Brahma Singh · K.V. Peter *Editors*

New Age Herbals

Resource, Quality and Pharmacognosy

Springer

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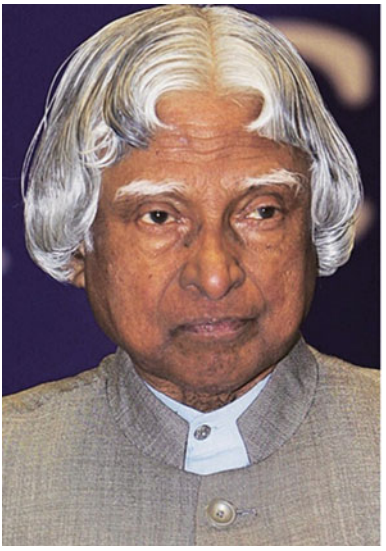
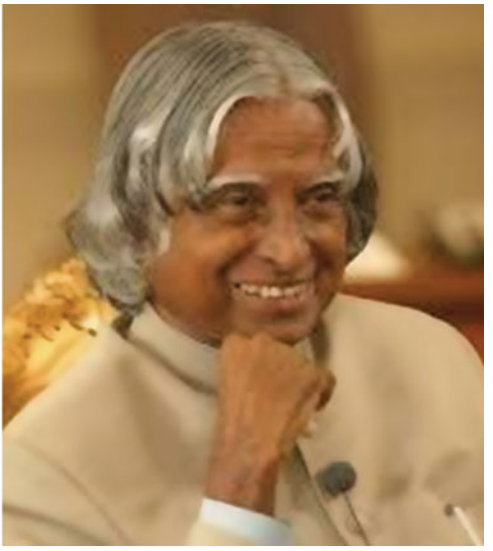
This publication is dedicated to the 11th President of India (2002–2007) – popularly known as People's President; former Secretary, Department of Defence Research and Development, Government of India; and Director General of Defence Research and Development Organization, New Delhi – late Dr. A.P.J. Abdul Kalam, Bharat Ratna, who had enormous interest in new age herbals.





Dr. A. P. J. Abdul Kalam

Foreword

I pleaded for "Evergreen Revolution" not only for food and nutrition security but also for a zero-hunger society free from food-related diseases and disorders like diabetes, obesity, cardiovascular diseases, the currently rampant and spreading "depression" and others. Vedic health and wellness promoting practices of ancient India given in *Sushruta Samhita* and *Charaka Samhita* have more relevance today than ever before. The World Health Organization estimates plant-based medicines cater to nearly 80% of the world population to meet the primary health requirements. Every plant in the universe – terrestrial, aquatic, marine and micro-cellular – has one or more wellness properties and uses but about 800 flowering plants, 650 lichens, 650 algae, 200 pteridophytes and 150 bryophytes are attributing medicinal properties and being used in Indian systems of medicine – Ayurveda, Unani, Siddha, Homeopathy (AYUSH), Tribal and Amchi/Tibetan. Incredible knowledge on phytomedicines is acquired in non-coded form by tribals and rural community, and the resultant "folk medicine" acquired national and international attention. "Holistic medicine" involving management of mind-body interaction, meditation, yoga, use of wellness foods – herbal tablets, capsules, syrups, nutritional formulations – and formulation of Food Safety Standards and its implementation are making visible changes in the life expectancy of people in India. Clean drinking water and hygienic standards for food and plant medicines/herbals processing are vital steps to be followed.

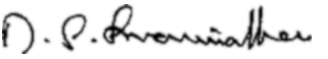
India stands 10th among the plant genetic resources rich countries encompassing 15 agro-climatic zones with a medicinal plant wealth of 15,000–20,000 and harbors two of the 25 hotspots of the world – Eastern Himalayas and Western Ghats. I am happy to note that this book covers not only the plant genetic resources on herbs but their quality aspects of raw material and processed formulations as well. Knowledge base on the use of medicinal plants and their varied formulations is deep-rooted in AYUSH systems of Indian medicine.

Modern lifestyles, kitchen-less homes, ready-to-eat foods, human migration and alien food styles are exposing people to new-age health problems. These newer problems need newer solutions. The book "New Age Herbals: Resources, Quality and Pharmacognosy" with its 21 chapters authored by the well-known, experienced and established scientists and science managers answer a few of the above queries. Noni (*Morinda citrifolia* L.) is one of such new age shrubs carrying more than

200 nutraceuticals. Its fruits, leaves and bark are used in medicine and dye industry. Sea buckthorn (Brahmphal) is another high-altitude, arid desert bush rich in pharmaceuticals and nutraceuticals. It is naturally growing in Ladakh (J&K) and Himachal Pradesh. The seabuckthorn getting global importance and attention besides being storehouse of phytomedicines is useful in checking soil and water erosion and fixing atmospheric nitrogen to enrich the soil. Similarly, other chapters on plants like *Rhodiola*, *Artemisia*, ashwagandha, Indian hawthorn (ghingharu) and medicinal mushrooms particularly *Cordyceps* along with veterinary phytomedicines covered in this book are quite informative providing potential lead for research and production of pharmaceuticals, cosmeceuticals, radioprotectors, adaptogens and nutraceuticals. The new age herbal research and development in progress in Indian scientific organizations like CSIR, ICAR and DRDO besides certain universities have been adequately covered in this book, which I consider a wise and useful compilation for the benefit of stakeholders.

I congratulate Dr Brahma Singh, Padma Shri Awardee and Former Director, Life Sciences, DRDO, New Delhi, for conceiving the idea of a book on new age herbals, for convincing experienced authors to write elaborate 21 chapters and for devoting time in editing them. I appreciate the technical and academic support given by Prof KV Peter to the Editor for a useful publication. I compliment all the authors who have put considerable time and effort to compile these informative chapters.

I also compliment Springer, Singapore, for publishing the book.



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Chennai, India

M. S. Swaminathan

Preface

It is a fact that majority of the global population depend upon herbs and herbal-based treatments for their primary health care and wellness. India has inherited one of the world's richest treasure troves of medicinal plants. The diversity of India in medicinal flora is unmatched with the presence of 16 different agro-climatic zones, 10 vegetation zones and 426 biomes. Conspicuous adverse side effects of modern drugs used in treatments of new lifestyle ailments – diabetes, cancer, cardiovascular diseases and obesity – and use of modern nano- and micro-analytical tools monitoring the chemistry and mode of action of plant molecule(s) have resulted in renewed interest and importance of herbs and medicinal plants. These advances facilitated the validation and revalidation of claims of phytomedicines mentioned in several ancient literatures or traditional systems of medicines practiced particularly in India and China like "Charaka Samhita", "Sushruta Samhita" and later "Ayurveda", "Siddha", "Unani", "SOWA-RIGPA-Amchi" (Tibetan, Bhutan), "Naturopathy", "Yog", "Homoeopathy" and "tribal medicine". The interest is not only to validate claims but to find out newer medicines and molecules for treatment of several emerging diseases. The research on bio-medicine triggered further research and development on medicinal plants on a fairly large scale in India and other countries. It is an established fact that management of health problems be affordable, less cumbersome and with no side effects, wherever herbs play a major role. Human food and nutrition are major contributors to human health and wellness. The Greek philosopher Hippocrates stated, "Food is thy medicine". Herbs and medicinal plants are now being authentically documented with empirical proof for their immunity modulation, pharmaceutical, nutraceutical, cosmeceutical, adaptation, radioprotection traits and other health-associated properties. Research and development reported and under progress on above aspects in India are compiled through contribution of chapters by scientists of repute. Some of them devoted their lifetime to research on particular aspects of certain medicinal plants.

Many questions and queries are raised on quality and availability of raw herbs and phytomedicines. This book covers the availability of quality herbs both from wild and from farmer's fields raised by adopting modern production technologies and good agriculture practices (GAP) and use of known plant variety or particular genotypes (to avoid variation in different lots of medicine). Maintenance of quality of raw materials and phytomedicines are covered in two chapters elaborately. The

21 chapters are categorized under plant resources, quality, pharmacognosy and new age herbals keeping in view the interests of practitioners, students and readers.

With the advent of intensive and inquisitive herbal research and development, extraordinary pharmaceutical, nutraceutical and other traits of underutilized and underexploited plants like sea buckthorn/brahmphal, noni, rosewood, Indian hawthorn and sweet wormwood are being revealed. Chapters on these new medicinal plants are quite informative having several leads for further research to deal with newer health problems. Research and development on certain novel herbs in Indian science and industry's laboratories are covered in this compilation. All the stakeholders in herbal and medicinal plants would find the book informative with updated scientific knowledge. The book will be a pathfinder for disease and disorder-free world.

Chennai, India — K. V. Peter
New Delhi, India — Brahma Singh

Acknowledgements

We, the editors, express our gratitude to all the authors of 21 chapters who have spent a lot of time in writing the interestingly useful chapters. We appreciate their time, patience, efforts and stringless co-operation. We are grateful to Prof. MS Swaminathan "Father of Green Revolution in India" for the Foreword.

We enjoyed working with the publisher Springer particularly Dr Madhurima Kahali, New Delhi, and Ms Reshmi Rema, Project Coordinator (books), Chennai, and impressed with their pleasant managerial dealings. We sincerely acknowledge assistance and contribution of all concerned with this publication particularly Mr Biswal Atma. Our families, especially spouses Saroj Singh and Vimala Peter, supported us in this academic venture which we thankfully acknowledge with love and affection.

Chennai, India — K. V. Peter
New Delhi, India — Brahma Singh

Contents

Introduction

Brahma Singh

India, China, and many other countries in the world follow a living tradition with herbs and herbals. In fact, India can be called an emporium of herbs. World Health Organization's estimates quoted time and again by press, book writers, and others indicate that still 80% of world population surprisingly depends on herbals for their primary health care. Herbs being an important part of healthy life, human life, and herbals seem inseparable. The curative and preventive capabilities of herbs in most of the ailments or medicinal plants are known and documented amply too in ancient literature of different civilizations in India and abroad. The enormous literature emphasizes that nature has created a perfect system to keep human healthy. If health problem is there, herbs are also there in good number to overcome it. One has to find it out (research) and document. In this context it is further emphasized that nature has provided natural solution to most of the health aspects of human being. Herbs are identified by hit and trial method since the inception of life on planet earth. Ancient literature do have mentions of herbal medicines for much-talked-about age-related and other difficult-to-cure diseases, namely, memory loss (dementia), tremor, immunity loss, osteoporosis, osteoarthritis, diabetes, liver disorders, and several others for which still few or no modern medicines with high percentage of relief and subsequent management are available.

Before the advancement of bio- and chemo-sciences as well as technologies, treatment of diseases was dependent mainly on herbs and the herbalist knowledge which was either inherited or studied in isolation. Interestingly the knowledge is based on empirical findings (without documenting data due to lack of organized health system as that of present day) deploying human subjects with remarkable more or less complete success (almost 100%). This is a marvelous and lasting way of dealing with health issues without knowing the chemistry, chemical constituents of the medicine, and their mode of action. With the advancement in modern medical

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science, which has done wonders in medical surgery and others, the importance of herbal treatment has significantly not only suffered, but research on the system had gone in low key if not very low or forgotten. The place of traditional phyto-medicines/herbals has obviously been taken over by synthetic medicines. This system popularly known as allopathy is flourishing world over despite side effects caused by synthetic drugs and late development of resistance in pathogen to antibiotics. Abuse to synthetic drugs and molecules leading to several side effects, toxicity and inefficiency besides suppressive effect, have compelled the health maintenance system to relook and selectively adopt the ancient herbal system, generally termed as traditional system of medicine, with present-day health science at least in India. The introduction of synthetic aspirin (basically understood herbal) and use of plant-based drugs like cinchona, cocaine, codeine, morphine, digitoxin, quinine, artemisinin, and others played a significant role in creation of belief among human health professionals in the effectiveness of enormous natural herbal wealth. The formula "Take a handful of one-year-old wormwood, soften it in twice as much water, squeeze the plants out and drink the juice" from a fourth-century Chinese medical text inspired the 84-year-old Chinese pharmacologist Youyou Tu to research the medicinal effects of sweet wormwood (*Artemisia annua*) and develop a new malarial treatment, artemisinin. It has saved over 1.5 million lives around the world and bagged Tu recently the Nobel Prize in Medicine. The editor considers this system as "new age herbals." Here herbals mean plants or botanicals used as medicines both preventive and curative. As stated earlier herbals obviously were quite popular before the development of modern medical science. In India new age herbals are applicable to Ayurveda, Unani, Siddha, Amchi, Homeopathy, and others. In fact medicinal plant-based system of medicine (both preventive and curative) providing lead and support to modern medical system is also new age herbals. Accordingly ethnomedicinal uses of plant species documented in different countries are being followed to take clue to develop synthetic drugs wherever possible. As per estimates of Ministry of Environment, Forests and Climate Change, Government of India, 4635 ethnic communities in India use more than 7500 species of plants for human and animal health care (http://www.devalt.org/newsletter/jan98/of_2.htm). These data indicate the vast subject of herbals having researchable potential areas in management of human and animal as well as food crop health. Dietary supplements and nutraceuticals out of herbs have become popular round the globe which can otherwise be termed as phyto-medicines or botanicals. "In the last century, more than 121 pharmaceutical products were formulated based on the traditional knowledge on medicinal plants obtained from various sources" (Hasan et al. 2009). Safe drug or medicine is obviously an important issue. Phyto-medicines are considered safe because of their very long time-tested practice mainly on human subjects. In developed world now phyto-medicines are getting increasing consideration in treatment of chronic diseases such as cancer, diabetes, asthma, and end-stage renal disease because they have proven safety by their continuous use over the centuries. As stated earlier treatment with medicinal plants is considered very safe as there are no or minimal side effects. Herbal remedies are in synchronization with nature,

which is considered an advantage. The distinct advantage is that the use of herbal treatments is independent of age groups and gender.

Despite lack of systematic research data, the use of herbal medicines is on the increase these days as has been stated earlier. Realizing the potential and importance of herbs, Indian Council of Medical Research (ICMR), New Delhi (mainly deals with modern system of medicine), has started compiling information on them in the form of "Book Review Indian Medicinal Plants." Several such volumes have already been published by the council for the use of researchers, students, and those owning the knowledge (intellectual property rights). The ICMR monograph on each plant/herb deals with a wide range of information under major heads – general information, pharmacognostic, chemical, pharmacological and biological, clinical, and toxicological studies with complete references of cited work (Tandon 2011). This is of great help to convince the scientific community and health practitioners to consider the usefulness of herbs and herbals and undertake research on medicinal plants. In fact medicinal plants are storehouses of a large number of useful bioactive ingredients which have enormous potential for use in synthetic drugs or modern medicine development. Medicinal plants have also contributed in development of human cultures around the whole world exhibiting the way to healthy life. Certain plants, rich in nutrition and capable of overcoming common and uncommon nutrient deficiencies and associated diseases in the body, are recommended for therapeutic purposes. Some of these plants include turmeric, ginger, lemon, green tea, walnuts, aloe, pepper, beetroot, carrot, and others which are otherwise also part of human diet in India and other countries.

Herbs, world over, have gained popularity in biological control of agriculture pests and residential and workplaces because of organic way of life and environment concern making them important sources for pharmaceutical manufacturing.

Commonly Used Herbs

The ancient and present knowledge on some of the herbs have gained popularity not only among herbalists or Ayurveda practitioners but in households. Some plants like Ashwagandha (*Withania somnifera*), asparagus, *Ephedra*, and others have now a number of known bioactive ingredients which are being used in synthesis of drugs to overcome diseases and disorders. Some of the popular herbs with some of their long time-tested medicinal values/food supplements are as follows:

Antacid: marshmallow, aloe, cardamom, fennel, dandelion, flax seeds
Antipyretic herbs: *chirata*, black pepper, sandalwood, and safflower
Antiseptic: aloe, sandalwood, and turmeric
Appetizer: cinnamon and cardamom, peppermint and clove
Asthma, bronchitis, and other respiratory ailments: ginger, clove, black pepper, cardamom, eucalyptus, and wild cherry
Astringents: cinnamon and sandalwood
Blood toxins: dandelion, basil, neem, and red clover

Cardiac stimulants: chamomile, calamus, ajwain, basil, cardamom, chrysanthemum, coriander, fennel, peppermint, spearmint, cinnamon, ginger, and turmeric
Herbs with antibiotic property: turmeric, acacia, aloe, cryptolepsis, *Echinacea*, eucalyptus, garlic, ginger, goldenseal
Sedatives: chamomile, lavender, red clover, St. John's wort, hops, ashwagandha (*Withania*), passionflower, and California poppy
Snake bite: mongoose (*Ophiorrhiza mungos*) and Russell's viper (*Daboia russelii*)
Sores, boils, and wound: black pepper, cinnamon, myrrh, aloe, sandalwood, ginseng, red clover, burdock, bayberry, safflower, turmeric, marshmallow and licorice
Stimulants: cayenne, red chili, myrrh, camphor, and guggul
Tonic: aloe, goldenseal, wood apple (bael), barberry, *chirata*, sea buckthorn, noni, goji berry, gooseberry, and giloy

Traditional therapies or phytotherapies use single or multiple plant extracts or dry powder of plant parts, at times mixed with non-herbal too. These are combination of several pharmacologically active constituents or the selected drug combinations with the known mode of actions of each single constituent. Such plant combinations do activate the body defense system instead of eradicating/inactivating directly the causal agents as in prevailing allopath system of treatment. Now this concept of multiple drug therapy has become common in modern clinical system for treatment of acquired immune deficiency syndrome (AIDS), cancer, malaria, asthma, hypertension, and others. This is because of rich but undocumented experience on the therapeutic experiences on multiple herbs or their bioactive ingredient therapeutics. It is believed that low-dose plant extracts singly or generally in combination exhibit synergetic or additive effects of bioactive curative ingredients in management of most of the complex diseases, often termed as incurable, without the fear of the side effects. Here long time-tested experience rather than known mode of action of different natural chemicals is important to achieve the goal of successful treatment of ailment. Scientific understanding of this natural multi-chemical therapy in most of the cases is still awaited.

Herbs Availability

Herbs are still mainly collected from wild/forest/nature. Like any other natural resource, herbs are bound to become endangered species or get extinct in the absence of appropriate concerted and timely measures used to conserve and propagate them. Fortunately the Government of India is aware of this fact and accordingly initiated several national programs on conservation and multiplication of venerable plant resource/herbs. Establishment of National Medicinal Plants Board with State Medicinal Plants Boards under the Ministry of AYUSH (Ayurveda, Yoga and Naturopathy, Unani, Siddha, and Homeopathy) is one such major initiative for development of medicinal and aromatic plants. AYUSH would be developing education and research in Ayurveda, Yoga, Siddha, Unani, and Homeopathy in the country.

Biodiversity parks and herbal gardens are being added not only in India but around the world to conserve natural bio-resource including herbs. In fact in a complete turnaround, modern system of medicine is now getting more interested in medicinal plant therapies or phyto-medicines. This obviously has resulted in an increased popularity of primary health care for a number of human diseases and related issues on epidemics like that of dengue. At the same time, plant-based drug laboratories are now multiplying world over more so in China and India – the pioneer on herbals. Another good aspect of the subject is that some of the lifesaving drugs are from herbs besides being of a mention of sanjeevani booti (lifesaving herb) in Ramayana, one of the ancient religious popular books in India. Another example is reserpine from *Rauvolfia* which is an effective tranquilizer and high blood pressure normalizer. Interestingly there are personalized herbs particularly in tribal belts of India providing relief to the people but never ever documented.

As mentioned earlier over 90% of the medicinal plants used in Indian pharmaceutical/cosmeceutical and essence industries are collected from natural forests. With modern research and technology, development confirming medicinal value of certain plants of interest in plant medicines is increasing. This has obviously resulted in commercial overexploitation of certain medicinal plants leading to endangered particular species level. This overexploitation of certain herbs has obviously led to habitat destruction necessitating their protection, education, and awareness on collection, conservation, and cultivation. Due to rapid urbanization and spread of modern primary health care to remote and rural areas, traditional herbal knowledge is being gradually lost. This loss of traditional herbal knowledge has accelerated loss of medicinal plant wealth too as their conservation and multiplication have been adversely affected. This further emphasizes the need to conserve and multiply herbal wealth. Efforts in this direction are in progress throughout the country but need their streamlining. Conservation efforts in the absence of regulation on the subject generally do not show desirable results. Hence a long-term strategy coupled with national policy is required without delay. There is no national policy on medicinal plants in India. Efforts in this direction are through seminar, symposia, making recommendations, and gathering public opinion to draft national policy on medicinal and aromatic plants.

Conservation Strategy

Like other plants, herb conservation encompasses both in situ (on site) and ex situ (off site) conservation. Both, in situ and ex situ conservation, are important. Well-documented plant diversity hot spots in the country are being protected to great extent which helps in in situ conservation of herbal wealth. Major role, in in situ conservation, is to be played by state forest departments and surrounding villages. These departments are to be enriched with physical resource and knowledge not only for themselves but to disseminate with villagers around the forest. This would require regulation on extraction and harvest of herbs from protected areas. The National Biodiversity Authority and NMPB with their state units are contributing

to achieve desired goals. At the same time ex situ conservation is to be strengthened by establishing medicinal plant gene banks, herbal gardens, and cryopreservation at educational and research institutions both at state and center government level. Different agriculture science departments in India including department of biotechnology have taken up projects of establishing gene banks and parks of medicinal plants in urban and rural as well as forest areas including cryopreservation/conservation through using liquid nitrogen and permafrost.

Cultivation

Of late, cultivation or multiplication of medicinal plants is being promoted to check nonavailability or erosion of medicinal germplasm from nature. Besides, farming of medicinal plants provides better return to growers when compared with other commercial crops in vogue. It helps in providing uniform raw material essential to develop drugs or formulations from these for claimed results/performance. Production technology of medicinal plants is being developed rightly at regional basis by state agriculture universities, research institutes, and central research laboratories. Suitable varieties of medicinal plants are being developed in public sector institutions besides private sector. Medicinal plants being large in the number of production protocols of important few, required continuously in bulk, are available and are being updated and added too mostly to match the regional climate and soil conditions. Out of around 400 plant species used in AYUSH system of treatment, around 30 species are under commercial cultivation as per reports of NMPB. Research on development of production technology of certain medicinal plant is vigorously pursued by the Council of Scientific and Industrial Research, Indian Council of Aricultural Research (ICAR), and several State Agricultural Universities. Since demand of certain medicinal plants is on the steady increase, their cultivation on a large scale is being promoted. However development of cultivation protocol of these plants is not easy due to the priority rightly given to development of production protocols for important food crops. Unavailability of quality planting material and very little or no crop improvement program along with lack of standardized agronomic practices are major bottlenecks. This is an important aspect requiring great attention of planners. Besides climatic conditions or the environment under which the herb is raised has significant effect on the quality and quantity of active ingredient in the plant. Medicinal plants have been of great interest to tribes who consume them on daily basis as preventive health management, something worth emulating. Another issue in multiplication of herbs is that these plants have commercial value to different communities, but being in remote area and sometimes inaccessible areas, they get much less than the prevailing price of produce in the market. To enhance their income from medicinal plants, value addition by simple processing techniques such as drying, cleaning, crushing, powdering, grading, and packaging as well as labeling organic after certification needs to be popularized among the tribes.

Herbal Science

Herbal science includes disciplines, both major and fundamentals, of botany, economic botany, organic chemistry, pharmacology, and others. Botany includes the identification (taxonomy of herb), genetics (improvement in herb potential – varieties/hybrids), biotechnology (biomedical engineering, bioinformatics), cultivation of plants (economic botany/agronomy), conservation of herbs, and others. Chemical characterization of herbs includes identification, isolation, and quantification of bioactive ingredients in different plant parts at different stages of growth. Pharmacology is the science which covers study of the biochemical reactions that the bioactive ingredients in medicinal plants have on cell cultures, animals, and humans. Keeping in view the history of plant medicines, there is a great need of studies in the field of pharmacognosy (study of crude drug). From a practical perspective, such study includes mainly three aspects, namely, herb quality (correct identity, high purity, consistency – more or less same quality and quantity of bioactive ingredient in each batch), efficacy (therapeutic indications, clinical studies, pharmacological investigations), and safety (adverse reactions, ingredient interactions, contradictions, precautions). These as well as associated aspects have been covered under different chapters of this publication.

Herbal/Botanicals

Herbal or herb chemicals or botanicals are naturally occurring chemical compounds in plants. Estimation and characterization of these have been possible with the advancement of analytical chemistry popularly known as phytochemistry/analytical chemistry which is the study of phytochemicals in quality, quantity, presence, absence, etc. The major classes of pharmacologically active phytochemicals as categorized in literature are listed below.

Alkaloids

Alkaloids are nitrogenous organic compounds mostly of plant origin. These are characterized by pronounced physiological and pharmacological activities and actions in human. Alkaloids are bitter in taste. Medicinally important alkaloids includes atropine, scopolamine, and hyoscyamine (all from nightshade), berberine (from *Berberis* and *Mahonia*), caffeine (*Coffea*), cocaine (*coca*), ephedrine (*Ephedra*), morphine (opium poppy), nicotine (tobacco), psilocin (many psilocybin mushrooms), reserpine (*Rauvolfia serpentina*), quinidine and quinine (*Cinchona*), vincamine (*Vinca minor*), vincristine (*Catharanthus roseus*), and others (https://en.wikipedia.org/wiki/Alkaloid).

Glycosides

A glycoside is a molecule in which a sugar is bound to another functional group via a glycosidic bond (https://en.wikipedia.org/wiki/Glycoside). Inactive glucosides are there in many medicinal plants which are enzymatically hydrolyzed by breaking off the sugar part. This makes the chemical active and available for use in medications. Some of the glycosides are anthracene, phenol, steroid, flavonoids, cumarin, saponin, aldehyde, and others. Senna, rhubarb, and aloe contain anthraquinone, and foxtail lily contains digoxin and digitoxin glycosides. Sea buckthorn plant contains flavonol glycosides.

Polyphenols

Polyphenols include the colorful anthocyanins, hormone-mimicking phytoestrogens, astringent tannins, and others. Their several classes are commonly found in plants. In Ayurveda, the astringent rind of the pomegranate is used as a medicine, while polyphenol extracts from plant materials such as grape seeds are sold for their potential health benefits despite the lack of evidence. Plants containing phytoestrogens have been used for centuries to treat fertility, menstrual, and menopausal problems. Polyphenols rich plants are *Pueraria mirifica*, kudzu, fennel, anise, and many more (https://en.wikipedia.org/wiki/Medicinal_plants).

Terpenes

Terpenes and terpenoids (built up from terpene building blocks) of many kinds are found in resin yielding plants such as the conifers. Terpenes may be classified by the number of isoprene units in the molecule. Terpenes being strongly aromatic and repulsive to plant pests are hence used extensively in pesticides. Their scent makes them useful in essential oils, whether for perfumes such as rose and lavender or for aromatherapy. Steroids in mammals are products of terpenoid metabolism. Some of the terpenoids are azadirachtin (neem tree), artemisinin (*Artemisia annua* Chinese wormwood), and tetrahydrocannabinol (*Cannabis sativa*) (https://en.wikipedia.org/wiki/Terpene).

Research Hurdles in Phyto-medicines

There is renewed attention and demand of natural medicines. This is quite encouraging and welcome happening from practical and scientific point of view. The efficacy of natural medicines in several cases has been proved over a long period of time. The mode of action of herbals is complex. The mechanism of action of single bioactive factor is easy to work out and understand but is complicated collectively adding the factor of additive or cumulative effects and synergy. Plant

extracts used in treatment are either unfractionated or partly fractionated. In most of the cases, medicine for treatment is often mixtures of different constituents. In most of the cases, synergism is expected to play a major role which is absent in single bioactive molecule. Single bioactive molecule remains ineffective for wants of co-bioactive molecule/factor or filler or environment. It is not only difficult but at present not possible to characterize such mixtures for identification of bioactive factors for evaluation on the pattern of modern drugs. It is a major challenge in research on herbs to compete with modern medicines on account of this. Of late questions on safety aspects of herbs have been rightly raised in the absence of studies on the subject in the past. This aspect has attracted the attention of herbal scientists and pharmacies to label the safety aspect after evaluation or rules on the subject. With technology advancement and refinements in analytical tools as well as bioinformatics, now it has become possible to ascertain safety aspect of herbal extracts and their bioactive factors/chemicals. This very factor would be helpful in using phyto-medicines/preparation with certain amount of confidence in the effectiveness of the disease management. Hence herbs would now contribute to the society correspondingly more than they have ever been previously. Better future for pharmacognosists can now be anticipated without much of doubt.

Constraints Associated with the Dealing of Herbal Medicines

As elaborated earlier herbs and their extracts contain cocktail of complex chemicals such as alkaloids, glycosides, terpenes, fatty acids, flavonoids, tannins, saponins, lignans, peptides, oligosaccharides, and many more organic molecules. It is still problematic to pick up bioactive and useful one(s) out of the mix as singly they are ineffective. This adds to the volume of drug and other associated processing and handling factors.

Pharmacological activity of bioactive factor of medicinal plants during processing, heating, boiling, dying, and so on is likely to be altered. Sometimes their dissolution rate is also affected. The quality and quantity both of bioactive factors are affected by the host of production environment such as soil, photoperiod, rain and humidity, temperature fluctuations, shade, frost, altitude, and similar others.

Other factors such as infections, infestation, agronomic practices followed in production other than a known variety/hybrid, and many more production and processing unavoidable variables can play an important role leading to batchwise variation. Such variations are being taken care of by way of standardization of raw herbs discussed in detail in subsequent chapters. There is a limit to supply raw material of medicinal plants; the supply is difficult on mass scale all the year round. An aspect of adulteration, greed for easy money, is also cropping up with increase in demand of raw herbs, which is being tackled by developing detective techniques and tests.

Cosmeceuticals

Globally over the last few years, herbal cosmetic business has emerged as new growth frontier as everybody obviously wants to look young forever. The cosmetics mainly phyto-cosmeceutical or new age herbal company are fetching billions of dollar in revenues helping present-day population to look young and healthy. Keeping in view the cost-effectiveness of phyto-medicines coupled with no or little adverse effects in comparison with synthetic drugs/cosmetics, reputed pharmaceuticals are investing greatly in herbo-cosmeceuticals and phyto-medicines.

Nutraceuticals

Besides therapeutic and cosmeceutical nature of herbs, they are potential source of nutraceuticals and are in increasingly great demand as nutraceuticals or food supplements world over besides phyto-medicines. This is because of regulations on their use as medicine but not as food supplement in advanced countries globally. We are rightly heading toward an era where scientifically worked-out food/diet partly or fully would be the medicine for human.

India New Age Herbal Potential

World market for herbal supplements and remedies is expected to reach more than USD 115 billion by 2020. This covers major herbals. For India to be the major supplier of phyto-medicines, there is a very late realized need to combine India's traditional wisdom of healing and other systems of healing with modern pharmaceutical research and manufacturing capabilities. There should be robust clinical trials besides inclusion of the subject in curriculum of medical institutes and universities. Beyond any doubt plant-based drugs have unparalleled chemical diversity and an incredible potential of new drug development as nature has given solution to most of health problems. The modern medical system is recognizing and resorting to a system based on the combination of therapies as a leading science to deal with the wisdom that lies in botanicals and herbs. However, many folds are still most wanted to explore unseen secrets of curative potentials of herbs to treat dreaded diseases. To get more significant results, phytopharmacological screening coupled with phyto-chemical studies in the light of modern technologies involving characteristics of new substances, history and pattern of use, any adverse reaction, biological action, toxicity, carcinogenicity, and clinical trials is much needed. Issues mentioned in this chapter have been addressed in subsequent chapters in this publication.

National Policy

As stated earlier there is hardly any regulation on exploitation of medicinal plants in places other than protected areas in the country. Most of the banned plant species for export are because of their RET (rare, endangered, and threatened) status and not that of their therapeutic value. There is thus need of national policy on medicinal plants covering all aspects of collection, production, processing, value addition, incentives, storage, transportation, marketing, etc. Efforts in this direction in the country are on. National Biodiversity Authority (NBA), Chennai, does have regulation on access and benefit on the use of herbs from the wild. NMPB has advisory role on a set of medicinal and aromatic plants. Besides the public sector, private sector (major user of medicinal plants or raw material) is to be roped in promotion of production and processing of herbs and contribute to their conservation and multiplication in addition to large-scale extraction/collection from nature under a well-thought, debated, and formulated national policy on the subject.

The Book Coverage

Of late there is positive outlook on herbals or phyto-medicines. The time-tested traditional knowledge on herbs have partly been understood scientifically with present day analytically and synthesizing tools and techniques. A beginning to complement and supplement modern medical systems has also been made. This publication covers topics listed under contents and cover views and opinions of scientists working on the subject for decade(s) highlighting importance of herbals not only in treatment of diseases and disorders but helping human to maintain their energy, look, and beauty for the life besides the overview of scientific knowledge available. We hope the contents and opinion expressed by scientist authors on the new age herbals would benefit researchers, students, and other stakeholders on the subject.

