanich V (2009) Collaborative platform for multicultural herbal information creation. In: Proceedings of the 2009 ACM SIGCHI international workshop on intercultural collaboration, IWIC'09, Palo Alto, Feb 2009. pp 13–21

Mustaffa S, Ishak RZ, Lukose D (2012) Ontology model for herbal medicine knowledge repository. CCIS 295:293–302

Ram S (2011) Research output on Artemisia (Artemisia annua): a bibliometric study. Ann Libr Inf Stud 58:237–248

Ramos MA, Melo JG, Albuquerque UP (2012) Citation behavior in popular scientific papers: what is behind obscure citations? The case of ethnobotany. Scientometrics 92:711–719

Saxton JD, Owen DJ (2005) Developing optimal search strategies for finding information on herbs and other medicinal plants in Medline. J Altern Complement Med 11:725–731

Senthilkumaran P, Amudhavalli A (2007) Mapping of spices research in Asian countries. Scientometrics 73:149–159

Serra Da Cruz SM, De Macêdo Vieira AC (2010) Managing semantic annotations on medicinal plants. In: 2010 international conference on information society, i-Society, London, June 2010. pp 554–559

Singh G, Ahmad M, Nazim M (2008) A bibliometric study of *Embelia ribes*. Libr Rev 57:289–297

Vadivu G, Hopper SW (2012) Ontology mapping of Indian medicinal plants with standardized medical terms. J Comput Sci 8:1576–1584

Wieland LS, Manheimer E, Berman BM (2011) Development and classification of an operational definition of complementary and alternative medicine for the Cochrane collaboration. Altern Ther Health Med 17:50–59

Younger P, Boddy K (2009) When is a search not a search? A comparison of searching the AMED complementary health database via EBSCOhost, OVID and DIALOG. Health Info Libr J 26:126–135

Chapter 18
International Trade of Medicinal and Aromatic Plants

Klaus Dürbeck and Teresa Hüttenhofer

Abstract During a time period of more than 500 years the history of international trade of medicinal and aromatic plants (MAPs) has witnessed numerous twists and turns according to human needs and societal preferences. The challenges facing the trade can be articulated as botanical identification, product and process documentation, and need for rural income generation in origins, as basis of international trade.

In many countries the processing of plant extracts (vegetable oils, essential oils, solvent extracts for food, cosmetics and pharmaceuticals) using plant raw material from wild collection, cultivation, horticulture and forestry has led to the bottleneck in commercialization of value added ingredients on global scale. The market development for MAPs, natural ingredients and final products are subject to continuous changes of legislation framework and market drivers.

The demand for wild collected plant species is increasing by the day. At the same time wild collection has become a risk not only to nature, but as well to humans, due to disregard of process and product standards (Good Practices), resource – and risk management tool, amongst others. Likewise, cultivation and domestication is lacking a broad, international basis regarding understanding and definitions which leads in total to a dramatic shortage of raw plant material for value addition and trade – a worldwide issue.

Process and product quality aspects got expanded by quality of documentation and (intercultural) communication in the context of international trade of MAPs. Not only the (cap)abilities on the seller's and buyer's side are crucial, but also the communication within the communities – knowledge management. For sustainable trade, rural income generation in the countries of origin and consumer safety cannot be ignored.

Depending on the viewpoint MAPs as a whole trade sector is a forerunner in terms of strategic important natural resources. Trade promotion programs in Europe actively promote the value chains and secure their direct access to MAPs sources and natural ingredients by strengthening the producer, the exporter side in the partner country.

K. Dürbeck (✉) • T. Hüttenhofer
Klaus Dürbeck Consulting, Rufstrasse 5, 83064 Raubling, Germany
e-mail: K.Duerbeck@duerbeck.de

Á. Máthé (ed.), *Medicinal and Aromatic Plants of the World*, Medicinal and Aromatic Plants of the World 1, DOI 10.1007/978-94-017-9810-5_18

International guidelines as well as national and regional legislation, which implement basically the Good Practices and can be certified by a private standard, represent the need for harmonization and cross-referencing in the sector.

To subsume, the trade of MAPs and natural ingredients has overcome the technological bottlenecks from the past. The limitations of today's trade in MAPs and natural ingredients are principally the quality aspects such as product documentation, consumer safety and intercultural communication. The sustainable supply of raw material constitutes the principle bottleneck for the global trade.

Keywords Rural income generation • Fair trade • Value chain • Natural ingredients • Sustainable procurement

1 Introduction

For longer than 500 years, the history of international trade of medicinal and aromatic plants (MAPs) has witnessed many twists and turns according to human needs and societal preferences. As prominent example may serve the need to explore traditional medicine as relief for tropical diseases, like malaria relating to the use of *Cinchona calisaya* from Peru, or the benefit of tropical spices from Indonesia specifically of pepper was instrumental for strategic health care during times of peace and war.

The challenge at that point is described by the amount of plant species to be included for traditional medicine and supply chains amounting to estimates of more than 70,000 plant species in traditional medicine and more than 2,000 major species in international trade for applications in food, cosmetics and pharmaceutical preparations. Estimates indicate that from all the plant species less than 700 species were subject to domestication and plant breeding. For the rest difficulties in botanical identification and proper labelling are dominant, not to talk about re-labelling and adulteration of goods in the process of international trade.

2 Origins and Resources

The market development for MAPs, natural ingredients and final products are subject to continuous changes of legislation framework and market drivers. The basic forces catering for changes origin out of considerations primary health care for access to plant derived traditional medicine. At the same time support to consumer safety consideration is required to take action in quality including botanical identification and documentation of species concerned.

In many countries the processing of plant extracts (vegetable oils, essential oils, solvent extracts for food, cosmetics and pharmaceuticals) using plant raw material from wild collection, cultivation in agriculture, horticulture and forestry has led to the bottleneck in commercialisation of value added ingredients on global scale.

3 Wild or Spontaneous Species

The increasing success of the implementation of far reaching World Health Organization (WHO) guidelines on traditional medicine is causing increasing demand for an increasing number of wild collected plant species. Wild collection, habitually, was organized by traditional healers and shamans who were capable of linking the properties to related species. Today the use of MAPs is not benefitted by the close link between the person collecting and applying the species in the context of traditional medicine. In the best case scenario traditional medicine companies have employed traditional healers and/or shamans to advise the company on the species and traditional medicine relationship in terms of identification of species, safety or efficacy. Wild collection has become a risk not only to nature but as well to humans. The situation becomes more difficult like in Europe when the market partners are still looking for inexpensive options for raw material procurement – putting price before quality.

Today trade, processing and manufacture of medicinal and aromatic plants, natural ingredients and traditional medicine benefit largely from the new system of Good Practices (GxPs), process and product standards, and risk management tools (Burger et al. 2008). The Good Agricultural and Collection Practices (WHO 2003) are complemented by risk management and mutually agreed rules (standards). The important consequence of good collection practices for sustainable wild collection is that the responsibilities are divided in responsibilities for the resource owner and the resource user. The resource owners of wild collection areas often fail to implement their respective activities on resource assessment and resource management before issuing contracts and permits to the resource users meaning individuals, communities or productive sector.

This fact has numerous consequences on collection areas, take-out rates, but as well on cost and price calculations resulting in wrong decisions of resource management and large impacts on rural income generation. Today positive and dynamic trade development takes place where the institutional framework is promoting value addition and rural income generation in the country of origin. At the same time the plant resources are absorbed through the utilization of wild collected resources in national and regional markets, but as well in expanding south-south trade. Here the trade relations with BRICS countries need to be mentioned as strong competitors for the traditional destination in the “northern” markets like Europe, North America or Japan.

In summary the tools for trade in sustainably procured plant raw material (GxPs, risk management, and the process and product standards) are available; what is sometimes missing are the incentives and the commitments for/by resource owners and users to implement afore-named. Emerging as the crucial bottleneck to implement sustainable wild collection the social and economic aspects are getting in the focus of stakeholders involved. Value chains for MAPs attractive to all actors concerned will prevail. If not, the value chains are migrating to more fragile states where the business proposal is more attractive for people concerned. Stating this is reality in many conflict and post conflict countries, but at the same time not acceptable when trade partner countries are demanding the implementation of human rights or UN organisations are promoting social standards. Predominant examples may be derived from the trade practices in liquorice and liquorice ingredients.

Here an important observation is frequent when it comes to migration of collection (and cultivation) into fragile states. Examples can be named as wild collection on the Balkans or Central Asia, otherwise the migration cultivation of aromatic plants and processing of natural ingredients like essential oils from lavender or rose to countries, like Ukraine or Afghanistan.

4 Cultivated Species

Today reasonable amount of literature is published on domestication and cultivation, horticulture and forestry of medicinal and aromatic plants. What is surprising is the variation in the definition of terms used. In short, there is no harmonised language (despite the GACP) used by service providers, including consultants and academia, and productive sector in different countries. The characteristics used by the authors are related to a process of botanical identification, plant breeding and crop management which requires a planning of minimum 10 years and the respective resources allocated. In the recent past domestication of plant species was not documented on a regular basis on global level and with different understanding and definitions resulting that the number of species has not increased to a sizeable amount.

Today we witness a dramatic shortage of raw material for value addition and trade. This holds true not only from the European perspective, but is visible even in countries of origin with important developments in traditional medicine and cosmetics, and importance as food.

5 Human Resources and the Value Chain

As regularly observed incentives are missing to continue with raw material production and procurements. Moreover, the knowledge base gets lost among the producer communities and along the supply chains giving rise to the need for training and awareness rising, less for the research and development needs.

Knowledge management is a significant aspect of benchmarking in supplier, seller and buyer audits. An integral part forms the process and product documentation given rise to considerations on tracking and traceability.

As a new aspect the definition of quality aspects in the global trade has developed. Process and product quality aspects are broadened by quality of documentation and (intercultural) communication. Intercultural communication is getting in the forefront of international, global trade of MAPs with specific focus on languages and attitudes of sellers and buyers. In today's more liberal trade environments occasionally the seller's side is more educated and knowledgeable than their counterparts on the buyer side. Here the new set of tools provided by United Nations and legislators open harmonized new ways to communicate along the value chains strongly guided by the human factors and service providers. From today's perspective many in-transparent trade models can be explained as "black box" scenarios, especially the low price supply chains leaving little room for value addition in the countries of origin. In short, still too many human resources are bound in little transparent cross border and transit trade scenarios, very attractive for the supply chain manager, but with little contribution to rural income generation in the countries of origin, and with consumer safety.

6 Options for Value Addition

In recent years the industrial utilization of medicinal and aromatic plants was supported by value addition through processing spearheaded by UNIDO since 1980s (Wijesekera 1991), and Good Practices and documentation (WHO and the Good Agricultural and Collection Practices (GACP)). More recently the GACP were supported through legislative driven and private standards (like FairWild Standard). Today the international trade of medicinal and aromatic plants is to be characterized by new discussion points like "Good Practices (GxPs)", risk management procedures and the issues stemming from the implementation of the Good Manufacturing Practices for applications as food, cosmetics or pharmaceuticals with special references to Hazard Analysis and Determination of Critical Control Points (HACCP). Since early 2000s different interest groups including NGOs and the productive sector were working in parallel to provide rules and references for the implementation of sustainable production of plant raw material for local, regional and international supply chains and later for value chains.

The options for value addition in the origins are twofold covering technical value addition through processing of natural ingredients (Wijesekera et al. 1997), and establishing product documentation for ever increasing needs for audits, verification and certifications to substantiate claims concerning traceability or efficacy.

7 The International Trade

7.1 Setting the Scene for Strategic Important Resources

Depending on the viewpoint medicinal and aromatic plants as a whole trade sector is a frontrunner in terms of strategic important natural resources. More so since in the Western world we have identified nutrition and health as the main limiting factors for economic development. On the other side ample activities could be noticed after 1980 to provide the framework conditions for use of MAPs and the ingredients thereof in traditional medicine, pharmaceuticals, cosmetics and food spearheaded by United Nations Organizations like WHO, UNIDO, FAO and respective joint committees like Codex Alimentarius and UN Economic Commissions. Manuals and standards provide guidance to member governments and the productive sector, furthermore, the United Nations contributed as well to the development and implementation of regional and national legislation and norms.

What has been witnessed in recent years is the change of paradigms along the individual chain of plant raw material and natural ingredients. Trade literature describing these changes originates basically from the programmes of trade development to name the most prominent ones, like International Trade Centre (ITC), UNCTAD Biotrade, Food and Agriculture Organization (FAO) or United Nation Industrial Development Organization (UNIDO) with funding from UNDP or Global Environment Fund (GEF). The mentioned UN initiatives were supported by bilateral Trade Promotion Programmes focusing as well on MAPs and natural ingredients, like GTZ/Protrade, Germany, since 1988, CBI, The Netherlands, since 2000 (CBI, 2000–2014), SIPPO, Switzerland, since 2001, or more recently the Import Promotion Desk from Germany, since 2012. JAICA, Japan, is developing similar export and market entry programmes with trade fair participation for Japan. Other countries exhibit fewer initiatives to actively promote the value chains and the direct access to sources of MAPs and natural ingredients.

All mentioned trade promotion activities use specialized trade fairs in Europe as communication platforms, with differing degrees of intensity for preparation for trade fairs, matchmaking before and during trade fairs, and trade fair follow-up activities. Common for all the mentioned market entry activities is the strengthening of the producer, the exporter side from partner countries. Since the late 1990s several countries adopted the Public-Private-Partnership mechanisms with a specific focus on engaging service providers, companies and industries in economic development activities in respective partner countries. Public-Private-Partnerships (PPP) did not visibly improve the overall access to scarce and strategic resources leading in Germany to the development of the Import Promotion Desk strongly supported by both sides, the associations of the productive sector (access to scarce resources) and the agencies (needing new, old tools for accessing markets).

7.2 International Guidelines and Regional and National Legislation

Support was needed to implement international agreed and harmonized Good Practices (GxPs) and the risk management of the companies focusing on procurement of natural ingredients which gave rise to a large number of Private Standards from NGOs and wholesalers for raw material procurement from sustainable wild collection and sustainable use of Biodiversity, like Biotrade Principles and Criteria, FairWild Standard as management and certifiable standard, UEBT Standard for self-audit and verification. Latter ones are implementing the Good Agriculture and Collection Practices (GACP) and the UNCTAD/Biotrade Principles and Criteria exhibiting the well understood need for harmonization and cross-referencing taken up by regional sector associations like EUROPAM. The private standards include a larger number of NGOs and universities active in environmental protection leading to an improved communication between the interest groups on protection and conservation on one side and the initiatives exhibiting the thinking of the productive sector on sustainable use of natural resources on the other side.

8 Cultural Geography

As described the global trade of medicinal and aromatic plants and natural ingredients was missing additional expertise and knowledge which led increasingly to the match of resource management and use with inputs from cultural geography. Advanced thinking in resource planning and management was in need of knowledge about the natural and geographical resources in the origins. The observed migration of sourcing areas (wild collection and cultivation) from established societies to fragile states left a wide field to identify, document and use human and natural resources for rural income generation.

From the market entry activities an additional issue emerged to be addressed under the term “intercultural communication”. Cultural geography is considered the key tool to cope with today’s communication challenges in a world of fast changing business environment, including the drivers behind the global trade of medicinal and aromatic plants.

9 Conclusion

Currently, the trade of MAPs and natural ingredients has overcome the technological bottlenecks from the past. All nature extraction technologies are available like distillation of essential oils (Wijesekera et al. 1997), cold press of vegetable oils and solvent extraction of active principles, like colours, thickeners and flavour besides active principles from medicinal plants.

The limitations of today's trade in natural ingredients are principally on product documentation and intercultural communication. The increasing shortage of raw material and ingredients is leading to a competition between national and regional markets (south-south trade) as well as the export of natural ingredients to establish markets in North America, Europe and Japan.

Over recent years China has entered the market as supplier of natural ingredients; however, quite a number of raw materials are coming from outside of China. Not only China but also the BRICS countries are facing nowadays similar challenges in access to raw materials. The mayor supplier countries in Latin America, Africa and Asia have established own processing capacities to offer value added ingredients to manufacturers of pharmaceuticals, cosmetics and food. The sustainable supply of raw material constitutes the principle bottlenecks for the global trade. Companies from the origins are less willing to offer plant derived raw material – MAPs – except value addition options are possible, likewise for rural income generation in the origins.

As consequence of the international guidelines national and regional authorities are covering all necessary aspects of traditional medicine as competent authorities (often far away from collection areas and knowledgeable human resources) to become institutionalized among new and sometimes less experienced countries and actors.

References

Burger J et al (2008) Ecocultural attributes: evaluating ecological degradation in terms of ecological goods and services versus subsistence and tribal values. In: Greenberg M (ed) Risk analysis, vol 28(5). Official Publication of the Society for Risk Analysis

Centre for Promotion of Imports from Developing Countries CBI (ed) (2000–2014) EU strategic marketing guide. Natural ingredients for pharmaceuticals. Volume I and successive editions. Compiled by ProFound – Advisers in Development and Klaus Dürbeck

Wijesekera ROB (ed) (1991) The medicinal plant industry. CRC Press, Boca Raton\Ann Arbor \Boston\London

Wijesekera ROB, Ratnatunga CM, Dürbeck K (1997) The distillation of essential oils. Manufacturing and plan construction handbook. Protrade, Department of Foodstuffs and Agricultural Products GTZ

WHO (2003) WHO guidelines on good agricultural and collection practices (GACP) for medicinal plants. WHO, Geneva

Chapter 19
Medicinal Plants Conservation Strategies for Primary-Healthcare and Livelihood at Local Level: An Examination of Initiatives in South Asia

A.K.M. Shahidullah and C. Emdad Haque

Abstract This chapter examines initiatives to conserve medicinal plants in Bangladesh and India. These initiatives formed part of development interventions seeking to improve livelihoods and primary healthcare services in poor rural communities. The intervention projects studied were carried out in two districts of Bangladesh and one in Odisha state, India. Case study and participatory field methods were adopted to gather data and information on the medicinal plants conservation components of those projects. The study found that the initiatives, which combine both healthcare and livelihood objectives, have been successful not only in conserving medicinal plant resources, but also promoting their status and use. As a result, threats to medicinal plant stocks are being redressed, better livelihoods are being created, and primary healthcare situations are being enhanced. This study also revealed that the most successful strategies for conserving medicinal plant species comprised of a mix of *ex-situ*, *in-situ,* and production system approaches.

Keywords Medicinal plants • Conservation strategies • Primary health care • Livelihood at local level • Initiatives in South Asia

1 Introduction

Any plant containing a useful concentration of medicinally-active substance is considered a medicinal plant. These plant resources have been under threat since the eighteenth century, and already many species have become extinct - leading to a corresponding loss of biodiversity. At the same time, the demand for medicinal plants is rapidly increasing, coinciding with a global resurgence of traditional

A.K.M. Shahidullah • C.E. Haque (✉)
Natural Resources Institute, University of Manitoba, 70 Dysart Road, Winnipeg, Manitoba R3T 2N2, Canada
e-mail: umshahia@myumanitoba.ca

Á. Máthé (ed.), *Medicinal and Aromatic Plants of the World*, Medicinal and Aromatic Plants of the World 1, DOI 10.1007/978-94-017-9810-5_19

medicine. The global market for herbal medicine is estimated to be increasing by 15–20 % annually (Subrat 2002; Rahman 1999; Ghani 2003).

There exists a general lack of diverse opportunities for income generation in rural South Asia. Most livelihoods are based on agriculture and the exploitation of common-property natural resources (FAO 2004). While medicinal plant plays a role in livelihood generation, overexploitation of these from natural habitats severely limits such opportunities (Shahidullah 2007). Striking an effective balance between development and conservation is thus vital to introducing new sources of income generation and maintaining their stability.

Efforts to conserve medicinal plant resources and promote their development must simultaneously balance ecological sustainability, social factors, and economics (Parrotta 2002). As Lange (1998) has noted, in today's world of globalization, medicinal plant projects must incorporate a sound understanding of the economic situation at both the local and international levels. Viewing about medicinal plants conservation strategies in South Asia Karki (2000) and Vedanand (2002) suggest that growers and promoters should work toward a market-responsive production system in order to promote cultivation, homestead plantation, and other *in-situ* and *ex-situ* efforts. However, criteria for such a market-responsive production system are not specified in their literature. Seeking to fill this knowledge and methodological gap, our study examined current efforts by medicinal plant growers and promoters to foster environmental conservation, livelihood generation, and primary healthcare at the local level in the South Asian context.

1.1 Medicinal Plants and Their Significance at Local Level in South Asia

Nearly 70–80 % of the world's population relies on medicinal plants and other forms of traditional medicine as a primary source of healthcare (Fransworth and Soejarto 1997; Shengji 2001; Bodeker 2002). Consequently, the importance and value of medicinal plants as a resource has been well-recognized in both developed and developing economies (Wheelright 1974). South Asia is home to nearly 8,000 plant species with known medicinal properties; so the region has a long and rich history of traditional medicine systems (Switzer et al. 2003).

The earliest written description of medicinal plants is found in the *Rig Veda* (4500–1600 BC) – the oldest book in existence - and the *Ayurvedic* (5000 BC), *Unani*, *Siddha,* and *Tibetan* systems remain important sources of healthcare for tens of millions of people (Switzer et al. 2003). Medicinal plants and traditional medicine systems also underpin many livelihood-generating enterprises in rural areas. *Arya Vaidya Sala* (AVS) and *Kabirajghar* in South Asia are excellent examples of combining traditional medicine and modern business practices. Many unemployed youth and rural poor are learning traditional herbology from their parents and peers and turning this knowledge into successful enterprises (Karki 2000). Such enterprises can thus not only strengthen the social fabric and preserve the traditional knowledge and culture, but also bolster local economies.

1.2 Conservation: Threats, Issues and Strategies

Threats to global medicinal plant stocks have been of increasing concern to conservationists as well as the primary users of these resources. Uncontrolled trade, over-exploitation of wild plants, destructive harvesting techniques, and the destruction of natural habitats are pushing many South Asian regions to the brink of ecological, economic, and social catastrophe (Subrat 2002). The vast majority of these plants are exported to industrialized countries, which often restrict the use of their own native species for medicine (Lange 1998).

Weak or nonexistent regulation is a major contributor to this crisis. Mulliken (2000) argues that lack of transparency coupled with weak law-enforcement encourage illegal cross-border trading within India, Bangladesh and Nepal. Furthermore, while medicinal herbs were traditionally collected only to satisfy immediate, local demand, the expansion of global trade has lead many individuals and families to harvest large quantities of plants for export. This combination of lax regulation and the lure of short-term profits has lead to the over-exploitation of wild medicinal plant stocks, with little consideration given to sustainability (Karki 2002).