Keeping in view the richness of herbs as phytopharmaceuticals, nutraceuticals, and cosmeceuticals and the recent knowledge of important herbs and herbals and related issues causing hindrance in their promotion and progress, the publication has been written. An attempt has been made to compile rich experience of scientists working on herbs in this proposed book *New Age Herbals*. New age single plant species having multiple medicinal traits worth exploiting, i.e., *Hippophae rhamnoides* (sea buckthorn) and *Morinda citrifolia* (noni), also find place as full chapters in the book.

Indeed, the future of phyto-medicine in modern medicine manufacture (develop and multiply) looks very promising, as long as scientists and health practitioners keep a curious and objective mind, without prejudice or favor toward the concept of "herbals." The author is of the view that there is need to integrate phyto-medicines into modern health-care management system and vice versa without causing threat to

each other by updated appropriate regulations keeping in view the merits of each and interest of patient or user. Since primary health care is important and people prefer phyto-medicines, as stated earlier, there is need to strengthen primary health care with phytopharmaceuticals world over.

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Part I

New Age Medicinal Plants

Research and Development on *Artemisia annua* in India

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Introduction

Artemisia annua (qinghao in Chinese), belonging to the family Asteraceae, is a short-day, annual, cross-pollinated medicinal plant native to China. Over a period of time, it has been naturalized and grows wild in many other regions of the world. It produces an array of secondary metabolites including sesquiterpenoids, triterpenoids, monoterpenoids, phenolics, coumarins, steroids, lipids, flavonoids, and aliphatic compounds (Bhakuni et al. 2001). However, it derives its importance from the antimalarial phytomolecule, artemisinin (qinghaosu in Chinese), which is an endoperoxide sesquiterpene lactone (Hsu 2006). For the multidrug-resistant strains of *Plasmodium falciparum*, the malarial parasite, artemisinin, and artemisinin-based combination therapies (ACTs) are globally recognized and established as antimalarials. Presently, the plant remains the only commercial source of artemisinin.

The disease burden due to malaria is huge. According to the WHO estimates, released in December 2016, there were 212 million cases of malaria in 2015 and 429,000 deaths. In spite of many decades of intense research and development effort, there is no commercially available malaria vaccine in hand (WHO 2017). Despite sporadic reports related to occurrence of resistance against artemisinin (Dondorp et al. 2009, 2011), the importance of artemisinin and related drugs will be retained until a highly efficient vaccine for malaria materializes or next-generation antimalarials are discovered. Interestingly, the *A. annua* plant still retains its prominence for the commercial production of artemisinin despite the recent entry of

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Fig. 1 *Artemisia annua* growing in the farm of CSIR-CIMAP at Lucknow

semisynthetic artemisinin into commercial production (Paddon and Keasling 2014; Paddon et al. 2013).

In India, *A. annua* (Fig. 1) was introduced by CSIR-CIMAP in early 1980s, from the Royal Botanic Gardens, Kew, UK (Kumar et al. 2015; Srivastava 1999). Intensive research efforts carried out by Indian scientists, especially those from CSIR laboratories like CIMAP and CDRI, fructified into improved plant varieties and processing technologies, which in turn made artemisinin and related drugs affordable and produced a number of patents (Tripathi and Dikshit 2015). Here we have tried to present a snapshot of the research carried out on *A. annua* in Indian laboratories since then.

In planta Artemisinin Biosynthesis

Glandular secretory trichomes are responsible for the biosynthesis and sequestration of artemisinin in *A. annua*. In the plant, artemisinin is biosynthesized from the precursor, isopentenyl pyrophosphate (IPP), via farnesyl pyrophosphate (FPP) (Fig. 2). FPP forms amorpha-4, 11-diene through amorpha-4, 11-diene synthase (ADS), which commits the metabolic flux toward the biosynthesis of artemisinin. CYP71AV1 converts amorpha-4,11-diene to artemisinic aldehyde (in two steps) or artemisinic acid (in three steps). Further, artemisinic aldehyde gets converted to dihydroartemisinic aldehyde [by artemisinic aldehyde $\Delta^{11,13}$ reductase (DBR2)], which in turn forms dihydroartemisinic acid [through the action of aldehyde dehydrogenase 1 (ALDH1)]. The enzymes for most of the steps are known now. However, it is believed that the final steps of the pathway between dihydroartemisinic acid and artemisinin are possibly nonenzymatic, caused by the slow spontaneous autoxidation of dihydroartemisinic acid to artemisinin. It is a matter of research whether these steps occur nonenzymatically *in planta* also or there are enzymes (most likely peroxidases) present to catalyze them (Nair 2015). Thus a certain degree of gene prospecting related to artemisinin metabolism is still required to be done in *A. annua*.

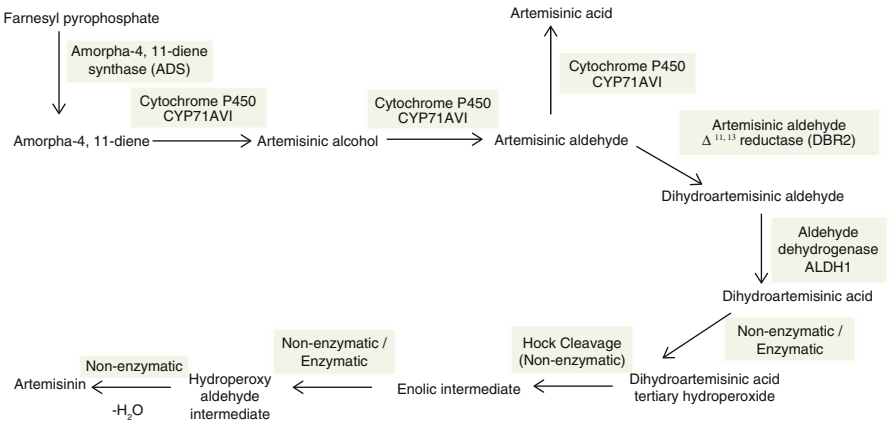

Fig. 2 Artemisinin biosynthetic pathway in *Artemisia annua*, as best understood through presently available literature

Genetic Resources and Elite Genotypes of *A. annua*

A. annua is a diploid and cross-pollinating species, where selection in the available germplasm and genetic breeding toward high artemisinin production have been the two primary modes for improving the deficit in the artemisinin supply chain (Xie et al. 2016). Artemisinin is found in aerial plant parts (leaves, inflorescence, stem) but has not been detected in pollen and roots, with the range of its content being 0.01–1.2% in different genotypes (Jain et al. 1996; Khanuja et al. 2005; Liu et al. 2006). It is evident that there is still scope for further enhancement of *in planta* artemisinin content (as observed recently in the case of *A. annua* plant *Apollon* launched by Mediplant with artemisinin content touching 1.6%) (Carlen and Simonnet 2015; Simonnet et al. 2011). Plants rich in artemisinic acid (0.8%) have also been isolated (Gupta et al. 1996).

The prerequisite for efficient gene prospecting related to artemisinin metabolism is the availability of stable high artemisinin-yielding genotypes. Researchers at CSIR-CIMAP, Lucknow, have done tremendous amount of work in this direction and were able to improve upon early low artemisinin and low artemisinic acid genotypes like *Asha* toward the development of varieties rich in artemisinic acid [*Suraksha* (Kumar et al. 1999)] or artemisinin [*Jeevanraksha* (Kumar et al. 1999) and *CIM-Arogya* (Khanuja et al. 2005)]. *CIM-Arogya*, in particular, stably produces high amount of artemisinin (1.0–1.2% on dry weight basis) like the European variety *Artemis* [an F_1 hybrid (population) variety developed by Mediplant (Conthey, Switzerland) (Graham et al. 2010)]. It has also been successfully tagged using a sequence-characterized amplified region (SCAR) marker (Khanuja et al. 2005; Khanuja et al. 2008, US Patent No. 7,375,260, Shasany et al. 2007). It has been very well accepted by the Indian farmers and industry and is best suited for gene

prospecting. Enhancement in artemisinin content was achieved through the employment of recurrent selection (four cycles) with relation to heritability, correlation, as well as molecular marker in the plant (Paul et al. 2010). Genetic improvement was carried out through gene pool exploitation using polycross design, whereby it was found that high artemisinin-yielding plants favor oval canopy (Paul et al. 2014).

The optimal plant development stage and leaf ontogeny level for maximal artemisinin accumulation have been identified (Nair et al. 2013). Under North Indian conditions, the artemisinin content increases from undetectable levels at the seedling stage (6-day old; February) to a maximal level at the pre-flowering stage (6-month old; August) and declining beyond that stage. It has also been observed that artemisinin content is maximal in the uppermost leaves of the secondary branches. The artemisinin content in the seed, seed husk, and stem was found to be 1/35, 1/3, and 1/10, respectively, of that in the leaves. Padalia et al. (2011) have studied the ontogenic variability in the volatile constituents of *A. annua* (*CIM-Arogya*). Higher yields of artemisinin are obtained by multi-harvest/ratooning of *A. annua* crop (Gupta et al. 2002; Kumar et al. 2004). Under subtropical agroclimatic conditions prevailing in the North Indian plains, it has been recommended that *A. annua* should be cultivated at significantly high plant density (2.22×10^5 plants/ha) for obtaining higher yield of artemisinin (Ram et al. 1997).

Characterization of Genetic Elements from *A. annua*

The genome of *A. annua* is not yet available, but a genetic map (Graham et al. 2010) and transcriptomic data (Wang et al. 2009) have been generated. A representative transcriptomic resource from the elite Indian genotype, *CIM-Arogya*, has also been generated and utilized for development of a small-scale custom microarray, which in turn has been used for identifying candidates for gene bioprospecting and their downstream characterization (Nair 2015; Nair et al. 2013). The *A. annua* custom array was successfully employed to identify differentially expressed genes during growth stages of *A. annua* that are contrasting for their *in planta* artemisinin content. These efforts will be instrumental in facilitating discovery of new genes and elucidating the mechanism of regulation involved in artemisinin metabolism in *A. annua*. Besides, an efficient method for *Agrobacterium*-mediated transformation of *A. annua* has also been developed (Alam et al. 2014), which will help in characterization of plant genes. Till date, many genes and genetic elements have been characterized from *A. annua* (Table 1).

However, till date there is no report of transgenic plants that may be of agricultural importance for sourcing artemisinin, which could be probably due to multifarious factors like metabolic flux competition, issues with gene expression levels, trichome size and number limitation, unknown final biosynthetic steps, etc. (Xie et al. 2016). In fact, any transgenic or genetic modification (GM) approach will need to compete with the semisynthetic approaches for better economics.

Table 1 Genetic elements of *Artemisia annua* studied in Indian laboratories

S. No.	Genetic element	References
1	Sequence-tagged QTLs	Sangwan and Sangwan (2001)
2	MicroRNA and their mRNA targets	Pani et al. (2011)
3	HMG-CoA reductase; amorpha-4,11-diene synthase	Alam and Abdin (2011)
4	ISSR markers	Kumar et al. (2011)
5	RAPD markers	Kumar et al. (2011) and Sangwan et al. (1999)
6	Cytochrome P450 monooxygenases	Misra et al. (2012)
7	Intron-flanking EST-specific markers	Kumar et al. (2012)
8	EST-derived SSRs	Kumar et al. (2014)
9	Peroxidases	Nair (2015)
10	Sterol C-4 methyl oxidase	Singh et al. (2015)
11	*AaGL2* and *AaMIXTA-Like1* gene promoters	Jindal et al. (2015)
12	Cinnamate-4-hydroxylase	Kumar et al. (2016)

Responsiveness of *A. annua* to Biotic and Abiotic Factors

The synergistic compatibility between *Glomus mosseae* and nitrogen-fixing *Bacillus subtilis* (Daz26 strain) for use as an efficient microbial consortium in *A. annua* for enhancing plant biomass yield, growth, as well as artemisinin content and yield has been demonstrated (Awasthi et al. 2011). The potential use of the consortium of a root endophyte [*Piriformospora indica* strain DSM 11827] and a nitrogen-fixing bacterium [*Azotobacter chroococcum* strain W-5] for increasing overall productivity and sustainable agriculture has also been studied in *A. annua* (Arora et al. 2016). The role of bioinoculants (*Streptomyces* sp., *Bacillus megaterium*, *Trichoderma harzianum*) as an efficient tool for the enhancement of growth, nutrient uptake, antioxidant, as well as artemisinin content in the plant has also been studied (Gupta et al. 2016). Altered trichome density and enhanced jasmonic acid (JA) level-mediated transcriptional patterns have been implicated for increased artemisinin content in *A. annua* colonized by *Rhizophagus intraradices* (Mandal et al. 2015). A decrease in artemisinin concentration in plants after treatment with ibuprofen (JA biosynthesis inhibitor) also implicated JA in artemisinin production.

A link between the phenylpropanoid/lignin and artemisinin biosynthetic pathways through salicylic acid caused by accumulation of trans-cinnamic acid due to the block at the step catalyzed by cinnamate-4-hydroxylase has been demonstrated using a RNAi-based strategy (Kumar et al. 2016). Artemisinin content as well as glandular trichome density and size have been found to be reduced under water stress conditions (Yadav et al. 2014). There is a possibility of having *A. annua* plant adapted for growth under saline conditions toward agriculture-related techno-economic benefit (Yadav et al. 2017).

UV-B exposure increases artemisinin content in the plant, possibly through the overexpression of DBR2 due to UV-B-caused DNA hypomethylation and UV-B-mediated epigenetic activation of additional WRKY-binding site(s) in the promoter of the gene (Pandey and Pandey-Rai 2015). In another study, Co-60 gamma-rays irradiated sodium alginate along with phosphorus has been found to significantly enhance leaf (dry) yield, leaf artemisinin concentration as well as overall artemisinin yield (Aftab et al. 2014). Existence of an interactive regulatory network between primary and secondary metabolism as well as tolerance toward arsenic has been indicated in *A. annua* using a proteomics-based approach (Rai et al. 2014). Triacontanol and chlormequat (2-chloroethyltrimethylammonium chloride) have been found to enhance herbage and artemisinin yields in *A. annua* (Shukla et al. 1992).

Other Uses

- Cytokinins have been isolated from *A. annua* (Shukla et al. 1994).
- The oil from *A. annua* has been found to be having repellent (behavioral) as well as toxic (physiological) activity on *Tribolium castaneum* (Herbst) and *Callosobruchus maculatus* (L.) that are stored-product beetles (Tripathi et al. 2000).
- Extracts of *A. annua* and its isomeric flavonoids (casticin and chrysosplenetin) have been shown to act as insect growth inhibitors against the African pod borer, *Helicoverpa armigera*, and appear to have potential for developing novel biopesticides (Anshul et al. 2013).
- *A. annua* plant material has also been found to be a potential nitrification inhibitor for increasing fertilizer nitrogen use efficiency (Kiran and Patra 2003).
- The antileishmanial efficacy of *A. annua* leaves and seeds against *Leishmania donovani* is due to direct leishmanicidal action and triggering of Th1-biased protective cell-mediated immunity along with memory generation. Thus there exists a possibility for use as adjunct therapy for leishmaniasis treatment, either singly or in form of a combination with other antileishmanials (Islamuddin et al. 2015).
- Artemisinin has also been shown to possess strong activity against the peptic ulcer-causing pathogen, *Helicobacter pylori* (Goswami et al. 2012).

Some Recent Developments

- Recently, compartmentalized metabolic engineering in tobacco for artemisinin biosynthesis at clinically meaningful levels and effective malaria treatment by oral delivery of plant cells has been shown, which will make artemisinin therapy cheaper for the global population living in endemic regions (Malhotra et al. 2016).
- Production of artemisinin by plant hairy root cultures has also been attempted in liquid- and gas-phase bioreactors (Patra and Srivastava 2015; Patra and Srivastava 2016).

- A rapid HPTLC-based method for simultaneous analysis of artemisinic acid and artemisinin in *A. annua* has been developed (Khan et al. 2015).
- Development of CIM-Sanjeevani, a high artemisinin (1.2%) yielding population of *A. annua* from polycross progenies between *Jeevanraksha* and *CIM-Arogya* varieties by CSIR-CIMAP (Gupta et al. 2016). An agreement has also been signed between CSIR-CIMAP and M/s IPCA Laboratories, Ratlam, M.P., for transfer of technology of CIM-Sanjeevani (Hindustan Times, 25 October 2016).

Societal Impact

CSIR-CIMAP, in association with CSIR-CDRI, Lucknow, developed an artemisinin derivative, α,β-arteether, for easy parenteral treatment of severe and complex cases of cerebral malaria. As an advantage, the epimeric mixture of α- and β-arteether has higher solubility in oil and is cost-effective for production on a large scale. The technology for the production and distribution of α,β-arteether, an indigenously developed antimalarial drug, has been transferred to the Mumbai-based firm, M/s Themis Medicare, and distributed under the trade name E-Mal (Jain et al. 2000). α,β-arteether also found a place in the national drug policy for the control of malaria formulated by the Ministry of Health and Family Welfare, Government of India, in 2007 (Tripathi and Dikshit 2015). *A. annua* cultivation by Indian farmers has led to enhanced incomes in the rural sector. CSIR-CIMAP has facilitated public-private partnership (PPP) mode for *A. annua* cultivation by bringing together farmers and industry. For example, contract farming of *A. annua* (*CIM-Arogya*) is being done by M/s IPCA Laboratory, Ratlam, under agreement with CSIR-CIMAP. Presently, the crop is cultivated in states like Uttar Pradesh, Bihar, Madhya Pradesh, Uttarakhand, and Gujarat under contractual buy-back arrangement with the industry (Kumar et al. 2015). In 2012–2013 it was estimated to cover an area of 2670 acres and provided an attractive return to the growers (Rs. 65,759 per hectare in about 4 months).

CSIR-CIMAP's technologies have generated employment opportunities worth 4.5 lakh man-days from cultivation of the crop alone. Many industrial clients like M/s Themis Medicare, Mumbai; M/s Biotech International Ltd., New Delhi; M/s Vital Health Care Pvt. Ltd., Mumbai; M/s Scimitar Biotec, Chandigarh; M/s Sanat Product Pvt. Ltd., Sahibabad; M/s Gujarat State Fertilizer & Chemicals Ltd., Vadodara; M/s IPCA Laboratory, Ratlam; M/s Bharti Society, Salem; and M/s Disinfecto Chemical Industries Pvt. Ltd., Lucknow, have benefited from the technology package (CSIR-CIMAP realized an amount of Rs. 76.17 lakh as a result of technology transfer during 2004–2012).

Presently, IPCA is the Indian market leader in antimalarials across all dosage forms. It has succeeded in full backward integration, which covers the complete chain from growing the *A. annua* crop in the farm to the manufacture of APIs and formulations on industrial scale. Presently, around 18% of the company's turnover comes from antimalarials, which sums up to around US$ 60million (IPCA Website 2017).

Conclusion

In 2015, the global demand for antimalarial drugs was around 1.3 billion treatment courses, and it is expected to reach 1.4 billion by 2018 (Nylang 2016). Presently, only a small fraction of the Indian requirement of *A. annua* is produced in the country, and mostly it has to be imported. Indian researchers seem to be geared up to meet this challenge. Taken together, the research on *A. annua* in India has been well balanced on the basic and applied fronts. It provides a successful example of "lab-to-land" as well as "farm to pharma" approaches, whereby end-to-end components have been taken care of for establishing a societal value chain. The "A" (agriculture), "B" (biology), and "C" (chemistry) domains have synergistically combined to translate the research for the benefit of mankind (Table 2).

Table 2 Brief chronology of major milestones in *A. annua*-related research in Indian laboratories

S. No.	Milestone/implication	Period/ year	References
1	Introduction of *A. annua* in India from the Royal Botanic Gardens, Kew, UK, by CSIR-CIMAP	Early 1980s	Kumar et al. (2015) and Srivastava (1999)
2	Development of viable variety *Asha* having low artemisinin and low artemisinic acid	1989	Tripathi and Dikshit (2015)
3	α,β-arteether developed by CSIR-CIMAP and CSIR-CDRI licensed to Themis Medicare Ltd., Mumbai	1997	CDRI Website (2017)
4	CSIR-CIMAP and CSIR-CDRI jointly won the CSIR process technology shield for developing the α,β-arteether processing technology	1998	Tripathi and Dikshit (2015)
5	The then Prime Minister of India, Sri Atal Bihari Vajpayee, launched E-Mal (α,β-arteether) from Themis on the occasion of the first National Technology Day (11 May 1999)	1999	Themis Medicare Website (2017)
6	Development of varieties *Suraksha* (artemisinic acid-rich) and *Jeevanraksha* (artemisinin-rich). *Suraksha* was released on the annual day of CIMAP by the then DG CSIR, Dr. Raghunath A. Mashelkar. The then Prime Minister of India, Sri Atal Bihari Vajpayee, dedicated *Jeevanraksha* to the nation on the occasion of the first National Technology Day (11 May 1999)	1999	Kumar et al. (1999)
7	Primers and a screening method developed for early identification of *A. annua* plants having high artemisinin content	2004	Khanuja et al. (2009)
8	Genetically tagged artemisinin-rich improved variety *CIM-Arogya* developed	2005	Khanuja et al. (2005)
9	CSIR-CIMAP's *Artemisia* biovillage program launched	2005	Tripathi and Dikshit (2015)

(continued)

Table 2 (continued)

S. No.	Milestone/implication	Period/ year	References
10	The then President of India, Dr. A.P.J. Abdul Kalam, mentioned *A. annua*-related technology as one of the most significant technologies of the year in his address to the nation on the National Technology Day.	2006	Tripathi and Dikshit (2015)
11	α,β-arteether found a place in the national drug policy (for malaria control) of the Ministry of Health and Family Welfare, Government of India.	2007	Tripathi and Dikshit (2015)
12	CSIR Technology Award – 2012 awarded to CSIR-CIMAP for development and commercialization of *A. annua* technology package	2012	Tripathi and Dikshit (2015)
13	Introduction of oil-free E-Mal (α,β-arteether) injection in ampoules and pre-filled syringe by Themis	2013	Themis Medicare Website (2017)
14	In July, CSIR-CIMAP and the Meghalaya Basin Development Authority signed a MoU to connect farmers of Meghalaya to the herbal industry so as to provide them with a better livelihood	2015	Nylang (2016)
15	Development of artemisinin-rich variety *CIM-Sanjeevani* and agreement between CSIR-CIMAP and M/s IPCA Laboratories, Ratlam, M.P., for transfer of its technology	2016	Gupta et al. (2016) and Hindustan Times (25 October 2016)

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Indian Sea Buckthorn

Brahma Singh

Introduction

The sea buckthorn (SBT; *Hippophae rhamnoides*) in the family Elaeagnaceae is a deciduous shrub with a vast area of natural growth in temperate regions of the world, mainly in Mongolia, China, Tibet, Russia, Canada, India, Pakistan, and Nepal. The ancient Greeks noticed that horses fed with the leaves and new branches of SBT exhibited shiny hair and skin and a significant visible gain in weight. This resulted in the naming of the genus *Hippophae* (from hippo [horse] and phaos [shine]) (Singh 2005).The most common species of the genus *Hippophae* is *rhamnoides*, which is known by various names, such as Siberian pineapple, sand thorn, sea berry, and sallow thorn. In India the importance and widespread occurrence of SBT has been documented, pioneered by the author, over the past 25 years, in the cold deserts of Ladakh (State of Jammu and Kashmir) and in Lahaul and Spiti (Himachal Pradesh), where it is known by many local names, such as Sastalulu, Shangti, Dhurchuk, Chumma, Tarwaa, Sirmaa, Chhurmak, and Leh berry. On September 23, 2015, a renowned Indian yoga guru, Baba Ram Deo of Patanjali Yogpeeth, Haridwar, Uttarakhand, named the plant Brahmaphal, in a transfer of technology function at the Defence Institute of High Altitude Research (DIHAR), Leh (Jammu and Kashmir), acknowledging the contribution of the author in finding this plant in Ladakh, an area known as a barren cold desert, and popularizing the plant in scientific and business communities in India. Hence, the Hindi name for SBT is also given as Brahmaphal. The author has seen it growing wild in other Indian Himalayan states such as Uttarakhand, Sikkim, West Bengal (Darjeeling hills), and Arunachal Pradesh. It mostly grows wild along river beds or wherever there are small water streams and even irrigation channels. Sea buckthorn is one of the future

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crops. The shrub has a remarkable lifespan of more than 100–150 years, and it has a number of eco-environmental and commercial benefits (Rana and Verma 2011).

Author's Association with Sea Buckthorn

The author has had interesting and lucky associations with the SBT. In 1992, he was director of the Field Research Laboratory (FRL), now the Defence Institute of High Altitude Research (DIHAR), Leh, of the Defence Research and Development Organization (DRDO), New Delhi, India. While in the office he got a message that a group of farmers had come to discuss their problems with him. He invited them into his office as a priority. Once they were comfortable he listened to them, and astonishingly, heard that they wanted to get rid of a perennial thorny plant they called a weed that was spreading in their barley and wheat fields—the two major crops of the cold arid Ladakh area. They brought a plant sample of the so-called weed with them; the author could not identify the plant and hence asked them to give him 1 week to provide a response. On referring to the literature he found that this "weed" was the golden bush—SBT. He studied it and considered that it was God's gift to the area. After a few days he requested the farmers not to weed it out but to allow it to proliferate, to profit them. To obtain benefits with this plant he and his colleagues at DRDO developed harvesting, post-harvest handling, and value-added technologies for dealing with the tiny orange fruits of the SBT. The provision of raw material (pulp and seeds) to various industrialists earned several crore of rupee/ several million U S Dollar (Rs one crore = 10 million US Dollar) annually in Leh and the Nubra Valley of Ladakh alone. Compact Food Limited (CFL), Leh, the pioneering company established in the 1990s by Mr. D. K. Mittal, has made a great contribution to the popularization of SBT in India. In 2015 Patanjali Yogpeeth (a renowned food and herbs company in India) used DRDO technologies for the production of SBT tea and other beverages and soft gel capsules from SBT fruit pulp and seeds procured from Ladakh; these raw materials were worth three crores of rupees/30 million US Dollar to the local population. Large quantities of SBT fruit pulp and seeds are now being exported annually to benefit the local populace. Over a dozen SBT fruit pulp factories with different capacities now operate in Leh and the Nubra valley. This is a success story of WEED TO WEALTH in a cold arid tribal region of India. Sea buckthorn (a previously unknown fruit) has become popular in India since DIHAR, Leh, developed and commercialized the technology for preparing beverages from its fruit pulp; DIHAR, Leh also found that the SBT natural growth area covered more than 11,500 ha in Ladakh (Dwivedi et al. 2009). Now more than 35 science laboratories in India are working on this plant for its antioxidant, immunomodulatory, anti-inflammatory, radioprotective, nutraceutical, pharmaceutical, and cosmetic characteristics, besides developing production and post-production technologies and environmental conservation strategies. Being a thorny shrub SBT is an ideal bio-fence plant for farmers to protect their crops from stray animals. Rare wild life in the area, from birds to the double-humped camel, survive on SBT.

Author in a natural growth of SBT in Leh

The Shrub

The SBT (*Hippophae rhamnoides*) shrub attains a height of 0.5–6 m in general, and it may reach 10 m. The leaf arrangement in stems/branches is observed as alternate or opposite. The leaves are lanceolate, distinct silvery-green, and 3 to 8 cm in length. The width of the leaf is less than 7 mm. The branches are stiff, dense, and full of thorns. Sea buckthorn has separate female and male plants, i.e., it is dioecious. The male plants bear brownish flowers that are rich in pollen. The pollen is distributed by wind; thus SBT is anemophilous. Juicy oil-rich fruits, popularly called berries, are produced by female SBT plants. The fruits are initially green and subsequently red/orange/yellow in color, depending on the characteristics of the variety/hybrid. Sea buckthorn has a deep root system which spreads extensively and rapidly. Sea buckthorn is a non-leguminous plant, but it fixes atmospheric nitrogen at the rate of 180 kg per ha via the presence of *Frankia* actinomycetes, which are present in

nodules on the roots, similar to the rhizobium in legumes. *H. salicifolia* (willow-leaved SBT) and *H. tibetana* are also found in a sizeable area in Ladakh, but are yet to be exploited (Chaurasia and Singh 1996).

Importance

The area of SBT natural growth and commercial plantations worldwide is estimated to be 3.0 million ha, 90% of which is in China, Mongolia, Russia, Northern Europe, and Canada. The area under SBT growth in India, mainly consisting of natural growth, is approximately 13,067 ha in total; in Ladakh (Jammu and Kashmir), 9267 ha; Himachal Pradesh, 1000 ha; Uttarakhand, 2000 ha; and Sikkim, 800 ha. Commercial plantations or orchards are yet to be established in India, excluding some efforts in Himachal Pradesh and Ladakh. SBT is an excellent plant for controlling soil, wind, and water erosion in cold desert areas, and it is ideal for fencing fields, to prevent the entry of stray animals, as well as to indicate that someone is in possession of barren but potentially useable land. The SBT is also an excellent bio-fuel. After seeing the results of research carried out by DRDO and other science laboratories, some of India's horticulture departments in hilly regions are now realizing the plant's importance and have begun to cultivate the shrub on a large scale with locally developed technology. The shrub is not damaged by long and severe winters. It thrives mainly on the banks of rivers, streams, and rivulets, and on river islands and foothills that have plenty of sunshine. In India the natural growth of SBT is confined to elevations of up to 12,000 ft/3657.6 m above mean sea level; it can face temperatures of minus 50 °C because its cell sap acts as antifreeze. It cannot tolerate waterlogging caused by heavy precipitation or irrigation (Singh 2005).

The Fruit

The fruit of SBT consists of skin, pulp, and a single seed. All these three parts are storehouses of useful and rare bioactive substances that can be used in nutraceuticals and pharmaceuticals. After pressing, the fruit consists of juice, 74.5%; seed, 6.54%; and residue/pomace, 19.45%. As reported by Dwivedi et al. (2009) and others, the fruit is rich in ascorbic acid (vitamin C); vitamin E (mixed tocopherols); folic acid; and carotenoids, including beta carotene, lycopene, and zeaxanthin. The fruit is yellow, orange, or red in color and is rich in omega fatty acids (omega, 3, 6, 7, and 9) and contains saturated oils and sterols, mainly β-sitosterol; organic acids, such as ascorbic acid, quinic acid, and malic acid, and flavonoids. Dwivedi et al. (2009) have reported that "One hundred gram of sea buckthorn fruit juice contain 49 kcal".

The physicochemical characteristics of SBT are given below in Table 1.

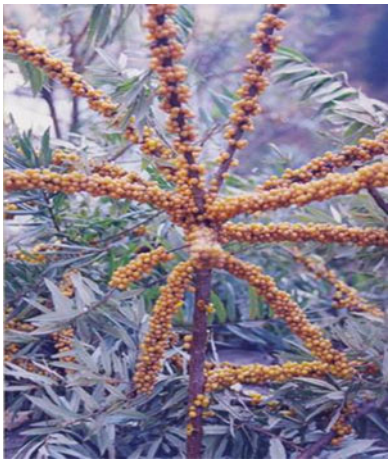
Table 1 Physicochemical properties of ripe fruits of *Hippophae rhamnoides* of Ladakh

Physical characteristics	
Length (mm)	7.34
Width (mm)	5.91
Shape	Round, oval, ovoid, oblong
Color	Yellow, orange to orange red, Green when immature
Seed (%)	6.54
Residue/pomace (%)	19.45
Juice yield (%)	74.5
Average weight of 100 fruits (g)	14.07
Chemical characteristics	
Total soluble solids (°Brix)	14.3
Acidity, as malic acid (%)	2.54
pH of juice	2.15
Total sugar (%)	1.03
Reducing sugar (%)	0.96
Non-reducing sugar (%)	0.07
Moisture (%)	74.58
Ash (%)	1.8
Crude protein (%)	2.64
Crude fiber (%)	3.54
Total carbohydrates (%)	20.56
β-Carotene (mg/100 g)	12,839.67
Vitamin C (mg/100 g)	424.8
Vitamin B_1 (mg/100 g)	2.664
Vitamin B_2 (mg/100 g)	6.227
Pectin (%)	0.48
Fat (%)	1.54
Energy (kCal/100 g)	106.66
Minerals (mg/kg)	
Sodium	41.28
Potassium	1499.96
Calcium	383
Iron	11.68
Magnesium	47.7
Zinc	0.94
Phosphorus %	0.02

Source: FRL/DIHAR (DRDO), the sea buckthorn (2006)



Hippophae rhamnoides



H. salicifolia

H. tibetana

The percentages of unsaturated fatty acids in the seed, pulp, and pomace are 87%, 67%, and 70%, respectively. The pomace (residue after extraction of seed and juice, which includes the outer skin), is rich in carotenoids, flavonoids, and vitamin E. The seeds contain very high percentages of unsaturated fatty acids and sterols (Stobdan et al. 2010).

The Leaf

Sea buckthorn leaves are good fodder/feed for 51 types of birds and 29 types of animals. In Ladakh, the endangered double-humped camel, and yaks, sheep, goats, cattle, donkeys, and poultry survive on the dried leaves of SBT mainly during the sub-zero winter months when absolutely no other green fodder is available in the region. Green as well as dry SBT leaves make nutritive fodder for animals. The leaves are a good source of protein, ether extract, carbohydrates, crude fiber, and total ash, as is evident from Table 2.