Loss of natural habitats is considered as the greatest threat to medicinal plant resources (Hamilton 1997); for example, the destruction of mangrove swamps for aquaculture or forests for cultivation in Bangladesh and India resulted to the extinct or threatened status of many species (Miththapala 2006). Climate change also affects natural habitats and threatens species with low tolerance to climatic variability (Hachfeld and Schippmann 2000). Natural genetic changes (mutation, speciation, and evolution) as well as human genetic modifications (intended to increase concentrations of active medicinal compounds) are also having profound impacts (WWF and IUCN 1997).

In recent years, the shortcomings (i.e. side effects and non-holistic nature) of modern allopathic medicine - and a realization that technology alone cannot solve the world's healthcare problems - have lead to a global resurgence interest in traditional herbal medicines. This is especially true in South Asia, where the high cost of modern medicines make it difficult for government to meet increasing public healthcare demands. This demand, along with the region's rich history of traditional medicine, had brought issues of over-exploitation and conservation of medicinal plants to the forefront of public discourse (Switzer et al. 2003).

Shahai (2002) raised the issue of protection of indigenous intellectual property as well as access and benefit sharing, having found a large number of patents on genetic resources and knowledge obtained from South Asian countries. Kaushik (2002) asserts that the most relevant issues regarding the conservation and sustainable use of biodiversity and associated traditional knowledge are the prevention of bio-piracy and misappropriation of this very knowledge, and the fair and equitable sharing of the associated medical benefits.

Shahidullah and Haque (2010) argue that if modern sustainable business practices can be coupled with traditional medical knowledge, not only can this knowledge be preserved and local healthcare needs met, but a large number of jobs can be created as well. Agarwal and Narain (1990) suggest that development policies and programs should address the needs of traditional resource-users at the grass-roots level; for example, in tribal areas where primary healthcare is largely herbal-based, the focus should be on protection of species with local value.

In order to conserve these medicinal plants resources, a multitude of strategies are being practiced by development promoters and partners, and include both *in-situ* and *ex-situ* measures. Among these strategies are the enhancement of cultivation efforts, improved management of wild populations, public awareness campaigns, trade monitoring, and national and international legislation, law enforcement, and regulation. From a 'systems' viewpoint, Hamilton (2004) argues that the general medicinal plants conservation strategy can be seen as comprised of four sub-systems: (i) production systems and *in-situ* conservation; (ii) commercial systems; (iii) the *ex- situ* conservation, propagation, domestication, and breeding of crop varieties; and (iv) new product discovery. In practice, all these subsystems can be closely connected.

The *in-situ* conservation approach seeks to ensure that the plants continue to grow and evolve in their natural habitats. Meanwhile, probably not beyond the definitional boundary of *in-situ* approach, field cultivation of medicinal plants and growing in home gardens are also useful strategies to promote medicinal plants. These are complementary means of providing accessible cures for common ailments and additional sources of income (Schippmann 2001). Many experts favour the commercial-systems approach; for example, Shahidullah and Haque (2010) suggest nexus between cultivars, development NGOs, and pharmaceutical manufacturer in order to promote cultivation and thereby enhance conservation efficiency and rural livelihoods.

Conservationists can engage in various strategic ways such as helping to formulate and promote appropriate standards, supplying relevant information to involved parties (collectors, growers, traders, manufacturers, and consumers), and facilitating communication between these parties (Hamilton et al. 2003). Apart from *in-situ* and production system, *ex-situ* approaches to conservation, propagation, domestication, and breeding of medicinal plants are also gaining popularity. In such programs, the seeds or vegetative parts of the rare medicinal plants are collected from the wild and bred in a controlled environment in order to ensure adequate stocks. This approach often involves the preservation of samples in botanical gardens, seed banks, and gene banks (Shengji 2001).

2 Research Methods: Intervention Projects as Case Studies

This study applied *qualitative case study* and *participatory* methods for field investigation (Yin 2003; Chambers 1997). Three sites were studied: two in the districts of Gopalganj and Natore in Bangladesh, respectively, and one in the Cuttack district of Odisha state, India. All three cases were selected based on their similar programmatic focus i.e. medicinal plant-based development interventions. The three programs were entitled as: (i) the Sustainable Environment Management Program (SEMP) (ii) Livelihoods, Empowerment and Agro-Forestry (LEAF) and (iii) the National Program on Promoting Conservation of Medicinal Plants and Traditional Knowledge for Enhancing Health and Livelihood Security.

Medicinal plant conservation constituted only one facet of each program; specifically, this study examined the 'Medicinal Plants Conservation through Community Participation' component of SEMP (herein referred to as Case 1), the 'Medicinal Plants Production for Livelihood Improvement and Poverty Reduction' component of LEAF (Case 2), and the 'Promoting Conservation of Medicinal Plants and Traditional Knowledge for Enhancing Health and Livelihood Security' component of the UNDP Country Cooperation Framework (CCF-II; Case 3).

The investigation process in Cases 1 and 2 involved mainly participatory rural appraisal (PRA) tools which included: direct observation of medicinal plants conservation initiatives at village level; key informant interviews (KII) with semi-structured questionnaire, where the subjects were herbal healers and practitioners, NGO managers, and community leaders; focus group discussions (FGD) with *union*-level (the lowest tier of public administration) stakeholders; household surveys of 30 farmers and growers of medicinal plants; and a review of published and unpublished academic literature.

The investigation process in Case Study 3 involved the use of Rapid Rural Appraisal (RRA) techniques, including secondary literature reviews (mostly obtained from the project- implementing NGOs), direct observation of the conservation initiatives at the site level, view exchanges in multi-stakeholder workshops, and key informant interviews (KII). The field study duration was 5 months in Cases 1 and 2, and two weeks in Case 3. A brief overview of the studied sites and conservation programs is given below.

2.1 Case Study Sites and Implementing Partners

Case Study 1 examined SEMP's community-supported medicinal plants conservation initiative in Gopalganj District, Bangladesh. Satpar *union* at Gopalganj Sadar Upazila was selected as the field study site (Fig. 19.1). Satpar is a floodplain area characterized by a large number of medicinal plants species, which have traditionally played a significant role in the primary healthcare of the local people. A total of 135 *Kabiraj* (Ayurveda practitioners) and traditional plant-dependent

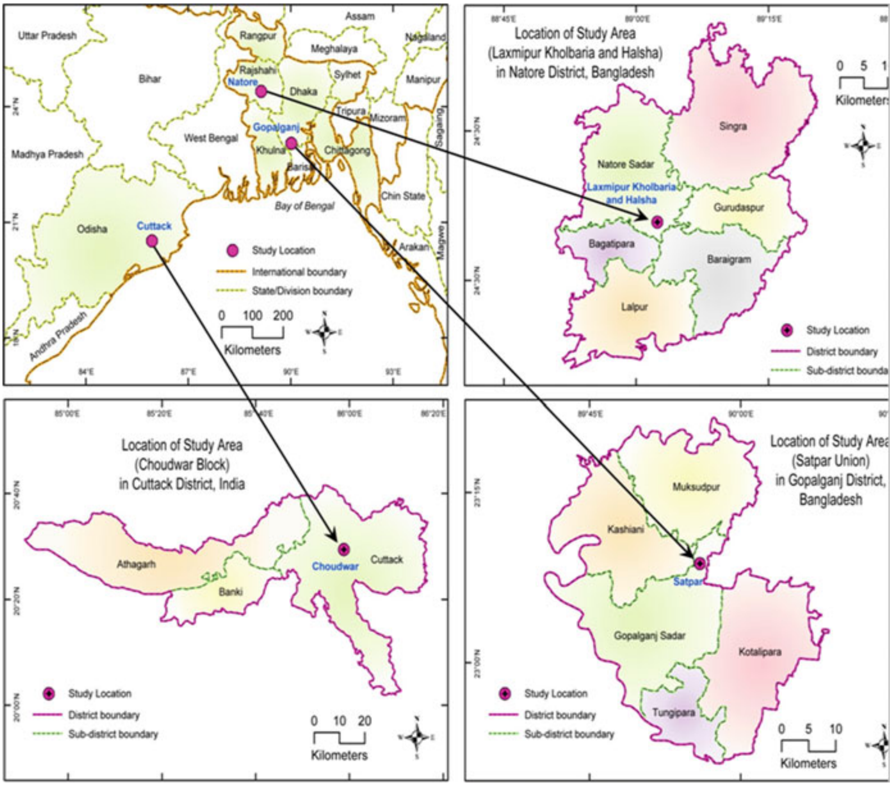


Fig. 19.1 Map of the case study sites in Gopalganj and Natore districts of Bangladesh, and Cuttack District of Odisha State, India

healers live in and around the locality. IUCN, as one of the implementing partners of SEMP, was responsible for promoting community-based floodplain resource management, and received field implementation assistance from the Bangladesh Center for Advanced Studies (BCAS) in the Madhumati floodplain areas of Gopalganj district. One of the primary objectives at this site was to enhance the capabilities of the *Kabirajs* in order to sustain local traditions of plant-based primary healthcare.

Case Study 2 examined conservation efforts via medicinal plant cultivation and production assistance at Natore District, Bangladesh. Intercooperation, a Swiss Development Agency, implemented LEAF's medicinal plants-based livelihood improvement and poverty- reduction program at Laxmipur-Kholabaria and Halsha *unions* of Natore Sadar district with implementing assistance from local NGO namely LUSTRE. In order to assess the production and local management of these medicinal plants and the livelihood dynamics of the producers, five villages were studied (3 in Laxmipur-Kholabaria *union* and 2 in Halsha *union)* (Fig. 19.1).

In **Case Study 3**, the intention was to obtain a rapid assessment of conservation efforts for medicinal plants under the CCF-II program in Cuttack District, Odisha, India. With the guidance of the Foundation for Revitalization of Local Health Traditions (FRLHT), the medicinal plants conservation component of the program was implemented in three Indian states namely: Andhra Pradesh, Odisha, and West Bengal. The field-level implementation of the Odisha component was carried out by SAMBAND, a local NGO based in Bhubaneshwar. We selected Tangi-Choudwar Block in Cuttack District, Odisha (Fig. 19.1) to assess the conservation initiatives of SAMBAND.

3 Results

In analyzing the results of this study, we sought to determine the specific threats facing medicinal plant stock, and the specific strategies adopted to implement associated conservation, livelihood-generating, and healthcare-oriented initiatives.

3.1 Threats to Medicinal Plants Resources at the Local Level

The nature of the threats facing medicinal plant resources and their management is very context-specific. For example, plants growing in floodplains are vulnerable to extensive flooding while plants in Odisha hills are vulnerable to climatic variability or anthropogenic damage. In all sites, 85 % of medicinal plants were found in forests, bamboo groves, marginal lands, hillsides, canal banks, homestead bushes, and along backyard boundaries (Thompsen and Alam 2006). However, these natural stocks are rapidly declining, the main reasons for which cited by the research participants being unrestricted commercial exploitation, deforestation, collection for fence-building and firewood, and damage by cattle. This depletion was compounded by lack of awareness among the local people of the plants' properties and value.

Local practitioners of traditional herbal medicine reported that in the past they could sustainably collect medicinal plants from local sources, but are now increasingly becoming dependent on external supplies due to depletion of local species through indiscriminate collection. Inadequate knowledge and the lure of short-term profits by collectors are the main causes of such unsustainable practices. Collection practices vary depending on the collectors, who can range from traditional collectors to seasonal collectors, occasional collectors, and collecting agents (employed by commercial herbal medicine manufacturers). Traditional collectors have better knowledge of sustainable collection as their livelihoods depend on maintaining adequate wild stocks of plants.

Most harmful collectors are the 'collecting agents' employed by wholesalers or processors. Such agents typically travel between medicinal plants species rich areas to assess availability and return with teams of labourers to extract plants based on commercial demand. They are usually highly knowledgeable about plant species and properties, but largely uninterested in sustainability, often bribing forestry department personnel in order to access protected plant stocks (Shahidullah and Haque 2009).

3.2 Conservation Strategies by the Intervention Projects

The intervention projects examined by this study sought to address both local healthcare and livelihood needs while conserving medicinal plant species and related traditional knowledge.

This task presented many challenges and required the implementation of a variety of site-specific strategies. Most of these strategies consisted of common Natural Resource Management practices, but in certain cases more extensive multi-level cooperative strategies were necessary. Broadly speaking, the studied conservation approaches fell into two categories: *in-situ* (e.g. local cultivation) and *ex-situ* (e.g. collection and breeding of species for off-site cultivation). However, according to Hamilton's (2004) 'systems' framework, production systems can be seen as merely an extension of *in-situ* conservation measures. A summary of the studied conservation strategies, their categorization, and results are presented below in tabular form (Tables 19.1, 19.2, and 19.3)

3.3 Support to Primary Healthcare

The medicinal properties and common uses of many plants have been extensively catalogued by several studies (e.g. Mati 2005; Ghani 2003). In order to complement this research with more site-specific data, we interviewed herbal practitioners at the SEMP Gopalganj site. Common local species and their use are summarized in the Table 19.4:

As reported by the herbal practitioners, most of the patients they receive are from poor and middle socioeconomic strata of the locality. Most successful treatments they are delivering with these plants are for the diseases pertaining to gastric disorder, sexual health, and worms. Affluent people hardly visit them only except for skin diseases and sexual health. The size of population suffering from jaundice, diabetes and asthma is increasing in the locality and they are also relying more on the herbalists. Fever and cold-related disease treatment by them are not as popular as before because of the availability of cheaper and rapidly curing allopathic medicines. However, they still receive considerable number of patients with stomach disorder.

In CCF-II, Odisha site, the users' guide booklet and the poster in local language depict photographs of prioritized medicinal plants for primary healthcare such as:

Table 19.1 *In-situ* approaches to medicinal plants conservation

In-situ conservation system			
Strategy	Adopted by		Outcome
Biodiversity conservation area (BCA)/medicinal plants Conservation area (MPCA)	SEMP	√	SEMP-IUCN established eight BCAs (averaging five acres each) where succession and regeneration of about 40 medicinal plants species have been recorded. These areas are well-marked with signboards, and people are aware of their existence and purposes through community meetings and discussions
	LEAF		
	CCF-II	√	Under CCF-II, Samband's has established six MPCAs each 200 acre size. These were developed through participatory ways. The areas belong to State Forest Department (SFD) of Odisha, but co-managed after Samband's intervention. Distinct boundaries, signs and site-specific management bodies are in place. Floristic survey by Samband recorded occurrence of 300 medicinal plants in those MPCAs
Floral sanctuary/medicinal plants conservation plot (MPCP)	SEMP	√	Floral sanctuaries are relatively small plots considered as medicinal plants hotspots for a group of species or a particular species. These are strictly demarcated protected areas may be situated inside BCA or MCA. IUCN-BCAS established 12 floral sanctuaries (average 1.2 acres) in *khas* lands in the study area. In CCF-II, Samband identifies floral sanctuaries as MPCPs. It has established around 18 MPCPs in Cuttack district of Odisha
	LEAF		
	CCF-II	√	
Swamp forest restoration	SEMP	√	Floodplain areas characterized by rivers, canals, and *beels* having adjacent raised or marshy lands. These lands were naturally covered with wetland plant species and used to be a good habitat for many medicinal plant species. IUCN-BCAS intervention restored many of such patches with physical intervention, such as: canal excavations, erosion prevention, and others. It is believed that species will regenerate and sustain in those habitat again. LEAF and CCF-II areas have a small percent of wetlands, therefore, no such measure chosen by the projects in those areas
	LEAF		
	CCF-II		

(continued)

Table 19.1 (continued)

In-situ conservation system			
Strategy	Adopted by		Outcome
Riparian area protection	SEMP	√	IUCN-BCAS protected fallow areas along river banks and adjoining areas with erosion proofing measures and helped regenerating the riparian vegetation. LEAF and CCF-II intervention areas did not adopt this as a measure
	LEAF		
	CCF-II		

Aloe vera, Andrographis paniculata, Asparagus racemosus, Butea monosperma, Carica papaya, Coleus amboinicus,, Eupatorium triplinerva, Hibiscus roasa-sinensis, Hollarhena pubescens, Justicia adhatoda, Kalanchoe pinnata, Mentha piperita, Moringa oleifera, Nyctanthes arbor-tristis, Punica granatum, Saraca asoca, Terminalia bellirica, Terminalia chebula, and *Tinospora cordifolia* and preparation methods of various herbal medicines from them (Table 19.5).

In order to ensure the abundance of above species, Samband established three nurseries at three different *blocks* in Cuttack district Odisha. Each nursery grows seedlings of 18 plant species that are supplied to the HHGs. Thus, the necessary plants for treating the above ailments become available in the HHGs and primary healthcare is ensured. At Sambandh's field Campus at Choudwar *block* in Cuttack, Odisha, a centralized nursery of these selected medicinal plants has been established (Fig. 19.2) to sustain the effort.

3.4 Enhancing Livelihood Security

In Bangladesh, the intervention of the LEAF program has proven successful in encouraging and enabling poor households to take initiative in cultivating and planting medicinal plant species to improve their livelihoods. In 2003, as part of its poverty reduction initiative, LEAF selected poor households within the Case Study 2 site and assessed their specific needs in order to develop customized livelihood-enhancing strategies. Most group members opted to grow medicinal plants, especially *Aloe vera,* whose cultivation was already popular among their neighbours. LEAF then provided training on production, processing, and marketing, as well as on micro-site analysis and homestead space planning in order to ensure improved and profitable production practices for medicinal plants. Finally, the project provided flexible financial credit options to help farmers establish their cultivation operations.

In 2006, in the studied five villages we found 160 households engaged in medicinal plants cultivation. Among them, 30 were interviewed in order to determine the extent to which this cultivation was contributing towards viable

Table 19.2 Production system approaches to medicinal plant conservation

Production system			
Strategy	Adopted by		Outcome
Cultivation	SEMP		Cultivation of commercially traded medicinal plants has gained immense popularity in the LEAF intervention area. In the five villages of the LEAF Natore study site, a total of 160 farmers are cultivating around 10 varieties of medicinal plants in their agricultural land. Within CCF-II, Samband also introduced medicinal plants cultivation in the tribal communities of Cuttack district, Odisha. A handful of species are being grown on contractual farming basis for supplying to the nearby herbal medicine manufacturing plant. With increased market awareness and knowledge, increased numbers of farmers are becoming engaged in cultivation in those areas
	LEAF	√	
	CCF-II	√	
Home herbal garden	SEMP	√	Establishment of Home Herbal Garden (HHG) at the household level for providing health security at the doorstep was found to be the most popular and common strategy in all the study sites. At SEMP study area, all the *Kabiraj* (herbal medicine practitioners) established a home herbal garden for professional purpose. At LEAF site it was found that many families dedicated a large part of the yard for growing several plants species both for healthcare and economic benefits. In CCF-II site, Samband reported that it has helped establishing around 3,000 HHGs in the district of Cuttack. Each HHG has invariable 15 species which are suitable to treat most frequently occurring diseases in those localities
	LEAF	√	
	CCF-II	√	
Plantation in homestead or restored fallow areas	SEMP	√	Apart from planned HHGs, people in those sites were also found planting high-value medicinal plants around their homesteads. LEAF project constituted 'Tree Farmers Group (TFG)' by training who now possesses specialized-knowledge to helped individual households to plant and grow high value medicinal plants in their homesteads. Similar group is also present in SEMP and CCF-II structure, SEMP has Village Resource Management Committee (VRMC) and CCF-II has Gaon Kalyan Samity (GKS) – meaning village welfare cooperative, where plantation of medicinal plants around homestead is on of the prime agenda
	LEAF	√	
	CCF-II	√	
Pond-side plantation	SEMP	√	Bio-physical characteristics of both the SEMP and LEAF areas are endowed with innumerable ponds. Many households are growing medicinal plants in those pond-side edges both for economic return as well as self-use. Such practice however, was not observed in CCF-II site
	LEAF	√	
	CCF-II		

Table 19.3 *Ex-situ* approaches to medicinal plant conservation

Ex-situ conservation system			
Strategy	Adopted by		Outcome
Nursery	SEMP	√	Nursery raising by individual household has been found predominant in all the three study sites. Nurseries help propagate the species in the locality and beyond and provide economic returns to the owners. LEAF helped establishing around 50 nurseries in the study area, while SEMP established nursery for more than 70 herbal practitioners at individual level. Under CCF-II, Samband established 12 nurseries in Cuttack, Odisha for the traditional healers (locally known as *Baidays*)
	LEAF	√	
	CCF-II	√	
Community nursery	SEMP	√	Community nursery is usually developed under group ownership at village level. SEMP has at least one community nursery in each village. Seedlings of at least 50 species are produced in these nurseries. Similar to SEMP, CCF-II in Cuttack Odisha also established community herbal garden (CHG) at village level. Samband established 6 such CHGs in the study area (while it has established 112 CHGs in Odisha state)
	LEAF		
	CCF-II	√	
Seed bank	SEMP	√	A seed bank is a tool for *ex-situ* conservation of plants and genes. A seed bank is only applicable with seeds of long viability. But many indigenous practices have the ability to store seeds of even very short viability. SEMP project sought knowledge on indigenous technologies from the community knowledge leaders to store seeds as part of the participatory action and thereby established seven seed banks. In CCF-II study site we found 2 seed-banks located in their knowledge information centres
	LEAF		
	CCF-II	√	
Plantation in common areas	SEMP	√	In both SEMP and CCF-II sites, the project implementers brought many common areas under medicinal plants plantation; these areas include: front and backyards of educational institutions, churches, temples, mosques, local marketplaces, block plantation in the wastelands, roadside strip plantation, and plantation at river and canal banks
	LEAF		
	CCF-II	√	

livelihoods. Among the interviewed respondents, 53 % agreed that their livelihood had significantly benefited from medicinal plant cultivation, 20 % replied it was moderately supporting their livelihoods, and 27 % said the mere cultivation of medicinal plants did not ensure their livelihood security.

A farmer (in Fig. 19.3) in LEAF area explained: *With aloe vera leaves yielding every 15 days from my field I am now leading a viable life. My college-going son assists me in activities like land preparing, planting, harvesting, and irrigation time to time. Ours is a four-member family. We have two fields, 18 and 12 decimal in size respectively. Earlier I used to cultivate paddy, wheat or mustard in these but with those harvests I was in hardship to maintain my family. Now I am not that bad because of medicinal plants production.*

SEMP and CCF-II also carried out programs for improving the livelihood security of traditional healers (*Kabiraj* and *Baidya*). SEMP contacted 135 herbal

Table 19.4 Local primary healthcare usage of medicinal herbs

Disorder/ ailments	Healing herbs (scientific name)	Usefulness[a]	User group[b]	Demand[c]
Fever (old & new)	Azadirachta indica, Andrographis paniculata, Aegle marmelos	√	L	→
Stomach (dysentery, diarrhoea)	Phyllanthus emblica, Terminalia chebula, Terminalia bellirica, Centella asiatica, Aegle marmelos	√√	L, M	↗↗
Gastric disorder	Phyllanthus emblica, Terminalia bellirica, aloe vera, Terminalia chebula, *Bryophyllum pinnatus*	√√√	L,M	↗↗
Sexual health	Polyalthia longifolia, Abroma Augusta, Bombax ceiba, Curchuligo orchiodes	√√√	A,L, M	↗↗
Cough and cold	Ocimum gratissimum, Justicia adatodha, Ficus religiosa, Zingiber officinale, Solanum surrattense	√√	L,M	→
Skin disease	Azadirachta indica, Vitex negundo, Achyranthes aspera, Swertia chirata, Hemidesmus indicus, Andrographis paniculata	√√	A,M	↗↗
Worm	Ananus comosus, Andrographis paniculata, Tinospora cordifolia Miers, Polyalthia longifolia, Leucas aspera,	√√√	L,M	↗↗
Jaundice	Pigeon pea, Aphanamixis polystachya, Leucas aspera, Ficus religiosa	√√	L,M	↗↗
Diabetes	Azadirachta indica, Trigonellafoen umgraceum	√√	L,M	↗↗
Asthma	Zingiber officinale, Glychiryza Licories, Justicia adatodha	√√	L,M	↗↗

[a]moderately useful- √, very useful- √√, extremely useful- √√√
[b]Poor people and lower class- L, Lower middle and middle class-M, Affluent people- A
[c]unchanged or slight increase reported- →, increasing considerably- ↗↗

healers in the *Gopalganj* district and organized meetings and workshops to learn about the essential plants needed for their practice. Based on the healers' needs, SEMP established medicinal plants nurseries and gardens in the locality in order to secure their profession. In addition, these healers received grant money to help them expand their gardens and medical practices.

Under CCF-II, Samband has succeeded in establishing a community-owned enterprise called *Healing Heritage Producers Company Ltd* (HHPCL) in Odisha, India. The HHPCL has been able to provide alternative sources of livelihood to over 1,500 producers by procuring raw material directly from local collectors and growers at market price, thus involving local people in value addition. A total of 17 medicinal products are currently being produced by the HHPCL for curing common ailments. The company markets its products using its large network of local healers (*Baidyas*) in all the districts of Odisha state.

Table 19.5 Common ailment and herbal treatment practices by the local people in Cuttack, Odisha, India (derived and reproduced from Samband's Document)

Ailment (local name)	Common name	Suggested formulation	Use-intensity by locals[a]
Taral Jhara	Diarrhoea	Leaf juice of Coleus amboinicus along with black pepper	√√
Jwara	Fever	Juice or decoction of Nyctanthes arbor-tristis, Tinospora cordifolia & Andrographis paniculata along with black pepper	√
Sweta pradar	Leucorrhoea	Root paste of *Hibiscus rosa-sinensis* along with raw milk or leaf pulp of *Aloe vera*	√√√
Nala Rakta Jhada	Dysentery	Leaf juice of *Kalanchoe pinnata* along with black pepper	√√√
Raktahinata	Anaemia	Fruit pulp of Carica papaya or leaf soup of Moringa oleifera	√√
Thanda kasha	Cold cough	Leaf juice of *Justicia adhatoda* along with black pepper	√√
Gastrics	Gastritis	Leaf juice of Mentha piperita or Wedellia chinensis along with black pepper	√√
Poda Gha	Burn wounds	Leaf pulp of Aloe vera application externally	√√
Munda bindha	Headache	Leaf pulp of Aloe vera application externally	√
Kachhu Kundia	Skin disease	Leaf paste of Andrographis paniculata	√√√
Katagha	Cut and wound	Leaf juice of Eupatorium triplinerve	√√√
Krumi	Worm infestation	Leaf Juice of Androghraphis paniculata	√√√

[a]Most local people use- √√√, approximately half of the local people use- √√, less than half of the local people use- √

4 Discussion

Examination of three projects in Bangladesh and India revealed that medicinal plants and traditional healing techniques are the source of primary healthcare for many people in rural areas. In Bangladesh, the importance of these resources is well-recognized, and since 2000 the conservation of medicinal plant species has become one of the highest-priority areas for intervention by development agencies. In India, similar projects such as CCF-II have also focused on medicinal plant conservation as a means of enhancing primary healthcare resources and livelihood security in rural states such as Odisha.

Income generation in rural South Asia is primarily limited to agricultural cultivation and exploitation of common property natural resources (FAO 2004). Over-extraction of medicinal plant resources from common property areas through indiscriminate collection practices poses a significant threat to the sustainability of

Fig. 19.2 Samband's medicinal plants nursery under CCF-II project at Cuttack, Odisha

Fig. 19.3 A farmer at his *Aloe vera* field at Kholabaria Village, Natore District, Bangladesh

many species. Unchecked deforestation, livestock ranching and other human activities further contribute to this threat. While natural forces such as floods and climate change-related events are harder to mitigate, anthropogenic pressures such as lax regulation and ignorance can be effectively addressed through programmatic intervention.

Singh (2002) stressed that the medicinal plant sub-sector of South Asia's economy must be reorganized and redirected towards a more people-centered, livelihood-focused, and market-oriented production-cum-conservation system. This view was reflected in the programs in Bangladesh and India, which saw overwhelming support and participation by local communities. All three projects studied were able to undertake different conservation strategies through participatory practices. A multitude of strategies were at play in all three sites within *in-situ*, *ex-situ*, and production system approaches. The livelihood and production aspects of the intervention successfully brought traditional herbal practitioners, farmers and other local stakeholders together to put their collective efforts in conserving and promoting the medicinal plants resources.

In-situ conservation strategies by SEMP and CCF-II projects through the establishment of Biodiversity Conservation Area (BCA) and Medicinal Pants Conservation Areas (MPCA) were the large-scale conservation measures. Such measures are common and offer best results in terms of resource protection and ecological sustainability if managed efficiently. To ensure optimum result out of such measures, the management mechanism often requires active involvement of multi-stakeholders, as in the developing country contexts, public management of natural resources have been proven less effective than the joint management (Nayak 2006).

Floral sanctuary and conservation plots by SEMP and CCF-II were found to be highly productive in enhancing regeneration, succession, and propagation of many species including the rare ones. However, such sanctuaries and plots require fencing, intense observation, maintenance, and other resources, and thus pose a considerable challenge to maintain; many projects have failed following the termination of active development (Ferraro and Subhrendu 2006; Oates 1999). As people's livelihoods are at stake, it is imperative that such challenges be addressed.

Measures for restoring ecosystems through physical intervention (e.g. erosion proofing and canal digging carried out by SEMP) can help prevent species loss. Though such specific, targeted efforts can prove costly, they can also be integrated into other environmental and natural resources initiatives. CCF-II and SEMP projects' efforts to convert open access and common areas into medicinal plant plantations is another unique *ex-situ* conservation strategy, but one suitable for trees only; shrubs, herb, and vines categories are too vulnerable to damage by cattle and human activities.

Planned or commercial production of medicinal plants was virtually non-existent in Bangladesh or in some cases it was developed only nominally. Forests or other natural ecosystems provide around 90 % of the overwhelming bulk of the medicinal plants (Ghani 2003) used as raw materials, mostly in traditional systems of medicines. Local collectors or collecting agents used to collect and supply these plants, but livelihoods support from the collection job has never been remarkable. It is only the local herbal medicine practitioners who had a conventional dependence on 'nature' around them for collection of plants to sustain their profession, but medicinal plant cultivation was never a significant feature of the local economy.

Since 2000 there has been a paradigm shift as nearly 200 farmers in the LEAF project-site at Natore District, Bangladesh have succeeded in deriving the majority of their livelihood from the cultivation of around 10 medicinal plant species - chief among which being *aloe vera* - rather than conventional grains and vegetables. Many poor and marginal farmers even converted their homesteads into medicinal plant gardens or seed-beds. While most farmers reported significant improvements to their livelihoods, those without large cultivation areas found they could not entirely depend on medicinal plants for livelihood stability. Thus land availability has a significant impact on the effectiveness of medicinal plant cultivation - as does the specific species cultivated, as fast-growing varieties are preferred for field cultivation Dold and Cocks (2002).

Notably, medicinal plant production prompted many households to restore fallow lands and make use of pond edges and riverbanks, thus creating synergy between habitat conservation and generation of income opportunities. Similarly, Home Herbal Gardens (HHGs) though an *ex-situ* conservation strategy and primarily designed for serving healthcare purposes of the household, but in SEMP area they served as a raw materials production base for the herbal practitioners while in LEAF area people sold the produce from these gardens. Thus privately-owned gardens are integrated into the larger production and supply system.

The nursery strategy gained substantial popularity in all three study areas. Most nurseries raise species other than medicinal plants for their commercial viability. At the community level, however, the nurseries raised collectively by groups (i.e. village resource management committees (VRMC) in SEMP and village welfare cooperative (GKS) in CCF-II play key roles in the conservation and promotion of the medicinal plants species, raising hundreds of species, distributing saplings to fellow community members, and providing knowledge and advice on plant cultivation.

Heywood (2000) emphasized the role of such nurseries in expanding conservation efforts at the community levels, an emphasis shared by both SEMP and CCF-II. Evidence from the studied projects in Bangladesh and India suggests that primary healthcare in rural areas is heavily dependent upon the medicinal plants. Most of the local people rely on herbal plants or treatment by herbal practitioners for most of their diseases. Herbal practitioners (*Kabiraj* and *Baidya*) are still the primary authorities consulted for their cures. Conservation of medicinal plants, as demonstrated by SEMP, LEAF, and CCF-II in South Asia, exemplified the efficacy of development agencies in enhancing the well-being of rural communities.

Of all the components of these conservation efforts, *capacity building* of herbal medicine practitioners (*Kabiraj and Baidya*) demands further and renewed attention. As a *Kabiraj* (in Gopalganj, Bangladesh) expressed, *the training that we were provided by IUCN-BCAS was helpful. I found it very useful in terms of gaining more knowledge on herbal treatment, plant properties, their processing, and their* *importance of conservation*. Most *Kabirajs and Baidyas* do not have formal training, having learned the art of herbal medicine from their parents or mentors (Khan and Rashid 2006). The valuation and sustainability of these traditional practices requires policy attention, particularly in South Asia.

5 Conclusions

Medicinal plants have been an important natural resource to the people of South Asia since time immemorial. The importance of preserving this resource has become even more apparent in recent decades as many species became scarce due to indiscriminate collection practices and unchecked deforestation. This scarcity threatens not only the health of local people, but also the livelihood security of the herbal practitioners. Since 2000, sensing this looming crisis, multiple international development agencies have launched cooperative ventures to conserve and promote the development of these resources in various regions of South Asia.

Few of these projects adopted the conventional conservation strategies of fencing and fining, instead option for a variety of *in-situ*, *ex-situ*, and production-system approaches which simultaneously addressed the goals of promoting medicinal plant preservation and enhancing local livelihoods. With unmet demand of herbal practitioners and growing demand from herbal manufacturers for medicinal plants raw materials, the efforts to protect these resources are unlikely to succeed. Therefore, the lateral entry of production sub-system in the conservation system bears an immense significance and needs to be fostered in order to sustain the medicinal plants resource-base, as it not only increases the supply but also reduces the pressure on wild-stock.

One of the most significant advantages of production system is support to the rural livelihoods. In the past, only traditional herbal practitioners and local collectors were primarily dependent on medicinal plants for their livelihoods. Studies in Bangladesh, however, have revealed that medicinal plant production can successfully constitute the sole source of income for rural farmers and marginal households. The most crucial element in achieving success was the tying of livelihood generation to conservation goals; when conservation efforts promise livelihoods opportunities to marginal rural farmers, they are more likely to succeed.

We conclude that the issue of medicinal plant conservation is not singularly grounded in healthcare demands; rather it requires consideration of multiple dimensions such as local livelihoods, global markets, and specific local conditions. Yet another dimension, probably the most important aspect in medicinal plants conservation, is the issue of preserving and sustaining 'traditional knowledge'. Herbalists complained, as the plant vanishes so does the knowledge on it eventually; therefore, in the face of consumptive market systems, protection of the plants are essential for protecting the knowledge in order to meet emerging healthcare needs, not only in South Asia but around the world.

References

Agarwal A, Narain S (1990) Towards green villages: a strategy for environmentally sound and participatory rural development. Centre for Science & Environment, Delhi

Bodeker G (2002) Medicinal plants: towards sustainability and security. IDRC Medicinal Plants Global Network Sponsored Discussion Paper for WOCMAP III, Chiang Mai

Chambers R (1997) Shortcut and participatory methods for gaining social information for projects. In: Sustainable development: social organization, institutional arrangements and rural development, vol 6. IICA, San Jose, pp 177–208

Dold AP, Cocks ML (2002) The trade in medicinal plants in the Eastern Cape Province, South Africa. S Afr J Sci 98(11 & 12):589

FAO (2004) Bangladesh country overview, yearly country report. FAO, Dhaka Office

Farnsworth NR, Soejarto DD (1997) Global importance of medicinal plants. Cambridge University Press, Cambridge, UK

Ferraro PJ, Subhrendu KP (2006) Money for nothing? A call for empirical evaluation of biodiversity conservation investments. PLoS Biol 4:4

Ghani A (2003) Medicinal plants of Bangladesh: chemical constituents and uses. Asiatic Society of Bangladesh, Dhaka

Hachfeld B, Schippmann U (2000) Conservation data sheet 2: exploitation, trade and population status of *Harpagophytum procumbens* in Southern Africa. Med Plant Conserv 6:4–9

Hamilton AC (1997) Threats to plants: an analysis of centres of plant diversity. In: Touchell DH, Dixon KW (eds) Conservation into the 21st century. Kings Park and Botanic Garden, Perth, pp 309–322

Hamilton AC (2004) Medicinal plants, conservation and livelihoods. Biodiv Conserv 13(8):1477–1517

Hamilton AC, Shengji P, Kessy J, Khan AA, Lagos-Witte S, Shinwari ZK (2003) The purposes and teaching of applied ethnobotany. WWF, Surrey

Heywood V (2000) Management and sustainability of the resource base for medicinal plants. In: Honnef S, Melisch R (eds) Medicinal utilization of wild species: challenge for man and nature in the new millennium. WWF/TRAFFIC Germany, Hanover

Karki MB (2000) Commercialization of natural resources for sustainable livelihoods: the case of forest products. In: ICIMOD (ed) Growth, poverty alleviation and sustainable management in the mountain areas of South Asia. ICIMOD, Kathmandu, pp 293–320

Karki MB (2002) MAPPA's role. Paper presented at a workshop on Wise practices and experiential learning in the conservation and management of Himalayan medicinal plants, Kathmandu, Nepal, 15–20 December 2002, supported by the Ministry of Forest and Soil Conservation, Nepal, the WWF-Nepal Program, Medicinal and Aromatic Plants Program in Asia (MAPPA), IDRC, Canada, and the WWF-UNESCO People and Plants Initiative

Kaushik N (2002) Bio-prospecting biodiversity in the search for alternative pesticides. In: Proceedings of second international conference on alternative control methods against plant pest organisms. Lille, 4–7 March 2002

Khan NA, Rashid AZMM (2006) A study on the indigenous medicinal plants and healing practices in Chittagong Hill Tracts (Bangladesh). Afr J Trad Complement Altern Med 3(3):37–47

Lange D (1998) Europe's medicinal and aromatic plants: their use, trade and conservation'. TRAFFIC Europe International, Cambridge, UK

Mati A (2005) Nirog Thakar Upai (ways to remain free of diseases). Modern Publications, Dhaka

Miththapala S (ed) (2006) Conserving medicinal species: securing a healthy future. IUCN, Ecosystems and Livelihoods Group, Colombo

Mulliken TA (2000) Implementing CITES for Himalayan medicinal plants Nardostachys grandiflora and Picrorhiza kurrooa. TRAFFIC Bull 18:63–72

Nayak PK (2006) Politics of co-optation: self-organized Community Forest Management and Joint Forest Management in Orissa, India, Masters Thesis, University of Manitoba

Oates JF (1999) Myth and reality in the rain forest: how conservation strategies are failing in West Africa. Univ of California Press, Oates
Parrotta J (2002) Conservation and sustainable use of medicinal plant resources – an international perspective. Paper presented at the World Ayurveda Congress, Cochin, 1–4 Nov 2002
Rahman F (1999) Bangladesh risk assessment and management-bio safety capacity building initiative of IUCN Asia. Working paper, Ministry of Environment and Forest, Dhaka
Sahai S (2002) Protection of indigenous knowledge and possible methods of sharing benefits with local communities. Multi-stakeholder dialogue on trade, intellectual property and biological and genetic resources in Asia, BRAC Centre for Development Management, Rajendrapur
Schippmann U (2001) Medicinal plants significant trade study. German Federal Agency for Nature Conservation, Bonn
Shahidullah AKM (2007) The role of medicinal plants in livelihood improvement and ecological sustainability in Bangladesh: an application of a participatory approach to management and marketing. In: Masters Abstracts International 45(5)
Shahidullah AKM, Haque CE (2009) Medicinal plant production and livelihoods: a participatory approach to management and marketing. VDM Publishing, Saarbrucken
Shahidullah AKM, Haque CE (2010) Linking medicinal plant production with livelihood enhancement in Bangladesh: implications of a vertically integrated value chain. J Transdis Environ Stud 9(2):1–18
Shengji P (2001) Ethnobotanical approaches of traditional medicine studies: some experiences from Asia. Pharm Bot 39:74–79
Singh A (2002) Non-timber forest products market study for Pithoragarh, Tanakpur, Haldwani and Ram Nagar. CECI-India, New Delhi
Subrat N (2002) Ayurvedic and herbal products industry: an overview. Paper presented at a workshop on wise practices and experiential learning in the conservation and management of Himalayan Medicinal Plants, Kathmandu, Nepal, 15–20 December 2002, supported by the Ministry of Forest and Soil Conservation, Nepal, the WWF -Nepal
Switzer H, Karki M, Vedavathy B (2003) Commercialization of natural resources for sustainable livelihoods: the case of forest products. In: ICIMOD (ed) Growth, poverty alleviation and sustainable management in the Mountain Areas of South Asia. ICIMOD, Kathmandu, pp 293–320
Thompsen S, Alam J (2006) Medicinal and aromatic plants industry development. A sectoral study report on medicinal and aromatic plants, conducted for Swiss Development Corporation and Intercooperation, Dhaka. Intercooperation, Dhaka
Vedanand (2002) Emerging global markets in medicinal plants products: challenges and opportunities for Indian herbal drug industries. In: Karki M, Johari R (eds) The role of medicinal plants industry in fostering biodiversity conservation and rural development. IDRC, New Delhi, pp 3–5
Yin RK (2003) Case study research design and methods, 3rd edn, Applied social research methods series, 5. Sage, Thousand Oaks
Wheelright GE (1974) Medicinal plants and their history. Dovers Publications, New York
WWF & IUCN (1997) Centers of plant diversity: a guide and strategy for their conservation. IUCN Publications Unit, Cambridge, UK

Chapter 20
Phytogenic Feed Additives in Animal Nutrition

Tobias Steiner and Basharat Syed

Abstract Antibiotic growth promoters (AGPs) were – and in many countries are still – continually included in animal diets in sub-therapeutic concentrations in order to achieve better feed conversion and higher growth rates by reducing the activity of the microbiota in the digestive tract. However, the routine use of AGPs in animal diets was associated with the development of bacterial resistance towards several antibiotic substances. While scientists continue their research on the actual impact of the extensive use of AGPs in animal feeds on microbial resistance in humans, consumers are becoming increasingly aware of the negative effects of the so-called "superbugs" (bacteria highly resistant to antibiotics).

A complete ban of AGPs was implemented in Europe in 2006 as a consequence of growing public concern. In the meantime, the ban of AGPs has become a worldwide trend where many countries outside the European Union are on their way towards restricting or banning the use of AGPs in animal feed. However, solutions are not only sought to replace AGPs in animal feeds, but also to reduce the overall use of veterinary antibiotics in animal agriculture. Thus, current research is directed towards measures to help animals maintain good health and reach their growth potential.

The feed industry has recognized the potential of plant-derived substances for different animal species in the last few years. Phytogenic feed additives (PFAs) referring to essential oils, spices, herbs or plant extracts, combine bioactive ingredients and flavouring substances. Hence they are categorised as 'sensory additives' according to European legislation. PFAs improve growth rate, nutrient digestibility and gut health in animals. These properties of PFAs project them as a suitable alternative to AGPs in animal production.

Keywords Phytogenic feed additives • Growth promoters • Performance • Animal nutrition • Antibiotic resistance

---

T. Steiner • B. Syed (✉)
BIOMIN Holding GmbH, Industriestrasse 21, 3130 Herzogenburg, Austria
e-mail: basharat.syed@biomin.net

Á. Máthé (ed.), *Medicinal and Aromatic Plants of the World*, Medicinal and Aromatic Plants of the World 1, DOI 10.1007/978-94-017-9810-5_20

1 Introduction

Global meat production has been increasing steadily over the last decades. Considering a steady growth in the human population, this trend is projected to continue into the near future (FAO 2012). Thus, modern animal agriculture relies on efficient production techniques in order to supply high-quality, safe animal products in a sustainable way while maintaining profitability for producers. An increasing demand for animal protein on the one hand, and stricter regulations in terms of animal welfare and environmental protection on the other, make further improvements in current production techniques mandatory. In particular, feed conversion, calculated as the amount of feed consumed per unit of live weight gain, eggs or milk production, is a key parameter that needs to be further improved in the coming decades. The contribution of feed cost to overall production cost is 60–70 %. Thus, optimizing the utilization of feed is key to sustainable and profitable animal production. The animal requires a certain level of nutrients to meet its requirement for metabolic processes such as basic body functions and the accretion of muscle tissue, or the production of eggs or milk. In most parts of the world, energy, protein and phosphorus are the most expensive feed ingredients. It is, therefore, mandatory to utilize them in the most efficient way possible in order to optimize feed efficiency.