Almost all parts of SBT plants are important, as is evident from their uses, given in Table 3 below (Singh 2008).

Table 2 Approximate chemical composition of sea buckthorn (SBT) mature green leaves

Constituents	(%)
Moisture	52–69
Total ash	1.76
Crude protein	2.2–2.4
Crude fiber	4.6–4.85
Ether extract	6.6–6.94
Total carbohydrate	32–37
Calcium	69 mg/100 g

Source: FRL/DIHAR (DRDO), the seabuckthorn (2006)

Table 3 Sea buckthorn plant parts and their uses

Serial Number	Plant part	Uses
1	Roots	Fuel, fixes atmospheric nitrogen (Frankia), controls soil erosion
2	Bark	Pharmaceuticals and cosmetics
3	Leaves	Pharmaceuticals, cosmetics, tea, food, animal and poultry feed, fodder
4	Fruit	
	Juice	Sport drinks, health drinks, other beverages, wines, food
	Oil	Pharmaceuticals, nutriceuticals, and cosmetics
	Pomace	Animal feed, food colors, nutraceuticals
	Seed oil	Pharmaceuticals, nutraceuticals, and cosmetics
	Seed cake	Food, feed, nutraceuticals
5	Twigs	Fuel, fencing for fields, fodder

Traditional Medical Uses

Sea buckthorn fruits in the form of concoctions have been used in the Tibetan system of medicine for more than 1000 years (Singh 2008). The concoctions are applied topically to treat skin problems caused by solar irradiation/sunburn, as well as bedsores. These concoctions are also useful in the treatment of stomach ulcers. The concoctions have been reported as being astringent, anti-diarrheal, stomachic, antitussive, and anti-hemorrhagic. In central Asia, the leaves are used to treat skin disorders and rheumatoid arthritis. In Mongolia, colitis and enterocolitis in both humans and animals is treated with extracts of SBT branches and leaves. Ancient Tibetan and Chinese medicinal literature documented the use of SBT berries for treating fevers, hepatic diseases, circulatory disorders, inflammation, toxicity, abscesses, coughs, colds, ischemic heart diseases, metabolic disorders, tumors (particularly in the stomach and esophagus), and gynecological diseases; for clearing sputum and aiding digestion and blood purification; and for their laxative effect. The Amchi system of medicine (Sowa Rigpa) in Ladakh uses SBT leaves, berries, pulp oil, seed oil, etc., in different herbal formulations. Sea buckthorn has been used for

centuries in European and Asian countries as the main ingredient in some nutraceuticals, pharmaceuticals, and cosmeceuticals. Dwivedi et al. (2006) have reported that SBT oil has general nourishing, revitalizing, and restorative actions. Skin problems such as acne, dermatitis, irritated dry itching skin, skin soreness, ulcers, burns, scalds, cuts, and tissue regeneration are effectively treated with SBT concoctions.

Feed, Fodder, Fuel, and Bio-Fencing

Sea buckthorn is a favorite fodder and feed for high-altitude animals such as the double-humped camel, pashmina goats and sheep, yaks, and donkeys. The fruit of SBT, particularly the seeds, provides feed for many rare bird species found in high-altitude cold deserts and other places of natural SBT growth. The leaves of SBT, as well as its fruit, are rich sources of protein, vitamins, and minerals. The seeds and pomace (fruit parts other than juice) are excellent feed for poultry and cattle. The phytochemicals present in SBT foliage, fruit fibers, and seeds, mentioned earlier, make these plant parts nutritionally rich feed and fodder for animals and birds.

The dry twigs and roots of SBT make excellent fuel for villagers. Living and non-living SBT bio-fences are not uncommon for crop fields and forests in the areas of its habitation. As a thorny plant it is an ideal bio-fence.

Phytochemicals

Several studies in India and abroad have indicated that SBT fruits are rich in vitamins, minerals, and other bioactive biochemical, such as steroids, terpenoids, phenolics, and fatty acids (Dwivedi et al. 2009 and Lu 1992). Leucocyanidin, catechin, flavonol, and trace flavanones are the flavonoid components noted in SBT fruits and leaves. The mineral contents of SBT have great nutritional value. The iron content of SBT is in the range of 22–282 mg kg^{-1}, making it good for people with anemia. Gallic, p-coumaric, ferulic, protecatechuic, p-hydroxybenzoic, and ellagic phenolic acids are present in both the fruits and leaves. From the Leh valley of India, Stobdan et al. (2010) and other scientists working in Indian science laboratories have reported that natural growth SBT has high contents of many vitamins, e.g., vitamin C (275 mg 100 g^{-1}), vitamin A (432.4 IU 100 g^{-1}), vitamin E (3.54 mg 100 g^{-1}), riboflavin (1.45 mg 100 g^{-1}), niacin (68.4 mg 100 g^{-1}), pantothenic acid (0.85 μg 100 g^{-1}), vitamin B_6 (1.12 mg 100 g^{-1}), and vitamin B_2 (5.4 μg 100 g^{-1}). Similarly, natural growth SBT has high levels of minerals, e.g., potassium (647.2 mg L^{-1}), calcium (176.6 mg L^{-1}), iron (30.9 mg L^{-1}), magnesium (22.5 mg L^{-1}), phosphorus (84.2 mg L^{-1}), sodium (414.2 mg L^{-1}), zinc (1.4 mg L^{-1}), copper (0.7 mg L^{-1}), manganese (1.06 mg L^{-1}), and selenium (0.53 mg L^{-1}). The same authors, in 2011 and 2015, reported more than 40 volatile compounds in the fruits and leaves of SBT. Eight aliphatic esters, nine aliphatic alcohols, and 10 aliphatic hydrocarbons were obtained on steam distillation of SBT fruits. Decanol, ethyl octanoate, ethyl dodecanoate, and

ethyl decanoate are the primary constituents that produce the aroma of SBT. Sea buckthorn leaves contain the tannins hippophaenins A and B. The peel of the stems and fruits contains 5-hydroxytryptamine (5-HT), a rare occurrence in the plant kingdom. 5-HT existing in a free or grouped state can regulate human emotion, blood pressure, body temperature, and hormone levels. It also acts as a neurotransmitter and can function as an anti-cancer, anti-irradiation, and anti-infective agent. It can also promote coagulation by transforming fibrinogen to fibrin.

Some potentially bioactive molecules (phyto-medicines) in SBT (Patil and Chaudhary 2016) are:*Phytosterols*: Regulate inflammatory processes, and are anti-ulcer, anti-cancer, and anti-atherogenic, and improve microcirculation in the skin.

Polyunsaturated fatty acids: Have immunomodulatory, neuroprotective, and anti-tumor effects.

Organic acids: Have anti-ulcer, wound-healing, and anti-arthritis effects. Lower the risk of heart attack and stroke.

Tocopherols: Are antioxidants, minimizing lipid oxidation, and have pain-relieving effects.

Vitamin C: Sea buckthorn fruits and leaves are rich in vitamin C, which is an antioxidant and sustains cell membrane integrity. Vitamin C accelerates collagen synthesis.

Carotenoids: Help in collagen synthesis and epithelialization, besides being antioxidants.

Vitamin K: Promotes wound healing, prevents bleeding, and has an anti-ulcer effect.

Vitamin B complex: Known for cell repair and nerve regeneration.

Zinc: Aids in cell proliferation, boosts utilization of vitamin A; acts as a cofactor for enzymes, and stimulates blood circulation.

Coumarins and triterpenes: Regulate sleep, appetite, learning, and memory.

Polyphenolic compounds: Have antioxidant, cytoprotective, cardioprotective, and wound-healing effects.

Sea Buckthorn Oil

As stated earlier, SBT fruit pulp and seeds contain a substantial quantity of oil, which is reported to be helpful for human health. Some scientific work has been done in India on SBT oil. Parimelazhagan et al. (2005) reviewed the literature on the subject and reported that both the pulp and the seed oil have biochemicals of medicinal value. Based on the literature it can be stated that the oil content of seeds of different genotypes from different altitudes ranges from 9.50% to 23.36%. The oil content of the seeds is greater than that of the pulp. The maximum oil content of the pulp has been reported to be 18.75% in *H. neurocarpa*, while in the seeds of *H. rhamnoides* sub-species *tibetana* the oil content is 17.85%. Carotenoids, e.g., α-, β-, and γ-carotene; lycopene; β-cryptoxanthin zeaxanthin; zeaxanthin dipalmitate, and zeaxanthin palmitate, have been reported in the pulp and seed oil, and because of their antioxidant activity, they are known to minimize the risk of age-related macular degeneration.

As reported by Kumar et al. (2011), SBT seeds contain 8–10% oil. The dried fruit pulp contains 20–25% oil, while the pomace contains 15–20% oil. The unsaturated fatty acids reported in the seed oil are linolenic acid (omega-3; 20–23%), linoleic acid (omega-6; 40–43%), oleic acid (omega-9; 19–22%), and palmitoleic acid (1–3%). The major saturated fatty acids reported are palmitic (7–9%) and stearic (3–4%). The pulp and seed oils have different physicochemical and biochemical properties. The pulp oil is rich in carotenoids. Phytosterols, tocopherols, and tocotrienols are found in substantial quantities in both the pulp and seed oils. In the seed oil, the monounsaturated and saturated fatty acid content is as high as 65%. Vitamin E and β-sitosterol are found in both the pulp and seeds of SBT. The oil of SBT seeds contains high amounts of carotenoids, tocopherol, tocotrienol, and sterols (Kumar et al. 2011). The biochemical constituents of SBT have been reviewed by Stobdan et al. (2017).

Seed Oil Extraction Techniques

There are four seed oil extraction methods; namely, solvent extraction using petroleum-ether, screw pressing, aqueous, and supercritical carbon dioxide extraction. Important heat-sensitive compounds can be extracted in natural form without degradation by a supercritical carbon dioxide technique. Being non-toxic and non-explosive, this technique is environmentally friendly. Supercritical carbon dioxide is available in food-grade quality of high purity, and can easily be removed from food products. Hence, seed oil extracted by this technique is preferred in food, pharmaceutical, and other industries.

Chemical Profile of Sea Buckthorn Oil

Sea buckthorn oil, present in both seeds and pulp, is a rich source of carotenoids, vitamins E and K, fatty acids, and phytosterols. It is also a rich source of as many as 28 trace elements, e.g., calcium, magnesium, iron, zinc, selenium, and iodine. Palmitoleic, palmitic, and oleic acids are found in high amounts in the pulp oil, while linoleic, α-linoleic, oleic, palmitic, stearic, and vaccenic acids are found in high amounts in the seed oil. The seed oil of the SBT is reported to be the only seed oil that contains linolenic acid (omega-3) and linoleic acid (omega-6) at a 1:1 ratio in the natural state. The seed oil of SBT has a level of 32% omega-3 (linolenic acid), as shown in recent studies (Stobdon et al. 2017; Suryakumar and Gupta 2011), and it could be an important part of a balanced diet, an ideal we have lost through our 'modern diet'. Omega-3 and 6 are essential fatty acids for the human body and they carry fat-soluble vitamins, i.e., vitamins A, D, E, and K and also promote cognitive function and bone health. Studies have shown that increasing omega-3 fatty acid levels in the human diet leads to significant improvements in several neurological disorders, such as depression, Alzheimer's disease, memory loss, and schizophrenia in people of all ages. Oleic acid, which has been shown to reduce blood cholesterol

levels, is also found along with other beneficial fatty acids in SBT seed oil (Dwivedi et al. 2009; Kumar et al. 2011).

As reported by Kumar et al. (2011), the tocopherols and tocotrienols present in SBT oil are collectively known as vitamin E. α-Tocopherol has the highest antioxidant activity and is the most abundant tocopherol, comprising approximately 76–89% of the tocopherols in SBT fruit. The quantity of all tocopherol groups, except for β-tocopherols, is higher in SBT seed oil than in the pulp oil. Tocotrienols are observed in higher concentrations in the pulp oil. The content of vitamin E, a strong antioxidant, in SBT seed oil, makes it a strong scavenger of free radicals and helps inrepair of body miscles and proper functioning of body organs, enzymatic activities and neurological process.

Carotenoids Sea buckthorn pulp oil contains a high quantity of carotenoids (527.4 mg/100 g) (Cenkowski et al. 2006; Kumar et al. 2011). Vitamin A precursors of β-carotene and lycopene are the most common carotenoids in SBT pulp. Carotenoids have many physiological functions. Besides being free-radical scavengers they enhance the immune system in vertebrates. Certain human foods contain high levels of carotenoids; most of these have antioxidant activity. People with a high intake of β-carotene have a significantly reduced risk of lung cancer, as revealed by epidemiological studies (Yuan et al. 2003). However, paradoxically, diet supplementation with high doses of β-carotene in smokers results in an increase in cancer risk. This is possibly caused by excessive beta-carotene leading to breakdown products that reduce plasma vitamin A, which exacerbates the lung cell proliferation induced by smoking.

Sterols Cenkowski et al. (2006), Suryakumar and Gupta (2011), and several others have reported the presence of obtusifoliol, sitosterol, isofucosterol, campsterol, citrostadienol, avenasterol, cycloartenol, 24-methylenecycloartanol, and stigmastanol in SBT seed and pulp oil. The fruit pulp contains 1–3% sterols, while the seed oil has 1–2% sterols.

Health Benefits of Sea Buckthorn

As is evident from the text above, SBT is a rich source of several pharmaceuticals. It is reported to be a strong antioxidant and has anti-stress and anti-aging effects. It slows down skin aging and promotes wound healing. Its liver-protective effect is significant. Sea buckthorn has also been reported to be useful in the treatment of coronary heart disease Suryakumar and Gupta (2011). DRDO research has indicated that SBT fruit juice helps acclimatization in high altitudes and maintains good health, particularly in people from low-altitude regions. Sea buckthorn products help in promoting stomach, ocular, brain, skin, and blood health, besides being useful in the treatment of cancer, diabetes, aging, and coughs and colds (Singh 2008; Korekar et al. 2011). Sea buckthorn leaves contain significantly greater amounts of

hydrophilic than lipophilic phenolics and antioxidants (Dolkar et al. 2017), and these hydrophilic products are useful for several health problems.

Sea buckthorn has been reported to be of help in the treatment of the following health conditions/diseases (Singh and Chaurasia 2000a, b; Kumar et al. 2011).

Cancer Therapy Radiation and chemotherapy in cancer treatment have adverse effects on the health of the patient; SBT oil is reported to reduce these adverse effects. SBT provides a rich and comprehensive supply of nutrients to the patient and helps to improve their condition. It also removes stasis and eliminates waste, improves gastrointestinal function, increases appetite, promotes tissue growth, avoids infections at operative sites, and helps in restoring liver and kidney function, leading to good health (Kumar et al. 2011). The inhibitory effects of SBT oil on gastric acid and abnormal increases in gastric proteinase prevent the spread of ulcers and metastasis (Kumar et al. 2011).

Cardiovascular Health The considerable amounts of omega fatty acids in SBT have tonic effects on the cerebral and cardiovascular systems of the human body. Arteriosclerosis, caused by high blood fat levels, is considered to be the main cause of cardiovascular health problems. Sea buckthorn oil lowers blood fat levels, and improves the quality of the blood vessels. The fatty acids in SBT oil perform different functions. Linolenic acid promotes metabolism, reduces blood pressure, and dissolves accumulated fat. Linoleic acid regulates blood pressure, reduces serum cholesterol, and prevents heart arrhythmia. Oleic acid reduces cholesterol. Together, these unsaturated fatty acids prevent thrombosis and inhibit platelet agglutination. Vitamin E and other antioxidants remove injured and dead cells from the blood, prevent arterial wall damage caused by waste products, and remove waste products, including peroxides. The flavonoids in SBT improve heart function and increase the anti-hypoxia under normal or below normal blood pressure by increasing the blood flow of the coronary artery and increasing nutrients in the blood supplying the heart muscles; this lowers oxygen consumption and strengthens muscle contraction. 5-Serotonin (5-HT) and betaine in SBT are helpful in coordinating and regulating the nervous, endocrine, and immune systems to normalize cardiovascular function (Kumar et al. 2011).

Immune System The seed oil of SBT contains immunity-building factors such as flavonoids, glucosides, phenols, terpenes, vitamins, and trace elements, e.g., zinc, selenium, iron, and manganese (Kumar et al. 2011).

Skin Problems Skin conditions such as eczema, poorly healing wounds, burns, and sunburns, as well as burns caused by cancer radiation treatment and cosmetic laser surgery, are treated with SBT oil and its products. As reported by Kumar et al. (2011), Russians and Tibetans prepare several drugs using SBT oil for the treatment of chills, fevers, edema, furuncles, stomach tumors, tissue regeneration, skin grafts, inflammation, bacterial infections, pain, burns/injury caused by cosmetic laser surgery, corneal wounds, and abscess obstruction by sputum. Kumar et al. (2011) have also

emphasizedthat palmitoleic acid, which is present in SBT oil, is a component of the skin. As a topical agent palmitoleic acid is helpful in healing wounds and for the treatment of burns and scratches. This fatty acid can also nourish the skin as desired when taken orally.

Antioxidant Activity As stated earlier, SBT oil is rich in carotenoids (e.g., β-carotene), fatty acids (1:1 ratio of omega-3 to omega-6), oleic acid and lower saturated fatty acids, tocopherols (α- tocopherol, γ-tocopherol), and tocotrinol, as well as phytosterols; the oil also contains 28 trace elements (iron, zinc, calcium, magnesium, selenium, iodine, and others). All of these entities are known antioxidants. They have very low molecular weights, which is helpful in the neutralization or scavenging of free radicals. With SBT seed oil, superoxide dismutase is activated to eliminate free radicals. As a natural immunity enhancer, the seed oil eliminates mutant and dead cells, enhances macrophage phagocytosis, and kills cancerous cells (Kumar et al. 2011; Stobdon et al. 2017).

Ice Nucleating Agents

The fruits of SBT are a plant resource for ice nucleating agents (most biological nucleating agents are found in microorganisms or insects). Aqueous extracts of SBT fruits are added to other products to increase their freezing points. The fruit juice of SBT has a high freezing point and has been found to be ideal for areas that experience sub-zero temperatures for considerable periods. The addition of SBT berry juice and aqueous leaf extracts is helpful in increasing the freezing point of food products by providing an ice nucleating agent. The anti-nucleating agent is a protein and protein-lipid aggregate. This can be obtained by extracting the tissue with an aqueous solution containing a saccharide, particularly pectin alone or in pectin in combination with another saccharide (US Patent 5637301 A).

Feel-Good Factors

A certain percentage of the population, according to the World Health Organization, is in a sub-optimal health condition despite all their body physical and chemical health parameters being normal. Under such conditions a person does not feel good and has many problems. This condition may include lack of interest in work, lack of concentration, poor appetite, endocrine disorders, lung infection or respiration problem, fatigue, palpitations, and lack of strength after recovery from serious illness. The person is not ill but has little interest in work and other life activities. The person is not affected by pathogens, and has no physio-bio-chemical disorders, but their efficiency is low as they do not enjoy their work or life. Products from the SBT provide various nutrients and bioactive substances that are needed by the body, such as unsaturated fatty acids, carotenoids, polysterols, amino acids, vitamins, trace elements, and others. These entities regulate and harmonize the

endocrine, circulatory, autonomic nervous, and digestive systems and correct mood enhancer balances (http://www.healthkingenterprise.com/v2/product/other/seabuckthornseedoil.asp). Beverages and other preparations made from SBT fruits do help in overcoming the above problems. Regular consumers of SBT fruit juice may not face these problems at all because of the immune-enhancing effects of SBT.

Cultivation of Sea Buckthorn

Except for experimental orchards at horticultural hill universities and DIHAR and a few farmers in Himachal Pradesh, SBT cultivation has not yet been established in India. This is because of the non-utilization of the large quantity of available forest produce. With increasing awareness, the demand for SBT fruits would increase and facilitate the undertaking of commercial cultivation.

Soil and Climate

Sea buckthorn grows in a variety of soils, including the sandy soils prevalent in Indian cold-climate deserts, which are not rich in nutrients and have poor water-holding characteristics. Sea buckthorn has an extensive root system that goes very deep in the soil and provides soil binding in areas that are prone to wind and water erosion. The plant is tolerant to alkaline as well as acidic soil conditions, the optimum pH being 6–7. Hence it is very hardy bush. It has Frankia (an actinomycetic nitrogen-fixing, nodule-forming endophyte of woody trees and shrubs) nodules on its roots capable of fixing atmospheric nitrogen at the rate of around 180 kg per ha annually. *H. salicifolia* has aerial Frankia nodules.

Varieties

At DIHAR three high-yielding SBT selections; namely, FRL/DIH Selection-1, FRL/DIH Selection-2, and FRL/DIH Selection-3, are available to farmers. These selections were made from the 19 different selections made earlier from the 200 selections originally collected locally. Less thorny, big-fruited, and short-growth-duration selections from abroad are being evaluated at Kukumseri, the Lahaul Valley, Himachal Pradesh, and at Leh (Jammu and Kashmir).

Propagation

Sea buckthorn can be propagated by various methods. Based on experiments conducted at DIHAR, Leh, and Himachal Pradesh Agriculture University Palampur, brief descriptions of various methods of its propagation are given below (Dwivedi et al. 2001).

Seeds

Sea buckthorn can be propagated successfully with seeds, which are viable for a period of 2 years, after which they rapidly lose viability. Sea buckthorn seeds are small (4.5 mm in length) with a brown hard seed coat. One gram of seeds may contain about 100–105 seeds. Freshly harvested seeds have a short physiological dormancy and thus cannot germinate immediately after harvesting. May is the best time for the sowing of seeds in nursery beds. Before sowing, the mature seeds should be stratified for about 25 days by keeping them in a box in alternate layers of sand and seed. These boxes are kept in cool, shady conditions for about 25 days. This method gives a germination rate of 85%. Seeds can also be sown directly in nursery beds in November. Small seeds have shown poor germination. This method is useful only for raising large-scale nurseries for the afforestation of mountainous wastelands. Propagation through seeds leads to the problem of maintaining the desired ratio of male and female plants in the orchard. The use of seedlings multiplied through root suckers, soft wood cuttings, and tissue culture overcomes this problem.

Suckers

Sea buckthorn has a profuse suckering habit and has been reported to yield 13–40 suckers per plant depending upon the growth conditions and age of the plants. These suckers are true to type and can be separated, along with the roots, from the mother plants and planted directly at the planting site. The best time for the separation of suckers and planting is in March before bud break.

Stem Cuttings

Hard Wood

Sea buckthorn can be propagated successfully by stem cuttings, and this method has been recommended for commercial propagation. It helps in the development of a proper plant canopy and maintenance of the male/female ratio in the orchard. One- to 2-year-old stem cuttings of pencil thickness are ideal for SBT propagation. The stem cuttings are taken from identified male and female plants to obtain elite planting material. The best time for taking cuttings is in March. The side branches and thorns are removed to make 30-cm-long cuttings. The cuttings are then placed in running water for a period of 2–3 days. Sea buckthorn cuttings are not easy to root on their own, thus the rooting hormone indole butyric acid is applied at 500 ppm before the cuttings are planted in the nursery beds. The best time for the planting of cuttings has been found to be April. Planting should be done in rows 30–45 cm apart and with 15-cm spacing between the cuttings. Sea buckthorn normally takes 60–75 days for rooting, depending upon the soil and climatic conditions. A success rate of about 85% has been recorded for the rooting of stem cuttings under Leh conditions. These cuttings are ready for plantation in the main field in March of the following year.

Soft Wood

DIHAR has successfully standardized the propagation of SBT through soft wood cuttings (1-year-old branches) using naphthalene acetic acid at 200–300 ppm. The technique has yielded over 90% rooting in cuttings, with excellent root quality under moist conditions with coarse sand as the rooting medium. The plantlets are readied for transplantation in poly bags within 90–100 days after the planting of the cuttings. This method is much faster than that with hard wood cuttings.

Planting and Care

Sea buckthorn prefers sunny, well drained, deep soils with assured irrigation facilities. It has a deep root system (3–5 m) and for its optimal growth soil must be 2–3 m in depth and should be free from stones/hard rocks, etc. It is recommended that SBT be planted in a hedgerow system that provides a greater space between rows and a lesser space between plants. Single or double hedgerow systems can be adopted, and these need to provide enough space to approach each plant easily for training, pruning, harvesting, and other cultural operations. In a single hedgerow system, the distance between rows is maintained at 2–4 m for pure cultivation and 4–5 m for intercropping. The SBT plants are normally planted in rows at a distance of 1.0 m. However, the method and distance of planting can be modified according to the climatic conditions, fertility status of the soil, varietal features, orchard management practices adopted, and harvesting requirements.

The pits for plantation should be of 60-cm diameter and 60–80 cm deep so that stones and roots can be removed from the root zone. The best time for the digging of pits is the end of October or early November. If the pits are not dug during this period, they must be dug at least 45 days before planting. After the digging of pits the soil should be cleaned properly, and well aged farmyard manure, at 2 kg per pit, should be mixed in properly before filling the pits. The plants are to be grown organically or naturally. In heavy soils, sand should also be mixed in to make the soil light and porous. The pits should be opened for solarization for about 10–15 days and thereafter they should be filled properly with the prepared mixture. Sea buckthorn can be planted in autumn (October–November) and spring (March–April) after the onset of dormancy and before the sprouting of buds, respectively. Spring planting has been found to give better results with regard to plant establishment and its survival. At the time of planting, it must be ensured that at least 10% of male plants must be planted in the orchard, distributed uniformly all over the orchard to ensure proper pollination. Sea buckthorn is a drought-tolerant shrub. After establishment in the first year it can tolerate even severe drought. For higher yields of fruit and leaves, light irrigation, at 15- to 20-day intervals, is desirable during the summer months (June–August).

Pruning and Training

The basic aim of pruning is to strike a balance between the roots and shoots (vegetative) growth of the plant by heading back or thinning out the excessive vegetative growth of the plant. Pruning is also practiced to induce fresh growth, to encourage regeneration, to remove diseased or dried branches, to promote flowering/fruiting, to reduce crop load, to improve the quality and color of the fruits, to provide shape and strong structure to the plant, and to remove undesirable growth and interfering branches. Data on SBT pruning is not available since no systematic study has been conducted. However, pruning is essentially required for canopy management, the removal of profuse suckers, and the induction of new growth, which is essential to induce flower buds in the next season. For the production of quality fruits, SBT should be pruned regularly for ease in harvesting and for plant exposure to the sun. The best time for pruning SBT in Ladakh is March, before sprouting of the plant in spring. Pruning should be done with sharp secateurs or a saw. Pruning should not be done once the plant/buds start sprouting. The intensity of pruning depends upon the age and growth of the plant and the soil conditions. However, 50% of the growth of 3- to 4-year-old shoots should be removed.

For the quality production of fruits, training is an essential operation to provide a desirable plant shape to make cultural operations easy, and also to provide a strong and scientific structure for the plant. Training helps to expose the fruits to the sun for better color development and enables the plant to bear the maximum crop load. Evaluation of different systems of training needs to be done for SBT, since there is no standard system recommended for the crop. However, it has been observed that SBT, being a thorny shrub, should be restricted to a plant height of less than 2 m for ease in harvesting. Normally, properly managed SBT plants develop a crown that is 2–3 m in diameter at the age of 4 years after planting.

Insect Pests

The death hawk moth, *Acherontia styx*, has been reported to cause serious damage to SBT (Stobdan et al. 2017). Defoliating beetles, *Brahmina cariacea*, have also been noted. *Holotrichia longipennis Br.* damages the early growth of SBT. The Indian meal moth, *Plodia interpunctella (Huber)* damages SBT fruits during storage. Aphid attack of SBT has been noted in Uttarakhand. Several insect pests of SBT have been reported in China and Russia (Stobdan et al. 2017).

Diseases

Forty-seven pathogenic species belonging to 42 genera have been reported to infest SBT in Russia. In India there are very few reports on the diseases of SBT. Root-rot caused by *Rhizoctonia solani* Kuhn has been observed in nurseries at Gangotri and

Ranichauri in Uttarakhand, and *Verticilium sp.* and *Fusarium sp.* infesting SBT have also been observed there.

Yields

Vegetatively propagated SBT plants normally start bearing fruits at the age of 3–4 years after plantation, while seedlings take 5–6 years to come into fruiting. Sea buckthorn orchards start commercial production only 8 years after plantation. Yields of SBT plants vary according to their age, management practices adopted, and variety. Cultivated varieties have been reported to yield about 10–15 ton/ha fruit under properly managed orchard conditions. However, under Ladakh conditions, the yield of ripe fruits ranges from 0.6 to 2.0 kg per plant, since the plants are growing wild under natural conditions. From 8-year-old SBT growth or plantations 200–-300 quintal per ha of fresh leaves can be harvested.

Post-Harvest Management

Sea buckthorn is a non-climacteric fruit which ripens on the plant and cannot be ripened after harvesting. Maturity indices of the crop have not yet been worked out. Ripening of fruits generally sets in by the middle to end of August under Ladakh and Lahaul conditions. Ripening of fruits can be judged on the basis of surface color, texture, juice content, and total soluble solids. A sharp increase in the total soluble sugar of the fruits has been recorded after ripening and it reaches 12°Brix and even above. The fruit juice content can exceed 70%. Ripe fruits remain laden on the plants even throughout winter, up to April and even beyond, if not harvested. However, it is advisable to harvest the fruits before the onset of winter (sub-zero temperatures), which will damage the texture and quality of the fruits. The fruits of the SBT are very small and are soft, delicate, and juicy; they are highly perishable and cannot be transported over long distances. Thus, after harvesting, the fruit needs to be processed as soon as possible. Ripe fruits should be processed within 24 h of harvesting. The fruits are collected in plastic baskets, which should have a capacity of not more than 10 kg in order to avoid pressure damage to the. The ripe fruits of SBT also have a musky odor, which must be reduced before processing. Washing with cold water helps in reducing the odor of the fruits. For storage and distance transportation of ripe fruits the recommended temperature is 4–6 °C to reduce post-harvest losses. Quick freezing of the fruits and storage at −20 °C has also been found to be effective for their long-term storage. More than two dozen fruit juice extraction units with sizeable capacity now operate in Leh and the Nubra valley. In Lahaul and the Spiti Valley there are many home-scale pulping units, and their numbers are increasing.

The National Medicinal Plants Board (NMPB), Ministry of Ayurveda, Yoga and Naturopathy, Unani, Siddha and Homoeopathy (AYUSH), Government of India, New Delhi, provides 50% of the cost of the cultivation of *H. rhamnoides*

(474.69 USD). The estimated annual return per hectare from SBT cultivation in India is 30,884 USD (Stobdon et al. 2017).

Commercial Sea Buckthorn Products

Sea buckthorn oil soft gel, pulp oil, seed oil, fruit powder, leaf powder, tea, juice, juice concentrate, juice powder, and frozen fruits are commercial products in India. The ripe fruits are used for making various products. The mature fruits are pressed/pulped and separated into juice and seed plus skin/fiber. The juice is centrifuged and the pulp oil is decanted off. The oil-less juice can be processed as fruit juice, a mixed beverage, or pure SBT juice. It can be stored at low temperatures for spraying or freeze drying to make juice powder, or for other purposes; potassium metabisulfite is added as a preservative (Stobdon et al. 2017).

The remaining fruit part, the pomace, consisting of the seed, skin, and other solids, is processed by sun drying to separate the seeds. The solids and skin can be used as is in animal feed, or the pigments/carotenoids can be separated out before the pomace is used in animal feed. The seed is subjected to oil extraction, preferably using CO_2 extraction (supercritical fluid extraction). Sea buckthorn seed oil is used in phytomedicines and in the manufacture of health products (Kumar et al. 2011). The non-oil solids or seed cake are used to make biscuits or snacks or animal feed (Bawa et al. 2002). Dehydrated sun-dried or cabinet-dried fruits make good snacks. Leaves from both male and female plants are used in making herbal tea, poultry feed, animal feed, and fodder for the double-humped camel (author's observation).

Some Indian SBT products are shown below:

Soaps

Dehydrated berries

Omega-7 capsules

Oral SBT Oil/drops

Juice

Nectar

Seed oil

Ultraviolet sunscreens

Some Indian SBT products are shown above

Indus Berry-fruit juice (IIIM; Jammu)

SBT Products of Prima Natural Care Pvt Ltd, Kailash Colony, New Delhi

Sea-buckthorn pills/tablets

The Life Science laboratories of DRDO have developed the following products, using mainly the fruits and leaves of *H. rhamnoides* (Singh 2015; Stobdan et al. 2015):

DIHAR, Leh
Beverages (Leh Berry, Ladakh Berry, Power Berry, Sindhu Berry, and Sepricot); fruit jams, pickles, herbal tea, antioxidant herbal supplements, soft gel capsules, herbal appetizers, UV protective oil, adaptogenic herbal appetizer, and poultry feed.

Defence Food Research Laboratory (DFRL), Mysore
Sea buckthorn biscuits, fruit jelly, health drinks (squash), wine, food colorant, and yoghurt.