Antibiotic growth promoters (AGPs) were – and in many countries still are – continually included in animal diets in sub-therapeutic concentrations in order to achieve better feed conversion and higher growth rates by reducing the activity of the microbiota in the digestive tract (Dibner and Richards 2005). However, the routine use of AGPs in animal diets was associated with the development of bacterial resistance towards several antibiotic substances (DANMAP 2011; Marshall and Levy 2011). While scientists continue their research on the actual impact of the extensive use of AGPs in animal feeds on microbial resistance in humans, consumers are becoming increasingly aware of the negative effects of the so-called "superbugs" (bacteria highly resistant to antibiotics). A complete ban of AGPs was implemented in Europe in 2006 as a consequence of growing public concern. In the meantime, the ban of AGPs has become a worldwide trend where many countries outside the European Union are on their way towards restricting or banning the use of AGPs in animal feed. In 2011, for example, Korea banned the use of AGPs in animal feed. However, solutions are not only sought to replace AGPs in animal feeds, but also to reduce the overall use of veterinary antibiotics in animal agriculture. Thus, current research is directed towards measures to help animals maintain good health and reach their growth potential.

An increasing number of reports have confirmed that supplementation of diets with plant-based, i.e. phytogenic feed additives (PFAs) resulted in improved zootechnical and animal health parameters, hence indicating the potential of PFAs in animal nutrition.

2 Current Use of Phytogenic Feed Additives

The feed industry has recognized the potential of plant-derived substances for different animal species in the last few years. Thus, PFAs are used to an increasing extent, mainly in feeding programs for swine and poultry. Commercial products that are available in the market today differ substantially with regards to the ingredients, physical appearance and level of complexity in their formulations. Products include simple formulations based on a single (e.g. oregano or thyme oil) or few raw materials as well as highly complex formulations utilizing a large number of ingredients. Phytogenic substances utilised in PFAs include herbs, spices, essential oils and non-volatile extracts, from, for example, clove, anise, thyme, fennel or melissa, and many others (Table 20.1) (Máthé 2007).

PFAs are either applied in solid powdered, granulated or liquid forms. Solid PFAs are usually incorporated in premixtures or complete feeds. Recent developments include encapsulation techniques that aim to protect the active substances against thermal impacts, mask their strong odours or delay their release in the digestive tract. Liquid PFAs are suitable for drinking water or milk replacer supplementation, as well as for spray application to hydro-thermally processed feed, such as pelleted or extruded diets.

The fine art of formulating PFAs lies in a suitable combination of plant materials. This requires a deep understanding of (1) the flavouring properties, as well as (2) the biological effects of plant compounds in the animal organism. The combination of different plant materials allows for utilising a wider range of properties that plants offer. It is assumed that an interaction of all constituents will work harmoniously together, making a well-formulated PFA more potent than the sum of its individual parts.

Table 20.1 Herbs and parts thereof used in feed additives

Common name	Latin name	Parts utilised[a]
Anise	*Pimpinella anisum*	Seeds
Caraway	*Carum carvi*	Seeds
Cinnamon	*Cinnamomum verum*	Bark
Chamomile	*Matricaria recutita*	Flowers
Citrus	*Citrus sp.*	Peel
Clove	*Syzygium aromaticum*	Buds
Fennel	*Foeniculum vulgare*	Seeds
Garlic	*Allium sativum*	Bulb
Ginger	*Zingiber officinale*	Rhizome
Melissa	*Melissa officinalis*	Leaves
Onion	*Allium cepa*	Bulbs
Oregano	*Origanum vulgare*	Leaves
Peppermint	*Mentha piperita*	Leaves
Rosemary	*Rosmarinus officinalis*	Laeves
Sage	*Salvia officinalis*	Leaves
Thyme	*Thymus vulgaris*	Leaves
Valerian	*Valeriana officinalis*	Root, rhizome

[a]Parts either used in complete, ground form or to obtain extracts

3 Mechanisms of Action of Phytogenic Feed Additives in Animals

Phytogenic compounds have a complex mode of action, a fact which has been a big myth even for those using these substances as additives in animal feed. Therefore, a major objective of research and development in the last few years was to understand the role of PFAs in improving animal performance and health (Hippenstiel et al. 2011; Windisch et al. 2008; Máthé 2009). However, the vast number of phytogenic compounds and differences in the composition of PFAs that were used in the various studies make it difficult to postulate a general mode of action that is applicable to all commercial PFAs in the market.

In fact, the effectiveness of plant active ingredients has often been underestimated in recent years and it is not seldom that their mode of action has been misunderstood even by companies offering such products. It is often postulated that PFAs are anti-microbial. Indeed, many secondary plant ingredients and extracts do have such properties. The in vitro anti-microbial activity of plants and plant ingredients is well documented through scientific findings (Helander et al. 1998; Ultee et al. 2002; Burt 2004; Nikaido 2003; Preuss et al. 2005; Ouwehand et al. 2010) and plants themselves can respond to bacterial or viral attack for example by producing “phytoalexins” (Ahuja et al. 2012). The focus on anti-microbial effects is largely driven by the argument that PFAs are substitutes for AGPs. However, it would be inappropriate to limit the value of phytogenic substances in animal nutrition to an anti-microbial effect only.

Recently, more scientific data has been generated, which enables us to better understand the effects that PFAs have in the animal. The possible effects of PFAs are summarised in Fig. 20.1. With regards to an improvement in feed conversion, increased digestibility is considered a main effect of PFAs. Parameters influenced by PFAs include the secretion of digestive juices and enzymes, a modulation of the immune system, changes in the intestinal morphology, improvements in nutrient utilization and consequently, a higher level of performance. However, the above-mentioned parameters are interrelated with each other. Positive effects of PFAs on the morphology of the small intestinal tissues, for example, are postulated to increase nutrient digestibility. Furthermore, a stabilization of the intestinal microbiota results in reduced levels of microbial metabolites in the digestive tract, hence relieving the immune system and increasing energy available for muscle accretion.

4 Flavouring Effects of Phytogenic Feed Additives

Food preferences and food choices are influenced by food flavour (Clark 1998). Flavour is largely perceived through taste (gustation) and smell (olfaction). Describing how food tastes, human beings practically speak about the food flavour,

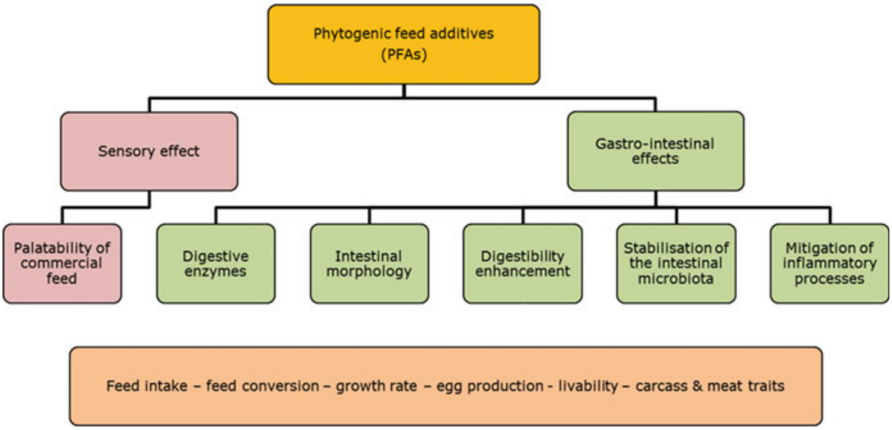


Fig. 20.1 Phytogenic compounds are effective at different levels in the digestive tract

but not the basic tastes like sour, sweet, bitter or salty. The texture and temperature of foods and the chemical burn of chili peppers and other irritants is also detected in the oral cavity. Taste receptors located in the oral cavity, particularly on the tongue, are responsible for perceiving the basic tastes. As confirmed by Drewnowski et al. (2002), components of flavour (such as the aroma of vanilla or orange), are airborne particles which enter the nose from the back of the mouth through the so-called retro-nasal transport or directly through the nasal passage. Since the sensation aroused by umami compounds such as the amino acid glutamate (often in the form of the sodium salt monosodium glutamate) occurring naturally in foods and providing flavour to many foods and cuisines around the world, is distinct from the other four taste qualities, scientists count its perception as a fifth basic taste quality (Beauchamp 2009).

In a scientific study, Bach et al. (2012) reported that lambs were able to sense the crude protein content in their diets and were accordingly able to modulate the short-term consumption of flavoured feeds based on their nutrient requirement.

Animals, particularly young piglets at the time of weaning, usually show insufficient feed intake, thus resulting in suboptimal growth performance. Among other factors, voluntary feed intake of young piglets and other animals is influenced by taste and olfaction. Therefore, animal nutritionists aim to provide highly palatable diets by including pleasant flavours. However, meaningful choice tests investigating the preference for certain flavours are scarce, indicating a certain preference for sweet, fruity and milky flavours in piglets (Jacela et al. 2010; Nofre et al. 2002; McLaughlin et al. 1983).

There are several types of flavours which can be added to feed to enhance or mask the natural flavour characteristics of feed and thus improve its palatability. However, there are differences between animal species, pigs and cattle being much more sensitive to taste compared with poultry (Chamorro et al. 1993; Ganchrow and Ganchrow 1987; Davies et al. 1979). Plant ingredients, such as herbs, spices, their extracts or single active principles, have pronounced flavouring properties. Their inclusion in diets, therefore, influences the sensory properties of these diets. Consequently, these compounds are authorized as flavouring substances in the European Union (Regulation EC No. 1831/2003).

5 Effects of Phytogenic Feed Additives on Digestibility

Digestibility refers to the extent to which nutrients contained in a diet are absorbed by an animal's body as it passes through the animal's digestive tract. Better digestibility of nutrients, or simply, better digestion, contributes to better feed efficiency. Poor digestibility of a diet is not only visible in sub-optimal feed efficiency, but also by the negative effects that impact the digestive tract directly. It is assumed that low digestibility of the diet is reflected in greater amounts of undigested feed in the gut, which is potentially subject to fermentation by the intestinal microbiota. Microbial fermentation takes place mainly in the large intestine, but also, to a lesser degree, in the small intestine. The presence of undigested nitrogenous compounds (i.e. proteins, amino acids) favours the formation of undesired metabolites, such as ammonia and biogenic amines. These metabolites are adverse not only because of their toxicity, but also because they are produced by decarboxylation of dietary essential amino acids (Kroismayr et al. 2008a). For example, cadaverine is made from lysine. Consequently, intestinal imbalances occur, resulting in enhanced inflammatory processes and accelerated turnover of the intestinal tissue, which results in poorer performance and potentially diarrhoea.

Improvements in digestibility were reported for different nutrients and amino acids in pigs (Maenner et al. 2011; Kong et al. 2009) and poultry (e.g. Mountzouris et al. 2011; Amad et al. 2011). Figures 20.2 and 20.3 indicate significant improvements in protein and amino acid digestibility, measured at the terminal ileum of weaning pigs (Maenner et al. 2011).

In another study with weaning piglets, PFA (oregano, anise and citrus essential oils) supplementation improved protein and organic matter digestibility (Zitterl-Eglseer et al. 2008). This was in concert with a reduction in the concentrations of aerobic and anaerobic bacteria in the gastrointestinal tract (Kroismayr et al. 2008a), which consequently indicates that the competition for nutrients between the host animal and its intestinal microbiota was decreased when the PFA was fed. Furthermore, intestinal concentrations of undesired microbial metabolites (i.e. ammonia and biogenic amines) were decreased in response to the PFA, which resulted in a relief from intestinal challenges, a stabilization of the digestive tract and ultimately, an improvement in weight gain, feed intake and feed conversion. Improvements in nitrogen digestibility were reported in pigs fed an essential oils mixture (rosemary, cinnamon, thyme, oregano and clove) at 5 and 7, but not at 9 weeks of age (Huang et al. 2010), indicating an influence of age.

Among other factors that will be discussed in the following sections, increased digestibility may be due to enhanced secretions of saliva, bile acids and digestive

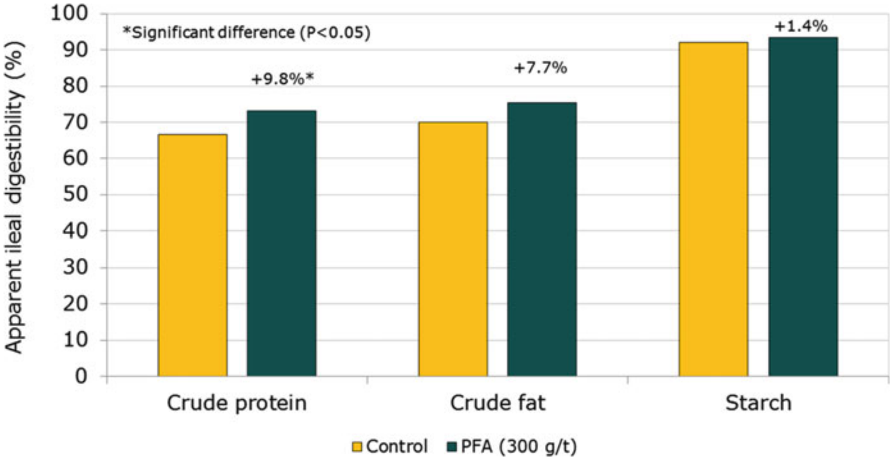


Fig. 20.2 Effect of a phytogenic feed additive on apparent ileal digestibility of crude protein, crude fat and starch in weaned piglets (Adapted from Maenner et al. 2011)

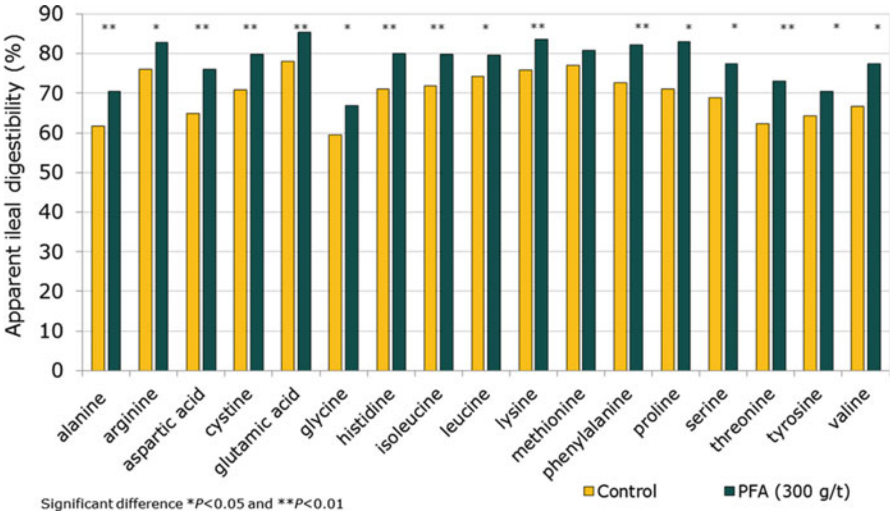


Fig. 20.3 Effect of a phytogenic feed additive on apparent ileal digestibility of amino acids in weaned piglets (Adapted from Maenner et al. 2011)

enzymes. However, studies investigating these effects are rather scarce, with a few examples in rats (Rao et al. 2003; Platel and Srinivaran 2000a, b, 2004) and broilers (Jang et al. 2004; Lee et al. 2003).

6 Anti-inflammatory and Anti-oxidative Effects of Phytogenic Feed Additives

It is well documented that infections lead to inflammation, hence the release of pro-inflammatory cytokines, chemokines and adhesion molecules (Collins et al. 1995). Therefore, restricting inflammation is of prime importance for proper animal health and growth. Cytokines not only activate immune cells, but also have an intense and deteriorating influence on animal health by reducing feed intake and promoting muscle tissue catabolism (Johnson and Escobar 2005).

The frequency and occurrence of inflammatory processes as a result of infections or feed-dependent changes are inversely proportional to animal health and growth performance, as outlined by Niewold (2007). The largely infamous metabolic inflammation as a consequence of feed intake is a normal physiological response of the intestines. Metabolic inflammation is influenced by specific feed constituents and is related directly to the energy value and the glycemic index of the feed consumed (Margioris 2009). In order to maintain the physiological coherence of the animal body, metabolic inflammation is firmly managed and systematised in the animal organism. In this way, the animal organism profits from a down-regulation of the intestinal inflammatory responses. However, in high producing commercial livestock, in spite of the firm physiological control of metabolic inflammation, the regulatory mechanism can be disturbed e.g. by large amounts of high-energy feed. Since phytogenic compounds have pronounced anti-inflammatory properties, this underlines the potential of PFAs to down-regulate inflammatory processes in the digestive tract.

The anti-inflammatory effects of phytogenic compounds were elucidated in a number of studies (Miguel 2010; Kroismayr et al. 2008b; Gbenou et al. 2013). The direct anti-inflammatory activity of a PFA (blend containing essential oils from peppermint, anise, clove and caraway) was examined at a cellular level in a test model with inflammation-induced intestinal cells (Gessner et al. 2013). Inflammation was induced by treating the cells with tumor necrosis factor α (TNFα). These cells were either incubated or not with an extract of the PFA. The first element to study was the nuclear factor κB (NF-κB), an important transcriptional factor that controls the expression of different genes [interleukin 8 (IL-8), intracellular adhesion molecule (ICAM-1) and monocyte chemotactic protein (MCT-1)] that are involved in the regulation of the pro-inflammatory response. The experiment revealed inhibitory effects of the PFA on IL-8, ICAM-1 and MCT-1 (Fig. 20.4). Thus, the PFA exerted a positive effect on the cellular inflammatory status by down regulation of NF-κB.

Protective effects of phytogenic compounds may also result from their anti-oxidative properties. Anti-oxidative effects were reported for a large number of plant substances (e.g. Miguel 2010; Wei and Shibamoto 2007). In this context, an important cellular element is the transcription factor Nrf2. Activation of the Nrf2 pathway leads to the induction of genes responsible for cellular defence against reactive oxygen species and detoxification of xenobiotics. The above mentioned

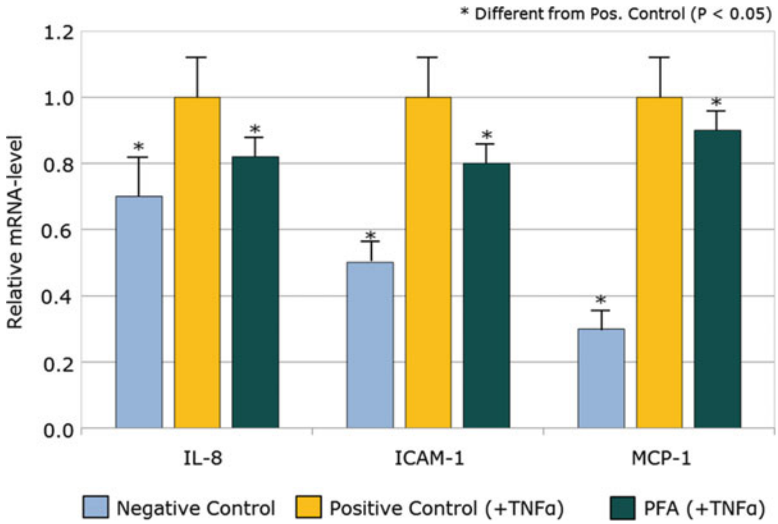


Fig. 20.4 Anti-inflammatory effect of a PFA (down-regulation of NF-κB target genes) (According to Gessner et al. 2013)

PFA was found to up-regulate Nrf2 target genes, i.e. cytochrome P450 isoform 1A1 (CYP1A1), heme oxygenase-1 (HO-1) and UDP glucuronosyltransferase isoform 1A1 (UGT1A1) (Gessner et al. 2013), hence providing further evidence for the protective effects of phytogenic compounds at the cellular level (Fig. 20.5). Therefore, regular supplementation of PFAs in the diet may act as prophylactic agents inflammatory reactions in the gastrointestinal tract by inhibiting the NF-κB pathway and stimulating the anti-oxidative factor Nrf2.

These findings are supported by experiments of Mueller et al. (2012), who examined the influence of different phytogenic substances (broccoli, turmeric, oregano, thyme, rosemary) on the regulation of xenobiotic- and antioxidant enzymes in the intestine and the liver of broilers. The authors found an up-regulation of the 'antioxidant response element' (ARE) genes in the small intestine, indicating reduced oxidative stress in the organism.

7 Effects of Phytogenic Feed Additives on the Intestinal Microbiota

An imbalance in the animal's gastro intestinal microbiota due to dietary or environmental changes is damaging for the growth, health and feed conversion of the animal. In contrast, an ideal, stabilized microbiota allows optimum growth performance (Schaedler 1973). As reviewed by Windisch et al. (2008), literature depicting the anti-microbial role of PFAs is ample. In in vitro studies, components derived from thyme and oregano plants, namely thymol and carvacrol, as well as

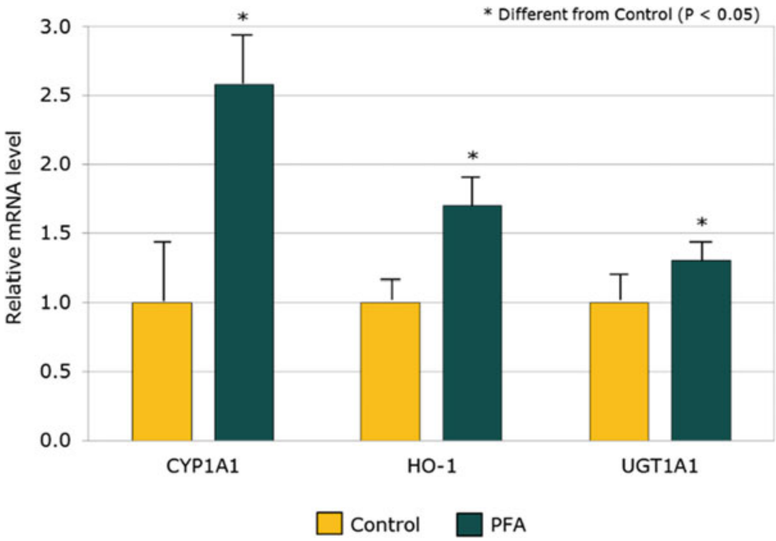


Fig. 20.5 Anti-oxidative effect of a PFA (up-regulation of Nrf-2 target genes) (According to Gessner et al. 2013)

many other compounds, demonstrated anti-microbial activities. Helander et al. (1998) reported that the effect is attributed to the lipophilic character of the active principles of phytogenic compounds, which permeate the cell membranes and mitochondria of the microorganisms, and inhibit the energy metabolism and the membrane-bound electron flow, leading to a collapse of the proton pump and draining of the ATP pool. The denaturation of cytoplasmic proteins and lysis of the cell membranes have also been attributed to high concentrations of essential oils.

The application of PFAs as alternatives to AGPs in livestock feeding seems logical because of their proven anti-microbial activity in vitro. Therefore, the intake of PFAs in animal feed is expected to influence the population as well as the composition of the gastrointestinal microbiota.

There is an increasing trend regarding in vivo studies on the influence of PFAs of the gut microbiota; however, the results are hard to correlate and interpret because of the diverse methodology employed during the investigations. Although methods to determine the activity vary, an impact on microbial communities can be tested in all parts of the intestinal tract. Some studies in broilers demonstrated a clear reduction of pathogenic bacteria such as *Escherichia coli* and *Clostridium perfringens* (McReynolds et al. 2009; Mitsch et al. 2004; Jamroz et al. 2005) and these effects are attributed mainly to their phenolic components and their action on microbial cells (Burt 2004; Si et al. 2006).

An anti-microbial effect of PFAs, however, is sometimes not observed in feeding trials, which may be due to optimal housing and hygienic conditions or the use of highly digestible diets (Hippenstiel et al. 2011). Taking into account that the dosages of PFAs in animal diets are usually lower than effective anti-microbial concentrations determined in vitro (Franz et al. 2010), a stabilising effect of PFAs on the gut microbiota is more likely than a direct bactericidal effect.

Indeed, it was shown that PFAs facilitate the proliferation of bacteria that are generally considered as beneficial, such as *Lactobacillus* (Mitsch et al. 2004; Jamroz et al. 2005; Mountzouris et al. 2011). Mountzouris et al. (2011) observed that inclusion levels of a PFA of 125 and 250 mg/kg diet resulted in a purportedly beneficial modulation of the caecal microbiota. There was a linear increase of caecal *Lactobacillus*, *Bifidobacterium* and Gram-positive cocci concentration with increasing PFA levels in 42 day-old broilers. In addition, caecal coliforms at 14 days of age were significantly lower at the inclusion levels of 125 and 250 mg/kg diet compared with the AGP Avilamycin. It is plausible that due to the reduction of undesired bacteria, including Clostridia, coliforms, Staphylococci and others, more space is available for lactobacilli to grow. Once the lactobacilli are established, they might selectively exclude pathogens from colonisation due to the fast proliferation, colonization and possibly acidification in the gut (McReynolds et al. 2009).

The inhibitory effect of PFAs on *Clostridium* is encouraging and paves the way for the removal of AGPs from diets. Clostridia, particularly *C. perfringens*, are responsible for necrotic enteritis in poultry although in the normal intestinal microbiota, *Clostridium* is detected irregularly and in small numbers (Barnes et al. 1972). Phytogenic compounds also inhibited the growth of clostridia in vitro (Briozzo et al. 1988; Dorman and Deans 2000). Mitsch et al. (2004) opined that PFAs stabilize the gut microbiota and thereby reduce the colonization of clostridia in the gut.

In a series of infection experiments in which broilers were orally challenged with *Clostridium perfringens*, birds fed a PFA (blend of essential oils from oregano, anise and citrus) showed less clinical signs of necrotic enteritis (i.e. reduced severity of necrotic lesions, lower levels of *C. perfringens* in the small intestine and lower mortality) compared to challenged, non-supplemented birds (McReynolds et al. 2009). Similar conclusions were drawn by Engberg et al. (2012). The authors carried out two experiments, in which the influence of increasing dietary concentrations of dried *Artemisia annua* leaves and n-hexane extract from fresh *A. annua* leaves on broiler performance was investigated. The results indicated that the extracts derived from *A. annua* could modulate the course of necrotic enteritis and compensate to a certain extent for disease-associated weight losses.

Numerous studies investigated the potential of phytogenic substances to prevent coccidiosis (Reisinger et al. 2011; Naidoo et al. 2008; Giannenas et al. 2004), one of the most costly diseases in commercial poultry caused by protozoan infections and currently controlled mainly by the routine use of anticoccidial drugs in the feed. Reisinger et al. (2011) observed that dietary supplementation with an essential oil-based PFA increased villus length and goblet cell density in the mid-ileum part of the small intestine of broilers exposed to a mild coccidial vaccine challenge. The authors concluded that PFA supplementation may have created an improved barrier against pathogens during this coccidial exposure. Alp et al. (2012), in a study designed to evaluate the effect of dietary oregano essential oil on performance, carcass yield, and serum IgG levels of broilers, concluded that the additive may provide an alternative to conventional anticoccidial additives in broiler feeds. A complete replacement of anticoccidial drugs, however, in the near future seems rather unlikely because of the lack of long-term, large-scale studies investigating the efficacy of PFAs for sustainable coccidiosis prevention.

8 Effects of Phytogenic Feed Additives on Intestinal Morphology

Morphological changes in the gastrointestinal tissues caused by PFAs may provide further information on possible benefits to the digestive tract. In general, PFAs caused an increase in height of villi across the small intestine in poultry (Hong et al. 2012; Reisinger et al. 2011; Peric et al. 2010). Similar effects of a PFA were reported by Namkung et al. (2004) in studies with pigs. This ought to increase the absorptive surface area and efficiency of digestion and absorption of nutrients. Greater villus height may also increase the activities of enzymes secreted from the tips of the villi, contributing to improved digestibility (Baurhoo et al. 2007).

An improved barrier function through higher numbers of goblet cells, as reported by Reisinger et al. (2011) is supported by Tsirtsikos et al. (2012) who carried out studies in which the duodenal mucus layer thickness of broilers showed a pattern of linear increase with increasing dietary PFA concentrations. Similar observations were reported by Jamroz et al. (2006), confirming the potential positive effect of PFAs on intestinal morphology.

9 Effects of Phytogenic Feed Additives on Animal Performance

Effects of PFAs on zootechnical parameters result from the mechanisms of action discussed in the above sections. In general, animal performance, e.g. body weight gain, feed conversion ratio and feed intake are influenced by several factors, namely genotype, feed composition, feeding systems, management, housing, environmental and hygienic conditions. The positive influence of PFAs on zootechnical parameters has been confirmed in a number of studies. An approximately 15 % improvement in body weight gain against a control group was observed by Çiftçi et al. (2005) in broilers. Mohamed and Abbas (2009) also recorded a 6 % increase in body weight gain in broilers by supplementing the diets with 1 g fennel per kg of diet. Nevertheless, certain studies have also depicted a negative influence of the phytogenic compounds on body weight gain. Cross et al. (2003) reported a tangible decrease in body weight gain when 5 g/kg thyme essential oil was added to the chicken feed. However, Cross et al. (2007), supplementing chicken diets with 1 g essential oil of thyme per kg, recorded a significant improvement in body weight gain. Similarly, Toghyani et al. (2010) reported an approximately 6 % improvement in body weight gain by supplementing broiler diets with 5 g thyme per kg of diet. Simsek et al. (2007) reported significant improvements in body weight, carcass characteristics and organoleptic analysis of meat in broilers by feeding 400 mg of anise essential oil per kg of diet.

A 5 % improvement in body weight was attributed to a favourable shift in intestinal microbiota by Tiihonen et al. (2010) in broiler chickens by supplementing the diets with a mixture of 15 mg thymol and 5 mg of cinnamaldehyde per kg of diet. A blend of essential oil from oregano, clove and anise improved the FCR in broilers by around 12 % against the control group and 8 % against a group of birds supplemented with AGPs (Ertas et al. 2005). Similarly, a 5 % improvement in FCR against the control group was recorded also by Ulfah (2006) by feeding broilers with a combination of the essential oils of oregano, thyme, cinnamon and eucalyptus. Hong et al. (2012) and Mountzouris et al. (2011) observed a 6 % and 7 % improvement in feed conversion, respectively, of broilers fed a blended PFA containing oregano, citrus and anise essential oils.

Similar results were obtained in studies with pigs. Supplementation of piglet diets with PFAs improved feed conversion by 3–5 % (Li et al. 2012; Maenner et al. 2011; Sulabo et al. 2007). Increased weight gain of piglets fed phytogenic compounds was also reported in different studies, as reviewed by Franz et al. (2010).

Improved feed conversion was reported in Holstein bull calves (Miller et al. 2012) when a blended PFA was included in the milk replacer. In addition, the authors also indicated reduced scour scores and fewer scour days.

10 Environmental Emissions

Among gases, ammonia is a concern in animal production because it negatively affects animal health and welfare. Furthermore, ammonia and odour emissions from animal production units in general are undesirable for people living close to farms. It is assumed that improvements in protein digestibility will result in better utilization of dietary amino acids, hence reducing the excretion of nitrogenous compounds in the slurry. As such, PFAs have the potential to reduce emissions from animal production units. Experiments investigating the influence of feed additives on gas emissions are rather scarce. In fact, such studies require strict experimental conditions, especially in terms of housing, temperature control, ventilation rates and measurements of aerial gas concentrations. Zentner et al. (2012), for example, reported a 24 % reduction in aerial ammonia concentrations in growing-finishing pigs fed a PFA based on oregano, thyme, anise and citrus essential oils. Furthermore, the authors found a reduction in odour emission, measured in odour units per cubic meter, by 29 %, indicating a close correlation between these parameters.

El-Deek et al. (2012), investigating the effects of feeding two levels of dietary crude protein (21 % vs. 23 %) in combination with or without two levels of green tea (1.5 and 3 g/kg diet) or one level of oxytetracycline at 0.1 g/kg diet, reported that decreasing the dietary crude protein level to 21 % had no adverse effects on the growth rate of broilers. Green tea supplementation at 1.5 g/kg diet increased growth rate and improved FCR by 10 %. The authors concluded that feeding broiler chickens a 21 % protein diet containing adequate amino acid levels when supplemented with green tea, had no negative effects on productive performance. This may contribute to decreased environmental pollution by decreased nitrogen excretion.

11 Effects of Phytogenic Additives on Meat Traits

Successful marketing of any food depends on the sensory characteristic of the product. In the marketing of meat, tenderness and succulence of the meat are of high importance. Unfortunately, the meat processor has only a limited influence on these factors. Therefore, knowledge about the possibilities of optimising the primary production of the meat on the farm is important for the food industry.

The potential efficacy of PFAs to improve overall meat quality attributes such as carcass yield, dressing percentage, fillet and tender yield, organoleptic cooked meat parameters, and the overall palatability and acceptability of meat is another area of scientific research. In a scientific study to investigate the potential of essential oils as growth promoting agents and as potential alternatives to AGPs with regards to carcass traits in broilers, Hong et al. (2012) observed that breast meat was more tender and thigh meat was juicier for birds in the essential oil group compared to the control and AGP groups.

Javan et al. (2012) evaluated the effect of dietary essential oil (*Zataria multiflora*) supplementation on the microbial growth and lipid peroxidation of broiler breast fillets during refrigerated storage. It was concluded that the essential oil delayed the peroxidation and microbial spoilage of chicken breast fillets. Similar results were reported for chicken meat (Avila-Ramos et al. 2012; Spernakova et al. 2007; Young et al. 2003; Botsoglou et al. 2002), turkey meat (Govaris et al. 2007) and fish (Giannenas et al. 2012) using different aromatic plants. In contrast, Simitzis et al. (2010) did not observe an influence of oregano oil on meat traits in finishing pigs, which may be attributed to the limited experimental duration of only 35 days.

Beneficial effects of dietary supplementation with PFAs on meat quality traits are mainly attributed to the anti-oxidative properties of phytogenic compounds. Supplementation of diets for Rainbow trout with either a carvacrol- or a thymol-based PFA significantly decreased malondialdehyde formation in the fillets, indicating enhanced oxidative stability of the fillet. In addition, glutathione-based

Table 20.2 Effect of PFAs on the antioxidant status of trout fillet after feeding the experimental diets for 8 weeks (antioxidant status was assessed during refrigerated storage at 0 or 5 days) (Adapted from Giannenas et al. 2012)

	Day (0)	Day (5)
Malondialdehyde (nmol/mg protein)		
Control	34.1[a]	49.1[#, b]
PFA-C	32.4[a]	38.6[#, b]
PFA-T	30.2	32.3[*]
Glutathione-S-transferase (mmol/min/mg protein)		
Control	2.12[#, a]	1.28[#, b]
PFA-C	2.82[*, a]	1.89[#, b]
PFA-T	2.84[*, a]	2.39[*, b]
Glutathione reductase (U/mg protein)		
Control	27.1[#, a]	16.6[#, b]
PFA-C	30.1[#, a]	21.4[*, b]
PFA-T	35.2[*, a]	24.5[*, b]

Phyto-C PFA based on carvacrol, *Phyto-T* PFA based on thymol
[*, #]Values in the same column with a different superscript symbol differ significantly ($p \leq 0.05$)
[a, b]Values in the same row with a different superscript letter differ significantly ($p \leq 0.05$)

enzyme activity at 0 and 5 days of storage was higher in fillets from both PFA-supplemented groups compared to the control, as shown in Table 20.2.

12 Future Considerations and Conclusions

The level of standardisation of PFAs has increased over the last 10 years. However, the different additives available in the market vary greatly in their composition, and thus in their in vivo effects as well. There are an increasing number of in vivo studies which focus on different aspects of incorporating PFAs in animal diets. Although, the results of these studies vary to a certain extent, positive results predominate in the conclusions.

Improvements were observed in the majority of the investigations to evaluate the influence of PFAs on digestibility, morphology of and inflammatory processes in the digestive tract, as well as modulation of the intestinal microbiota and, finally, the impact of PFAs on zootechnical parameters.

Despite a large number of scientific as well as field studies on the influence of PFAs on animal health and performance, the exact mode of action of these feed additives remains to be further elucidated. Aspects like undesired effects and effects of over-dosage need to be further investigated in both in vitro and in vivo trials to ensure the safe and productive use of PFAs.

An important aspect of future research should be to conduct standardised trials indicating the composition of the PFAs employed in the investigation, so that the observations could be easily compared. Potential synergistic effects of phytogenic compounds are likely; however, this needs to be studied in more detail and under standardised conditions.

Based on the literature available to date, PFAs comprising of single or combinations of components have significant potential to be considered as alternatives to AGPs. This is of increasing importance in view of the global trend to restrict or reduce the overall use of AGPs and antimicrobials in animal production.

Furthermore, the main focus of future considerations regarding the use of PFAs in the commercial livestock industry should be aimed at addressing the following issues necessary for sustainable animal production:

- Production efficiency: To organize a timely animal production that maintains profitability along the food chain.
- Animal health and welfare: To improve animal health, while reducing the use of therapeutic antibiotics.
- Consumer safety: To reduce the risk of bacterial resistance and with that, achieve a higher level of consumer security.
- Public acceptance: To improve the prestige and trust in commercial animal production utilizing plant-derived products.
- Sustainability: To conserve resources (protein, phosphorus, water and others) through improved feed efficiency.
- Environmental protection: Improving feed conversion and hence preventing environmental pollution with a simultaneous improvement in performance.

References

Ahuja I, Kissen R, Bones AM (2012) Phytoalexins in defense against pathogens. Trends Plant Sci 17(2):73–90

Alp M, Midilli M, Kocabağlı N, Yılmaz H, Turan N, Gargılı A, Acar N (2012) The effects of dietary oregano essential oil on live performance, carcass yield, serum immunoglobulin G level, and oocyst count in broilers. J Appl Poult Res 21(3):630–636

Amad AA, Männer K, Wendler KR, Neumann K, Zentek J (2011) Effects of a phytogenic feed additive on growth performance and ileal nutrient digestibility in broiler chickens. Poult Sci 90(12):2811–2816

Avila-Ramos F, Pro-Martínez A, Sosa-Montes E, Cuca-García JM, Becerril-Pérez CM, Figueroa-Velasco JL, Narciso-Gaytán C (2012) Effects of dietary oregano essential oil and vitamin E on the lipid oxidation stability of cooked chicken breast meat. Poult Sci 91(2):505–511

Bach A, Villalba JJ, Ipharraguerre IR (2012) Interactions between mild nutrient imbalance and taste preferences in young ruminants. J Anim Sci 90(3):1015–1025

Barnes EM, Mead GC, Barnum DA, Harry EG (1972) The intestinal flora of the chicken in the period 2–6 weeks of age, with particular reference to the anaerobic bacteria. Br Poult Sci 13:311–326

Baurhoo B, Phillip L, Ruiz-Feria CA (2007) Effects of purified lignin and mannan oligosaccharides on intestinal integrity and microbial populations in the ceca and litter of broiler chickens. Poult Sci 86:1070–1078

Beauchamp GK (2009) Sensory and receptor responses to umami: an overview of pioneering work. Am J Clin Nutr 90(3):723S–727S

Botsoglou NA, Florou Paneri P, Christaki E, Fletouris DJ, Spais AB (2002) Effect of dietary oregano essential oil on lipid oxidation in raw and cooked chicken during refrigerated storage. Meat Sci 62:259–265

Briozzo J, Nunez L, Chirife J, Herszage L, D'Aquino M (1988) Antimicrobial activity of clove oil dispersed in a concentrated sugar solution. J Appl Bacteriol 66:69–75

Burt S (2004) Essential oils: their antibacterial properties and potential applications in foods – a review. Int J Food Microbiol 94:223–253

Chamorro CA, de Paz P, Fernández JG, Anel L (1993) Fungiform papillae of the pig and the wild boar analyzed by scanning electron microscopy. Scanning Microsc 7(1):313–320

Çiftçi M, Güler T, Dalkiliç B, Ertas ON (2005) The effect of anise oil (*Pimpinella anisum* L.) on broiler performance. Int Poult Sci 4:851–855

Clark JE (1998) Taste and flavour: their importance in food choice and acceptance. Proc Nutr Soc 57(4):639–643

Collins T, Read MA, Neish AS et al (1995) Transcriptional regulation of endothelial cell adhesion molecules: NF-κB and cytokine-inducible enhancers. FASEB J 9:899–909

Cross DE, Svoboda K, McDevitt RM, Acamovic T (2003) The performance of chickens fed diets with or without thyme oil and enzymes. Brit Poult Sci 44:18–19

Cross DE, McDevitt RM, Hillman K, Acamovic T (2007) The effects of herbs and their associated essential oils on performance, dietary digestibility and gut micro-flora in chickens from 7 to 28 days of age. Brit Poult Sci 48:496–506

DANMAP (2011) Use of antimicrobial agents and occurrence of antimicrobial resistance in bacteria from food animals, food and humans in Denmark. ISSN 1600–2032. Report available at http://www.danmap.org

Davies OR, Kare MR, Cagan RA (1979) Distribution of taste buds on fungiform and circumvallate papillae of bovine tongue. Anat Rec 195:443–446

Dibner JJ, Richards JD (2005) Antibiotic growth promoters in agriculture: history and mode of action. Poult Sci 84:634–643

Dorman HJD, Deans SG (2000) Antimicrobial agents from plants: antibacterial activity of plant volatile oils. J Appl Microbiol 88:308–316

Drewnowski A, Coldwell SE, Rolls BJ (2002) Taste and smell. Encyclopedia of aging, 2002. Retrieved from Encyclopedia.com: http://www.encyclopedia.com/doc/1G2-3402200403.html

El-Deek AA, Al-Harthi MA, Osman M, Al-Jassas F, Nassar R (2012) Effect of different levels of green tea (*Camellia sinensis*) as a substitute for oxytetracycline as a growth promoter in broilers diets containing two crude protein levels. Arch Geflügelkd 76(2):81–87

Engberg RM, Grevsen K, Ivarsen E, Frette X, Christensen LP, Hojberg O, Jensen BB, Canibe N (2012) The effect of *Artemisia annua* on broiler performance, on intestinal microbiota and on the course of a *Clostridium perfringens* infection applying a necrotic enteritis disease model. Avian Pathol 41(4):369–376

Ertas ON, Güler T, Çiftçi M, Dalkilic B, Simsek UG (2005) The effect of an essential oil mix derived from oregano, clove and anise on broiler performance. Int Poult Sci 4:879–884

FAO (2012) FAO engages industry at world meat congress. In: 19th world meat congress, Paris, 4–6 June 2012

Franz C, Baser K, Windisch W (2010) Essential oils and aromatic plants in animal feeding – a European perspective. a review. Flavour Fragr J 25:327–340

Ganchrow JR, Ganchrow D (1987) Taste bud development in chickens (*Gallus gallus domesticus*). Anat Rec 218(1):88–93

Gbenou JD, Ahounou JF, Akakpo HB, Laleye A, Yayi E, Gbaguidi F, Baba-Moussa L, Darboux R, Dansou P, Moudachirou M, Kotchoni SO (2013) Phytochemical composition of *Cymbopogon citratus* and *Eucalyptus citriodora* essential oils and their anti-inflammatory and analgesic properties on Wistar rats. Mol Biol Rep 40(2):1127–1134

Gessner DK, Syed B, Steiner T, Eder K (2013) Influence of a phytogenic feed additive on inflammatory processes in intestinal cells. In: European symposium of porcine health management, Edinburgh, 22–24 May 2013

Giannenas I, Florou-Paneri P, Papazahariadou M, Botsoglou CE, Spais AB (2004) Effect of diet supplementation with ground oregano on performance of broiler chickens challenged with *Eimeria tenella*. Arch Geflügelkd 68:247–252

Giannenas I, Triantafillou El, Stavrakakis S, Margaroni M, Mavridis S, Steiner T, Karagouni E (2012) Assessment of dietary supplementation with carvacrol or thymol containing feed additives on performance, intestinal microbiota and antioxidant status of rainbow trout (*Oncorhynchus mykiss*). Aquaculture 350–353:26–32

Govaris A, Florou Paneri P, Botsoglou E, Giannenas I, Ambrosiadis I, Botsoglou N (2007) The inhibitory potential of feed supplementation with rosemary and/or α-tocoherol acetate on microbial growth and lipid oxidation of turkey breast during refrigerated storage. LWT Food Sci Technol 40:331–337

Helander IM, Alakomi HL, Latva-Kala K, Mattila-Sandholm TPI, Smid EJ, Gorris LGM, Wright A (1998) Characterization of the action of selected essential oil components on gram-negative bacteria. J Agric Food Chem 46:3590–3595

Hippenstiel F, Abdel-Wareth AAA, Kehraus S, Südekum KH (2011) Effects of selected herbs and essential oils, and their active components on feed intake and performance of broilers – a review. Arch Geflügelkd 75(4):226–234

Hong JC, Steiner T, Aufy A, Lien TF (2012) Effects of supplemental essential oil on growth performance, lipid metabolites and immunity, intestinal characteristics, microbiota and carcass traits in broilers. Livest Sci 144:253–262

Huang Y, Yoo JS, Kim HJ, Wang Y, Chen YJ, Cho JH, Kim IH (2010) Effects of dietary supplementation with blended essential oils on growth performance, nutrient digestibility, blood profiles and faecal characteristics in weanling pigs. Asian Aust J Anim Sci 23(5):607–613