Defence Institute of Physiology Applied Sciences (DIPAS), New Delhi
DIP-HIP (herbal adjuvant)

DIP-LIP (anti-atherogenic and hypolipidemic); DIP-ANTOX (antioxidant); and Herbo Healer.

Institute of Nuclear Medicine and Applied Sciences (INMAS), New Delhi
Herbal radioprotectants, UV sunscreens.

The author highlighted the following items while addressing an International Conference on sea buckthorn in Delhi, India (Singh 2015).

Some Interesting and Useful Facts on SBT

(a) The lifespan of this bush is reported to be 100–150 years.
(b) *Hippophae* means "shiny horse"; the ancient Greeks gave the name after observing that horses had a glossy coat after consuming SBT fruit and leaves. Legend has it that Pegasus (the flying horse) preferred eating buckthorn leaves to any other food.
(c) Sea buckthorn juice was the official team beverage for the Chinese Olympic team at the 1988 Seoul Olympics.
(d) Sea buckthorn fruit juice was used as a beverage and SBT oil was used as a radioprotective agent by Russian cosmonauts.
(e) Vitamin C requirements for the world population could be met from the natural growth of sea buckthorn.
(f) Genghis Khan, the great warrior, is believed to have relied on nutritious SBT year-round in his expeditions.
(g) In the eighth century, the medicinal value of SBT was recorded in the Tibetan medicinal classic *rGyud Bzi* ("The Essence of Nectar: The Manual of the Secret Teachings of the Eight Limbs" http://www.thlib.org/encyclopedias/literary/genres/genres-book.php#!book=/studies-ingenres/b27/#ixzz57pxSejy4).
(h) The SBT is the ideal plant to control wind and water soil erosion in cold arid regions.
(i) Besides supporting rare fauna, SBT fixes atmospheric nitrogen at 180 kg per ha annually through *Frankia* root nodules.

Requirements for Promotion of Sea Buckthorn

Indian

1. Development of harvesting technology to harness the huge natural growth of SBT in India.
2. Canopy management to facilitate the mechanical harvesting of fruits and leaves.
3. Establishment of facilities for the storage and primary processing of produce in the inaccessible SBT production areas of Ladakh, Himachal Pradesh, and Uttarakhand (for *H. salicifolia*).
4. Use of solar power to prepare fruit pulp and dehydrate leaves, pomace, and seeds.
5. Establish large-scale SBT plantations along river banks in the cold-climate desert of India to control soil erosion, conserve birds and biodiversity, fix atmospheric nitrogen, and provide livelihood security to the local populace.
6. It would be better to maintain organic production of SBT.
7. Make SBT a horticulture crop instead of minor forest produce by adopting modern production practices.
8. Undertake research and development on *Hippophae salicifolia* (willow leaf SBT), which has a vast natural growth area in Himachal Pradesh, Uttarakhand, Sikkim, and Arunachal Pradesh.
9. Examine State forest laws to make them SBT friendly for SBT utilization and proliferation.

Global

1. Study the SBT genome to identify useful genes to benefit other crops as well as SBT.
2. Develop scientifically proven health and cosmetic products from the SBT plant.
3. Make SBT known as a popular plant worldwide by popularizing its health and nutritional products and environmental benefits.
4. Establish a 'global advanced center of research on sea buckthorn' to harness the health and environmental improvement potential of SBT and to develop varieties for environmental protection and edible purposes.
5. Undertake scientific studies based on the development of SBT pharmaceuticals.

Summary

Sea buckthorn is an emerging but important horticulture crop in India, as it is in some other countries. There is a need for intensive research on its production, processing, and utilization, as well as a need to develop appropriate technologies for its exploitation in human health, wellness, and eco-environmental conservation. The nutritional and medicinal potential of SBT awaits exploitation, in the absence of (or inadequate) scientific studies and systematic clinical trials. Sea buckthorn has antioxidant properties, coupled with high nutraceutical and pharmaceutical value. Since SBT has been used for several thousand years in traditional systems of medicine in India, particularly the AMCHI system, for the treatment of various ailments, adverse effects on human health can be ruled out, as has been supported by scientific evaluation. The scientifically evaluated pharmacological effects of SBT are many: it has radioprotective effects, can be used for the treatment of frostbite and is effective in acclimatization to high altitudes and in accelerating wound healing, as well as in treating diseases of the liver. Scientific animal and human studies of SBT indicate that it is an antioxidant, and has anti-cancer, anti-inflammatory, cardioprotective, anti-atherogenic, immunomodulatory, antibacterial, and antiviral effects. Intensive research is needed to find the cellular and molecular mechanisms of these activities. Since SBT has been used for several thousand years in traditional systems of medicine in India, particularly the AMCHI system, for the treatment of various ailments, adverse effects on human health can be ruled out, as has been supported by scientific evaluation.

In India many institutes and laboratories of the Ministry of Agriculture and Farmer Welfare; the Ministry of Defence (Research and Development Organization); the Ministry of Science and Technology, Bio-technology, Forest and Environment; the Ministry of AYUSH; and the Health Ministry have established research and development projects on this plant according to their aspects of interest. The SBT plant will be a future high-altitude fruit crop in India and in very cold regions elsewhere in the world. India must establish an institute on SBT in Ladakh to develop sustainable potential technologies for providing ecological, economic, and environmental enrichment to the region. The organized cultivation of SBT is to be

vigorously promoted. The SBT plant is considered to be appropriate to lead us to a one-world, one-health system, and a one-medicine system for high-altitude cold-climate deserts.

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Noni (*Morinda citrifolia* L.): Research and Development

P. I. Peter and K. V. Peter

Morinda citrifolia L. popularly known as Indian noni or Indian mulberry (Family: Rubiaceae) (2n = 56) is an evergreen shrub which flowers and fruits throughout the year and grown in the tropical coastal regions of the world. John Britto (2008) reviewed the taxonomy of *Morinda* L. Rethinam and Singh (2008) published a global review on production technologies of noni (*Morinda citrifolia L.*). The species is generally found from sea level to 400 m amsl, although it adapts better to coastal regions. Noni, often found growing along lava flows in South East Asia, is an underutilised shrub and unknown to many people including botanists in spite of the fact that this miracle plant contains more than 200 nutraceuticals for health and wellness of people. In India, it is widely grown in Andaman and Nicobar Islands. John Britto (2013) studied ecotypic, morphological and genetic variations in accessions of *Morinda citrifolia* and its allied species from varied ecoclimatic habitats in Tamil Nadu using molecular tools (Fig. 1).

It grows throughout the coastal region along fences and walkways due to its wider adaptability to hardy environment. Veena Gupta (2016) conducted collection, conservation, documentation and genetic diversity studies of noni (Fig. 2).

In mainland India, it is found along the coasts of Kerala, Karnataka, Tamil Nadu, Gujarat, West Bengal, Odisha and Andhra Pradesh. Systematic cultivation in large area is very limited, and bulk of fruit supply comes from natural wild growth. Recently Rethinam (2016) reviewed global production and research in noni and indicated increasing global demand for noni-based products. Peter (2008a, b) published 'All About Noni' with 11 chapters and 9 appendices to meet the

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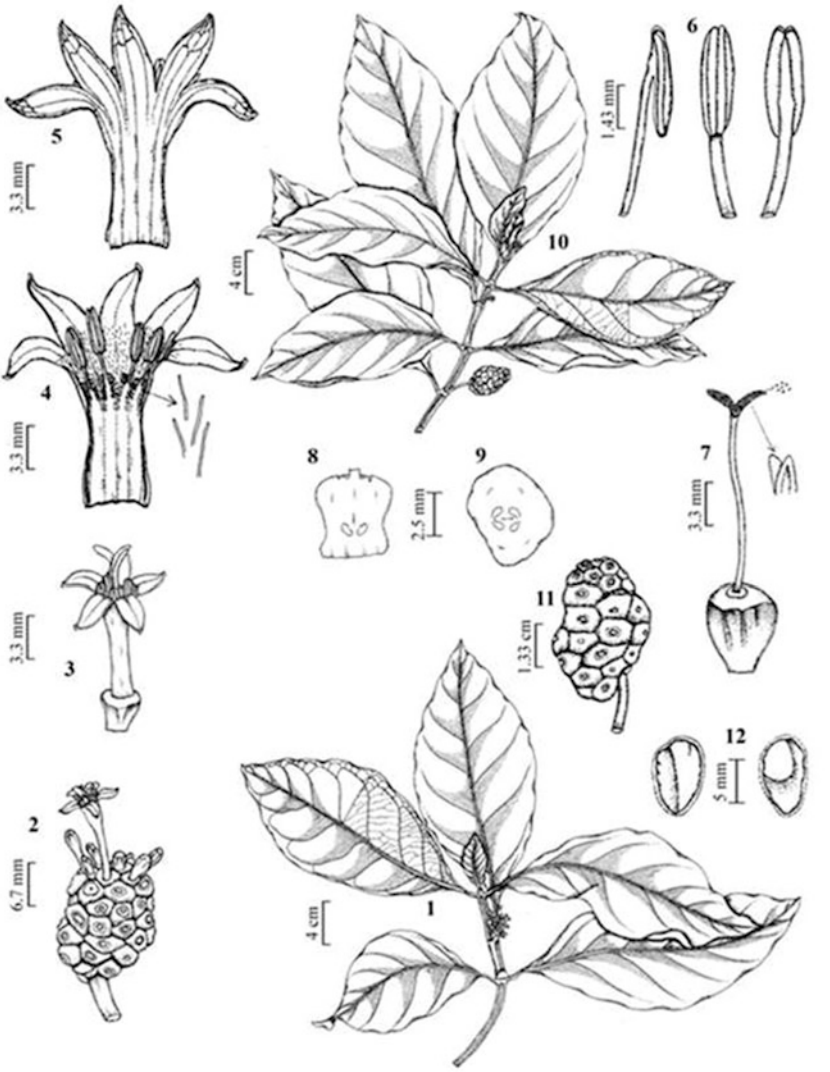

Fig. 1 Noni (*Morinda citrifolia* L.) (Source: John Britto 2008)

information requirements of noni enthusiasts and lovers. 'Hortus Malabaricus' names noni as 'Ada-pilava' and 'Macada-pala' and provides detailed description of the shrub. Noni is being described elaborately by Kirtikar and Basu in 'Indian Medicinal Plants' and by Rao et al. in 'Outlines of Botany'. Marimuthu and Peter (2010) published 'Noni (*Morinda citrifolia* L.)- A Text Book' dealing botany of *Morinda*, area, production, productivity, crop production technology, pre- and

Fig. 2 Noni (*Morinda citrifolia* L.) – fruit-bearing noni shrub

postharvest management, economics, pests, diseases, abiotic stresses, management practices and noni for health.

Noni Production

Rethinam (2014) reviewed good agricultural practices (GAP) of noni. Noni as a mixed crop in coconut performed well in different states under ICAR – All India Coordinated Research Project on Palms. The genus *Morinda* is distributed the world over, and the presence of as many as 115 species is reported. The presence of 19 species in New Caledonia (French island in South Pacific Ocean) is recorded. Survey of *Morinda* in South India revealed 12 species, or landraces of *Morinda* distributed throughout Tamil Nadu and Kerala alone. Two accessions, viz., IC 524021 and IC 524022, tolerant to saline soils and salt spray were identified by ICAR – Central Island Agricultural Research Institute, Port Blair. Two clonal selections 'Noni Kirti' and 'Noni Priya' are identified at the National Research

Centre on Noni, Chengalpet, Kanjeepuram District, Tamil Nadu. The above two varieties are getting registered as extant varieties at Protection of Plant Varieties and Farmers' Right Authority, New Delhi.

This shrub is sparsely distributed in coastal regions of Kerala and also in the Mangalore area of Karnataka. Recently an unidentified *Morinda* species with large and leathery leaves was reported in the Dandakaranya forest area of Malkangiri district in Odisha. The presence of *Morinda* sp. was also reported in the semi-arid ecosystem of Gujarat.

Seeds from the existing wild plants are collected, nurseries raised, and seedlings distributed for planting. Rooted stem cuttings are also used for propagation.

Planting Materials Production

Noni is relatively easy to be propagated from seeds, stem or rooted cuttings and air layering. The preferred methods of propagation are by seeds and cuttings from stem verticals. Arunachalam and Ashwita (2014) attempted with success wedge grafting on noni seedlings. The 'scion' flowered earlier to seedlings. Vegetative propagated planting materials maintain uniformity and high productivity. Subramani et al. (2007) attempted successfully micropropagation of noni for mass multiplication of elite clones. Micropropagation using tissue culture of axillary bud is the other standardised method of multiplication. Subramani (2008) reported callus and cell suspension studies of noni and demonstrated higher plant stand of TC noni.

Seeds

It is the best to collect noni fruits after they fall from the tree for seed extraction. Seeds are extracted from fruits, and sowing is done immediately. The seeds possess woody water coat which enables germination. Singh et al. (2006, 2007) conducted seed germination studies on noni and suggested measures to quicken germination. The seed requires scarification to reduce time to germinate and maintain uniformity. To scarify, the seed is cut, scratched or softened to allow ready penetration of water and air. Un-scarified seed usually takes 60 days and much longer (up to 6 months or more) for germination than scarified seed (3–4 weeks) depending upon the condition of germinating medium. Scarification can be done by a blending machine, nail cutter, etc. Seeds may be sown in nursery beds, trays or directly in light medium containers immediately after extraction from ripe fruits. They require hot and wet conditions for optimum germination. The warmest part of land provides better environment for higher seed germination. Noni seed has dormancy due to hard seed coat (water-repellant) thus delaying germination and needs 40–43 days to germinate. Seeds after drying in shade for 3–4 days can be stored in air-tight containers at room temperature. Preliminary seed storage experiments show that seeds have low viability but are orthodox in nature. The treatment with hot water at 40 °C for a period of 24 h and a treatment with sulphuric acid at 50% concentration

for 5 min were sufficient to overcome seed dormancy. The highest germination of seeds was obtained when the seeds were pricked and then treated with gibberellic acid (GA3 1000 ppm) for a period of 24 h. Seed treatment with hot water at 40 °C combined with seaweed (*Ascophyllum nodosum*) extract Biozyme and treatment with sulphuric acid (50%) for 5 min combined with Biozyme broke seed dormancy and gave better health and vigour to the germinated seedlings. Soaking seeds for 24 h with gibberellic acid (GA3 800 ppm) increased the germination percentage to 91.06 as against mere water treatment (51.4). The interaction of seed soaking and treatment with GA3 (800 ppm) increased high percentage of vigorous seedlings and number of leaves. Pretreatment of seeds with $NaHCLO_3$ (5% available chlorine) for 30 min increased germination up to 84%. Asha Sanker (2016) reported standardisation of agro-techniques for nursery and organic management techniques in noni in a multiple cropping system.

Planting Season

Ideal season for planting is from June to October. It can also be planted during January to March.

Pits for Planting

A pit of 0.6 m × 0.6 m × 0.6 m is dug, a few days before planting, and allowed to dry. The pit is filled with a mixture of topsoil, 2 kg of compost or farmyard manure, 2 kg of vermicompost and 1 kg of neem cake along with *Pseudomonas* sp., *Trichoderma*, VAM and *Azospirillum*. The pit is now ready for planting. Irrigation channels are to be formed, or drip systems need to be installed.

Spacing

The recommended spacing is 4 m × 4 m with a plant population of 700 plants/ha.

Weed Management

It is recommended to take up proper weed management till the plants are established and later adopt repeated mulchings along with intercropping with agathi (*Sesbania*), sunn hemp (*Crotalaria*), cowpea, horse gram or any fodder crop. In Hawaii, weeds like Guinea grass and sensitive grass (*Mimosa pudica*) compete with noni. It is also attacked by dodder (*Cuscuta* sp.), a parasitic weed. Manual removal of weeds from the field is recommended.

Nutrient Management

Noni has a deep taproot system and an extensive surface-feeding root system. The tree may not compete well in a landscape with plants which have aggressive, surface-feeding lateral roots like grasses. It can absorb the nutrients in manures and composts on the surface which is racked well coupled with irrigation. Organic manures are recommended for an organic noni fruit production. Since organic cultivation is promoted in India, the nutrient doses may be given in organic form from the approved organic sources. A tentative manure application for organic noni cultivation is suggested as below:

At 6th month, 2 kg. vermin compost or compost + ½ kg neem cake.
At 12th month, 2 kg. vermin compost or compost +1 kg neem cake + bio-fertilisers.
At 18th month, 2 kg. vermin compost +1 kg neem cake.
At 24th month, 5 kg. vermin compost +5 kg wood ash +1 kg neem cake +bio-fertilisers including *Trichoderma viride*, *Azotobacter*, phosphobacteria, *Pseudomonas* sp., etc.
After 24th month, the above dose is repeated every 6 months.

Noni displays abnormal foliar symptoms due to nitrogen, iron and phosphorous deficiencies like interveinal chlorosis, scorching of leaf margins, leaf curling, purpling and marginal necrosis. Nakeeran et al. (2009) reported exploitation of antibiotic producing plant growth-promoting rhizobacteria and fungal antagonists for the management of foliar diseases of noni. Further Nakeeran et al. (2015) reported management of dry and soft rot of noni fruits. Meena (2015) reviewed disease management in medicinal crops including noni. Sitanantham et al. (2016) reviewed organic pest control in cultivated medicinal plants, current Indian scenario and future research and development needs of noni.

Irrigation Management

The shrub thrives well with moderate irrigation and survives extended drought once established and matured. In dry condition when the trees are less than 2–3 years, irrigation once or more per week with 10 gallons of water per tree and for older trees more water with less frequency was suggested. Overwatering is not recommended. There is not much literature available on irrigation and quantity of water to be used. In India, irrigated noni is advocated for getting higher yield.

Planting pits are to be irrigated completely to wet the soil for 3 consecutive days. After planting, drip irrigation everyday for half an hour to supply 4 litres of water/plant/day for first 3 months, 8 litres of water/plant/day for next 9 months and there after 12 litres/plant/day up to 2 years is suggested. For adult plants after 2 years, 15 litres/tree/day are suggested. With good mulching at the base, this quantity of water is adequate depending upon the climate, soil conditions and canopy growth.

Green Manure Crops

Growing green manure crops like *Crotalaria* (sunn hemp), daincha, indigo, cassia, *Gliricidia*, agathi (*Sesbania* sp.), etc. produce adequate biomass for mulching for 2–3 months besides creating an ideal microclimate to the trees during summer. These crops also fix atmospheric nitrogen. Mulching reduces evaporation losses, prevents weed growth and conserves moisture. Upon slow decomposition in the basin, the physicochemical and biological properties of soil are improved, and water holding capacity and overall soil health improved.

Inter and Mixed Crops

Asha Sanker et al. (2016) reported economic success of intercropping noni gardens with zingiberaceous spices (ginger, turmeric) and medicinal plants. Growing green gram, black gram, cowpea, horse gram, groundnut, rain-fed millets, etc. during monsoon season utilising the available moisture is suggested as done by a few noni farmers. If irrigation facilities can be extended to the intercrops, the interspaces can be used for growing vegetables, flowers, medicinal plants and fodder crops which have good market potential in the area. Noni shrubs can be well fitted in coconut and areca nut plantations as mixed crop. In coconut, mango and cashew plantations, noni can be planted in between the rows. George Thomas (2010) reviewed the benefit of noni as an intercrop of coconut plantation. Coconut being a wide-spaced perennial crop remained committed to land for more than 65 or 70 years which provides ample scope for inter/mixed crop at various stages. Noni as a mixed crop is coming up well. Crops like banana, sugarcane, papaya, rose and similar other crops which require more water should be avoided as intercrop of noni as they may promote nematode population.

Harvest and Postharvest Processing

Noni bears fruits in about 9 months to 1 year after planting. Harvesting can be done throughout the year although there are seasonal patterns in flowering and fruiting. The commercial harvest can be done from 20 to 24 months after planting, and it is suggested to remove all flower buds up to 1 year and 6 months and then allow for flowering and fruit setting. Depending on the postharvest technologies adopted, the fruits may be harvested at different stages of development. After harvesting, the fruit ripens within a week at ambient temperature. Because of its short storage life, the fruits cannot be transported to distant places. To overcome this problem of perishability, the fruits with pedicel may be harvested to maintain better quality and market acceptability. The highest spoilage of fruits is observed in fruits harvested without pedicel. Fruits with pedicel performed well in terms of keeping quality, ascorbic acid and TSS. Among accessions SPG-2 recorded minimum loss of weight (2.90%) followed by Pbay-7 (3.74%) in 9 days during storage. Most of the processors buy

fruits harvested at the 'hard white' stage for juice extraction as the fruits become soft too quickly once this stage is reached. The change from stage 4 to stage 5 occurs very quickly (a few hours), and the pulp practically liquefies and turns from green to white, as well as develops the characteristic butyric smell. The fruits are individually selected on the tree and harvested by hand. At the 'hard white' stage, they are able to withstand transportation in baskets or containers, and exposure of the fruits to light or high temperatures immediately after harvest does not affect their overall quality. Before processing, fruits are ripened at room temperature for a day or more, depending on the end products (tee, juice, pulp, dietetic products, etc.).

Yield

Noni is a perennial shrub, and it is possible to find fruits at different stages of maturity on the same plant at the same time. A few farmers choose not to harvest in the first year, and they prune to let the bush grow stronger. In Hawaii, noni fruits are harvested throughout the year, although there are seasonal patterns in flowering and fruit bearing (meteorological factors, fumigation and irrigation). In India, the plants are allowed to grow for 2 years without any side growth by periodical pruning to make the plant sturdy. Noni plant is capable of giving yield up to 250–300 kg after 7–8 years of planting under better conditions of cultivation. In the initial stages, yield ranges from 30 kg to 40 kg per plant, and the well-grown tree produces an average of 99–100 kg. Effective life span of the trees will be up to 40–50 years, and the harvest can be done 6–7 times in a year.

In Hawaii, noni fruits are usually harvested two or three times per month, although fruit production is lower during winter. With a density of 638 plants per hectare with good soil fertility, drainage, and irrigation and appropriate pest, diseases and weed control, along with an appropriate manurial plan, it is possible to obtain yield from 7 tonnes/ha/year in the second year after planting to approximately 70 tonnes/ha/year after fifth year. With a juice extraction rate of approximately 50% (w/w), 1 ha can yield around 35 tonnes of juice. Many factors affect these yields, and most farmers do not obtain such good results because of diseases or poor agricultural practices. In Hawaii, an average annual yield of 50 tonnes/ha is generally recorded.

The cost of cultivation under Andaman and Nicobar conditions is worked out to Rs. 42,425 per ha. It was observed that 5-year-old plantations in Bay Islands gave a gross income of Rs. 4,68,750 per ha/− and a net income of Rs. 2,00,731/− per ha.

Storage

Ripened fruits can be stored up to 9 days, and juice needs to be extracted before 9 days of maturity. The effect of storage on different quality parameters was studied and reported that physiological loss of weight was the highest (28.26%) and spoilage

Table 1 Nutrient composition in plant parts of noni (*Morinda citrifolia*)

Parts of the plant	K	Ca	Mg	Fe	Cu
(ppm)					
Leaf	1219	5462	570.60	4.47	2.23
Wood	Trace	270.02	44.67	378.54	6.22
Bark	Trace	534.34	47.93	146.67	5.46
Fruit	1226	58.898	196.64	42.44	27.44

Noni contains approximately 200 nutraceuticals and phytochemicals including amino acids, vitamins, minerals, fatty acids, alcohols, phenols, anthraquinone glycosides, carotenoids, esters, flavonoids, iridoids, ketones, lactones, lignans, nucleosides, triterpenoids and sterols

was higher in non-pedicellate fruits. Fruits harvested with pedicel had maximum ascorbic acid and less spoilage.

Noni is getting attention of farmers in Andaman and Nicobar Islands, Karnataka, Andhra Pradesh, Odisha, Tamil Nadu and Kerala. It is included among *Future crops* of India for its value in wellness and good health. Murukesan Krishnapillai (2016) reported noni-based agroforestry systems as an adaptive strategy in a changing climate.

Noni-Based Wellness Products

Divine noni juice concentrate, noni juice capsules, morinda soap, and noni hair dye are a few wellness products (Table 1).

In language Hindi Noni is 'Aich' and in Sanskrit 'Aishka'.

Indian divine noni' is a popular noni juice concentrate proved for laxative, cellular cleansing and energising values. Mathivanan and Surendran (2007) studied chemical and biological properties of *Morinda* sp. and reported the presence of most of the essential elements in higher amounts in fruits of noni. Singh et al. (2007) analysed peptide and mineral profiles of noni fruits and leaves and reported the presence of above in higher quantities. Noni has proved to be a powerful stress reliever, allowing the body to create 'feel good chemicals' like serotonins and endorphins to counter the stress and its after effects. Paul Pandi et al. (2016) attempted identification of bioactive constituents in fruit extracts of *Morinda citrifolia* L. by HPLC method.

A disease is nothing but dis-ease of the body cells instead of being at ease. The main cause is lack of nutrition and stress leading to malfunctioning or ineffective functioning of cells. Even though every cell has its own desire to be healthy, it requires support system by a way of proper nutrition with the balance diet and a stress-free life. Diseases are nothing, but the cell of the particular organ or tissue is sick, damaged and functioning ineffectively. Somehow people have got a belief that disease is a part of the life, contrary to the truth.

Noni fruit juice facilitates the cells to be healthy with effective function and for good metabolism. When the cells are healthy and functioning properly, there is no room for diseases. Rem Abou Assi et al. (2015) published a comprehensive review on *Morinda citrifolia* L. – its industrial uses, pharmacological activities and clinical trials (Table 2).

Table 2 Important bioactive compounds and in vitro biological activity

Bioactive compound	Part	Class	Bioactivity
E-phyto	Fruit	Sterol	Anti-tuberculosis
Cycloartenol			
Stigmasterol			
Damnacanthal	Fruit	Anthraquinone	Anti-HIV
Isoscopoletin	Fruit	Phenolics	Antioxidant
Aesculetin			
Quercetin			
Americanin A	Fruit	Lignans	Antioxidant
Narcissoside	Fruit	Flavonoids	Antioxidant
2-Methoxy- 1,3,6-trihydro-xyanthraquinone(5)	Fruit	Anthraquinone	phase II enzyme inducer
Damnacanthol-3-O-β-D-primeveroside	Root	Anthraquinone	Anti-diabetic
Lucidin 3-O- β -D-primeveroside			
2-O-(β -D-Glucopyranosyl) -1-O-octanoyl-beta-D-gluropyranose	Fruit	Fatty acid ester	Anti-inflammatory
1,4-Dihydroxy-2-methoxy-7-Methylanthraquinone	Fruit	Anthraquinone	Wound healing activity
Austrocortinin			
(+)-3,4,3′,4′-Tetrahydroxy-9,7′alpha-epoxylignano-7 alpha,9′-lactone (+)-3,3′-Bisdemethyltanegnol	Fruit	Lignans	Cardiovascular activity
Quercetin			
Kaempferol			
Americanol A	Fruit	Lignans	Cardiovascular activity
3,3′-Bisdemethylpinoresinol			
Isoprincepin			
Morindolin			
Damnacanthal	Root	Anthraquinone	Anti-cancer
Morindone	Root	Anthraquinone	Anti-cancer
6-*O*-(β -D-Glucopyranosyl) -1-*O*-octanoyl-βb-D-glucopyranose	Fruit	Glycosides	Anticancer
Asperulosidic acid			

Noni being a powerful detoxifier, while the human body is toxified day in and day out by the food we eat, air we breathe, water we drink and cosmetics we use, toxins stay as fine layers on the cell membrane, blocking the cell membrane wall and preventing communication. Noni juice has proved to cleanse the toxic layers on the cell membrane, opening up the pores for the micronutrients to enter into the cells and for effective cell membrane communication.

Noni juice is a ‘feel good’ fruit juice; when you feel good, you make others feel good. And ‘feel good chemicals’ in the body help to be calm, peaceful and loving.

Research undertaken in more than 55 universities of the world showed the following benefits (Peter 2009):

- Food for living cells
- Detoxifier
- Helps pain management
- Enhances body energy levels
- Regulates immune system
- Friend of women
- Relieves stress
- Improves digestion
- Relieves constipation
- Alkaliser
- Improves memory and concentration
- Regulates body systems
- Enhances self-healing power
- Improves cell metabolism
- Enhances health of the skin, hair and nail
- Used for cuts, wounds and burns
- Manages weight
- Rejuvenates the body
- Revitalises cells
- Restores vitality
- Reduces inflammation
- Purifies the blood
- Enhances well-being

As a cellular food, noni builds the immune system and helps people with the following health conditions:

- Diabetes
- Blood pressure
- Arthritis
- Heart diseases
- Cancer
- Constipation
- Indigestion
- Kidney diseases
- Menstrual problems
- Obesity
- Paralysis
- Hair falling
- Headache and toothache
- Tuberculosis
- Itches and scabies
- Cold and cough
- Stomachache and ulcers

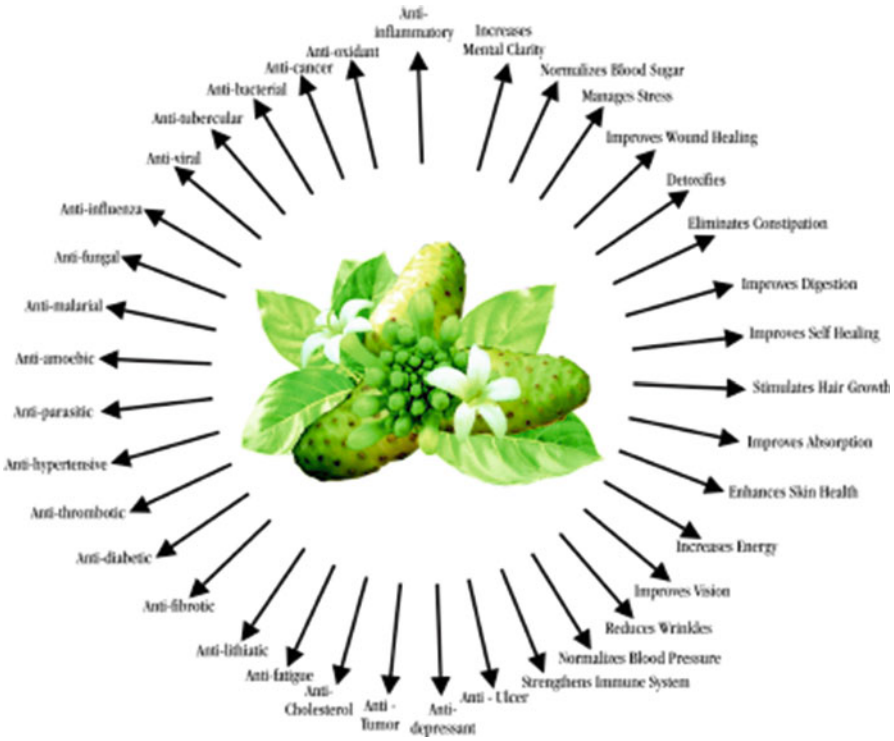

Fig. 3 Attributes of noni fruits

Sudhakar Konada et al. (2013) reported divine noni as a wonderful nutraceutical in preventing oxidative stress-induced cataract formation. Selvam (2013) investigated the safety assessment of noni juice of garcenia mix and reported safe. Sarvamangala (2016) reported role of noni fruit extract on oxidative stress-induced cataract formation in lens epithelial cells (Fig. 3).

World is moving towards wellness revolution which will impact everyone's life. Noni fruit – 'nature's gift to humanity' – has a great role to play in this wellness revolution, fulfilling the wishes and will of our creator bringing love, peace, harmony, happiness, good health and wealth to the deserving people of the world.

The chemistry of noni was investigated extensively by various scientific groups in over 54 universities and research institutes mainly in the USA, Europe, Australia, Canada, Japan, China, India, Cuba and Malaysia. Thyagarajan et al. (2015) elucidated pharmacological properties and clinical applications of *Morinda citrifolia* L.). The ripe fruit contains 90% water, and the pH is 3.72. The fruit pulp has a high level of soluble fibre (9.8%), soluble solids (8%), proteins (2.5%) and low level of lipids (0.15%). Fruit juice is a powerful detoxifier removing the toxins from antigens, metabolism, infections and cellular aberrations. The fruit juice stimulates production of T-cells, bacteriophages and thymocytes enhancing auto-immune system and function. Chemical analysis of fruits and leaves for potassium content

revealed higher quantity in fruits (1226 ppm) and leaves (1219 ppm), while it was negligible in wood and bark. Calcium and magnesium contents were higher in leaves (5462 ppm and 570.60 ppm, respectively). Magnesium content was higher in fruits (196.64 ppm) and lower in wood (44.67 ppm). Iron was higher in wood (378.54 ppm). Physicochemical analysis of fruits showed fruit weight (g) ranging 150–250, juice (%) 38.95–60.25, TSS (degree Brix) 8.40, acidity (%) 0.14 and vit. C (mg/100 g) 125–139. The reported amino acids (basic units of protein) in leaves are alanine, serine, threonine, tryptophan, tyrosine, valine, arginine, aspartic acid, cysteine, glutamic acid, glycine, histidine, isoleucine, leucine, methionine, phenylalanine and proline. Glucose and fructose are the abundant monosaccharides in fruits, while hexose and pentose are reported in root bark. Carotenoids-colouring pigments – having antioxidant property – are in substantial quantity in leaves, bark and fruits and have the potential to treat vitamin A deficiency leading to short/long sightedness. Noni seeds contain vegetable oil (12.49%) containing unsaturated edible fatty acids with medicinal properties.