Jacela JY, Joel M, DeRouchey MD, Tokach RD, Goodband JL, Nelssen DG, Renter SSD (2010) Feed additives for swine: fact sheets – flavors and mold inhibitors, mycotoxin binders, and antioxidants. J Swine Health Prod 18:27–32

Jamroz D, Wiliczkiewicz A, Wertelecki T, Orda J, Scorupinska J (2005) Use of active substances of plant origin in chicken diets based on maize and domestic grains. Brit Poult Sci 46:485–493

Jamroz D, Wertelecki T, Houszka M, Kamel C (2006) Influence of diet type on the inclusion of plant origin active substances on morphological and histochemical characteristics of the stomach and jejunum walls in chicken. J Anim Physiol Anim Nutr 90:255–268

Jang IS, Ko YH, Yang HY, Ha JS, Kim JY, Kang SY, Yoo DH, Nam DS, Kim DH, Lee CY (2004) Influence of essential oil components on growth performance and the functional activity of the pancreas and small intestine in broiler chickens. Asian Aust J Anim Sci 17:394–400

Javan AJ, Ghazvinian A, Mahdavi A, Vayeghan AJ, Staji H, Khaligh SG (2012) The effect of dietary *Zataria multiflora boiss*. Essential oil supplementation on microbial growth and lipid peroxidation of broiler breast fillets during refrigerated storage. J Food Process Pres 37:881–888. doi:10.1111/j.1745-4549.2012.00714.x

Johnson RW, Escobar J (2005) Cytokine regulation of protein accretion in growing animals. In: Burrin DG, Mersmann H (eds) Biology of metabolism in growing animals. Elsevier, Edinburgh, pp 83–106

Kong XF, Yin FG, He QH, Liu HJ, Li TJ, Huang RL, Fan MZ, Liu YL, Hou YQ, Li P, Ruana Z, Deng ZY, Xie MY, Xiong H, Yin YL (2009) Acanthopanax senticosus extract as a dietary additive enhances the apparent ileal digestibility of amino acids in weaned piglets. Livest Sci 123:261–267

Kroismayr A, Schedle K, Sehm J, Pfaffl M, Plitzner C, Foissy H, Ettle T, Mayer H, Schreiner M, Windisch W (2008a) Effects of antimicrobial feed additives on gut microbiology and blood parameters of weaned piglets. Die Bodenkultur 59:11–120

Kroismayr A, Sehm J, Pfaffl MW, Schedle K, Plitzner C, Windisch W (2008b) Effects of Avilamycin and essential oils on mRNA expression of apoptotic and inflammatory markers and gut morphology of piglets. Czech J Anim Sci 53:377–387

Lee KW, Everts H, Kappert HJ, Frehner M, Losa R, Beynen AC (2003) Effects of dietary essential oil components on growth performance, digestive enzymes and lipid metabolism in female broiler chickens. Brit Poult Sci 44:450–457

Li P, Piao X, Ru Y, Han X, Xue L, Zhang H (2012) Effects of adding essential oil to the diet of weaned pigs on performance, nutrient utilization, immune response and intestinal health. Asian Aust J Anim Sci 25(11):1617–1626

Maenner K, Vahjen W, Simon O (2011) Studies on the effects of essential-oil-based feed additives on performance, ileal nutrient digestibility, and selected bacterial groups in the gastrointestinal tract of piglets. J Anim Sci 89:2106–2112

Margioris AN (2009) Fatty acids and postprandial inflammation. Curr Opin Clin Nutr 12:129–137

Marshall BM, Levy SB (2011) Food animals and antimicrobials: impacts on human health. Clin Microbiol Rev 24(4):718–733

Máthé Á (2007) Essential oils as phytogenic feed additives. In: Franz C, Máthé Á, Buchbauer G (eds) Essential oils: basic and applied research. Allured Publishing Corporation, Carol Stream, pp 315–325

Máthé Á (2009) Essential oils: biochemistry, production and utilization. In: Steiner T (ed) Phytogenics in animal nutrition: natural concepts to optimize gut health and performance. Nottingham University Press, Nottingham, pp 1–18

McLaughlin CL, Baile CA, Buckholtz LL, Freeman SK (1983) Preferred flavors and performance of weanling pigs. J Anim Sci 56:1287–1293

McReynolds C, Waneck C, Byrd J, Genovese K, Duke S, Nisbet D (2009) Efficacy of multistrain direct-fed microbial and phytogenetic products in reducing necrotic enteritis in commercial broilers. Poult Sci 88:2075–2080

Miguel MG (2010) Antioxidant and anti-inflammatory activities of essential oils: a short review. Molecules 15:9252–9287

Miller BL, Earleywine TJ, Johnson TE (2012) The effect of essential oil/botanical product on performance and health of calves. J Anim Sci 90(Suppl 3):116 (M314)/J Dairy Sci 95(Suppl 2)

Mitsch P, Zitterl-Eglseer K, Kohler B, Gabler C, Losa R, Zimpernik I (2004) The effect of two different blends of essential oil components on the proliferation of *Clostridium perfringens* in the intestines of broiler chickens. Poult Sci 83:669–675

Mohammed AA, Abbas RJ (2009) The effect of using fennel seeds (*Foeniculum vulgare* L.) on productive performance of broiler chickens. Int J Poult Sci 8:642–644

Mountzouris K, Paraskeyas V, Tsirtsikos P, Palamidi I, Steiner T, Schatzmayr G, Fegeros K (2011) Assessment of a phytogenic feed additive effect on broiler growth performance, nutrient digestibility and caecal microflora composition. Anim Feed Sci Technol. doi:10.1016/ j.anifeedsci.2011.03.020

Mueller K, Bluma NM, Kluge H, Mueller AS (2012) Influence of broccoli extract and various essential oils on performance and expression of xenobiotic- and antioxidant enzymes in broiler chickens. Brit J Nutr 108(4):588–602

Naidoo V, Mc Gaw LJ, Bisschop SPR, Duncan N, Eloff JN (2008) The value of plant extracts with antioxidant activity in attenuating coccidiosis in broiler chickens. Vet Parasitol 153:214–219

Namkung H, Li M, Gong J, Yu H, Cottrill M, de Lange CFM (2004) Impact of feeding blends of organic acids and herbal extracts on growth performance, gut microbiota and digestive function in newly weaned pigs. Can J Anim Sci 84:697–704

Niewold TA (2007) The nonantibiotic anti-inflammatory effect of antimicrobial growth promoters, the real mode of action? A hypothesis. Poult Sci 86:605–609

Nikaido H (2003) Molecular basis of bacterial outer membrane permeability revisited. Microbiol Mol Biol Rev 67:593–656

Nofre C, Glaser D, Tinti JM, Wanner M (2002) Gustatory responses of pigs to sixty compounds tasting sweet to humans. J Anim Physiol Anim Nutr 86:90–96

Ouwehand AC, Tiihonen K, Kettunen H, Peuranen S, Schulze H, Rautonen N (2010) *In vitro* effects of essential oils on potential pathogens and beneficial members of the normal microbiota. Vet Med 55:71–78

Perić L, Milošević N, Žikić D, Bjedov S, Cvetković D, Markov S, Mohnl M, Steiner T (2010) Effects of probiotic and phytogenic products on performance, gut morphology and cecal microflora of broiler chickens. Arch Tierz 53:350–359

Platel K, Srinivasan K (2000a) Influence of dietary spices and their active principles on pancreatic digestive enzymes in albino rats. Food 44:41–46

Platel K, Srinivasan K (2000b) Stimulatory influence of select spices on bile secretion in rats. Nutr Res 20:1493–1503

Platel K, Srinivasan K (2004) Digestive stimulant action of spices: a myth or reality? Indian J Med Res 119:167–179

Preuss HG, Echard B, Dadgar A, Talpur N, Manohar V (2005) Effects of essential oils and monolaurin on *Staphylococcus aureus*: *in vitro* and *in vivo* studies. Toxicol Mech Meth 15:279–285

Rao RR, Platel K, Srinivasan K (2003) In vitro influence of spices and spice-active principles on digestive enzymes of rat pancreas and small intestine. Food 47:408–412

Regulation (EC) No. 1831/2003 of the European parliament and of the council of 22 September 2003 on additives for use in animal nutrition. Off J Eur Union L 268:29–43

Reisinger N, Steiner T, Nitsch S, Schatzmayr G, Applegate TJ (2011) Effects of a blend of essential oils on broiler performance and intestinal morphology during coccidial vaccine exposure. J Appl Poult Res 20:272–283

Schaedler RW (1973) The relationship between the host and its intestinal micro flora. Proc Nutr Soc 32:41–47

Si W, Gong J, Tsao R, Zhou T, Yu H, Poppe C, Johnson R, Du Z (2006) Antimicrobial activity of essential oils and structurally related synthetic food additives towards selected pathogenic and beneficial gut bacteria. J Appl Microbiol 100:296–305

Simitzis PE, Symeon GK, Charismiadou MA, Bizelis JA, Deligeorgis SG (2010) The effects of dietary oregano oil supplementation on pig meat characteristics. Meat Sci 84(4):670–676

Simsek UG, Çiftçi M, Dalkilic B, Guler T, Ertas ON (2007) The effects of dietary antibiotic and anise oil supplementation on body weight, carcass characteristics and organoleptic analysis of meat in broilers. Rev Med Vet 158:514–518

Spernakova D, Mate D, Rozanska H, Kovac G (2007) Effects of dietary rosemary extract and α-tocopherol on the performance of chickens, meat quality, and lipid oxidation in meat storage under chilling conditions. Bull Wet Inst Pulawy 51:585–589

Sulabo RC, Yacela JY, DeRouchey JM, Tokach MD, Neher F, Goodband RD, Dritz SS, Nelssen JL (2007) Effects of phytobiotics on nursery pig performance. J Anim Sci 85(Suppl 1):644

Tiihonen K, Kettunen H, Bento MHL, Saarinen M, Lathinen S, Ouwehand AC, Schulze H, Rautonen N (2010) The effect of feeding essential oils on broiler performance and gut microbiota. Brit Poult Sci 51:381–392

Toghyani M, Tohidi M, Gheisari AA, Tabeidian SA (2010) Performance, immunity, serum biochemical and hematological parameters in broiler chicks fed dietary thyme as alternative for an antibiotic growth promoter. Afr J Biotechnol 9:6819–6825

Tsirtsikos P, Fegeros K, Kominakis A, Balaskas C, Mountzouris KC (2012) Modulation of intestinal mucin composition and mucosal morphology by dietary phytogenic inclusion level in broilers. Animal 6(7):1049–1057

Ulfah M (2006) Essential oils as multi-function feed additive (MFA) to improve broilers performance, metabolism, dung consistency and efficiency of production. J Agric Rural Dev Trop Subtrop 88:50–55

Ultee A, Bennink MHJ, Moezelaar R (2002) The phenolic hydroxyl group of carvacrol is essential for action against the food-borne pathogen *Bacillus cereus*. Appl Environ Microbiol 68:1561–1568

Wei A, Shibamoto T (2007) Antioxidant activities and volatile constituents of various essential oils. J Agric Food Chem 55:737–1742

Windisch W, Schedle K, Plitzner C, Kroismayr A (2008) Use of phytogenic products as feed additives for swine and poultry. J Anim Sci 86(E. Supp):E 140–E 148

Young JF, Stagsted J, Jensen SK, Karlsson AH, Heckel P (2003) Ascorbic acid α-tocopherol and oregano supplements reduce stress induced deterioration of chicken meat quality. Poult Sci 82:1343–1351

Zentner E, Steiner T, Padoan D (2012) Impact of essential oils on ammonia and odor emissions of growing-finishing pigs. In: 4th European symposium of porcine health management, Brussels, 25–27 Apr 2012

Zitterl-Eglseer K, Wetscherek W, Stoni A, Kroismayr A, Windisch W (2008) Bioavailability of essential oils of a phytobiotic feed additive and impact of performance and nutrient digestibility in weaned piglets. Die Bodenkultur 59:1–4

Chapter 21
Multipurpose Herbs: Hidden Potentials and Dangers in the Garden

Ágnes Farkas

Abstract Several cultivated plants are used in a variety of ways: as ornamentals, spices, food plants, as well as medicinal plants. Other decorative plants may be dangerous if ingested or applied externally, due to their toxic compounds. Although the active principles and biological activities of several ornamentals are supported by scientific evidence, there is a growing need for further research. This chapter provides a brief overview of some multipurpose herbs, discussing ornamentals as edible and medicinal plants (e.g. *Lavandula*, *Mesembryanthemum*, *Punica*, *Salvia* etc.), their active compounds and physiological effects. Potential toxicity of ornamental plants is also discussed, highlighting plants containing cardiac glycosides (*Digitalis*, *Urginea*) and alkaloids (*Taxus*, *Datura*, *Laburnum*).

Keywords Antioxidant • Alkaloid • Bioactive • Edible • Glycoside • Ornamental • Poisonous • Polyphenol • Toxic

1 Introduction

Several plant species cultivated in our surroundings can be used for multiple purposes: they may serve as ornamentals, spices, food plants and medicinal herbs at the same time. Others, although appreciated for their aesthetic value and in some cases used by the pharmaceutical industry, may be dangerous if ingested or applied externally, due to their toxic compounds. Researchers all over the world strive to shed light on the hidden potentials – either beneficial or harmful – of garden plants, but there are still a large number of plant taxa that have to be investigated in depth. This chapter provides a brief overview of some multi-sided plants that are used for additional purposes besides decorating our homes and surroundings.

Á. Farkas (✉)
Department of Pharmacognosy, Medical School, University of Pécs, Pécs, Hungary
e-mail: agnes.farkas@aok.pte.hu

Á. Máthé (ed.), *Medicinal and Aromatic Plants of the World*, Medicinal and Aromatic Plants of the World 1, DOI 10.1007/978-94-017-9810-5_21

2 Ornamentals as Edible and Medicinal Plants

Lavenders (*Lavandula* sp.), oreganos (*Origanum* sp.), pot marigolds (*Calendula officinalis*) and sages (*Salvia* sp.) are widely known examples for plants used both for their aesthetic value and healing effect. Frequently, various parts of these herbs are also edible, most often used as spices. Plants with edible ornamental flowers include the genera *Chrysanthemum*, *Dianthus*, *Hemerocallis*, *Lavandula*, *Rosa* and *Tulipa*, as well as the species *Agastache foeniculum*, *Begonia* x *tuberhybrida*, *Calendula officinalis*, *Syringa vulgaris*, *Tagetes patula*, *Tropaeolum majus* and *Viola* x *wittrockiana* (Mlcek and Rop 2011). Examples for plants whose below-ground organs are used for food and medical purposes, and are cultivated also for their attractive flowers, include canna (*Canna edulis*) (Mishra et al. 2012) and turmerics (*Curcuma* sp.) (Prabhakaran Nair 2013).

A recent review on the health properties of Italian food plants concluded that several edible plants are promising for their potential health benefits. Some of these plants can also be grown as ornamentals. A notable example is *Phyteuma spicatum*, whose young leaves are consumed raw or cooked, is planted for its decorative value, and is used as a medicinal plant due to its tonic properties. However, it has never been investigated pharmacologically or biochemically and is a good candidate for future research (Guarrera and Savo 2013).

Punica granatum is another well-known representative of multipurpose plants. The juice pressed from the fruits is a popular drink in the Mediterranean region. At the same time the plant is frequently cultivated for decorative purposes, due to its flame-colored flowers, the extracts of which were shown to have antihyperglycemic and hypolipidemic effect (Bagri et al. 2009; Bhaskar and Kumar 2012). Various plant parts, including the flowers, fruits and leaves were shown to possess antioxidant activity (Ricci et al. 2006; Borochov-Neori et al. 2009; Celik et al. 2009; Bhaskar and Kumar 2012; Bekir et al. 2013). The fruits are highly valued for reducing the risk of cardiovascular disorders, which has been attributed to the exceptionally high antioxidant capacity, which in turn can be associated with the remarkably high content and unique composition of phenolic compounds (Borochov-Neori et al. 2009). Similarly, *Mesembryanthemum edule* has long been used in traditional medicine, as well as for food and ornamental purposes. The edible part is the fruit pulp, while the stems and leaves were found to exhibit strong antioxidant activity, which can be attributed to high levels of polyphenolic compounds (Falleh et al. 2011).

2.1 Bioactive Principles and Biological Activities of Ornamentals

Considerable amount of research has been carried out regarding the biologically active principles of ornamental plants (e.g. Yamagishi et al. 2010; Joselin et al. 2012), as well as their pharmacological effects, including antioxidant, anti-inflammatory and antimicrobial activities (e.g. Wetwitayaklung et al. 2008; Joselin et al. 2012; Orhan et al. 2012).

Héthelyi et al. (1987) and Vasudevan et al. (1997) reported that various species and chemovariants of the widely planted marigolds (*Tagetes* sp.) differed in the quality and quantities of active components. Polythienils, which are responsible for nematocidal activity, were detected in considerable amounts in the roots; whereas essential oils of the leaf and flower were dominated by terpenoids with repellent and biocidal activity. Marigold flowers are rich natural sources of carotenoids, widely used to extract lutein esters. A recent study found that even the extraction residue exhibited considerable antioxidant activity, which was significantly affected by the content of total phenolics and total flavonoids (Gong et al. 2012). Variation between cultivars in flavonoid components and radical scavenging activity has been reported also for rose petal teas (Vinokur et al. 2006) and edible chrysanthemum flowers (Sugawara and Igarishi 2009).

Kushad et al. (2004) detected 15 glucosinolates in varying concentrations in seven ornamental cabbage and six ornamental kale cultivars (*Brassica oleracea* var. *acephala*). Total glucosinolates were generally higher in ornamental cabbage than in ornamental kale, and significant varietal differences were measured within each *Brassica* group.

Our previous study (Böszörményi et al. 2009) compared the essential oil content and composition of two sage species, *Salvia judaica* and *S. officinalis*, and three horticultural varieties of the latter species. The essential oil yield of variety 'Kew Gold' was similarly high as in *S. officinalis*, whereas the other two varieties and *S. judaica* produced significantly lower volumes of essential oil. Each of the three ornamental varieties contained less α-thujone than *S. officinalis*, which is remarkable because high doses of thujone are considered as toxic (Lachenmeier and Uebelacker 2010). While the volatile compounds in the four *S. officinalis* taxa varied only in their ratio, the essential oil composition of *S. judaica* differed substantially from all other investigated taxa, having β-cubebene and ledol as its main compounds. The comparative analysis revealed that variety 'Kew Gold' can be recommended as an alternative of *S. officinalis*, considering both the yield and composition of essential oil (Farkas 2011).

Various ornamentals were shown to be rich sources of carotenoids, which are valued for their antioxidant properties. Lutein was determined as the main compound in *Narcissus pseudonarcissus* (Valadon and Mummery 1968) and *Tagetes erecta* (Moehs et al. 2001); antheraxanthin, violaxanthin, lutein, β-carotene and capsanthin were detected in *Lilium* species (Yamagishi et al. 2010); zeaxanthin, β- and ζ-carotene in *Crocus sativus* (Castillo et al. 2005); and astaxanthin in *Adonis aestivalis* (Neamtu et al. 1966; Cunningham and Gantt 2011).

Sabandar et al. (2013) have reviewed the bioactive components and pharmacological activities of the genus *Jatropha*, whose representatives are widely used in traditional medicine, and employed as ornamentals and energy crops. A variety of compounds, including cyclic peptides, alkaloids and diterpenes have been reported from this genus. Extracts and pure compounds of various taxa have been found to possess cytotoxic, antimicrobial, antiprotozoal, anticoagulant, immunomodulating, anti-inflammatory and antioxidant activities.

Snapdragon (*Antirrhinum majus*) seeds were found to be a good source of oil, containing neutral lipids in the highest amounts, followed by glycolipids and phospholipids. Snapdragon seed oil is characterized by high levels of linoleic and oleic acid, phytosterols and tocopherols. The radical scavenging activity of *A. majus* oil was found to be higher than that of extra virgin olive oil (Ramadan and El-Shamy 2013).

Recently, the leaves and bulbs of more than 100 ornamental varieties of daffodil (*Narcissus*) have been screened for their galanthamine content as well as their acetylcholinesterase inhibitory activity. Significant varietal differences were detected in the levels of the alkaloids galanthamine and sanguinine (Torras-Claveria et al. 2013).

Due to high demands for plants with both medicinal and ornamental potential, some of these species might become endangered and vulnerable, which might necessitate the implementation of various methods for conservation. Along these lines, micropropagation of several genera, including *Agapanthus*, *Albuca*, *Aloe*, *Crinum*, *Gloriosa*, *Hypoxis*, *Podocarpus*, *Salvia*, *Sandersonia* and *Scilla* has been elaborated (Finnie and van Staden 1989; Appleton and van Staden 1995; McCartan and van Staden 1998; Abrie and van Staden 2001; Fennel et al. 2001; Kowalski and van Staden 2001; Chukwujekwu et al. 2002; Huang and van Staden 2002; Bairu et al. 2007; Ascough and van Staden 2010; Grace 2011; Moyo et al. 2011; Baskaran and van Staden 2013).

3 Toxicity of Medicinal/Ornamental Plants

Numerous ornamental plants are rich in bioactive compounds with dose-dependent physiological effect: low doses are suitable for therapeutic purposes, while high doses exert a toxic effect. Frequently, the therapeutic range of such drugs is narrow, having little difference between the therapeutic and the toxic dose. Poisonings can be avoided by administering only the standardized products of the pharmaceutical industry under medical supervision and quitting the practice of using these drugs as home remedies.

Intoxication may result also from the lack of botanical knowledge: e.g. edible wild garlic (*Allium ursinum*) can be mixed up with cardiac glycoside containing lily-of-the-valley (Frohne and Pfänder 1997) or meadow saffron (*Colchicum autumnale*), which is a rich source of the highly toxic alkaloid colchicine (Klintschar et al. 1999; Sundov et al. 2005; Wollersen et al. 2009).

Children are particularly exposed to the danger of plant poisoning, partly due to their innate curiosity towards everything in their surroundings, and partly due to the fact that most adults, including child-care workers, are not familiar with toxic plants. Our survey conducted in 15 Hungarian child-care facilities revealed that potentially poisonous plants were present in all of the studied institutions, their proportion ranging from 7 to 20 % and 8 to 32 % in nurseries and kindergartens, respectively. The majority of toxic plants were shrubs, like common privet (*Ligustrum vulgare*), snowberry (*Symphoricarpus rivularis*), firethorn (*Pyracantha coccinea*), rockspray cotoneaster (*Cotoneaster horizontalis*), various junipers (*Juniperus sabina*, *J. virginiana*) and arborvitae species (*Thuja orientalis*, *Th. occidentalis*). In single cases specimens of common yew (*Taxus baccata*), common mistletoe (*Viscum album*) and golden chain (*Laburnum anagyroides*) were also found (Farkas et al. 2009; Farkas 2011).