The classical work of Dr. Ralph Heinicke detected a natural precursor for xeronine-proxeronine – in noni juice which gets converted to xeronine in human body by an enzyme proxeroninase. Xeronine has a wide range of biological activities, stimulation of hormones like prostaglandin, insulin, adrenalin, etc. Anthraquinones are anti-inflammatory, antiparasitic, antineoplastic and antibacterial in biochemical action. These compounds have anti-inflammatory and analgesic properties. Anthraquinones along with synergistic action of other ingredients of noni juice inhibit the COX-2 enzyme production leading to anti-inflammatory and analgesic properties. Damnacanthal, rubiadium, nor-damnacanthal, morindone and lucidin are anthraquinones with properties to inhibit enzymes which promote tumour formation. Anti-carcinogenic properties of damnacanthal are well established and well administered to patients. Noni juice is rich in terpenes having antioxidant and anti-carcinogenic properties. Eugenol, another terpene, has active germicidal and muscle relaxer properties. Betacarotenes in noni juice have proven antioxidant properties. Ursolic acid in noni has antimicrobial properties against several strains of *Staphylococci* and to fungal infection by *Candida albicans* and *Microsporum lanosum*. Phytosterols in noni-like (beta) sistosterol, stigmasterol and campesterol slow down intestinal absorption of cholesterol and thereby lower total plasma and LDL cholesterol levels. In animal studies, (beta) sterol is anti-inflammatory, anti-neoplastic and antipyretic and balances the immune system. Glycosides are organic compounds found in abundance in plant kingdom. One of the important glycosides in noni is asperuloside, traditionally used for dieresis, treating inflammation and varicose veins and phlebitis. Research indicates that it is anticlastogenic (prevents breakage of chromosomes) and anti-mutagenic. Scopoletin in noni has hepatoprotective activity including antibacterial against *Staphylococcus aureus*, *Streptococcus* sp., *Proteus mirabilis*, *Pseudomonas aeruginosa* and *Haemophilus influenza*. It is antipyretic, antifungal and hypotensive. Noni juice contains pectins, a polysaccharide involved in regulation of glucose absorption and bind bile acids and cholesterol. Proteins which are the building material of human body consist of 22 amino acids both essential and non-essential. Noni juice contains 17 amino

acids including all the 9 essential ones – histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan and valine – and biological activities of noni are elucidated by study of chemistry of noni juice. Antibacterial activity against infectious bacterial strains like *Pseudomonas aeruginosa*, *Proteus morganii*, *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli*, *Salmonella* and *Shigella* are reported. Dr. Ralph Heinicke accentuated that the antimicrobial effect observed may be due to the presence of phenolic compounds like aucubin, lasperuloside and alizarin in the fruit and other anthraquinone compounds in roots. The acetonitrile extract of the dried fruit inhibited the growth of *Pseudomonas aeruginosa*, *Bacillus subtilis*, *Escherichia coli* and *Streptococcus pyrugene*. It helps in stomach ulcer through inhibition of the bacteria *H. pylori*. The ethanol and hexane extracts of the dried fruit provide protection against *Mycobacterium tuberculosis*. E-phytol, cycloartenol and stigmasterol were identified in the hexane extract of noni fruit juice. Antifungal activity of noni juice was also reported. The methanol extract of the dried fruit inhibited *Tricophyton mentagrophytes* (79.3%), while approximately 50% activity was recorded against *Penicillium* sp., *Fusarium* sp., and *Rhyzopus* sp. Antiviral activity of noni juice was attributed to the presence of damnacanthal which inhibits Vpr-induced apoptosis. Antioxidant activity of extracts of the root, fruit and leaf was established in ferric thiocyanate (FTC) and thiobarbituric acid test (TBA). Alcoholic extract of tender leaves of noni exhibited anthelmintic activity against human *Ascaris lumbricoides*. Hepatoprotective activity of noni juice as nutritional supplement was shown. Antiobesity and hypoglycaemic effects; analgesic, anxiolytic and sedative effects; anti-inflammatory activity and wound-healing activity of noni were reported. Selvam et al. (2012) studied cytotoxicity of noni (Noni *Garcinia cambogia* mix) against human liver cancer cells (HepG2 Cells). Vidya et al. (2012) further reported efficacy of noni fruit extracts and scopoletin on a preponderant panel of human tumour cell lines.

Chemistry of noni is chemistry of health and wellness and demands further detailed studies on clinical and pharmacological properties which have far-reaching consequences on new lifestyle diseases like diabetes, cardiovascular diseases, obesity and arthritis and ultimately health and wellness of man.

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Rhodiola: An Overview of Phytochemistry and Pharmacological Applications

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Introduction

Plants, on the planet earth, are one of the most important natural resources available for the human life. They provide oxygen, food, essential pigments, and ornamental and medicinal components that provide support to humans for sustenance of life and combat with the harmful and life-threatening conditions on the planet. There are more than 300–315 species of medicinal plants which have been identified by the various botanists of the world (Sundriyal et al. 2004). The main and foremost use of the plant by humans is in the form of medicines. Since ancient times it has been recognized that there are many plant species which have huge medicinal value and have healing potential against various fatal diseases. Plants like *Azadirachta indica* (Neem), *Ocimum tenuiflorum* (Tulsi), *Mentha arvensis* (Menthol), *Psidium guajava* (guava), *Aleo vera*, *Rheum* sp., *Hypericum* sp., *Rhodiola* sp., etc. were not only used by the ancient people for treatment of diseases like stomachache, headache, paralysis, fever, etc., but in the present day also, they are being used in their raw as well as in the mixture form with other compounds for various human-related abnormalities like high free radical production in the body, inflammation, tumor, etc. (Sikkink 2009) The knowledge to use these plant species is available to us through various Vedas in which they are properly classified and designated for their potential use as medicine. Besides these well-known species of plants, there are many other species which are known for their high medicinal values and are maintained by various local traditional medicinal systems like Ayurveda, Siddha medicine, Unani, and Ancient Iranian medicinal system. All over the world, there are various practitioners who practice the medicinal plants for the treatment. In some African and Asian countries, around 80% of the their population relies on these traditional medicinal systems. Amchi system (sowa-rigpa) is also one of the well-developed medicinal system of

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India. In Amchi system of medicine, they have provided a very deep knowledge of medicinal uses of various plants which are found in the high mountain ranges of Ladakh region of Jammu and Kashmir. There are more than 200 species of plant species like *Rhodiola, Rheum, Podophyllum, Artemisia, Centurea,* etc. from these regions which have extensive application for the treatment against diseases like malaria, cancer, etc. and also show antimicrobial-, anticancer-, anti-inflammatory-, antihypotensive-, and anticholinergic-like activities. In various traditional formulations, these plant species were dried and mixed with the butter and applied as the ointment to relieve pain and swelling (Halldorsson and Grasnytjar 1783) and also being used as a supplement for long journey (Alm 2004). These plants contain a plethora of various classes of bioactive compounds like flavonoids, glycosides, phenylpropanoid, etc., which have a very high value toward the human health (Chaurasia et al. 2007). *Rhodiola* is one of the perennial herbs which belongs to the *Campanulaceae* family and also resembles to the sebum which is known as stonecrops. This genus is made up of about 93 species. It has a few distinguishing characters which includes the series of stamen and has a stout rhizome from which the plant arises. This plant makes a whorl and contain red or yellow color top which includes seeds of plants. *Rhodiola* has adapted to harsh and almost unforgiving climate. These *Rhodiola* species were reported to find in the northern hemisphere in countries like China, Mongolia, Korea, Sakhalin, the Kuriles, Japan, Sweden, Norway, Finland, India, and Pakistan. In China, it is distributed in the northwest and the southwest region, and it is locally known as Hong Jing Tian (Bassa et al. 2016). In India, it is distributed in Jammu and Kashmir region, Himachal Pradesh and Arunachal Pradesh, and almost in complete Himalayan belt. Tibet is one of the places which are rich in its production (Kumar et al. 2010a). *Rhodiola* species are also reported to find in Russia, the United States, and Canada (Lei et al. 2003).

In the Ladakh region of Jammu and Kashmir, *Rhodiola imbricata* and *Rhodiola heterodonta* have a diverse distribution. Out of the five different valleys of Ladakh (Suru, Zanskar, Nubra, Indus, and Changthang), these plants are available in Changthang, Nubra, Zanskar, and Indus valley. The major population of the plant found in the passes such as Khardungla pass (between Indus and Nubra valley), Changla pass (between Nubra and Changthang), Pensi-la pass (between Zanskar and Suru valley), etc. are present in between these valleys; they join these valleys to one another (Chaurasia et al. 2007). Few of the commonly known species of this family are *Rhodiola rosea* (known as roseroot), *Rhodiola imbricata* (recently known as a sanjivani), *Rhodiola heterodonta*, *Rhodiola quadrifida*, etc. All these species were reported to have very high medicinal values like antioxidant, anti-inflammatory, leprosy, antistress, etc. The kind of environment in which these plants grow is very harsh with a low temperature of around −10 °C and at a height of around 4000–5000 m above the sea level. In such hard survival conditions, these plants survive as well as propagate and have successfully adapted to that environment. The key component for their survival in those conditions is the adaptation of these plants and also the kind of compounds these plants produce in their biological mechanism

which are not only helpful to the plants but for human also. Historically, these plants have been used in various traditional medicine systems of India, China, Europe, etc. In the modern world, these plants of *Campanulaceae* genus are extensively used in various formulations and basically include the roots of these plants which contain a very high content of secondary metabolites. The key secondary metabolites which have been reported and extensively studied in these plants are rosin, rosavin, salidroside, etc. The present chapter discusses about the distribution of the plants in various countries, its bioactive compounds, and their bioactivity (Chaurasia et al. 2007).

Distribution of *Rhodiola* Species

Rhodiola rosea

Rhodiola rosea is an inhabitant of subarctic area of the northern hemisphere. It is mainly available in high altitudes over rocks and on Arctic sea cliffs in Europe, Asia, and North America, including Britain, further south in mountains, and China (Zhang et al. 2016). Mountain Altai and south region of foothill Altai, mainly in Ust-Kansky, Ust-Koksinsky, and Charishki regions, the availability of commercial roots and rhizome is in great abundance (Saratikov and Krasnov 2004).

Rhodiola imbricata

Rhodiola imbricata, found in Sinai Himalayas, Nepal, Qinghai, Xizang, and India, is also found in the hilly region of western Himalaya (Kanupriya et al. 2005). The major distribution of *Rhodiola imbricata* is in three different valleys (Zanskar (N33.95 and E76.46), Indus (N34.29 and E77.86), and Changthang (N 34.26 and E 78.14)) of Ladakh region of Trans-Himalayas (Chaurasia et al. 2007).

Rhodiola heterodonta

Rhodiola heterodonta species are endemic to the mountain range of Central Asia and mainly distributed in East Europe and Asia (Grace et al. 2009).

Rhodiola crenulata

The plant *Rhodiola crenulata* is native to the Qinghai-Tibet Plateau and the only original plant, according to the "*Pharmacopoeia of China*" (Chinese Pharmacopoeia Commission 2010). This plant has shown a distribution in Hengduan Mountains Region of China, Tibet, and Yunnan (Lei et al. 2003).

Rhodiola kirilowii

The plant is mainly prevalent in Gansu, Hebei, Qinghai, Shaanxi, Shanxi, Sichuan, Xinjiang, Xizang, and Yunnan (Kazakhstan, Myanmar). In Central Asia it is found in Narynskiy Range, Terskey Alatau, Alayskiy Range, northern China, and Tibet (Maximowicz 2007).

Rhodiola bupleuroides

The plant *R. bupleuroides* is aboriginal to western Tibet Autonomous Region, locally known as "Sheng-Chang Hong Jing Tian" or "Bu-Dan Hong Jing Tian, northwest of Yunnan, and Sichuan (Li et al. 2007). It is also found in Pakistan, Kumaon, Nepal, Sikkim, Bhutan, Myanmar, and SW China, at altitudes of 2750–3700 m (Hooker and Thomson 1998).

Rhodiola dumulosa

Rhodiola dumulosa is a perennial plant species that are found in various regions of China that includes Northern, Northwestern, and Central China. It is distributed as fragmented populations across Northern, Northwestern, and Central China (Hou and Lou 2011).

Rhodiola algida

Rhodiola algida is mainly distributed in the Qinghai Plateau in China (Qi et al. 2015). It is also present in large amount in Tibet. *Rhodiola algida* helps to improve oral mucositis which were induced in breast cancer patients (Loo et al. 2010).

Rhodiola sachalinensis

Rhodiola sachalinensis is a herbaceous plant (perennial) of the Crassulaceae family, predominantly found in the polar region of Arctic and Alpine (Seo et al. 2001) and high rocky mountain areas of East Asian countries (Ohwi 1984).

Rhodiola quadrifida

Rhodiola quadrifida is a grassy plant occurring predominantly in some highland regions of the East Siberia, former USSR *(Altai-Sayan)*, mountainous regions of China (Sichuan), and Mongolia (Hentii, Hangai, Hovsgol, Hovd, and Mongol Altai) (Wiedenfeld et al. 2007).

Rhodiola alsi

The data related to the distribution of this species is not extensively available, but it is only reported to be found in Qinghai-Tibet Plateau of China (Ma et al. 2008).

Chemoprofiling of *Rhodiola* Species

From age-old times, Asia and Europe have been utilizing *Rhodiola* species as medicinal resource which is endemic to the northern hemisphere's subarctic areas. Their usages include valuable functions as adaptogen, anti-inflammatory, and anti-depressant drugs. In the process to establish the therapeutic/pharmacological uses of these plants in modern medicine, the effects of *Rhodiola* sp. have been extensively studied. Out of all the species, *Rhodiola rosea* has been shown to possess greater amount of activities like angiomodulatory, antioxidant, antimicrobial, adaptogenic, antistress, immunomodulatory, and antitumoral effects. From a chemotaxonomical's view, eight compounds which include the phenylpropanoids rosarin, rosavin, and rosin, the phenylethanoids salidroside and tyrosol, the flavonol rhodionin, as well as catechin and gallic acid have been proposed as reference markers (Recio et al. 2016). These are monoterpene alcohols and their glycosides (cyanogenic and aryl glycosides), phenylethanoids and their glycosides, proanthocyanidins, flavonoids and gallic acid derivatives, flavonolignans. In chemical nature of the adaptogens, they are typically tetracyclic triterpenoids/steroids complex or phenolics. Salidroside (p-hydroxyphenethyl-β-D-glucoside), which is a major compound in *Rhodiola*, seems to be accountable for many observed with *Rhodiola* extract's effects (Table 1).

Figure 1 represents the structure of salidroside, rosavin, and rhodionin compounds (Panossian et al. 2010).

Rhodiola rosea

The phenolic compounds included in this species are based on phenylpropanoids and phenylethane derivatives class, such as rosavin, tyrosol, salidroside (rhodioloside), syringin, and triandrin. Few of the reported lignans are eleutherosid E and schisandrin B. Figure 2 represents the structure of important compounds from *Rhodiola rosea* (tyrosol, syringin, rosiridine, ginsinoside, sitoindoside) (Panossian et al. 2010).

Rhodiola imbricata

Rhodiola imbricata contains large amount of bioactive compounds. The Chemoprofile of *Rhodiola imbricata's* root revealed the presence of 63 phyto-chemotypes (Fig. 3), among them, 1-pentacosanol; hexadecanoic acid; 2-hydroxy-

Table 1 Chemical compounds of various *Rhodiola* species

S. No.	Plant	Compound
1.	*Rhodiola rosea*	Salidroside (rhodioloside); rosavin; syringing; triandrin; tyrosol; eleutherosid E and schisandrin B; syringin; rosiridine, ginsenoside; sitoindoside
2	*Rhodiola imbricata*	1-pentacosanol, stigmast-5-en-3-ol, (3β,24S); 1-tetracosanol; 1-hentetracontanol; 9,12,15-octadecatrienoic acid, 2,3-dihydroxypropyl ester, (Z,Z,Z); 17-pentatriacontene; bis (2-ethylhexyl) phthalate; 7,8-dimethylbenzocyclooctene; ethyl linoleate; 3-methoxy-5-methylphenol; camphor; methyl tri-butyl ammonium chloride; 1,3-benzenediol, 5-methyl dodecanoic acid, 3-hydroxy; octadecane, 1-chloro; ethanone, 1-(4-hydroxyphenyl); a-tocopherol; d-tocopherol; ascaridole; campesterol; heptadecane, 9 hexyl; 1-hentriacontane; 1-heptacosane; 1-tericosanol; 13-docosen-1-ol; 1,30-triacontanediol; Stigmast-3,5-dien-7-one; stigmastanol; 9,12-octadecadienoic acid(Z,Z)-,2-hydroxy-1-(hydroxymethyl)ethyl ester; 1-tetratetracontane; A-Tocopherol-β-D-mannoside;1-pentatriacontane; bacteriochlorophyll-c-stearyl; 1,3-dimethoxybenzene; a-D- glucopyranoside, O-b-D-glucopyranosyl-(1.fwdarw.3)-b-D-fructofuranosyl; benzene sulfonic acid, 4-amino-3-nitro; cholest-4-ene-3,6-dione; Cis-9- eicosen-1-ol; (4-carboxymethoxy) benzoyl, methanol; oleic acid; hexadecanoic acid, bis(2-ethylhexyl) ester; hexadecanoic acid, methyl ester; bacteriochlorophyll-c-stearyl; eucalyptol; 1-(2,6-dihydroxy-4-methoxyphenyl) ethanone; linalyl isovalerate; 1-chloro-2,4-dimethoxybenzene; Borneol;4-chlorobenezenethiol; thujone; phenol,3,5-dimethoxy acetate; 2,4-bis(1,1dimethylethyl) phenol; b-fenchyl alcohol; 5-pentadecyl −1,3-benzenediol; A-D-glucopyranoside,O-α-D-glucopyranosyl-(1.fwdarw.3)-β-D-fructofuranosyl; 1-pentatricontene; 3,7,11-trimethyl- 1-dodecanol; 1-dodecane; 3-methoxy-5-methyl phenol; di-butyl phthalate; 3,5-dihydroxybenzyl alcohol; 3-methoxy-5-hydroxybenzyl alcohol; orcinol; O-methylorcinol; *p*-hydroxybenzaldehyde; *p*-hydroxyacetophenone, *p*-hydroxybenzyl alcohol; 4-methoxyphenethyl alcohol, 3-hydroxy-5-methylphenyl-β-D-glucopyranoside; Stigmast-4-en-3-one; methoxyphenyl-β-D-glucopyranoside; 2-hydroxymethyl-6-methoxy- β-D-glucopyranoside; 13-tetradecen-1-ol acetate; phenyl- β-D-glucopyranoside,3,5-dimethoxyphenyl- β-D-glucopyranoside, trimethoxyphenyl- β-D-glucopyranoside, 3-hydroxy-2-(3-methyl-2-buten-1-yl)-benzoic acid, 2-(hydroxymethyl(−6-methoxy-3-acetylphenyl- β-D-glucopyranoside, 2-(hydroxymethyl)-6-methoxyphenyl β-D-glucopyranoside; 1-dotriacontane; 2-hydroxy-4-methylphenyl- β-D-glucopyranoside; hexadecanoic acid; benzenemethanol, 3-hydroxy, 5-methoxy
3	*Rhodiola heterodonta*	Salidroside; tyrosol methyl ester; mongrhoside; Rhodiocynanoside A; epigallocatechin gallate; viridoside; epigallocatechin-epigallocatechin-3-O- gallate; Tyrosol; heterodontoside and 3-O-galloyl-epigallocatechin- epigallocatechin-3-O gallate; (−)-EGCG-4β-benzylthioether and (−)- Epigallocatechin-3-O-gallate

(continued)

Table 1 (continued)

S. No.	Plant	Compound
4	*Rhodiola crenulata*	Salidroside; tyrosol; p-hydroxyphenacyl-b-D-glucopyranoside; icariside D2; rutin; lotaustralin; herbacetin-7-methyl ether; rhodiocyanoside A; crenulatin; rhodionin; daucosterol; b-sitosterol; geraniol; gallic acid; creosides I,II, III, IV, V; kenposide A; rhodioloside E; Isopentyl-3-O-β-glucopyranoside; rhodiooctanoside; dihydroconiferin; 4-hydroxypenzyl- β-D-glucopyranoside; triandrin; vimalin; caffeic acid; pollenitin; rhodiosin; kaempferol; 5,7,3′,5′-tetrahydroxydihydroflavone; luteolin; kaempferol-7-O-α-L-rhamnoside; ternatumoside II; crenuloside; (+)-isolarisiresinol; (+)-dihydrodehydrodiconiferyl alcohol; methyl gallate;2-(4-hydroxyphenyl) ethyl 3,4,5-trihydroxybenzoate; Clemastanin A; (7R,8R)-3-methoxy-8′-carboxy-7′-en-3′,7-epoxy-8,4′-oxyneolignan-4,9-diol; (7R,8R)-3-methoxy-8′-carboxy-7′-en-3′,8-epoxy-7,4′-oxyneolignan-4,9-diol; (7β,7′β",8α,8′α′)-3′-methoxy-9-oxo-7,9′,7,9″-diepoxylignan-3,4,4″-triol; icariside D2; rhodiolate; 4′-hydroxyacetophenone (4-HAP) Coniferoside; epicatechin-(4β,8)-epicatechin gallate (B2–3′-*O*-gallate); salidroside and *p*-tyrosol; (3R,5R,8R)-3-O-[α-l-arabinopyranosyl (1 → 6)-β-d-glucopyranosyl]-5-hydroxymegastigma-6,7-dien-9-one; (1R)-1-O-(β-d-glucopyranosyl)-phenylethylene glycol; n-octanol; 2-methyl-3-buten-2-ol; citronellol;,3-methyl-2-buten-1-ol; myteolp; picein and linalool
5	*Rhodiola kirilowii*	Tyrosol; digalloylproprodelphinidin B2 (rhodisin); Arbutin; epigallocatechin gallate; rhodiocyanoside A; fructopyran(1-4)-glycopyranose; lotaustralin; 3,3′- 3,3′-Digalloylprocyanidin B2; epicatechin-3-O-gallate; beta-sitosterol; trans-hydroxycinnamic acid; neryl beta-glucopyranoside; hexyl beta-glucopyranoside; gallic acid; rhodiolgin; isolariciresinol-9-O-beta-glucopyranoside; rhodiooctanoside; sacranoside B; geranyl beta-glucopyranoside
6	*Rhodiola bupleuroides*	Kaempferol-7-O-α-L-rhamnopyranoside; rhodiosin; quercetin; syringic acid; β-sitosterol; rhobupcyanoside; B gallic acid
7	*Rhodiola dumulosa*	β-sitosterol; sexangularetin; β-sitosterol glucoside; herbacetin-7-α-L-rhamnoside; kaempferol; gallic acid; kaempferol-3-O-beta-D-glucopyranoside-7-alpha-O-L-rhamnoside; rutin; kaempferol-7-O-α-L-rhamnoside; quercetin
8	*Rhodiola algida*	Salidroside; tyrosol; diacetylrhodalgin; rhodalgin; acetylrhodalgin; triacetylrhodalgin
9	*Rhodiola sachalinensis*	Salidroside; rhodiocyanosides; sacranosides; kaempferol, cinnamyl alcohol, and daucosterol
10	*Rhodiola qundrifida*	Rhodiacyanosides A and B, glycosides – octyl α-L-arabinopyranosyl (1-6)-β-D-glucopyranoside and gossypetin 7-O-β-D glucopyranosyl (1-3)-α-L-rhamnopyranoside, tricetin; quercetin; kaempferol, p-tyrosol, and rhodioloside

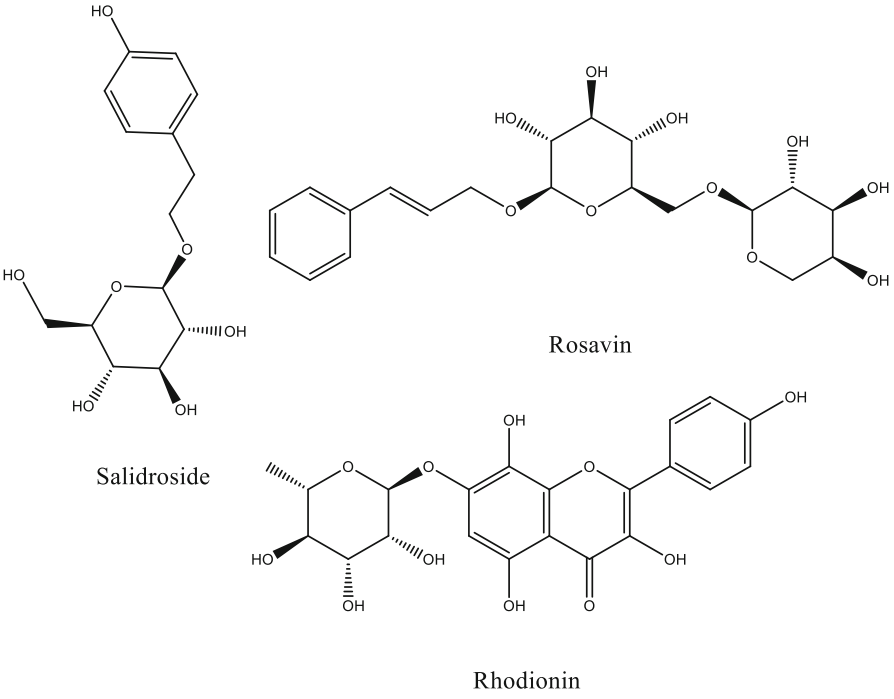


Fig. 1 Structure of salidroside, rosavin, and rhodionin reported by Panossian et al. (2010)

1-(hydroxymethyl)ethyl ester; stigmast-5-en-3-ol, (3β,24S); 1-tetracosanol; 1-hentetracontanol; 9,12,15-octadecatrienoic acid, 2,3-dihydroxypropyl ester,(Z,Z,Z); thujone; 9,12-octadecadienoic acid(Z,Z)-,; 17-pentatriacontene; 13-tetradecen-1-ol acetate; bis(2-ethylhexyl) phthalate; 7,8-dimethylbenzocyclooctene; ethyl linoleate; 3-methoxy-5-methylphenol; camphor; 1,3-dimethoxybenzene; methyl tri-butyl ammonium chloride; 1,3-benzenediol, 5-methyl; 1-heptacosane; benzenemethanol, 3-hydroxy, 5-methoxy; cholest-4-ene-3,6-dione; dodecanoic acid, 3-hydroxy; octadecane, 1-chloro; ethanone, 1-(4-hydroxyphenyl); a-tocopherol; d-tocopherol; campesterol; 1-dotriacontane; heptadecane, 9 hexyl;1-hentriacontane; 1-tericosanol; 13-docosen-1-ol; 1,30-triacontanediol; Stigmast-4-en-3-one; Stigmast-3,5-dien-7-one; stigmastanol; 1-tetratetracontane; 1-pentatriacontane; bacteriochlorophyll-c-stearyl; ascaridole; a-D- glucopyranoside, O-b-D- glucopyranosyl-(1.fwdarw.3)-b-D-fructofuranosyl; benzene sulfonic acid, 4-amino-3-nitro; Cis-9- eicosen-1-ol; (4-carboxymethoxy) benzoyl, methanol; oleic acid; hexadecanoic acid, bis (2-ethylhexyl) ester; hexadecanoic acid, methyl ester; bacteriochlorophyll-c-stearyl; eucalyptol; 1-(2,6-dihydroxy-4-methoxyphenyl) ethanone; linalyl isovalerate; 1-chloro-2,4-dimethoxybenzene; borneol; 4-chloro benzenethiol; phenol,3,5-dimethoxy acetate; 2,4-bis(1,1dimethylethyl) phenol; b-fenchyl alcohol; 5-pentadecyl −1,3-benzenediol; A-D-glucopyranoside,O-α-D-glucopyranosyl-(1.fwdarw.3)-β-D-fructofuranosyl; 1-pentatricontene; 3,7,11-trimethyl- 1-dodecanol; 1-dodecane;

3-methoxy-5-methyl phenol; and di-butyl phthalate were found to be present (Tayade et al. 2013). Figure 4, 3,5-dihydroxybenzyl alcohol; 3-methoxy-5-hydroxybenzyl alcohol; orcinol; O-methylorcinol; *p*-hydroxybenzaldehyde; *p*-hydroxyacetophenone, *p*-hydroxybenzyl alcohol; 4-methoxyphenethyl alcohol, 3-hydroxy-5-methylphenyl-β-D-glucopyranoside, methoxyphenyl-β-D-glucopyranoside,2-hydroxymethyl-6-methoxy-β-D-glucopyranoside, phenyl- β-D-glucopyranoside,3,5-dimethoxyphenyl- β-D-glucopyranoside, trimethoxyphenyl- β-D-glucopyranoside,3-hydroxy-2-(3-methyl-2-buten-1-yl)-benzoic acid, 2-(hydroxymethyl(−6-methoxy-3-acetylphenyl- β-D-glucopyranoside,2-(hydroxymethyl)-6-methoxyphenyl β-D-glucopyranoside,2-hydroxy-4-methylphenyl- β-D-glucopyranoside (Choudhary et al. 2015).

Rhodiola heterodonta

Rhodiola heterodonta contains a wide range of secondary metabolites. Figure 5 represents the structure of tyrosol, viridoside, salidroside, and tyrosol methyl ester. Heterodontoside, mongrhoside, and Rhodiocynanoside A were found in the ethanol extract (Grace et al. 2009). In case of the proanthocyanidins fraction, the class of compounds are epigallocatechin – epigallocatechin-3-O- gallate, epigallocatechin gallate, and 3-O- galloyl-epigallocatechin-epigallocatechin-3-O gallate. Figure 6 shows the chemical compounds reported by Yousef et al. (−)-EGCG-4*β*-benzylthioether and (−)- Epigallocatechin-3-O-gallate (Yousef et al. 2006).

Rhodiola crenulata

This species of *Rhodiola* also contain many medicinally important phytochemicals. A total of around 48 chemical compounds were found which includes 12 flavonoids and their glycosides, 5 flavanols and gallic acid derivatives, 26 alcohols and their glycosides, and 4 organic acids and 1 cyanogenic glycoside (Han et al. 2016). Figure 7 salidroside; tyrosol; p-hydroxyphenacyl-b-D-glucopyranoside; picein; icariside D2; rutin; lotaustralin; rhodiocyanoside A; daucosterol; crenulatin; rhodionin; b-sitosterol; gallic acid; creosides I,II, III, IV, V (Grech-Baran et al. 2015). Figure 8 kenposide A; rhodioloside E; isopentyl-3-O-β-glycopyranoside; rhodiooctanoside; coniferoside; dihydroconiferin; Icariside D2; 4-hydroxybenzyl- β-D-glycopyranoside; triandrin; vimalin; caffeic acid; pollenitin; rhodiosin; kaempferol; clemastanin A (Nakamura et al. 2008). The other various phenolic compounds identified from *R. crenulata* are (Fig. 9) 5,7,3′,5′-tetrahydroxydihydroflavone; luteolin; kaempferol-7-O-α-L-rhamnoside; ternatumoside II; crenuloside; (+)-isolarisiresinol; (+)-dihydrodehydrodiconiferyl alcohol; methyl gallate; (7β,7′β”,8α,8′α′)-3′-methoxy-9-oxo-7,9′,7,9”-diepoxylignan-3,4,4”-triol; (7R,8R)-3-methoxy-8′-carboxy-7′-en-3′,7-epoxy-8,4′-oxyneolignan-4,9-diol; (7R,8R)-3-methoxy-8′-carboxy-7′-en-3′,8-epoxy-7,4′-oxyneolignan-4,9-diol; 2-(4-hydroxyphenyl) ethyl 3,4,5-trihydroxybenzoate; herbacetin-7-methyl ether; and rhodiolate (Zhou et al. 2015). Some different phytochemicals isolated from *R. crenulata* includes 4′-hydroxyacetophenone;

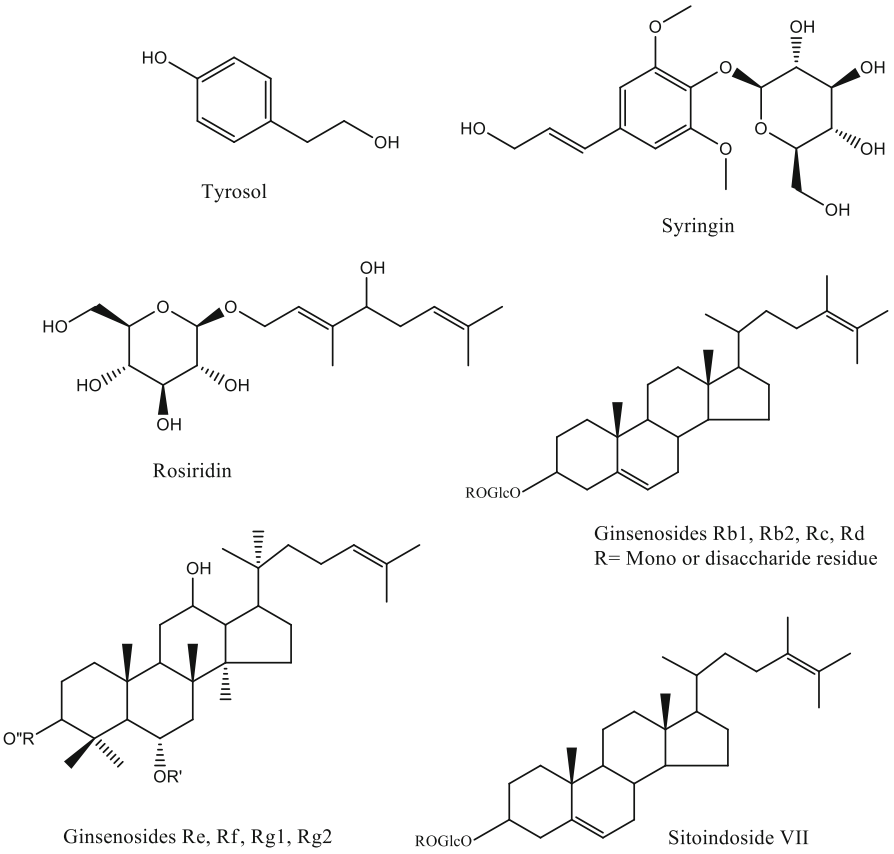


Fig. 2 Structure of some important phenylpropanoids and phenylethane derivatives reported by Panossian et al. (2010)

salidroside; *p*-tyrosol epicatechin-(4β,8)-epicatechin gallate (B2–3′-*O*-gallate) (Fig. 10) (Chu et al. 2014). (1R)-1-O-(β-d-glucopyranosyl)-phenylethylene glycol; (3R,5R,8R)-3-O-[α-l-arabinopyranosyl (1 → 6)-β-d-glucopyranosyl]-5-hydroxymegastigma-6,7-dien-9-one (Fig. 11) (Ma et al. 2008). n-octanol; 3-methyl-2-buten-1-ol ; 2-methyl-3-buten-2-ol; citronellol; myteolp ; Geraniol; and linalool (Fig. 12) (Lei et al. 2003).

Rhodiola kirilowii

The compounds isolated from *R. kirilowii* were arbutin, epigallocatechin gallate, rhodiocyanoside A, fructopyran(1–4)-glycopyranose, and lotaustralin (Fig. 13)

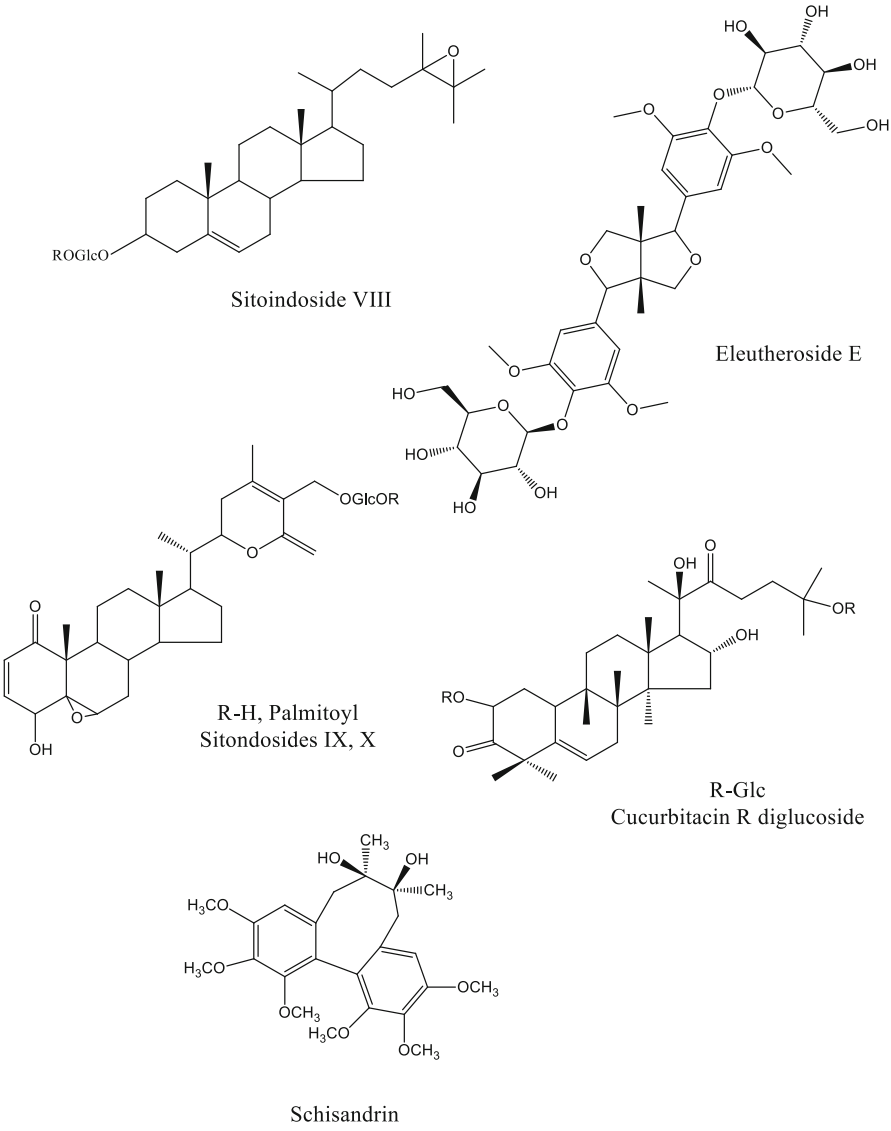


Fig. 2 (continued)

(Wiedenfeld et al. 2007). 3,3′-Digalloylprocyanidin B2; 3,3′-Digalloylproprodelphinidin B2 (Rhodisin); epicatechin-3-O-gallate (Fig. 14) (Wojcik et al. 2009). Beta-sitosterol; trans-hydroxycinnamic acid; geranyl beta-glucopyranoside; neryl beta-glucopyranoside; sacranoside B; hexyl beta-glucopyranoside; tyrosol; gallic acid; rhodiolgin; isolariciresinol-9-O-beta-glucopyranoside; rhodiooctanoside (Fig. 15) (Wong et al. 2008).

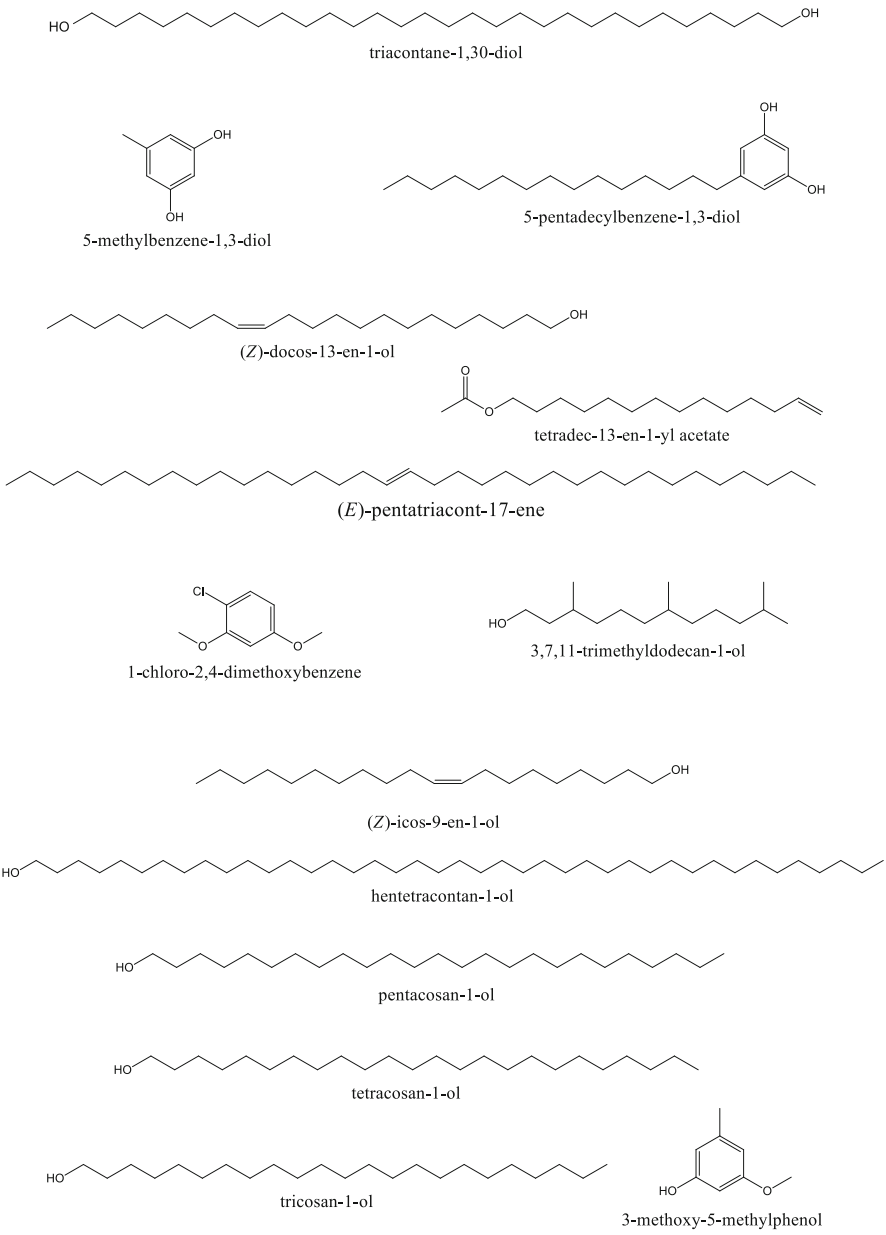
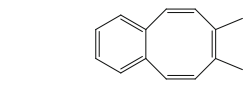
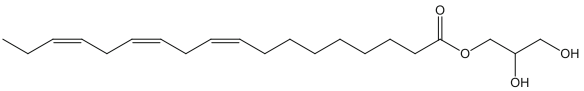


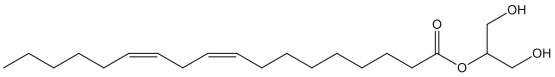
Fig. 3 63 Phyto-chemotypes reported by Tayade et al. (2013)



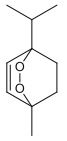
7,8-dimethylbenzocyclooctene



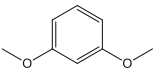
9,12,15-octadecatrienoic acid, 2,3-dihydroxypropyl ester, (Z,Z,Z)



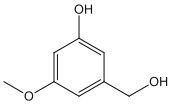
9,12-octadecadienoic
acid (Z,Z)-, 2-hydroxy-1-(hydroxymethyl)ethyl ester



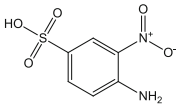
Ascaridole



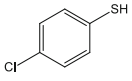
benzene, 1,3-dimethoxy



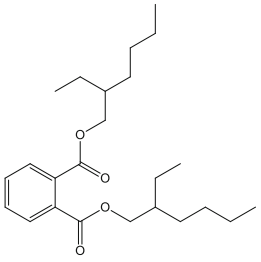
benzenemethanol, 3-hydroxy-5-methoxy



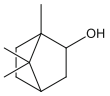
benzene sulfonic acid, 4-amino-3-nitro



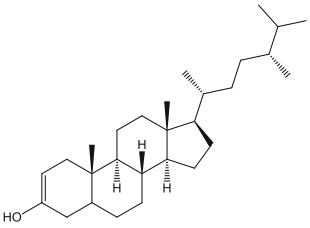
benzenethiol, 4-chloro



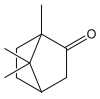
bis(2-ethylhexyl) phthalate



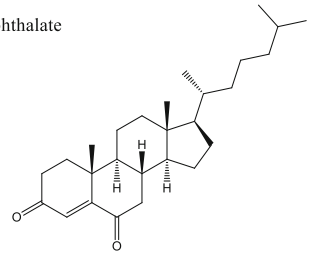
borneol



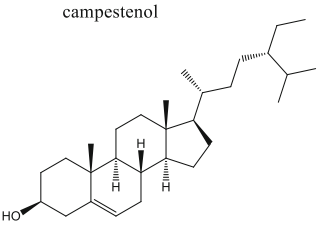
campestenol



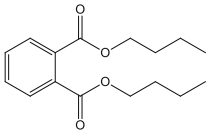
camphor



cholest-4-ene-3,6-dione



stigmast-5-en-3-ol, (3b,24S)



di-butyl phthalate

Fig. 3 (continued)

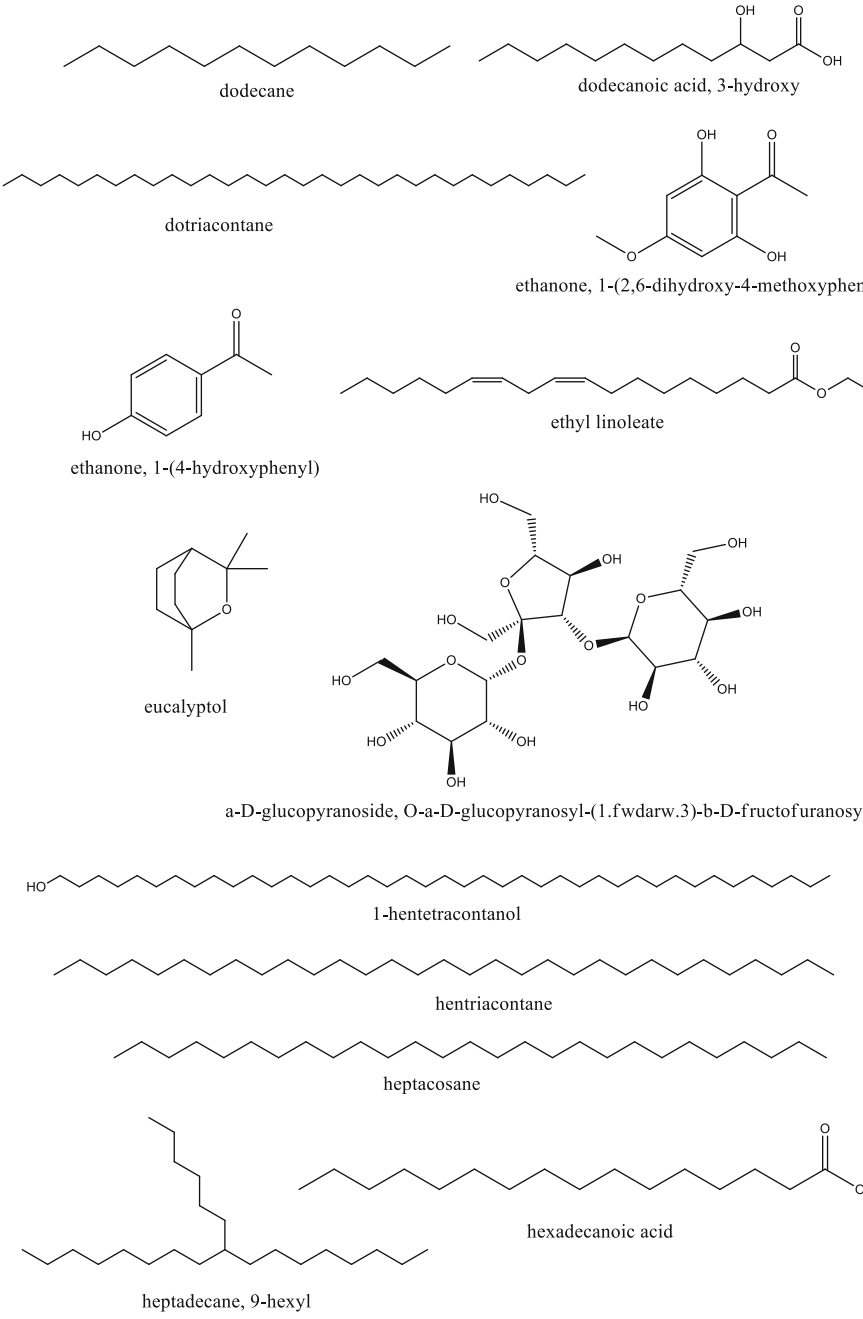
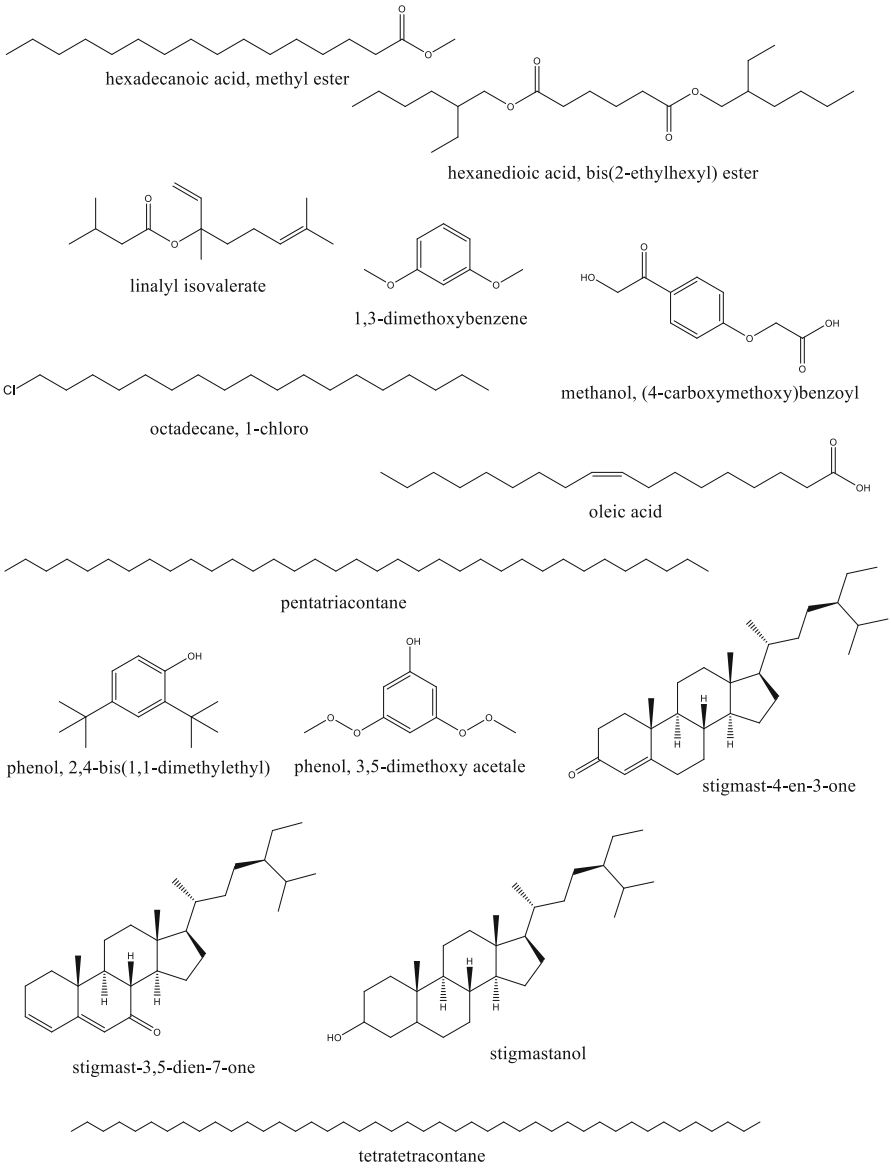


Fig. 3 (continued)



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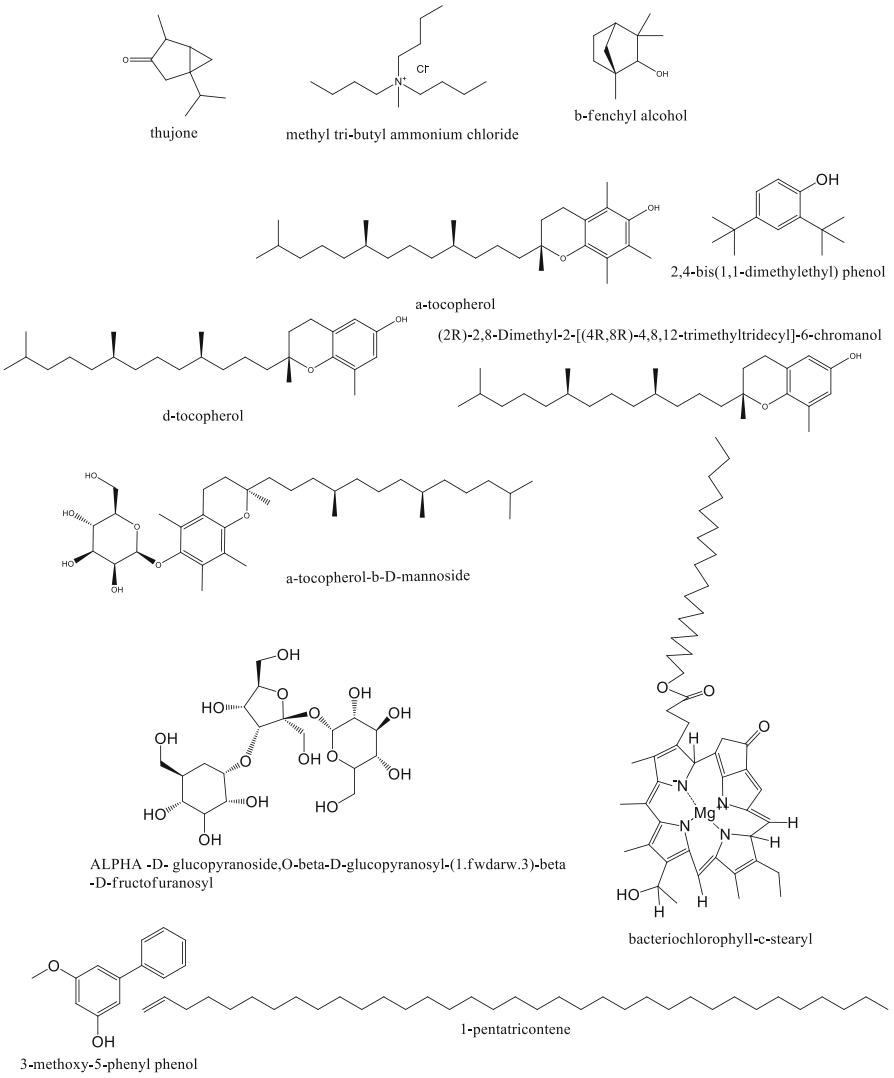

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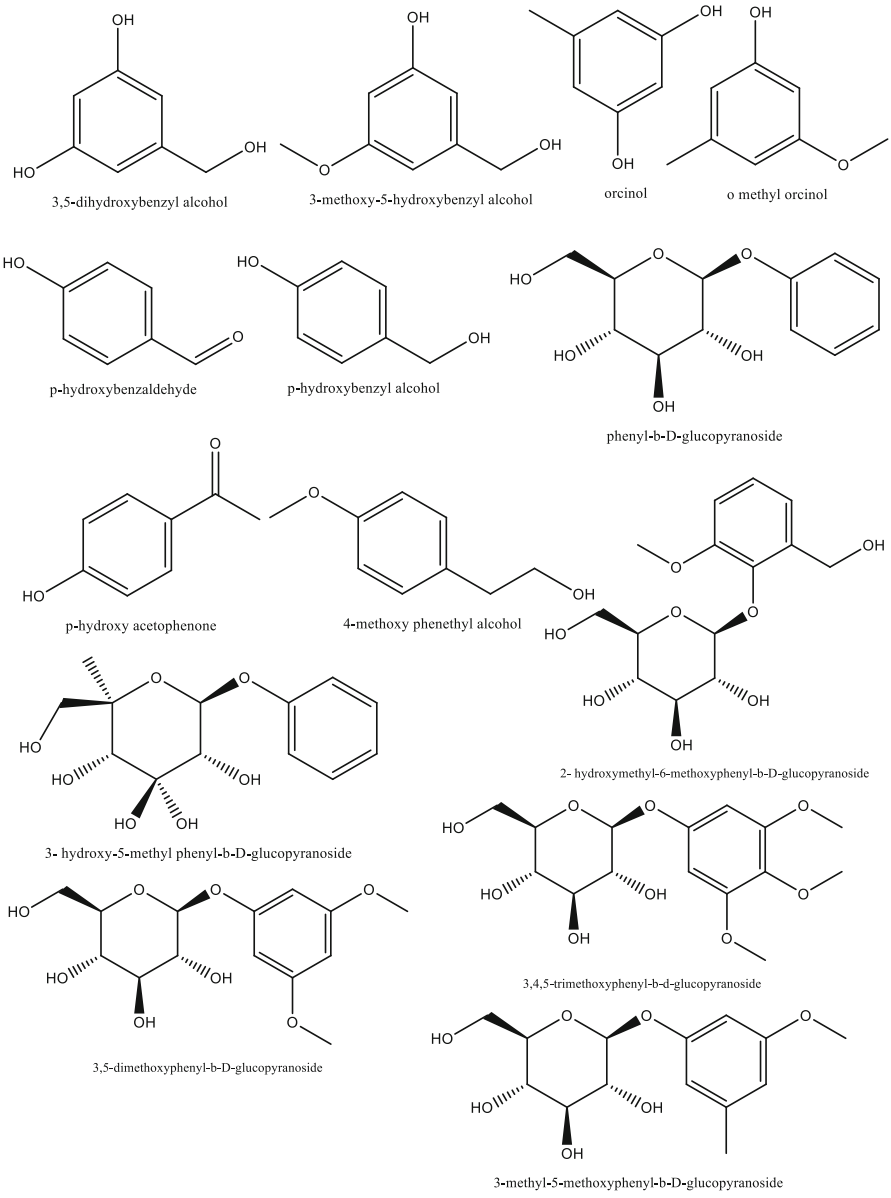


Fig. 4 Phytocomponents reported by Choudhary et al. (2015)

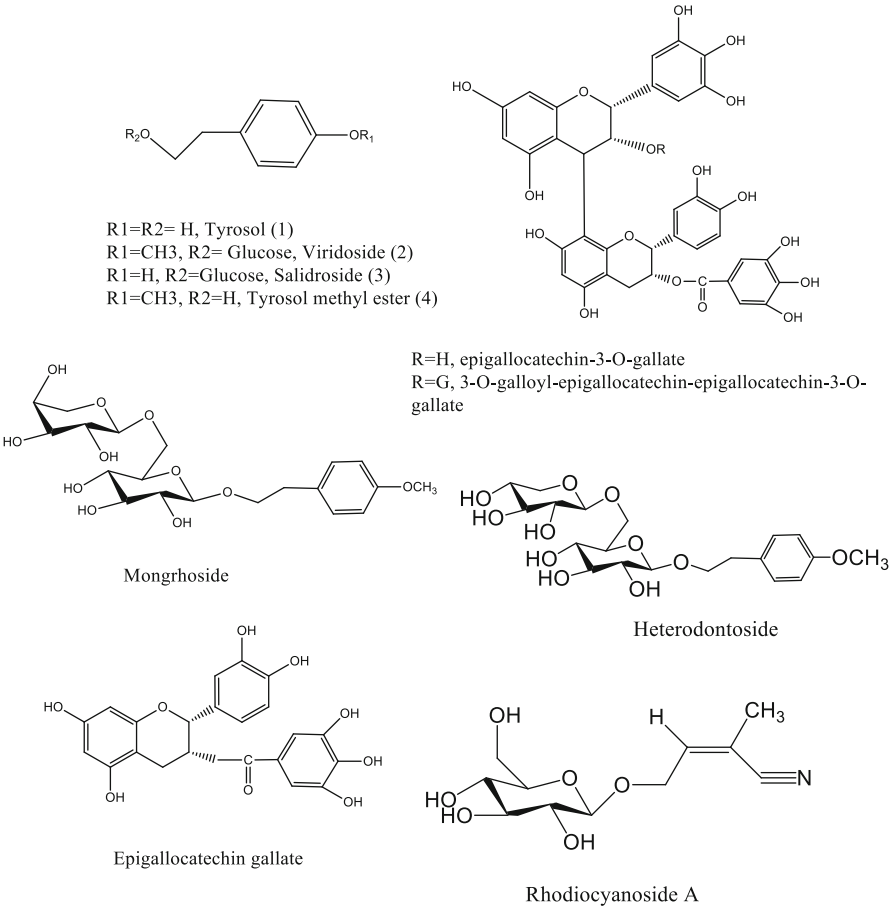


Fig. 5 Phytocomponents reported by Grace et al. (2009)

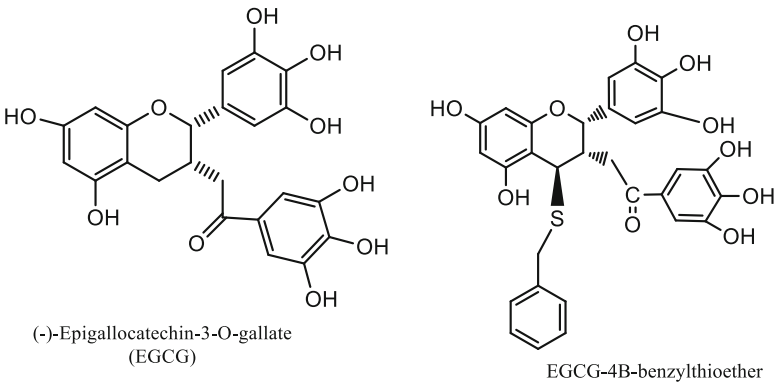


Fig. 6 Phytocomponents reported by Yousef et al. (2006)

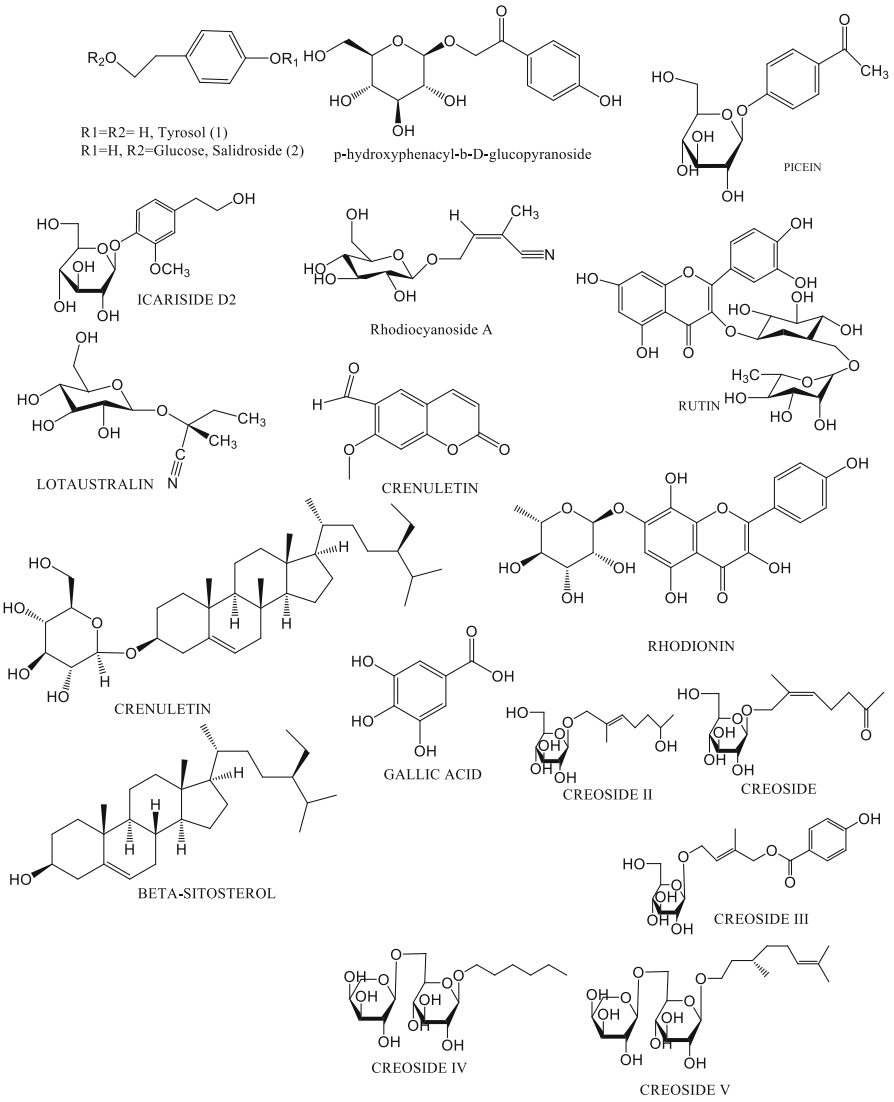


Fig. 7 Phytocomponents reported by Grech-Baran et al. (2015)

Rhodiola bupleuroides

The various compounds isolated from *R. bupleuroides* were gallic acid; kaempferol-7-O-α-L-rhamnopyranoside; rhodiosin; quercetin; syringic acid; and β-sitosterol (Fig. 16) (Li et al. 2007). Rhobupcyanoside B (Fig. 17) (Wang et al. 2016).