Accidental ingestion of various parts or other exposures to ornamental plants are among the leading causes of plant poisoning cases. In Hungary, in the period 2005–2011, 77 % (711 cases) of plant poisonings could be attributed to garden ornamentals like *Taxus baccata* (141 cases), *Convallaria majalis* (92), *Brugmansia suaveolens* (43), *Ligustrum vulgare* (39) and *Laburnum anagyroides* (26) (Knapp 2013). During the period 1966–1994 the Swiss Toxicology Information Center registered nearly 24,950 cases of contact with or ingestion of toxic plant material, and severe plant poisonings occurred in 152 cases. From the 24 plants involved, 14 species are frequently cultivated as ornamentals, including *Atropa belladonna*, *Brugmansia suaveolens*, *Dieffenbachia*, *Heracleum mantegazzianum*, *Laburnum anagyroides*, *Narcissus pseudonarcissus*, *Nerium oleander* and *Ricinus communis* (Jaspersen-Schib et al. 1996).

3.1 Plants Containing Cardiac Glycosides

Cardiac glycosides, used to treat heart failure, are frequently extracted from various foxgloves (*Digitalis* sp.), which at the same time are widely cultivated as ornamentals. To a lesser extent, the active compounds of other popular ornamentals, such as lily-of-the-valley (*Convallaria majalis*), sea squill (*Urginea maritima*), pheasant's eye (*Adonis vernalis*), oleander (*Nerium oleander*) and various hellebores (*Helleborus* sp.) are used in the treatment of cardiac failure, particularly in traditional medicine. However, these plants are poisonous if ingested or used inappropriately as tea drugs.

Aqueous extracts of *Urginea sanguinea* are traditionally used in Africa to treat a number of ailments, such as venereal diseases, abdominal pain, backache and hypertension; while the bulbs are used as expectorants, emetics, diuretics and heart tonics. Traditional healers are aware of the plant's toxicity, and prescribe low doses, which should not cause toxic symptoms. However, patients often use larger doses than prescribed, which may result in poisoning. The active principle of *U. sanguinea* was identified as scillaren A (transvaalin), a bufadienolide cardiac glycoside with a digitalis-like action (Marx et al. 2005). The same active compound can be detected in other *Urginea* species, like *U. maritima*, which is used in treatment of heart failure throughout Europe. Intoxication is the result of applying this drug inappropriately as a home remedy, in which case the required doses cannot be set properly.

3.2 Plants Containing Alkaloids

Data of the Hungarian Health Toxicological Information Service (http://www.okbi.hu/index.php/en/htis) reveal that the active principles of plants responsible for the largest number of poisonings belong to the class of alkaloids. The most frequently involved ornamentals include European yew (*Taxus baccata*, Taxaceae), angel's trumpet (*Brugmansia* and *Datura* species, Solanaceae) and golden chain (*Laburnum anagyroides*, Fabaceae). Similarly, the American Association of Poison Control Centers recorded various *Datura* species implicated in the highest number of plant exposures with a fatal outcome (Krenzelok and Mrvos 2011).

Various thorn-apples (*Datura* sp.) are rich sources of the tropane alkaloids atropine and scopolamine, which are applied in medicine as anticholinergic agents. Particularly the large-flowered species like *D. innoxia* and *D. metel* are popular ornamentals, being potential sources of intoxication if any plant part is consumed either intentionally or accidentally (Hanna et al. 1992; Birmes et al. 2002; Gupta et al. 2003; Steenkamp et al. 2004; Diker et al. 2007; Wiebe et al. 2008; Krenzelok 2010). Alkaloid content and composition largely varies depending on the *Datura* species and variety, the plant part and the season (Miraldi et al. 2001; Berkov et al. 2006; Doncheva et al. 2006; Iranbakhsh et al. 2006; Jakabová et al. 2012). We demonstrated that atropine and scopolamine are present not only in various plant parts, but also in the nectar produced by the flowers (Boros et al. 2010).

Taxus species like European yew (*T. baccata*) and Japanese yew (*T. cuspidata*) are widely used ornamental plants. Most people are not aware of their cardiac toxicity, due to the presence of taxines, which are diterpenoid alkaloids. There are reports on both fatal and non-fatal (Jambeih et al. 2012) taxine poisonings resulting from ingestion of various plant parts, most typically the leaves.

All parts, especially the bark and seeds of laburnum/golden chain (*Laburnum anagyroides*) contain the pyridine-like alkaloid cytosine, which exhibits pharmacological effects similar to nicotine. There are numerous reports of people getting poisoned with laburnum seeds, but serious (fatal) cases of poisoning are rare (Musshoff and Madea 2009).

4 Conclusion

The interest in multipurpose plants is on the rise: more and more species are used for their decorative value and simultaneously as food and/or medicinal plants. However, there are still numerous ornamental plant taxa which have to be investigated with regard to their active principles and biological activities. Phytochemical and pharmacological evidence will reveal if a certain plant can be applied in areas other than its traditional use, or should be avoided due to its toxic compounds. The examples provided in this chapter can direct our attention to the manifold – either beneficial or harmful – potential of plants surrounding us.

Acknowledgments Thanks are due to the Hungarian Health Toxicological Information Service for providing information on plant poisoning cases.

References

Abrie AL, van Staden J (2001) Micropropagation of the endangered *Aloe polyphylla*. Plant Growth Regul 33:19–23
Appleton MR, van Staden J (1995) Micropropagation of some South African *Hypoxis* species with medicinal and horticultural potential. Acta Horticult 420:75–77
Ascough GD, van Staden J (2010) Micropropagation of *Albuca bracteata* and *A. nelsonii* – Indigenous ornamentals with medicinal value. S Afr J Bot 76:579–584
Bagri P, Ali M, Aeri V, Bhowmik M, Sultana S (2009) Antidiabetic effect of *Punica granatum* flowers: effect on hyperlipidemia, pancreatic cells lipid peroxidation and antioxidant enzymes in experimental diabetes. Food Chem Toxicol 47:50–54
Bairu MW, Stirk WA, Doležal K, van Staden J (2007) Optimising the micropropagation protocol for the endangered *Aloe polyphylla*: can metatopolin and its derivatives serve as replacement for benzyladenine and zeatin? Plant Cell Tiss Org Cult 90:15–23
Baskaran P, van Staden J (2013) Rapid *in vitro* micropropagation of *Agapanthus praecox*. S Afr J Bot 86:46–50
Bekir J, Mars M, Souchard JP, Bouajila J (2013) Assessment of antioxidant, anti-inflammatory, anti-cholinesterase and cytotoxic activities of pomegranate (*Punica granatum*) leaves. Food Chem Toxicol 55:470–475. doi:10.1016/j.fct.2013.01.036
Berkov S, Zayed R, Doncheva T (2006) Alkaloid patterns in some varieties of *Datura stramonium*. Fitoterapia 77:179–182
Bhaskar A, Kumar A (2012) Antihyperglycemic, antioxidant and hypolipidemic effect of *Punica granatum* L flower extract in streptozotocin induced diabetic rats. Asian Pacific J Trop Biomed 2012:1764–1769
Birmes P, Chounet V, Mazerolles M, Cathala B, Schmitt L, Lauque D (2002) Self-poisoning with *Datura stramonium* – 3 case reports. Presse Med 31:69–72
Borochov-Neori H, Judeinstein S, Tripler E, Harari M, Greenberg A, Shomer I, Holland D (2009) Seasonal and cultivar variations in antioxidant and sensory quality of pomegranate (*Punica granatum* L.) fruit. J Food Comp Anal 22:189–195. doi:10.1016/j.jfca.2008.10.011
Boros B, Farkas Á, Jakabová S, Bacskay I, Kilár F, Felinger A (2010) LC-MS quantitative determination of atropine and scopolamine in the floral nectar of *Datura* species. Chromatographia 71:43–49. doi:10.1365/s10337-010-1524-y
Böszörményi A, Héthelyi É, Farkas Á, Gy H, Papp N, Szőke É, Lemberkovics É (2009) Chemical and genetic relationship among sage (*Salvia officinalis* L.) cultivars and Judean sage (*Salvia judaica* Boiss.). J Agric Food Chem 57:4663–4667
Castillo JA, Fernández L, Gómez-Gómez L (2005) Implications of carotenoid biosynthetic genes in apocarotenoid formation during the stigma development of *Crocus sativus* and its closer relatives. Plant Physiol 139:674–689
Celik I, Temur A, Isik I (2009) Hepatoprotective role and antioxidant capacity of pomegranate (*Punica granatum*) flowers infusion against trichloroacetic acid-exposed in rats. Food Chem Toxicol 47:145–149
Chukwujekwu JC, Fennel CW, van Staden J (2002) Optimization of the tissue culture protocol for the endangered *Aloe polyphylla*. S Afr J Bot 68:424–429
Cunningham FX Jr, Gantt E (2011) Elucidation of the pathway to astaxanthin in the flowers of *Adonis aestivalis*. Plant Cell 23:3055–3069. doi:10.1105/tpc.111.086827
Diker D, Markovitz D, Rothman M, Sendovski U (2007) Coma as a presenting sign of *Datura stramonium* seed tea poisoning. Eur J Int Med 18:336–338

Doncheva T, Berkov S, Philipov S (2006) Comparative study of the alkaloids in tribe *Datureae* and their chemosystematic significance. Biochem Syst Ecol 34:478–488

Falleh H, Ksouri R, Medini F, Guyot S, Abdelly C, Magné C (2011) Antioxidant activity and phenolic composition of the medicinal and edible halophyte *Mesembryanthemum edule* L. Ind Crop Prod 34:1066–1071

Farkas Á (2011) Friends or foes? Ornamentals – medicinal plants – poisonous plants. Acta Horticult 925:43–48

Farkas Á, Kapitány E, Szalai R, Molnár R, Pál R, Salamon-Albert É, Rudolf K (2009) Mérgező növények és gombák a pécsi bölcsődékben és óvodákban. Congressus Pharmaceuticus Hungaricus XIV, Budapest, 13–15 Nov 2009, Gyógyszerészet 2009/11. Suppl I. S. 107–108

Fennel CW, Crouch NR, van Staden J (2001) Micropropagation of the river lily, *Crinum variabile* (Amaryllidaceae). S Afr J Bot 67:74–77

Finnie JF, van Staden J (1989) *In vitro* propagation of *Sandersonia* and *Gloriosa*. Plant Cell Tiss Org Cult 19:151–158

Frohne D, Pfänder HJ (1997) Giftpflanzen. Wissenschaftliche Verlagsgesellschaft, Stuttgart

Gong Y, Hou Z, Gao Y, Xue Y, Liu X, Liu G (2012) Optimization of extraction parameters of bioactive components from defatted marigold (*Tagetes erecta* L.) residue using response surface methodology. Food Bioprod Process 90:9–16. doi:10.1016/j.fbp.2010.12.004

Grace OM (2011) Current perspectives on the economic botany of the genus *Aloe* L. (Xanthorrhoeaceae). S Afr J Bot 77:980–987

Guarrera PM, Savo V (2013) Perceived health properties of wild and cultivated food plants in local and popular traditions of Italy: a review. J Ethnopharmacol 146:659–680. doi:10.1016/j.jep.2013.01.036

Gupta SK, Peshin SS, Srivastava A, Kaleekal T (2003) A study of childhood poisoning at National Poisons Information Centre, All India Institute of Medical Sciences, New Delhi. J Occup Health 45:191–196

Hanna JP, Schmidley JW, Braselton WE (1992) *Datura* delirium. Clin Neuropharmacol 15:109–113

Héthelyi É, Dános B, Tétényi P, Juhász G (1987) Phytochemcial studies on *Tagetes* species, infraspecific differences of the essential oil in *T. minuta* and *T. tenuifolia*. Herba Hung 26:145–158

Huang LD, van Staden J (2002) *Salvia chamelaeagnea* can be micropropagated and its callus induced to produce rosmarinic acid. S Afr J Bot 68:177–180

Iranbakhsh A, Oshaghi MA, Majd A (2006) Distribution of atropine and scopolamine in different organs and stages of development in *Datura stramonium* L. (Solanaceae). Structure and ultrastructure of biosynthesizing cells. Acta Biol Cracov Ser Bot 48:13–18

Jakabová S, Vincze L, Farkas Á, Kilár F, Felinger A, Boros B (2012) Determination of tropane alkaloids atropine and scopolamine by liquid chromatography-mass spectrometry in plant organs of *Datura* species. J Chromatogr A 1232:295–301. doi:10.1016/j.chroma.2012.02.036

Jambeih RA, Shaheen WH, Li VY, Shaheen MH (2012) ST-segment elevation and ventricular tachycardia after ingestion of a common ornamental plant – a case report. Indian Heart J 6402:211–213

Jaspersen-Schib R, Theus L, Guirguis-Oeschger M, Gossweiler B, Meier-Abt PJ (1996) Serious plant poisonings in Switzerland 1966–94. Case analysis from the Swiss Toxicology Information Center. Schweiz Med Wochenschr 126:1085–1098

Joselin J, Brintha TSS, Florence AR, Jeeva S (2012) Screening of select ornamental flowers of the family Apocynaceae for phytochemical constituents. Asian Pac J Trop Dis 2(Suppl 1):260–264

Klintschar M, Beham-Schmict C, Radner H, Henning G, Roll P (1999) Colchicine poisoning by accidental ingestion of meadow saffron (*Colchicum autumnale*): pathological and medicolegal aspects. Forensic Sci Int 106:191–200

Knapp R (2013) Kerti dísznövények által okozott mérgezések Magyarországon 2005 és 2011 között (Plant poisonings caused by garden ornamentals in Hungary, from 2005 to 2011). DrPharm thesis, Pécs

Kowalski B, van Staden J (2001) Micropropagation of *Podocarpus henkelii* and *P. elongates*. S Afr J Bot 67:362–366

Krenzelok EP (2010) Aspects of *Datura* poisoning and treatment. Clin Toxicol 48:104–110

Krenzelok EP, Mrvos R (2011) Friends and foes in the plant world: a profile of plant ingestions and fatalities. Clin Toxicol 49:142–149. doi:10.3109/15563650.2011.568945

Kushad MM, Cloyd R, Babadoost M (2004) Distribution of glucosinolates in ornamental cabbage and kale cultivars. Sci Hortic 101:215–221. doi:10.1016/j.scienta.2003.10.011

Lachenmeier DW, Uebelacker M (2010) Risk assessment of thujone in foods and medicines containing sage and wormwood – evidence for a need of regulatory changes? Regul Toxicol Pharmacol 58:437–443

Marx J, Pretorius E, Espag WJ, Bester MJ (2005) *Urginea sanguinea*: medicinal wonder or death in disguise? Environ Toxicol Pharmacol 20:26–34

McCartan SA, van Staden J (1998) Micropropagation of the medicinal plant, *Scilla natalensis* Planch. Plant Growth Regul 25:177–180

Miraldi E, Masti A, Ferri S, Barni Comparini I (2001) Distribution of hyoscyamine and scopolamine in *Datura stramonium*. Fitoterapia 72:644–648

Mishra T, Das AP, Sen A (2012) Phytochemical screening and *in-vitro* antioxidant profiling of solvent fractions of *Canna edulis* Ker Gawler. Free Radicals Antioxid 2:13–20. doi:10.5530/ax.2012.2.1

Mlcek J, Rop O (2011) Fresh edible flowers of ornamental plants – a new source of nutraceutical foods. Trends Food Sci Technol 22:561–569. doi:10.1016/j.tifs.2011.04.006

Moehs CP, Tian L, Osteryoun KW, Dellapenna D (2001) Analysis of carotenoid biosynthetic gene expression during marigold petal development. Plant Mol Biol 45:281–293

Moyo M, Bairu MW, Amoo SO, van Staden J (2011) Plant Biotechnology in South Africa: micropropagation research endeavours, prospects and challenges. S Afr J Bot 77:996–1011. doi:10.1016/j.sajb.2011.06.003

Musshoff F, Madea B (2009) Fatal cytosine intoxication and analysis of biological samples with LC/MS-MS. Forensic Sci Int 186:e1–e4

Neamtu G, Tamas V, Bodea C (1966) Die carotinoide aus einigen *Adonis*-Arten. Rev Roum Biochim 3:305–310

Orhan IE, Erdem SA, Senol FS, Kartal M, Sener B (2012) Exploration of cholinesterase and tyrosinase inhibitory, antiprotozoal and antioxidant effects of *Buxus sempervirens* L. (boxwood). Ind Crop Prod 40:116–121. doi:10.1016/j.indcrop.2012.03.004

Prabhakaran Nair KP (2013) The agronomy and economy of turmeric and ginger. Elsevier, Amsterdam/Tokyo

Ramadan MF, El-Shamy H (2013) Snapdragon (*Antirrhinum majus*) seed oil: characterization of fatty acids, bioactive lipids and radical scavenging potential. Ind Crop Prod 42:373–379. doi:10.1016/j.indcrop.2012.06.022

Ricci D, Giamperi L, Bucchini A, Fraternale D (2006) Antioxidant activity of *Punica granatum* fruits. Fitoterapia 77:310–312. doi:10.1016/j.fitote.2006.01.008

Sabandar CW, Ahmat N, Jaafar FM, Sahidin I (2013) Medicinal property, phytochemistry and pharmacology of several *Jatropha* species (Euphorbiaceae): a review. Phytochemistry 85:7–29. doi:10.1016/j.phytochem.2012.10.009

Steenkamp PA, Harding NM, van Heerden FR, van Wyk BE (2004) Fatal *Datura* poisoning: identification of atropine and scopolamine by high performance liquid chromatography/photodiode array/mass spectrometry. Forensic Sci Int 145:31–39

Sugawara T, Igarishi K (2009) Cultivar variation in flavonoid components and radical scavenging activity of polyphenol fractions among edible chrysanthemum flowers. J Jpn Soc Food Sci Technol 56:600–604

Sundov Z, Nincevic Z, Definis-Gojanovic M, Glavina-Durdov M, Jukic I, Hulina N, Tonkic A (2005) Fatal colchicine poisoning by accidental ingestion of meadow saffron – case report. Forensic Sci Int 149:253–256

Torras-Claveria L, Berkov S, Codina C, Viladomat F, Bastida J (2013) Daffodils as potential crops of galanthamine. Assessment of more than 100 ornamental varieties for their alkaloid content and acetylcholinesterase inhibitory activity. Ind Crop Prod 43:237–244. doi:10.1016/j.indcrop.2012.07.034

Valadon LRG, Mummery RS (1968) Carotenoids in floral parts of a narcissus, a daffodil and a tulip. Biochem J 106:479–484

Vasudevan P, Kashyap S, Sharma S (1997) *Tagetes*: a multipurpose plant. Bioresour Technol 62:29–35

Vinokur Y, Rodov V, Reznick N, Goldman G, Horev B (2006) Rose petal tea as antioxidant rich beverage: cultivar effects. J Food Sci 71:42–47

Wetwitayaklung P, Phaechamud T, Limmatvapirat C, Keokitichai S (2008) The study of antioxidant activities of edible flower extracts. Acta Horticult 786:185–191

Wiebe TH, Sigurdson ES, Katz LY (2008) Angel's trumpet (*Datura stramonium*) poisoning and delirium in adolescents in Winnipeg, Manitoba: summer 2006. Paediatr Child Health 13:193–196

Wollersen H, Erdmann F, Risse M, Dettmeyer R (2009) Accidental fatal ingestion of colchicine-containing leaves – toxicological and histological findings. Leg Med 11:S498–S499

Yamagishi M, Kishimoto S, Nakayama M (2010) Carotenoid composition and changes in expression of carotenoid biosynthetic genes in tepals of Asiatic hybrid lily. Plant Breed 129:100–107

Glossary: With Selected Important Abbreviations

2,4-D *2,4-dichlorophenoxy acetic acid* is a synthetic auxin, a systemic herbicide, commonly used to control broadleaf weeds. It is also frequently used growth regulator in plant research, *in vitro* plant experiments

2D spectrum The result of Two-Dimensional Nuclear Magnetic Resonance Spectroscopy (2D NMR), where the observed data are plotted in a space defined by two frequency axes

2ip *2-isopentenyladenine* is a cytokinin used in plant biotechnology and *in vitro* plant micropropagation

ABI Database See: ABI/INFORM Global (ProQuest) database

ABI/INFORM Global (ProQuest) A comprehensive and diverse business database connecting business researchers with information needed in a faster way

ACT Artemisinin Combination Therapy is a recommended treatment for *Plasmodium falciparum* that causes malaria worldwide, since the effectiveness of chloroquine for *P. vivax* declines. Alternative therapies include ACTs, as they are also effective against *P. vivax* thus they could become the standard treatment for all forms of malaria

AFLP or AFLP-PCR Abbreviation for Amplified Fragment Length Polymorphism: a polymerase chain reaction (PCR) based technology in molecular biology used in DNA fingerprinting and genetic engineering.

AGPs Antibiotic Growth Promoters are antibiotics used in animal diets as growth promoters and in order to achieve better feed conversion and higher growth rates mostly by reducing the activity of microbiota in the digestive tract

AGRICOLA A unified hub for information covering all aspects of agriculture. It provides access to bibliographic records (journal articles, book chapters, theses, etc.) relating to the field of agriculture from the U.S. Department of Agriculture's National Agricultural Library (NAL)

AGRIS (FAO) International System for Agricultural Science and Technology is a global public database providing access to bibliographic information on agricultural science and technology. AGRIS covers all areas of interest to FAO, including food, nutrition, agriculture, fisheries, forestry, environment etc. and is

Á. Máthé (ed.), *Medicinal and Aromatic Plants of the World*, Medicinal and Aromatic Plants of the World 1, DOI 10.1007/978-94-017-9810-5

to improve the access and exchange of information serving the information-related needs of developed and developing countries on a partnership basis

APCI Atmospheric-pressure Chemical Ionization is a type of chemical ionization used in mass spectrometry (LC-MS) that vaporizes solvent and sample molecules by spraying the sample solution into a heater (heated to about 400 °C) using a gas, such as N_2.