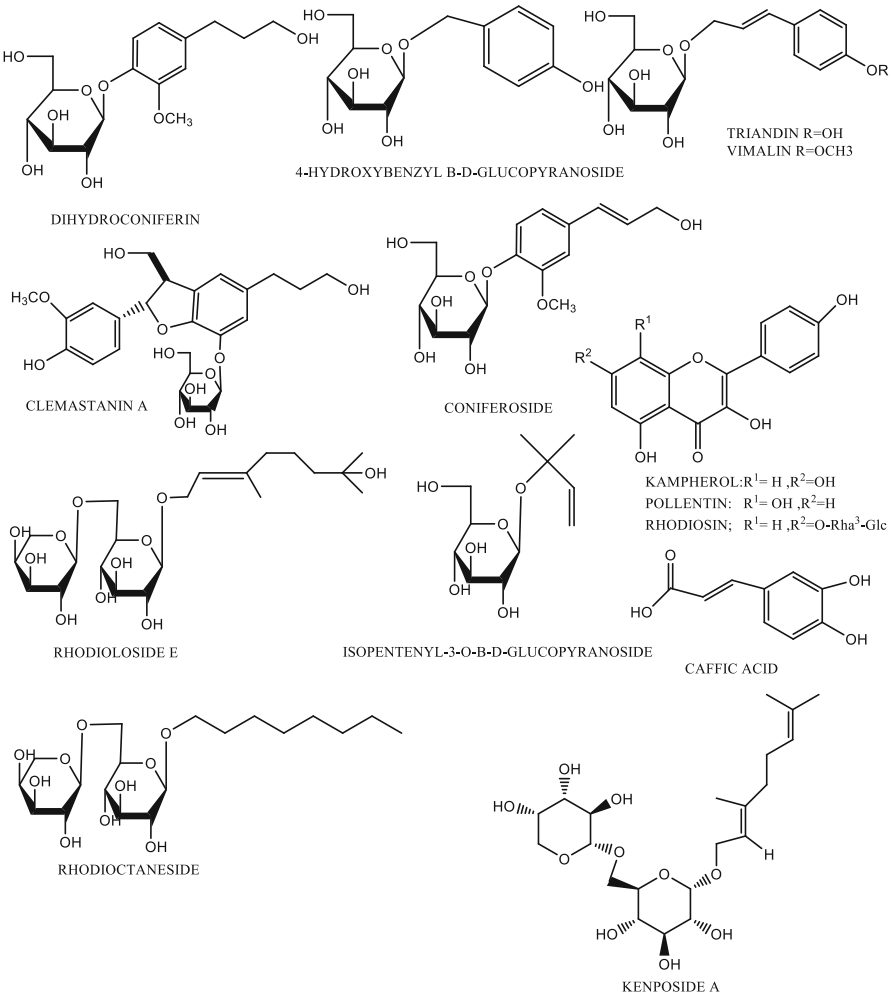


Fig. 8 Phytocomponents reported by Nakamura et al. (2008)

Rhodiola dumulosa

The various bioactive compounds isolated from *Rhodiola dumulosa* were β-sitosterol; sexangularetin; kaempferol-7-O-α-L-rhamnoside; herbacetin-7-α-L-rhamnoside; kaempferol ; and β-sitosterol glucoside (Fig. 18). The compounds which were obtained from this plant for the first time are (Dingqiang et al. 2005) quercetin; gallic acid; (±) -Isolariciresinol-3-alpha-O-beta-D-glucopyranoside; rutin; kaempferol-3-O-beta-D-glucopyranoside-7-alpha-O-L-rhamnoside (Fig. 19) (Liu et al. 2008).

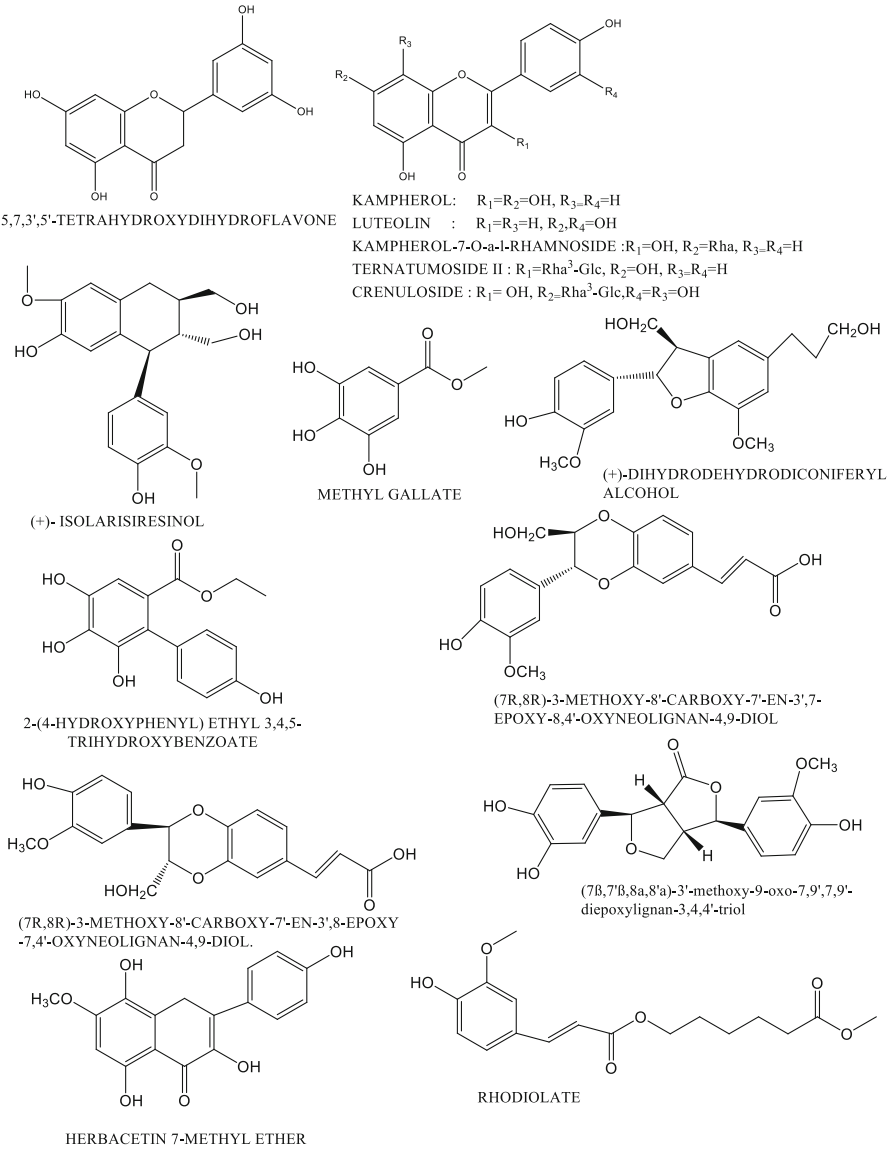


Fig. 9 Phytocomponents reported by Zhou et al. (2015)

Rhodiola algida

The marker compounds found in *Rhodiola algida* were salidroside and tyrosol (Fig. 20) (Lu et al. 2011). The other bioactive compounds reported in *Rhodiola*

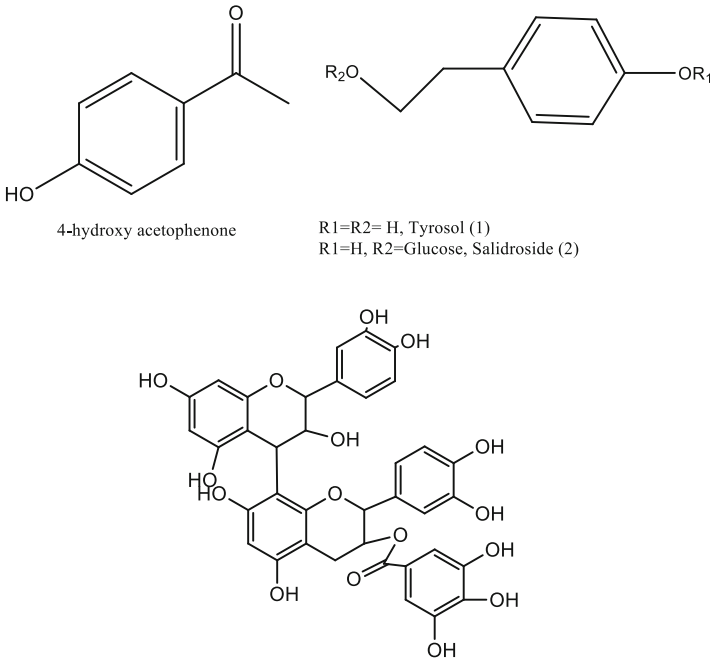


Fig. 10 Phytocomponents reported by Chu et al. (2014)

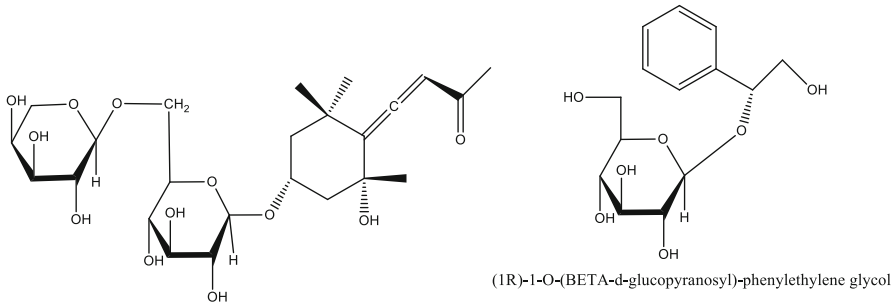


Fig. 11 Phytocomponents reported by Ma et al. (2008)

algida were rhodalgin, acetylrhodalgin, diacetylrhodalgin, and triacetylrhodalgin (Fig. 21) (Pangarova and Zapesochnaya 1975).

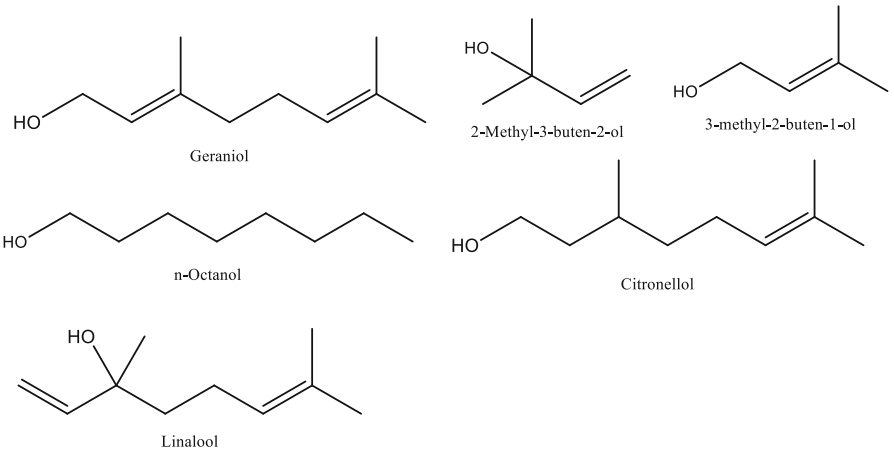


Fig. 12 Phytocomponents reported by Lei et al. (2003)

Rhodiola sachalinensis

The major active constituent of *Rhodiola sachalinensis* is salidroside (Li and Chen 2001). Several other bioactive compounds are glycosides such as rhodiocyanosides (Yoshikawa et al. 1995), sacranosides (Yoshikawa et al. 1997), and phenolic components (Fig. 22) (Lee et al. 2000). Kaempferol, cinnamyl alcohol, and daucosterol (Song et al. 2003).

Rhodiola qundrifida

Rhodiacyanosides A and B; octyl α-L-arabinopyranosyl(1-6)-β-D-glucopyranoside; tricetin and gossypetin 7-O-β-D glucopyranosyl(1-3)-α-L-rhamnopyranoside (Fig. 23) (Yoshikawa et al. 1995), two flavonols (quercetin and kaempferol); p-tyrosol and rhodioloside (Fig. 24) (Troshchenko and Kutikova 1967) were major compounds of this species of *Rhodiola.*

Bioactivity of *Rhodiola* Species

Rhodiola rosea

Antioxidant, adaptogenic, antistress, antimicrobial, immunomodulatory, angiomodulatory, and antitumor effects were the activities reported for *Rhodiola rosea*. *p*-Hydroxyphenethyl-*β*-D-glucoside is one of the major compounds found in *Rhodiola* that is responsible for many of the effects observed with *Rhodiola* extracts (Recio et al. 2016). Salidroside can be used as an effective agent against diabetes due

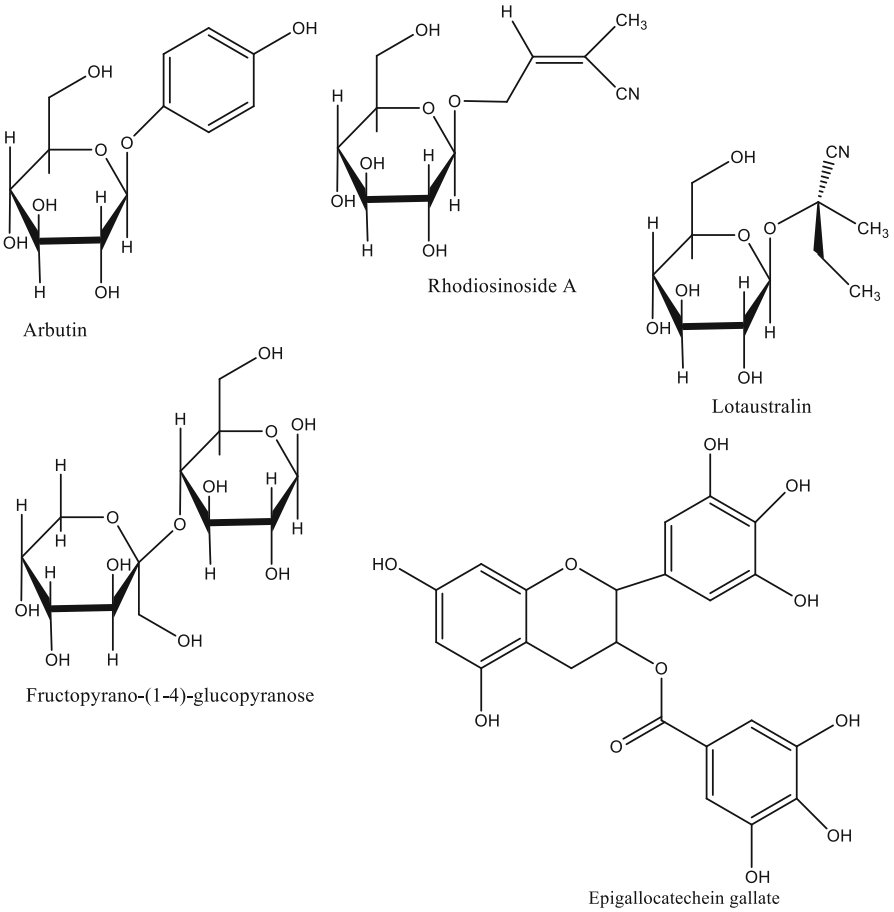


Fig. 13 Phytocomponents reported by Wiedenfeld et al. (2007)

to repression of adipogenesis and inflammation in eWAT and stimulation in hypothalamus of leptin signal transduction (Wang et al. 2016). The compounds from *Rhodiola rosea* showed RRL-induced protective effect on pulmonary fibrosis (PF) in rats. The treated rats had less lung fibrosis and inflammation than those in BLM-treated rats. Significant reduction of MMP-9 and α-SMA expression in the (bleomycin) BLM-induced PF rat mode was found after RRL treatment. Consistently, the expression of matrix TGF-β1 was inhibited significantly, while metalloproteinase-9 increased in the lungs of rats. These results strongly suggest that RRL attenuated BLM-induced fibrotic lung injury in rats (Zhang et al. 2016). The compound salidroside found to have protective effects toward the pulmonary arterial hypertension (PAH) induced chronic hypoxia. It has the potential to inhibit chronic hypoxia-induced pulmonary arterial smooth muscle cells (PASMCs) proliferation and reverse apoptosis resistance via AMPKα1-P53-P27/P21 pathway and via

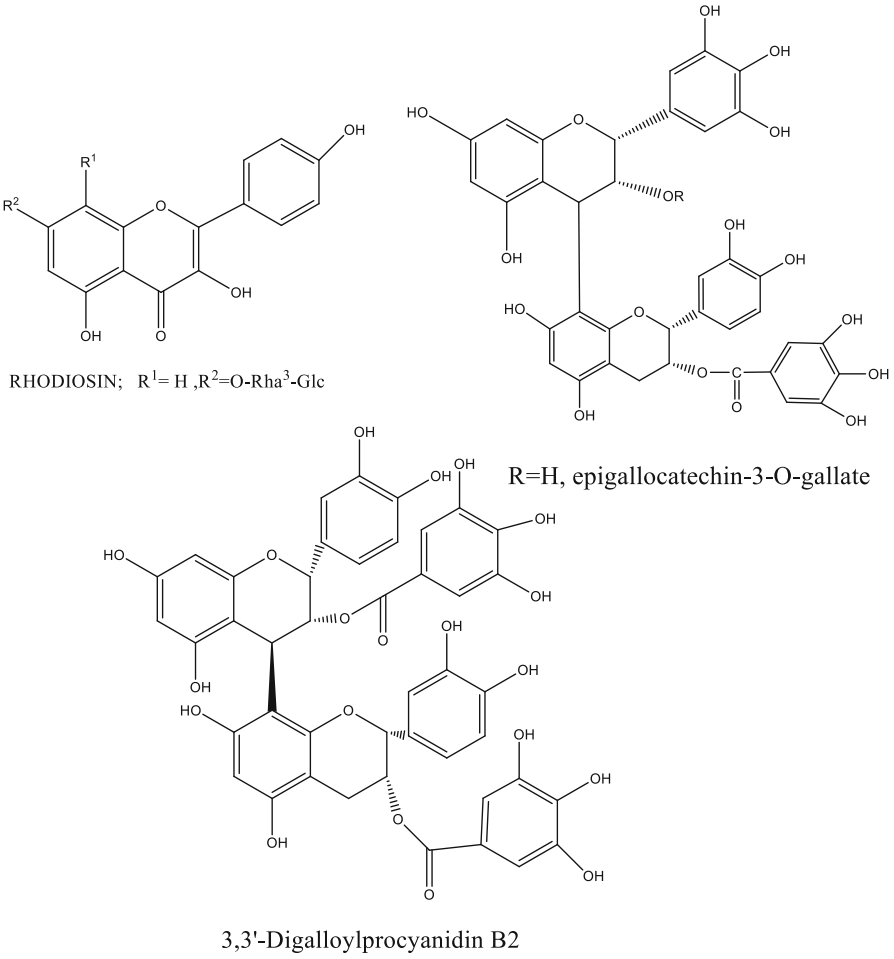


Fig. 14 Phytocomponents reported by Wojcik et al. (2009)

adenosine monophosphate-activated protein kinase (AMPK) α1-P53-Bax/Bcl-2-caspase 9-caspase 3 pathway (Chen et al. 2016). The extracts of *R. rosea* promote the host's immune response showing antitumoral properties, weak and medium-strength mutagens, and protecting tissues against free radicals. Even the *Rhodiola* extracts have the ability to inhibit angiogenesis. Extracts and salidroside stimulated specific and nonspecific immunity in in vivo as well as in vitro. It seems that they ameliorate immunity by enhancing Th1 cytokines without affecting the Th2 profile (Recio et al. 2016). The studies on *R. rosea* indicate that *R. rosea* extract was also characterized by unique pharmacological properties and stimulate positive effect on ATP synthesis in mitochondria of skeletal muscles in rat and stimulated reparative energy processes after intense exercise. *R. rosea* was found to be most effective for stimulating and increasing physical endurance. Treatment with *R. rosea* found to

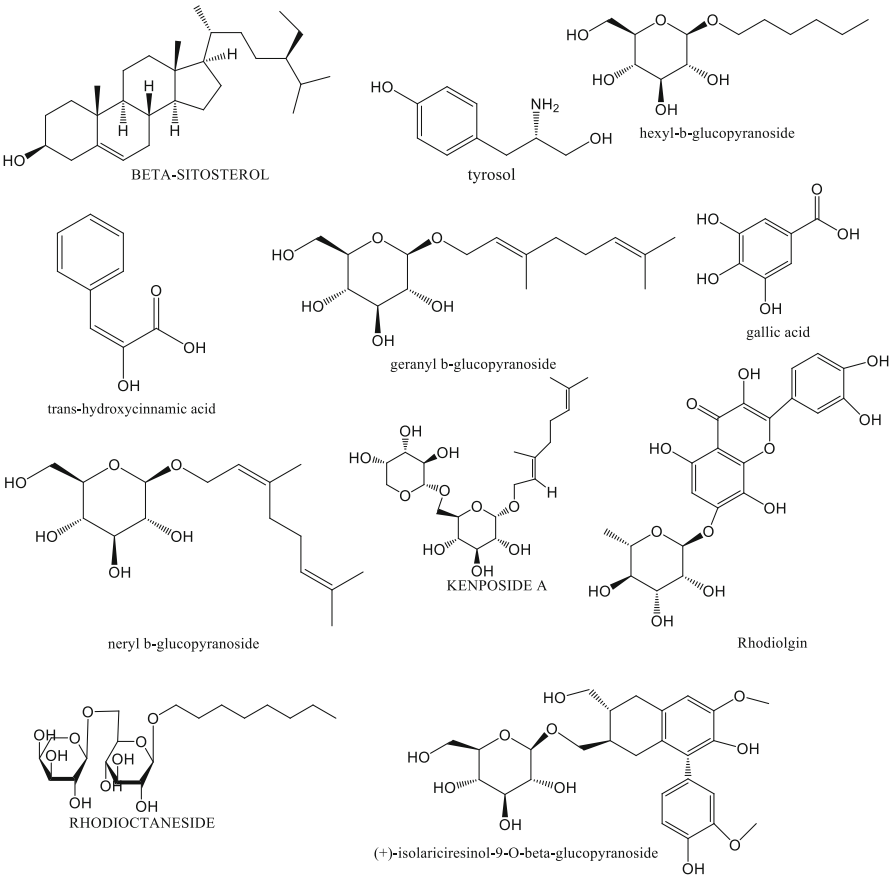


Fig. 15 Phytocomponents reported by Wong et al. (2008)

decrease the ammonia concentration in mouse muscles, thus reducing acidosis (Abidov et al. 2003). Based on numerous studies conducted over recent 35 years, *R. rosea* is recommended as the means of improving strength and endurance and replenishing the energy resources of the body (Seifulla 1999). *R. rosea* acts as an adaptogens by improving the physical endurance of male athletes, reducing blood lactate level, and accelerating recovery after exhausting exercise (Abidov et al. 2003; Azizov and Seifulla 1998; Maimeskulova et al. 1997). Administration of *Rhodiola rosea* (SHR-5) prior to acute stress produce favorable results and helps to prevent stress-induced disruptions in performance (Panossian et al. 2010). *Rhodiola rosea* extract found to have an anti-inflammatory effect and protected muscle tissue during exercise (Abidov et al. 2004). Various preclinical studies revealed the adaptogenic effect of *Rhodiola* root water-alcoholic extract (Abidov et al. 2003; Saratikov 1976; Saratikov et al. 1968; Aksenova et al. 1968; Panossian and Wagner 2005; Jafari et al.

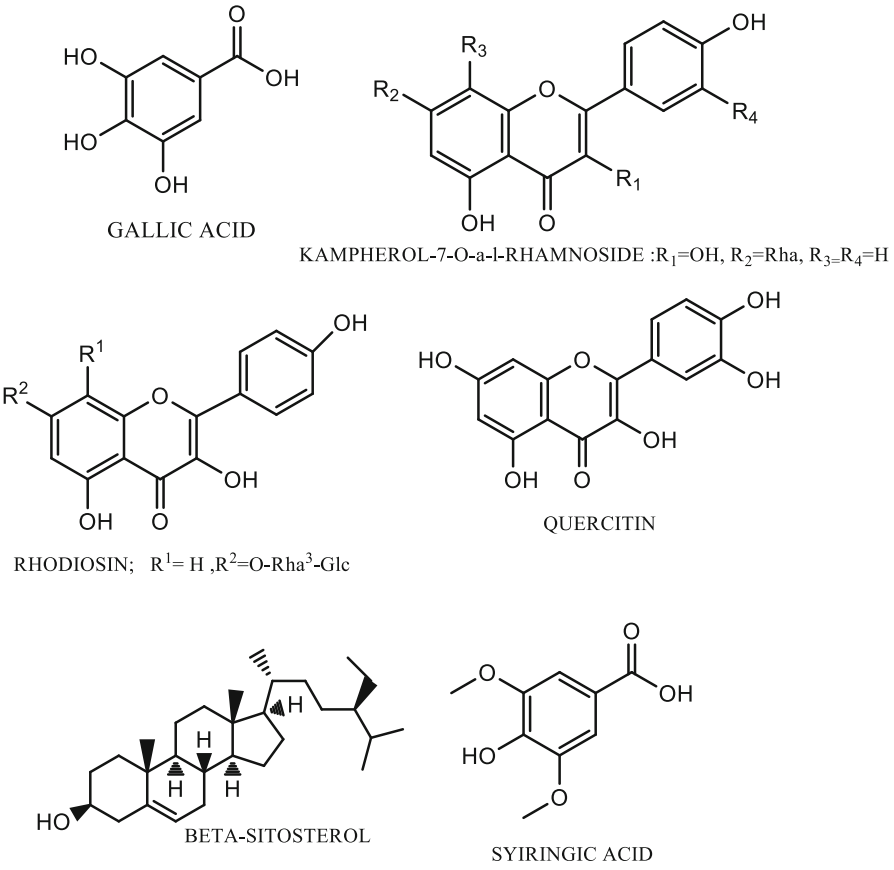
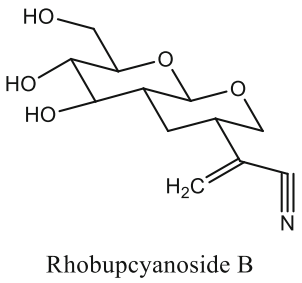


Fig. 16 Phytocomponents reported by Li et al. (2007)

Fig. 17 Phytocomponents reported by Wang et al. (2016)



2007; Perfumi and Mattioli 2007; Mattioli et al. 2008; Diermen et al. 2009; Qin et al. 2008; Siwicki et al. 2007; Wang et al. 2009; Pooja et al. 2009; Bany et al. 2009).

Many studies demonstrated that the regulation of key mediator like molecular chaperones (e.g., Hsp70) (Lishmanov et al. 1996; Prodius et al. 1997; Panossian

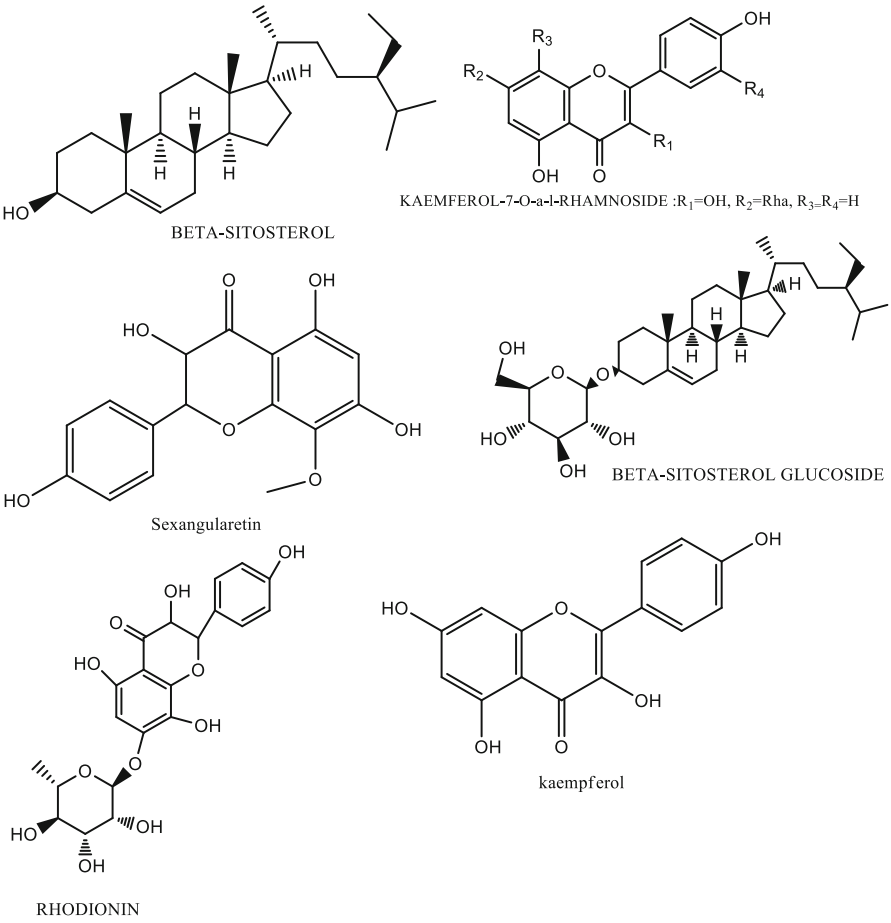


Fig. 18 Phytocomponents reported by Dingqiang et al. (2005)

et al. 2007, 2008, 2009; Wiegant et al. 2008; Olsson et al. 2009), cortisol (Olsson et al. 2009), nitric oxide (Panossian et al. 2007), Forkhead box O (FOXO) transcription factor DAF-16 (Wiegant et al. 2009), stress-activated c-Jun N-terminal protein kinase 1 (JNK1) (Panossian et al. 2007), and beta-endorphin by *Rhodiola rosea* is associated with the stress response (Lishmanov et al. 1987; Maslov et al. 1997; Arora et al. 2005). The studies reveal that the administration of *Rhodiola rosea* promotes a moderate increase in serum immune reactive beta-endorphin in rats under basal conditions which is equivalent to rats adapted to exercise. When *Rhodiola rosea*-treated rats were subjected to a 4 h period of nonspecific stress, the expected elevation in beta-endorphin was either not observed or substantially decreased. Consequently resulting in the characteristic perturbations of the

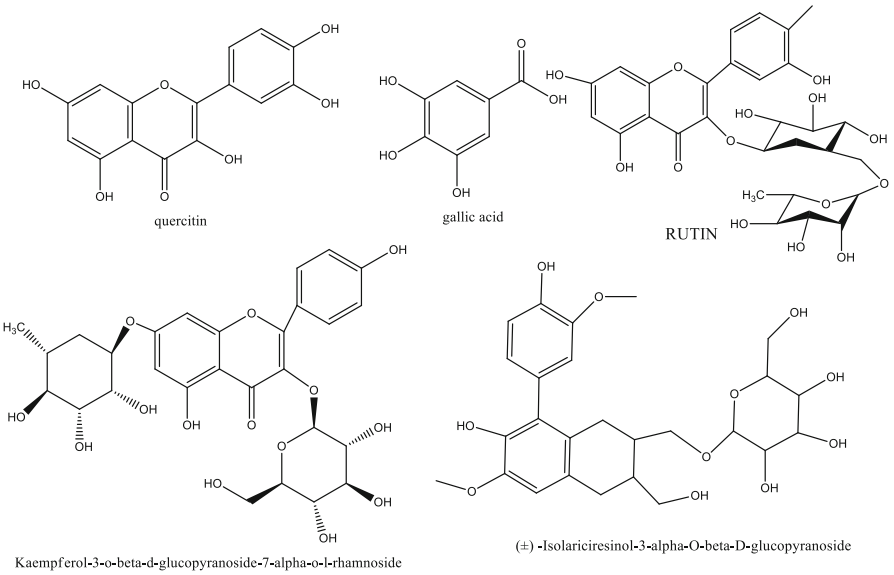
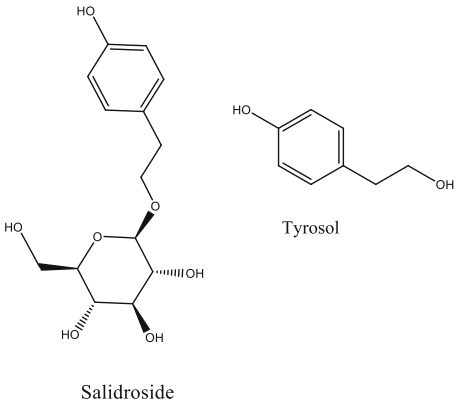


Fig. 19 Phytocomponents reported by Liu et al. (2008)