ARE Antioxidant Response Elements (AREs) mediate the transcriptional induction of a battery of genes which comprise much of this chemoprotective response system. The expression of genes encoding antioxidative and Phase II detoxification enzymes is induced in cells exposed to electrophilic compounds and phenolic antioxidants. Induction of these enzymes is regulated at the transcriptional level and is mediated by a specific enhancer. The antioxidant response element or ARE, is found in the promoter of the enzyme's gene

AYUSH Ayurveda, Yoga & Naturopathy, Unani, Siddha and Homoeopathy: successor to the Department of Indian Medicine and Homeopathy (ISM&H) created in 2003 with the aim to focus attention to development of Education and Research in Ayurveda, Yoga and Naturopathy, Unani, Siddha and Homoeopathy systems

B5 Gamborg et al. (1976) medium is used for *in vitro* plant culture of cell lines and tissues

BA 6-benzyladenine is a synthetic growth regulator used in *in vitro* plant culture. Its physiological effects include the regulation of cell division, stimulation of auxiliary and adventitious shoot proliferation, regulation of differentiation, inhibition of root formation, etc.

BCAS or NBCAS Biodiversity Conservation Area, or National Biodiversity Conservation Area denoting an environmentally protected area, in India, Laos, etc. NCASs have the main objectives: protect forests, wildlife and waters (a), maintenance of natural abundance and environmental stability (b), protection of natural beauty for leisure and research (c)

BELFRIT project An international project by the Belgian, French and Italian authorities to develop a common approach for the evaluation of botanicals

BRS Botanical Reference Substances (Standards) are authentic specimens chosen and verified on the basis of their suitability for intended use as prescribed in the Pharmacopoeia. These are authentic botanical reference materials to use in the development of internal quality control specifications. These materials are a necessity for laboratory testing of all botanical species.

CAB (CABI) Previously called Commonwealth Agricultural Bureaux, is a not-for-profit inter-governmental development and information organisation based in the United Kingdom that focuses primarily on agricultural and environmental issues in the developing world. CABI engages in projects that address agricultural and environmental issues worldwide. These focus on commodity crops, invasive species, and scientific communication. CABI is a leading global publisher producing key scientific publications, including the world renowned CAB Abstracts – the abstracting and indexing database in applied life sciences – as well as Compendia, books, eBooks and full text electronic resources.

CBD Convention on Biological Diversity (CBD) entered into force on 29 December 1993. It has three main objectives: The conservation of biological diversity (a), The sustainable use of the components of biological diversity (b), The fair and equitable sharing of the benefits arising out of the utilization of genetic resources (c). "Living in Harmony with Nature" the Strategic Plan for Biodiversity (2011–2020) will provide a flexible framework for the establishment of national and regional targets and for enhancing coherence in the implementation of the provisions of the Convention including the Global Strategy for Plant Conservation as well as the Nagoya Protocol on Access to Genetic Resources and the Fair and Equitable Sharing of the Benefits Arising from their Utilization.

CBME *Cannabis* Based Medicinal Extracts have recently become available for clinical research. Standardized CBMEs are claimed to have the potential to reduce previously intractable symptoms, including pain, spasms and spasticity in patients with MS and other neurological conditions with low levels of intoxication

CCD Charge-Coupled Device is a major form of technology in digital imaging. It is a device for the moving the electrical charge usually from within the device to an area where the charge can be manipulated (e.g.: converted into a digital value)

CCF II Second Country Cooperation Framework for the period 2001–2005 and UNDP Regional Cooperation Program for Europe, 2002–2005 of the United Nations Development Program (UNDP)

CEA Controlled Environment Agriculture is an advanced and intensive form of hydroponically-based agriculture, where plants are grown within a controlled environment so that horticultural practices can be optimized. Well-managed, CEA operations can provide fresh horticultural produce (including medicinal and aromatic plants) of high quality and free of agriculture chemicals

CITES Convention on International Trade in Endangered Species of Wild Fauna and Flora is an international agreement between governments with the aim to ensure that international trade in specimens (of wild animals and plants) does not threaten their survival. The text of the Convention was accepted in Washington, D.C., on 3 March 1973 and CITES entered into force on 1 July 1975.

Compendex (Ei/Elsevier) The broadest and most complete engineering literature database in the world that provides holistic and global view of peer reviewed and indexed publications (over 17 million records from 73 countries from 190 engineering disciplines)

CPMP Committee for Proprietary Medicinal Products is a former committee of the European Medicines Agency that was responsible for preparing opinions on questions concerning medicines for human use. It has been replaced by the Committee for Medicinal Products for Human Use (CHMP)

CRS Chemical Reference Standards elaborated to transfer accuracy as well as precision throughout large multi-laboratory measurement (networks)

CSA Sociological Abstracts a definitive and comprehensive sociology research database by ProQuest

DAD Diode Array Detector used to improve analytical capability in liquid chromatography. It permits qualitative information to be obtained beyond simple identification by retention time. It allows for the best wavelength(s) to be selected for actual analysis and is also helpful in determining if a peak represents a single compound or, is in fact, a composite peak

DNA *Deoxyribonucleic acid*. DNA carries most of the genetic information used in the development, functioning and reproduction of all known living organisms including many of the viruses

DOPA *3,4-Dihydroxy-L-phenylalanine* is a Natural isomer of the immediate precursor of dopamine; product of tyrosine hydroxylase

DOSY Diffusion Ordered Spectroscopy is a method used in NMR analysis of mixtures of organic chemicals. It is based on the different diffusion rates of chemicals through a solution and is directly related to the physical properties of the chemical components making up the mixture.

EEA European Economic Area that unites the EU Member States and the three EEA EFTA States (Iceland, Liechtenstein, and Norway) into an Internal Market governed by the same basic rules.

EMA European Medicines Agency is a decentralised agency of the European Union, located in London. EMA (formerly EMEA) is responsible for the scientific evaluation of medicines developed for use in the European Union. It began operating in 1995.

ESCOP European Scientific Cooperative on Phytotherapy was founded in June 1989. It is an umbrella organisation that represents national herbal medicine or phytotherapy associations in Europe, especially in their discussions with European medicines regulators. As a main activity, it publishes reviews (monographs) of the therapeutic use of leading herbal medicinal products, based on the latest evidence and on leading expertise across Europe.

ESI-IT-MS Electrospray Ionization – Ion Trap Tandem – Mass Spectrometry an ionization method used in the mass spectroscopy of biological macromolecules, especially proteins

ESRI methods ESRI is a Redlands, California based company that is engaged in developing GIS software used in various forms of imaging technology and management (e.g. spatial analysis, mapping and visualization, imaging and remote sensing, etc.)

EU European Union is an economic and political partnership between 28 European countries that covers a large part of the continent. It was created in 1958, in the aftermath of World War II. in the form of the European Economic Community (EEC) of Belgium, Germany, France, Italy, Luxembourg and the Netherlands. Its name was changed to EU in 1993.

Ex situ Latin word denoting out of position, the opposite of "*in situ*"

FairTrade International An international Fairtrade system made up of Fairtrade International and its member organizations. It represents the world's largest and most recognized fair trade system. Fair trade is an alternative approach to conventional trade and is based on a partnership between producers and traders, businesses and consumers

FairWild FairWild is a verification system that has offers a meaningful and comprehensive guidance framework and certification option for all sustainably collected wild plant, fungi and lichen species worldwide. Version 2.0 of the FairWild Standard covers both ecological sustainability and aspects of fair trade and social sustainability.

FAO Food and Agriculture Organization of the United Nations

FRLHT Foundation for Revitalization of Local Health Traditions, a Public Trust and Charitable Society, in India, founded in 1993. Its core activities include the conservation of threatened natural resources in use by Indian Systems of Medicine

FSTA (IFIS) An extensive specialist database. It contains carefully selected food, beverage and nutrition research and information data collated, summarised and indexed for efficient searching

FT-NIR approach Near-infrared spectroscopy (NIRS) is an analytical technique used in the pharmaceutical industry for the non-destructive analysis of dosage forms

GA(C)P Good Agricultural (Collection) Practice(s) is a combined abbreviation for GAP and GCP

GA_3 *Gibberellic acid* is a plant hormone that stimulates plant growth and development. It is a biologically active tetracyclic di-terpenoid compound stimulating seed germination, triggering transitions from meristem to shoot growth, juvenile to adult leaf stage, vegetative to flowering, determines sex expression and grain development, etc. GA_3 is known to be produced by the pathogenic fungus *Gibberella fujikuroi* from which three different types of gibberellins were extracted and characterized: $GA_{1,}$ GA_2 and GA_3

GAP Good Agricultural Practice(s) are a multiplicity of standards, codes and regulations developed to codify agricultural practices at farm level with the ultimate aim of ensuring safety and quality of produce in the food chain. Farther aims relate to market advantages, improving the use of natural resources, workers health and working conditions, etc.

GC-MS Gas Chromatography- Mass Spectrometry is used to separate chemical mixtures (the GC component) and to identify the components at a molecular level (the MS component). In the column of Gas Chromatograph filled with an inert gas (such as helium) a mixture is separated into individual substances, when heated. The separated substances flow into the Mass Spectrometer where compounds are identified by the mass of the molecules.

GCP Good Collection Practice(s) s are primarily intended to provide general technical guidance on obtaining medicinal plant materials of good quality suitable for the sustainable production of herbal products.

GEP Good Ethical Practice(s) is one of the guidelines used in the quality management (production, commerce) of medicinal and aromatic plant production.

GIS Geographical Information System is a basic data collection and decision support software system for precision plant production. It is a tool for thematic mapping in different layers in the same coordinate system, and therefore one can analyse the relationship of different characteristics in the field: between soil physical and chemical parameters and yield, topographic map and weed and pest distribution etc. The input data for GIS can be collected from soil and by different platforms (UAV's, airplanes or satellites etc.)

GLP Good Laboratory Practice(s) is one of the guidelines used in the quality management of medicinal and aromatic plant production and processing. Specifically it refers to a quality system of management controls for (research) laboratories

GMO Genetically Modified Organism(s) are living organisms whose genetic material has been modified (altered) using genetic engineering techniques. They are also known as transgenic organisms

GMP Good Manufacturing Practice(s) is one of the guidelines used in the quality management of medicinal and aromatic plant production and processing. Specifically it refers to a quality system of management controls for the manufacturing process so that products are consistently produced and controlled according to quality standards

GNSS Global Navigation Satellite Systems is a satellite system used to indicate (pinpoint) the geographic location of a user's receiver anywhere in the world

GPP Good Procurement Practice(s) is one of the guidelines used in the quality management of medicinal and aromatic plant production and processing. Specifically it refers to a quality system of management controls for the procurement of raw materials either of wild-crafted or cultivated origin

GPS Global Positioning System is a satellite aided positioning system for the determination of ground position of objects. It is the most important element of precision plant production. With the differential signal (DGPS) an accuracy of $\pm$1–3 m can be provided. It can be used for yield monitoring and mapping, weed mapping, variable rate fertilizing, variable rate irrigation, topography determination and defining boundaries etc.

GSE Grape Seed Extract is an industrial derivative of whole grape seeds. It is rich in antioxidants and oligomeric proanthocyanidin complexes (OPCs)

GSP Good Storage Practice(s) is one of the guidelines used in the quality management of medicinal and aromatic plant production and processing. Specifically it refers to a quality system of management controls in the process of storage, transport and distribution of plant substances and the conservation of medicinal and aromatic plants and their produce

HFR Heterocyclic Ring Fission or cleavage of the heterocyclic ring of molecules usually accomplished by using degradative procedures

HHPCL Healing Heritage Producers Company Ltd is a private company incorporated in India. It is a supplier and service provider of a wide range of herbal products

HMCP Committee on Herbal Medicinal Products is a committee of the European Medicines Agency that is responsible for preparing the Agency's opinions on herbal medicines (see: EMA)

HMP Herbal Medicinal Product, exclusively containing as active ingredients one or more herbal substances or one or more herbal preparations, or one or more such herbal substances in combination with one or more such herbal preparations (definition by EMA)

HPLC High performance liquid chromatography is an analytical chemistry technique used to separate, identify, and quantify each component in a mixture. A pressurized liquid solvent containing the sample mixture is pumped through a column filled with a solid adsorbent material. Each component of the sample interacts slightly differently with the adsorbent material, resulting in different flow rates of components and allowing for the separation of the components as they flow out the column. The use of various types of detectors allow for farther analytical options.

HPLC UV/ESI MS High-performance Liquid Chromatography coupled with ultra violet detectors (see: also HPLC, ESI-IT-MS, UV/ESI MS)

HPTLC High Performance Thin Layer Chromatography is a modern analytical tool for biological assays. It is equally suitable for qualitative and quantitative analytical tasks. In the form of visible chromatograms HPTLC produces complex information about the entire sample which is available at a glance

IAA *Indole-3-acetic acid* is a naturally-occurring, plant hormone of the auxin class. IAA is predominantly produced in cells of the apex (bud) and very young leaves of a plant, although several farther independent biosynthetic pathways are also known. Its plant physiological effects include inducing cell elongation and cell division (i.e.: plant growth and development).

IBA *Indole-3-butyric acid* is a plant hormone in the auxin family. As a plant growth regulator it is frequently an ingredient in many commercial horticultural plant rooting products.

ICMR Indian Council of Medical Research located in New Delhi (India) is the leading body in India dealing with the formulation, coordination and promotion of biomedical research

In situ Used for plants situated in the original, natural, or existing place or position. The opposite of *ex situ* (see: *ex situ*)

IPC Indian Pharmacopoeia Commission is an Autonomous Institution of the Ministry of Health and Family Welfare of the Government of India. IPC is created to set national standards of drugs

ISSC-MAP International Standard for Sustainable Wild Collection of Medicinal and Aromatic Plants that provides clear principles, criteria, indicators and verifiers for industry, resource managers, collectors and other stakeholders of MAPs to assess and monitor the sustainability of wild resources and collection practices. Under the auspices of FairWild Foundation it has been merged with the FairWild Standard version 1.0.

Kn *Kinetin* is a plant hormone, a type of cytokinin that promotes cell division. In plant tissue culture it is often used for inducing formation of callus (in conjunction with auxin) and to induce adventitious shoot tissues from callus

Lato senso A Latin word meaning “in the sense of”

LC PDA High-Performance Liquid Chromatography (HPLC) coupled with Photodiode Array detection

LC/MS High-Performance Liquid Chromatography (HPLC) coupled with Mass Spectrometry

LC/SPE NMR High-Performance Liquid Chromatography (HPLC) coupled with Solid Phase Extraction (SPE) and Nuclear Magnetic Resonance (NMR) spectroscopy

LEAF project Livelihoods, Empowerment and Agro-Forestry project a community agroforestry project in India that diversifies and sustains production for increased social, economic and environmental benefits for land users at all levels

Lipinsky's rule The rule of five by Lipinsky et al. on the physico-chemical properties of drugs according to which a compound is more likely to be membrane permeable and easily absorbed by the body if it matches certain criteria related to molecular weight, the compounds lipophilicity, the number of groups in the molecule and the number of groups that can accept hydrogen atoms to form hydrogen bonds

MAE Microwave Assisted Extraction is an extraction technique that combines microwave and traditional solvent extractions. Microwaves are applied for heating the solvents and plant tissues in the extraction process, which increases the kinetic of extraction. Its advantages include shorter extraction time, less solvent demand, higher extraction rate and lower cost

MAPs Abbreviation for medicinal and aromatic plants

MEDLINE (NLM) is the U.S. National Library of Medicine (NLM) premier bibliographic database

MeOH Methanol is a laboratory solvent. Methanolic extraction is commonly used plant analytics

MLWP Monographs and Lists Working Party on European Union Monographs and European Union List (MLWP) was established in 2006. It replaced the temporary Safety and Efficacy Drafting Group of the Committee on Herbal Medicinal Products (HMPC). Primarily it is engaged in carrying out the assessment in relation to the establishment of European Union herbal monographs and European Union list entries

MS Murashige and Skoog (1962) commonly used medium used in plant tissue cultures

N6 Chu (1976) nutrient medium used in plant tissue cultures

NAA *Naphthalene-1- acetic acid* is a growth regulator, a synthetic plant hormone of the auxin family. Used as a rooting agent it is an ingredient of commercial plant rooting horticultural products; It is also frequently used in the vegetative propagation (cloning) of plants from stem and leaf cuttings

NAL See: AGRICOLA

NAVSTAR GPS system Navstar is a network of U.S. satellites that provides global positioning system (GPS) services for navigation by both the military and civilians. It was developed by the U.S. Department of Defense (DoD). It is based on 24 satellites orbiting the Earth at a high altitude

NCCAM National Centre for Complementary and Alternative Medicine is the Federal Governments lead agency for scientific research on the diverse medical and health care systems, practices, and products that are not generally considered part of conventional medicine. It is one of the institutes of the US National Institute of Health (NIH)

NDVI Normalized Difference Vegetation Index is a graphical indicator used to analyze remote sensing measurements, typically but not necessarily from a space platform. It is used to assess whether the target observed contains live green vegetation or not.

NIH National Institute of Health of the United States is a part of the Department of Health and Human Services. It is the US medical research agency for making important discoveries that improve health and save lives

NMR Nuclear Magnetic Resonance (NMR) spectroscopy (see: LC/SPE NMR)

NOE Nuclear Overhauser Effect is a phenomenon observed by nuclear magnetic resonance (NMR) spectroscopy, when nuclear spin polarization from one nuclear spin population is transferred to another via cross-relaxation

PDA Photodiode-array Detector is used for obtaining spectral profiles from molecular mixtures or chromatographically separated samples. They provide ultra-fast, low noise spectral analysis; resolution determined by the number of diodes deployed over a specific wavelength range

PFA Phytogenic Feed Additives are feed additives of plant origin used in animal nutrition

PFG Pulsed Field Gradients (PFG) nuclear magnetic resonance (NMR) method is a well-established technique for studying molecular motion without disturbing the system under investigation

PFE Pressurized-Liquid Extraction or Pressurized Liquid Extraction (PLE) is a sample extraction method. It uses liquid solvents at elevated temperatures and pressures to prepare samples for gas chromatographic or liquid chromatographic analysis

PRA Participatory Rural Appraisal is a combination of approaches and methods that enable rural people to share, enhance and analyse their knowledge of life and conditions, to plan and act and to monitor and evaluate. In this process the role of the outsider is that of a catalyst, a facilitator of processes within a community which prepares them to alter their situation

RAPD Random Amplified Polymorphic DNA (RAPD) markers are DNA fragments from PCR amplification of random segments of genomic DNA with single primer of arbitrary nucleotide sequence.

RDA mechanisms Retro-Diels-Alder reaction is the microscopic reverse of the Diels–Alder reaction, the formation of a diene and dienophile from a cyclohexene

RRA technique Rapid Rural Appraisal is a flexible tool which can be used in a lot of different situations to achieve very different objectives. It usually involves collecting information by talking directly to people “on the ground” It uses a set of guidelines on how to approach the collection of information, learning from that information and the involvement of local people in its interpretation and presentation. Generally, it uses a set of tools (e.g.: exercises and techniques for collecting information, means of organising that information so that it is easily understood by a wide range of people, techniques for stimulating interaction with community members and methods for quickly analysing and reporting findings and suggesting appropriate action)

RTK Real Time Kinematic is a verified method of positioning in real time with the accuracy of roughly ±1 inch in the plant production. It is an efficient tool for steering of tractors (auto-steer systems) and other self-propelling machines (e.g. harvesters). RTK is an intercommunication system between base GPS station and rover units

SBAS Satellite-Based Augmentation Systems complement existing global navigation satellite systems (GNSS) and compensate for certain disadvantages of GNSS in terms of accuracy, integrity, continuity and availability

SciFinder "is a research discovery application that provides integrated access to the world's most comprehensive and authoritative source of references, substances and reactions in chemistry and related sciences"

SCOPUS (Elsevier) is an abstract and citation database of peer-reviewed literature: scientific journals, books and conference proceedings

SEMP Sustainable Environment Management Programs are conducted to educate how to maintain environmental sustainability i.e. the ability to maintain the qualities that are valued in the physical environment. As a rule, Environment and Sustainability Programs are interdisciplinary academic programs, research and scholarship, and community outreach activities that link environmental and sustainability initiatives

SFA Supercritical Fluid Extraction is the process of separating one component (the extractant) from another (the matrix) using supercritical fluids as the extracting solvents. Extraction is generally from a solid matrix

SFDA State Drug and Food Administration a regulatory body established in 1998, in China, with the task to supervise the duties and tasks of pharmaceutical drug registration and official control

SMart Nose® device An electronic nose is a device intended to detect odors or flavors. The stages of the recognition process are similar to that of human olfaction and include identification, comparison, quantification and data storage/retrieval. Recent types of electronic noses utilize mass spectrometry or ultra-fast gas chromatography as a detection system

SME Solvent Micro-Extraction

SPE Solid Phase Extraction is a sample preparation process. It is used by analytical laboratories to concentrate and purify samples for analysis. Int he extraction process the compounds dissolved or suspended in a liquid mixture are separated from other compounds according to their physical and chemical properties.

SPME Solid Phase Microextraction is a powerful sample preparation tool prior to mass spectrometric analysis. It is a very simple and efficient, solventless sample preparation method, invented by Pawliszyn, in 1989. The technique can be routinely used in combination with gas chromatography, high-performance liquid chromatography and capillary electrophoresis and is ideally suited for MS applications

TCM Traditional Chinese Medicine has its origins in ancient China. It has evolved over thousands of years. TCM practitioners use herbal medicines and various mind and body practices (e.g. acupuncture, tai chi) in treating or preventing health problems. In the US TCM is used primarily as a complementary health approach

TDZ *Thidiazuron* is a potent cytokinin used primarily woody plant tissue cultures. Its low concentrations can induce great axillary proliferation. At concentrations higher than 1 μM, it stimulates the formation of callus, adventitious shoots or somatic embryos

TM Traditional Medicine is – according to WHO – the sum total of the knowledge, skills, and practices based on the theories, beliefs, and experiences indigenous to different cultures, whether explicable or not, used in the maintenance of health as well as in the prevention, diagnosis, improvement or treatment of physical and mental illness

TRAFFIC International is a wildlife trade monitoring network, a leading non-governmental organization. It works globally on trade in wild animals and plants in the context of both biodiversity conservation and sustainable developmen

UNDP United Nations Development Program, established in 1965. It is the most important body of the United Nations operational activities on assistance (financial, technical etc.) for development purposes

UV/ESI MS method See: HPLC UV/ESI MS

Web of Science (Thomson Reuters) is a standard for research discovery and analytics. It connects publications and researchers through citations and controlled indexing in curated databases spanning every discipline. It offers over 100 year's worth of content that is fully indexed, including 2.6 million records and back-files dating back to 1898.

WHO World Health Organization of the United Nations with its headquarters in Geneva, Switzerland and 6 regional offices, WHO was founded in 1948

WPM Woody Plant Medium (McCown and Lloyd 1981) is a commonly used nutrient medium with woody plants

Index

Á. Máthé (ed.), *Medicinal and Aromatic Plants of the World*, Medicinal and Aromatic Plants of the World 1, DOI 10.1007/978-94-017-9810-5

T