Fig. 20 Phytocomponents reported by Lu et al. (2011)



hypothalamic-pituitary-adrenal axis was decreased or totally prevented (Lishmanov et al. 1987). *Rhodiola rosea*, with its potential to act as an anticancer agent, might be useful in conjunction with some pharmaceutical antitumor agents, and even supplementation of *Rhodiola rosea* extract inhibits the growth of both tumor types, extended survival times in rats with transplanted solid Ehrlich's adenocarcinoma and metastasizing rat Pliss lymphosarcoma and decreased metastasis to the liver. The studies reveal that the extract also directly suppressed the lung carcinomas (Udintsev and Shakhov 1991). *R. rosea's* protective effect against the antioxidant stress is not totally because of its antioxidant or prooxidant effects (Wiegant et al. 2008;

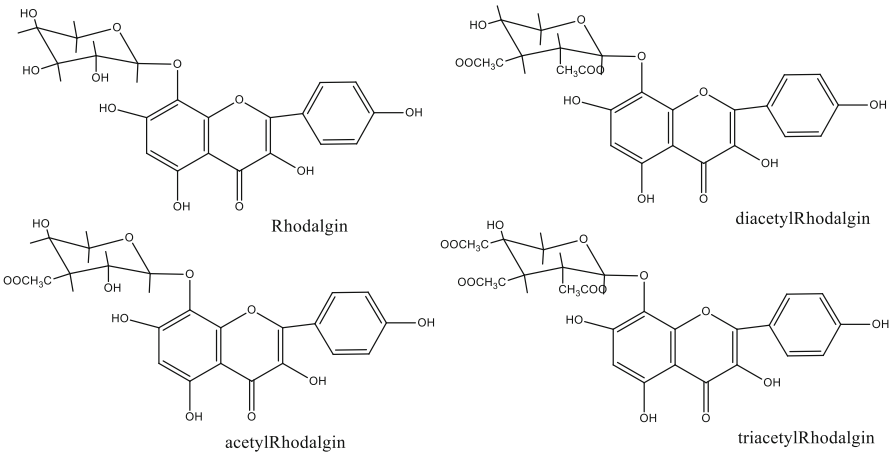


Fig. 21 Phytocomponents reported by Pangarova and Zapesochnaya (1975)

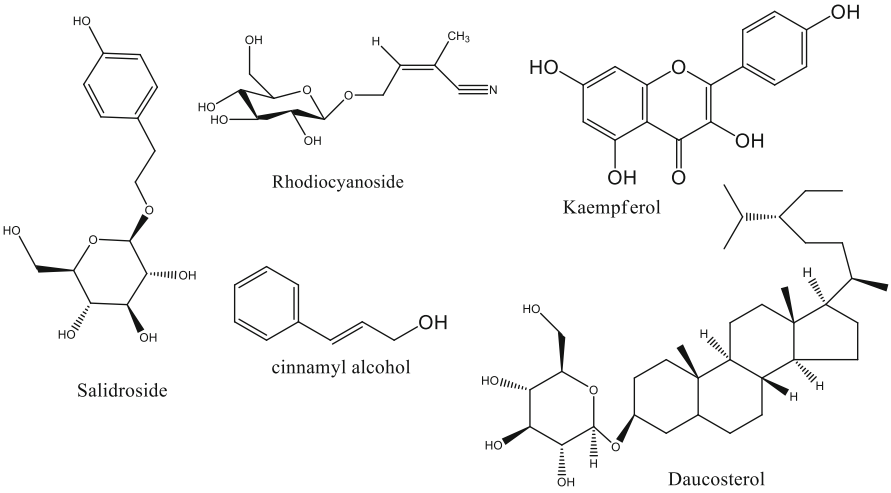


Fig. 22 Phytocomponents reported by Li and Chen (2001), Yoshikawa et al. (1995), Yoshikawa et al. (1997), Lee et al. (2000), Song et al. (2003)

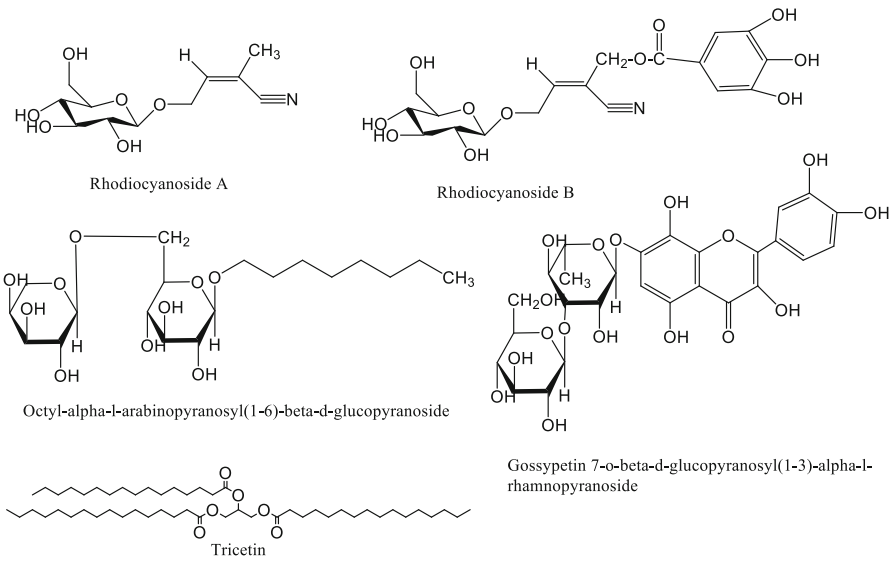


Fig. 23 Phytocomponents reported by Yoshikawa et al. (1995)

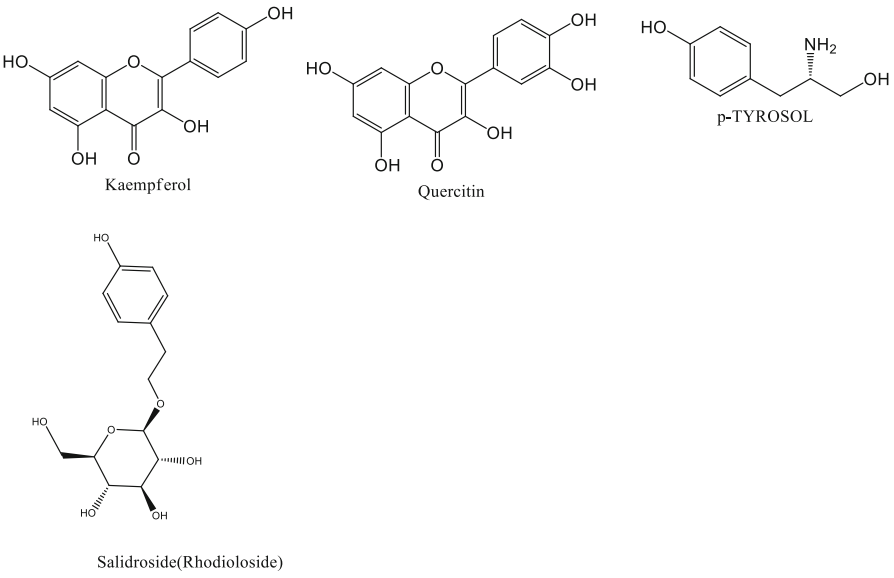


Fig. 24 Phytocomponents reported by Troshchenko and Kutikova (1967)

Schriner et al. 2009) because it does not elevate the major antioxidant defenses but due to activation of the antioxidant response element or degrading H_2O_2 (Schriner et al. 2009).

Rhodiola imbricata

Rhodiola imbricata is known to have many biological effects. There was a significant decrease in the cytotoxicity in comparison to control created by the tert-BHT (250 μM) by using the aqueous and alcoholic extract. Extracts have also reduced the ROS production which was developed by tert-BHT in the mitochondria which is comparable to the vitamin C. Addition of aqueous and alcoholic extract has no effect in GSH level in the tert-BHT-exposed macrophages. Treatment with the extract has six times increased the early apoptotic cells and three times increased the late apoptotic cells which were significantly low when treated with tert-BHT (500 μM). Comet assay revealed that 500 μM tert-BHT has applicably increased the single-strand break which has been reduced by the use of alcoholic and aqueous extract (Kanupriya et al. 2005). The DPPH assay study has reported to show significant inhibition of DPPH activity at 4.391 μg/ml in comparison to quercetin (3.824 μg/ml) and BHT (4.743 μg/ml) for 50% inhibition. The lipid peroxidation activity shows that *Rhodiola* aqueous extract has maximum scavenging activity at 500 μg/ml and minimum at 0.5 μg/ml where α-Tocopherol was used as a standard. The IC_{50} of extract was 5.12 and of standard was 4.89. For the superoxide ion radical, the IC_{50} of the extract, ascorbic acid, α-Tocopherol, and quercetin was 4.78, 3.36, 4.53, and 4.33 μg/ml. The aqueous extract has also reported to show ferric ion chelating activity; IC_{50} of the extract, α-Tocopherol, and quercetin was 5.33, 6.13, and 3.123 μg/ml. The hydrogen peroxide inhibition study revealed that the extract has a very high inhibition activity for hydrogen peroxide which is comparable to α-Tocopherol. The total flavonoid content in extract was reported to be 66.7 μg quercetin equivalent/mg, and total phenolic content was 240 ± 10 mg of gallic acid equivalent (Gupta et al. 2009). The acetone extract of *R. imbricata* was found nontoxic up to 2000 mg/ml and had shown no mortality in mice. This extract is reported to increase hematological count like RBC count, hemoglobin, hematocrit, MCV, MCH, MCHC, RDW, leukocytes, and platelets at conc. of 400 mg/ml. It also has shown a comparable recovery to standard silymarin for paracetamol-induced hepatic damage; it has decreased the SGPT (88.43 ± 0.3 U/l), ALP(193.53 ± 0.3 U/l), and SGOT(79.56 ± 0.3 U/l) liver marker and had increased the concentration of total protein and enzymatic antioxidants. It has also prevented the oxidation of the liver cells after the administration of paracetamol (Senthilkumar et al. 2014). It is reported that *Rhodiola* extract stimulates interleukin-6 (IL-6) and tumor necrosis factor-α (TNF-α) increase in human PBMCs and RAW 264.7 cell line. Reports also show an increase in the production of nitric oxide simultaneously which also activates the nuclear translocation of NF-κB in human PBMCs, which is comparable to the LPS which is a positive stimulant (Mishra et al. 2006). MTT assay of U87 cell line showed an increased survival of cells at doses between 25 and 125 μg/ml in case

of drug +radiation group. In vivo evaluation of revealed that intraperitoneal administration of hydro-ethanol extract rendered 83.3% survival (maximally effective dose, 400 mg/kg b.w.) 30 min prior to lethal (10 Gy) total-body γ-irradiation. The ability of hydro-ethanol extract to reduce the effect of lipid peroxidation induced by iron/ascorbate, radiation (250 Gy), and their combination was also analyzed, and it was found that it decreases in a dose-dependent manner. In a study aqueous extract of *Rhodiola* showed antiproliferative against K-562 cell line in 72 h incubation at a dose of 100 and 200 mg/mlin comparison to normal human peripheral blood lymphocytes or mouse macrophage cell line RAW-264.7 where there is no suppression. Aqueous extract was also found to induce intracellular reactive oxygen species which leads to apoptosis and arrested the cell progression at G2/M phase in K-562 cells. It also shows the anticancer activity which leads to an elevated NK cell cytotoxicity (Mishra et al. 2008). In vitro antioxidant activity was investigated by DPPH radical *R. imbricata* hydroalcoholic root extract which shows a greater correlation of inhibiting the free radicals with the increase in conc. of extract (Kumar et al. 2010b).

Rhodiola heterodonta

Study of the 80% EtOH extract of *R. heterodonta* (40 mg/kg) increased the survival rate of the treated mice under hypoxic conditions by 1.9 times compared with the untreated control have been reported. The survival time of mice under hypoxia increased by 192%, by injecting 80% ethanol extract of *R. heterodonta* which acts as an indication of adaptogenic activity (Grace et al. 2009). The research revealed that *Rhodiola heterodonta* extract has moderate adaptogenic effect and may be used as adaptogen. Also, low toxicity of *Rhodiola heterodonta* preparations has been demonstrated by histomorphological analysis of internal organs (Yunuskhodjaev et al. 2014). The total phenol and flavonoids of *Rhodiola heterodonta* root extract was found to be 79.21 ± 0.26 mg GAE/g and 269.3 ± 0.82 mg Qc/g, respectively (Kumar et al. 2010b).

Rhodiola crenulata

Rhodiola crenulata was reported to show increase in the glycogen synthesis and inhibits the lipogenesis by regulating the genes (glycogen synthase, glycogen synthase kinase 3β, CCAAT/enhancer-binding protein, fatty acid synthase, sterol-regulating element-binding protein 1c) related to the metabolism and process (Lin et al. 2016). It has been reported that the water and ethanol extract of *R. crenulata* has shown α-amylase inhibitory activity with an IC_{50} value of 98.1 μg total phenolic/ml and 120.9 μg total phenolic/ml. Besides this it also has reported to show an α-glycosidase inhibitory activity at an IC_{50} value of 60.3 μg total phenolic/ml and 60.2 μg total phenolic/ml (Kwon et al. 2006). A finding tells that its extract improved the functioning of the brain of rat model of Alzheimer's disease through protecting

neural stem cells by its key component salidroside which scavenged intracellular free radicals (Qu et al. 2012). *Rhodiola crenulata* was used in the treatment of chronic intermittent hypoxia-decreased cardiac fractional shortening and has shown a significant effective improvement, based on decreases in Fas, activated caspase 8, and FADD, activated caspase 3, compared to the hypoxia group. With treatment of *Rhodiola crenulata*, the cardiac mitochondrial-based apoptotic pathway in mice with chronic intermittent hypoxia was significantly decreased, which leads to decreases in pro-apoptotic protein levels like t-Bid, Bad, Bax, activated caspase-9, and activated caspase 3 as well as increases in anti-apoptotic protein levels p-Bad, Bcl-xL, and Bcl-2. Another pathway which is cardiac VEGF-related leads to a significant increase in protein p-PI3k, VEGF, and p-AKT level compared to the hypoxia group with treatment of *Rhodiola crenulata*, which is based on increased in pro-survival (Lai et al. 2015). *Rhodiola crenulata* extract and its bioactive components have reported to show a significant decrease in hypoxia-mediated endocytosis of the Na and K-ATPase because of the inhibition of the ROS-AMPK-PKC pathway in A549 cell line (Chen et al. 2015). It is reported that the extract also has estrogenic activity (Bassa et al. 2016). There is a reduction in proliferation, which stimulates differentiation and eliminates tumorsphere formation of in vitro glioblastoma multiforme cells with the effect of *Rhodiola crenulata*. The effects were associated with inhibition of Wnt/β-catenin signaling pathway (Guo et al. 2014). It also has effect on gluconeogenic gene expression by increasing the phosphorylation of AMPK level. It also reduced the plasma glucose level (Lee et al. 2015). Its treatment reduces the level of IFN-γ, high-sensitivity C-reactive protein, and CD8 (+) but increases in expression of CD4(+) CD25(+) FOXP3(+) and CD4(+) CD25(+) CD45(+) FOXP3(+) in the blood (Chen et al. 2015). A study for the survival rate of *Drosophila melanogaster* against the gut immunity raised by pathogen demonstrate that *R. crenulata* has increased the survival rates of adult flies and expression of antimicrobial peptide genes after pathogen or toxic compound ingestion. Moreover, it improved intestinal morphology and decreased levels of reactive oxygen species (Zhu et al. 2014). The compounds from *Rhodiola crenulata* extract were tested for xanthine oxidase (XO) inhibition activity in comparison to a known XO inhibitor allopurinol which has an IC_{50} value of 12.21 ± 0.27 μM. The compound B2-3′-O-gallate and 4-HAP reported an XO inhibitory effect, the half maximal inhibitory concentration values of compound were 15.62 ± 1.19 and 24.24 ± 1.80 μM, respectively, and there inhibition constants were 8.41 ± 1.03 and 6.16 ± 1.56 μM, respectively. These results suggest that β-2-3′-O-gallate and 4-HAP are potent XO inhibitors (Chu et al. 2014). Its use in Chinese prescription significantly decreases the level of serum glucose, lipid profile, blood urea nitrogen, urine albumin excretion, and urease activity which improves renal function. Chinese prescription could also affect oxidative stress. It could reduce the renal damage induced by hyperglycemia in type 1 diabetic rats. Its effects work by regulating the metabolism of glucose and lipid, the oxidative stress, and the microcirculation disturbance (Fu et al. 2013). *R. crenulata* phenolic-enriched extract was capable of

inhibiting the proliferation, motility, and invasion of human-derived MDA-MB-231 and mouse-derived V14 breast cancer cell lines. The extract also leads to death of the tumor cell lines by inducing autophagic-like vesicles but not the immortal human mammary epithelial cells (Tu et al. 2008). By activation of AMPK signaling, *Rhodiola crenulata* root extract (RCE) can regulate hepatic gluconeogenesis (Lin et al. 2016). The root extract of *R. crenulata* was found to improve insulin sensitivity and attenuate abnormal lipid metabolism in a rodent model of diabetes (Wang et al. 2012). Increase in glycogen synthesis and inhibition of lipogenesis, while regulating genes included in glycogen metabolism like glycogen synthase (GS), glycogen synthase kinase 3β (GSK3β), CCAAT/enhancer-binding protein (C/EBP), fatty acid synthase (FAS), and sterol regulatory element-binding protein 1c (SREBP-1c), have been reported by Lin et al. (Lin et al. 2016). The various phenolic compounds were found to be potent as antioxidants and could moderately stimulate IFN-γ expression (Zhou et al. 2015).

Rhodiola kirilowii

Rhodiola kirilowii extract was reported to protect against problems related to the heart and lungs while moving to high altitude, anticoagulative property and decrease the level of blood sugar (Zhang et al. 1989). *Rhodiola kirilowii* was found to have in vitro inhibitory activity against serine protease (NS3-SP). Serine protease is a potent target of antiviral screening against HCV (Zuo et al. 2007). It also have a great potential as cellular immunity enhancers. The in vitro studies revealed that the extracts stimulate activity of granulocyte and increased lymphocyte response toward mitogens, and in vivo experiment leads to enhance the ability of lymphocytes derived from parental strain mice which were fed with *R. kirilowii* aqueous and hydroalcoholic extracts, to induce local cutaneous graft-versus-host reaction in F1 hybrids (Wojcik et al. 2009). The in vitro activities against *Mycobacterium tuberculosis* of its extracts and pure components were evaluated by testing their minimal inhibitory concentration and minimal bactericidal concentration, and the compounds gallic acid and epigallocatechin gallate exhibited an in vitro inhibitory and bactericidal activities against *Mycobacterium tuberculosis* in different extent (Wong et al. 2008).

Rhodiola bupleuroides

The data available related to the species is very less. Still the report which is available in relation to this species shows that it has compounds which were evaluated for its inhibitory activity against α-glucosidase, and the results show that it has an IC_{50} of 278.28 ± 0.55 μM in comparison to the positive control (acarbose) at 210.40 ± 0.32 μM (Wang et al. 2016).

Rhodiola algida

Rhodiola algida found to increase immunity which was receiving chemotherapy post-mastectomy and also decreases oral ulcers. Thus *Rhodiola algida* has the potential to be used concurrently with chemotherapy to alleviate the occurrence of oral ulcers. The optimal concentration of *Rhodiola algida* favored the proliferation of lymphocytes (Loo et al. 2010). The clinical reports suggest that it regulates IL-2 in Th1 cells and IL-4, IL-6, and IL-10 in Th2 cells which effectively stimulate human peripheral blood lymphocytes, enhancing immune responses and its underlying immunomodulatory effects (Li et al. 2009). Its anticarcinogenic effect on MCF-7 breast cancer cells can lead to downregulation of protein levels of HIF-1α and HIF-2α, which are overexpressed under hypoxic conditions and increasing cell apoptosis. *R. algida* has a high potential to be antitumor agent (Iaremii and Grigoreva 2002). Beside its antitumor role, *R. algida* also have immunomodulatory agent (Li et al. 2009).

Rhodiola sachalinensis

The studies reported the hypnotic activity and sedative of salidroside from *Rhodiola sachalinensis* (Li et al. 2007). *Rhodiola sachalinensis* has found to have stimulating role for the nervous system, enhancing working efficacy, decreasing depression and microwave radiation, resisting anoxia, eliminating fatigue, improving sleep, preventing high altitude sickness, etc. (Khanum et al. 2005; Ming et al. 1988). Salidroside, which is a phenylpropanoid glycoside, has been reported to have anti-inflammatory activity (Lu et al. 2003). Crude extracts of *Rhodiola sachalinensis* was found to have high DDPH radical scavenging activity (Zhang et al. 2007). In the study with mice, salidroside showed that it enhances the sleep, by shortening the effect on the sleep latency and also prolonging the effect on the sleeping time in mice treated with hypnotic dosage of pentobarbital sodium (Li et al. 2007). The extract of *Rhodiola sachalinensis* was found to promote endurance and to increase the body's resistance against mental and physical stresses (Xu et al. 1998). The aqueous extract found to activate NF-ķB which enhances the induction of iNOS gene in RAW264.7 macrophages (Li et al. 2012). The root has been used to treat cold and flu-like symptoms, but the underlying mechanism is not known (Li et al. 2012). It has been also found to be hepatoprotective against cytotoxicity induced by tacrine in human liver-derived Hep G2 cells (Mishra et al. 2010). Kaempferol is a representative flavonol, and its derivative, kaempferol-6′-*O*-acetate, was reported to have hepatoprotective effect against cell death induced by TNF-α (Lu et al. 2003).

Rhodiola qundrifida

The plant extract was applied for the treatment of fatigue, blood pressure, dysentery, and genital diseases of women and as a stimulator of the nervous system (Saratikov

et al. 1967, 1978; Rohloff 2008; Mora et al. 2015; Yoshikawa et al. 1996). *Rhodiola qundrifida* reported to be antiallergic; rhodiacyanosides have inhibition effect on the histamine which were released from the rat peritoneal exudate cells which were sensitized with anti-DNP-IgE (Mora et al. 2015). The extract of *Rhodiola qundrifida* was found to be stimulatory in cell-mediated immunity (Rozewska et al. 2008a). *Rhodiola quadrifida* hydroalcoholic and aqueous extracts induce an immune modulatory effect on the mouse granulocytes activity which is evaluated by chemiluminescence test (Rozewska et al. 2008b). Its extract response toward the cell-mediated immunity is also evaluated by other tests like respiratory burst activity (RBA) and potential killing activity (PKA) tests (Siwicki et al. 2007; Rozewska et al. 2008a). *Rhodiola quadrifida* also have inhibitory in the highest (50 μg/ml) dose and granulocytes activity in lower doses (Wojcik et al. 2008).

In Vitro Propagation/Culture

In vitro study was done to develop the plant in lab condition to use for various medicinal purposes besides collecting the raw material from the wild environment and to develop the germplasm. The explant selection is a very crucial factor for the success of morphogenic potential of the isolated cells. There is an extensive use of biotechnological approach to increase the various metabolites of the plant (Grech-Baran et al. 2015). The explants will determine the organogenic as well as the genetic stability of the progeny after cloning. Leaves or leaf disks were the most potent and preferable explants for callus, shoot, and bud formation (Tasheva and Kosturkowa 2010). In a procedure for in vitro propagation of roseroots (*Rhodiola rosea*), a medicinal plant, was developed by using a RITA bioreactor system which includes liquid medium in combination with a gelled medium. Three clones were established on a basal medium (BM) for germinated seedlings on half strength Murashige and Skoog (MS) salts. Shoots of all three clones rooted in vitro in the growth regulator-free basal medium within 5–6 week of culturing with a frequency of 90–95% in all three clones. Plantlets obtained in vitro were adapted and transferred to soil with a survival rate of 85–90% (Debnath 2009). In a report a callus culture was established to produce the cinnamyl glycosides (Gyorgy et al. 2004). An in vitro micropropagation study was conducted for the plant in 24 modified Murashige and Skoog media (Tasheva and Kosturkowa 2010). A change in the media composition can effect in the metabolite production of the plant. The addition of methyl jamsonite in the callus culture of *Rhodiola sachalinensis* had increased the salidroside and polysaccharide content (Yu et al. 2011). A change in composition of media is also used for the establishment of the in vitro culture of *Rhodiola henryi* (Kang et al. 2010). The preservation study of callus has shown that the melatonin has improved the survival of callus *Rhodiola crenulata* (Zhao et al. 2011).

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Ophiocordyceps sinensis: The Medicinal Caterpillar Mushroom

Ranjit Singh, P. S. Negi, and Sanjai K. Dwivedi

Introduction

Ophiocordyceps sinensis, previously known as *Cordyceps sinensis*, popularly called as caterpillar mushroom is a non-toxic, medicinal fungus found growing in the Himalayan hills in India, Nepal, China and Tibet. In higher hills of Uttarakhand (India), this fungus is locally known as *yarsha gamboo* or *kira ghas*, since it parasitizes on the lepidopteran insect larvae of the caterpillar *Thitarodes* (*Hepialus*) *armoricanus* Oberthur of family Hepialidae under the soil (Huang 1999). The grasslands are habitat for *Thitarodes armoricanus* and hence for *Ophiocordyceps sinensis* because the former is the host insect for the later in alpine meadows just above the tree line. Its local name in Uttarakhand is *yarsha gamboo* which is derived from *yartsa gunbu*, a Tibetan name meaning summer grass winter worm.

For the first time, *Torrubia*, a Franciscan friar in Cuba in the eighteenth century, described it as the trees growing out of the bellies of wasp; due to this reason, the genus *Ophiocordyceps* is sometimes known as *Torrubia* in honour of its inventor (Christensen 1975). In China, this caterpillar mushroom is known as *dong chong xia cao*, and Japanese named it as *tochukaso*. *Ophiocordyceps sinensis* (Fig. 1) thrive on a number of grass root-boring caterpillars of ghost moth. The life cycle of the moth *Thitarodes* (*Hepialus*) *armoricanus* generally completes in 1–5 years, and major part of it is represented by caterpillar; the adult moth lives only for 15–20 days (Zhu et al. 1998). Nearly 40 species of *Thitarodes* (*Hepialus*) moths are recognized in the Tibetan Plateau region, and about 30 species can be infected by *Ophiocordyceps sinensis*. The genus *Thitarodes* accommodates the *Hepialus armoricanus* and other related species which were newly erected in 1968, and earlier they were placed in the genus *Hepialus* which is the host for *Ophiocordyceps sinensis* (Wang 1999; Sung et al. 2001, 2007b).

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Fig. 1 Natural specimen of *Ophiocordyceps sinensis*

In India, this fungus has got popularity during the 1990s when it was collected for the first time from the high altitude hills of Dharchula (Uttarakhand) in the Central Himalayas by some local people called Khampas (a Tibetan race). Initially Khampas used to collect this fungus for medicinal purpose, but later on they started to sell it across the borderline to Tibet (China). After a few years, local people came to know about this important fungus, and they also started its collection and selling it in the local market. Later on, large number of local people got involved in this business. In Central Himalayan hills, local people consume this fungus in both fresh and dried form (Arora 1986). They take it for maintenance of stamina of the body and relief from many human ailments. The dried powder of this fungus is boiled with milk or added to vegetables or mutton soup. Due to its high efficacy and potency in curing diseases, *Ophiocordyceps* was prescribed by the ancient medicinal practitioners as the *panacea of all ills*. Mainly it came in the limelight in the world during the year 1993 in which Chinese athletes shattered many world records in outdoor games in Olympics in Germany. At first, it was speculated that a performance-enhancing drug might be consumed by the athletes, but later it was revealed that their vigour had increased because of the regular consumption of a fungus *Ophiocordyceps sinensis*, which in the traditional Chinese system of medicine had been used for centuries to increase vigour and vitality of people (Zang and Kinjo 1998). The initial record of *Ophiocordyceps sinensis* as medicine is as old as the Qing Dynasty during the year 1757, which appeared in *New Compilation of Materia Medica* (Wang et al. 2000). It contains cordycepin, carbohydrate d-mannitol, vitamin B_{12}, six essential amino acids and unsaturated fatty acids.

Being a hormone stimulator, *Ophiocordyceps* is considered as one of the most important natural anti-ageing medicines. Monoamine oxidase formation is inhibited

by *Ophiocordyceps* which is an enzyme responsible for ageing in man. *Ophiocordyceps sinensis* was found beneficial in many human disorders like climatic age illness, neurasthenia, impotence, emission, cirrhosis, rheumatoid arthritis, etc.

Ophiocordyceps mycelium is composed of white threadlike hyphae intermingled inside the host insect body; the mycelium keeps consuming the internal organs of the insect until it completely fills the entire body leaving behind the outer exoskeleton only (Gao et al. 2003; Nielsen et al. 2000). When there is no further nutrient available for the mycelium to further multiply, *Ophiocordyceps sinensis* produces a fruiting body which comes out of the caterpillar body from mouth region. Fruiting body is generally 5–10 cm long and comes out of the soil for the dispersal of spores by wind so that the spores will be able to find a new host for further infection. The fruiting body, i.e. stroma, is generally double the length of the caterpillar. Spores take few weeks for maturation. The larger the size of the caterpillar, the larger the size of stroma as it consumes nutrients from the caterpillar body.

The specimens of *Ophiocordyceps sinensis* are found occurring rarely in higher hills from an altitude of 11,000 to 14,000 ft. above the mean sea level. The local people collect specimens of this fungus during the month of May and June just after the melting of snow. Most of the families residing in these remote localities were found busy in collection of this fungus throughout its period of growth. It has been observed that each family having five to six members engaged in collection of this fungus for about 35–45 days was able to collect approximately 1 kg of dried fungus specimens in one season. The complete drying of specimens takes approximately 1-week time since it is cold and humid weather prevailing all around that altitude. Semi dried specimens containing high amount of moisture content were spoiled by the contamination of other fungi.

Taxonomy

Ophiocordyceps sinensis belongs to subdivision *Ascomycotina* of kingdom fungi which is mainly characterized by the presence of sexually produced spores called ascospores formed within an organ called ascus. Having the characteristic features like ascomata surrounded by a peridial wall, unitunicate asci, flask-shaped perithecia and long narrow asci having thread like ascospores, this fungus is kept under the family *Ophiocordycipitaceae* of class *Pyrenomycetes* of subdivision *Ascomycotina*. There are about 400 species of *Cordyceps* (Sung et al. 2007). Till recently, this fungus is known by the name of *Cordyceps sinensis*, and the name has been changed to *Ophiocordyceps sinensis* ((Berk.) G.H. Sung) by Sung and his coworkers.

China is having maximum genetic diversity with 68 species followed by 33 species found occurring in the Tibetan Plateau and Himalayas. *Ophiocordyceps* generally parasitize moths and insects, and out of 400 species, only a few are being collected for their medicinal properties. *Ophiocordyceps sinensis* is the most commonly collected fungus followed by *Cordyceps militaris*, which is widely distributed in North America and Eurasia (Arora 1986; UNW 1996). However, *Ophiocordyceps sinensis* is the

costliest and most in demand fungus in the world due to its potent medicinal properties. According to recent investigations by Sung et al. (2007b) they have changed the name of genus *Cordyceps* to *Ophiocordyceps.*

A very few species of this fungus are also found growing on the underground fruiting bodies of another fungus. Out of the seven such mycophilus species of *Ophiocordyceps*, two species are found growing on *Claviceps*, and the rest five species grow on *Elaphomyces* under the ground (Dubey 1983). A new classification of *Ophiocordyceps* species has also been suggested on the bases of chemotaxonomy of partial nucleotide sequences of 18S rDNA obtained from four different species (Ito and Hirano 1997).

Life Cycle of *Ophiocordyceps sinensis* and Insect *Thitarodes armoricanus*

Ophiocordyceps sinensis needs a caterpillar of the moth *Thitarodes armoricanus* to complete its life cycle. *Ophiocordyceps* produces billions of spores in order to maximize the chances for finding caterpillar for infection and further completion of its life cycle (Kendrick 1992). Spores generally take 15 days for infecting the larva after landing on its body (Li 1993; Li et al. 1999, 2001). Spore germinate to produce mycelium which is a white micro-thread, which keeps on multiplying in the body of the larva and side by side keeps on consuming the internal organs of the larva, and when the entire organs were consumed and the body of the caterpillar is stuffed with mycelium, then this fungus generates a fruiting body which comes out from the head portion of the larva. The fruiting body further grows up to 3–10 cm in length. The fruiting body is brown in colour and club shaped and comes out of the soil for the dispersal of the spores. Precipitation and snowfall have also an impact on the size of the fruiting body, but excessive snowfall degrades the stromata and impacts the *Ophiocordyceps* spore production badly (Boesi 2003).

The ascospores or their fragments initiate the infection in the body of insect larvae. This inoculum puts out a germination tube which penetrates the body integuments and develops into the hyphae or mycelium within the body tissue of the caterpillar. This fungus produces an enzyme called cutinase, which helps in the digestion of larval body tissue. The hyphae break up into fragments and get distributed in the hemocoel. Further, these fragments bud off to produce more propagules which give rise to the complete hyphae. After some days of fungus infection, the body tissues of insect larvae get stuffed with the compact mass of mycelium. The mycelium modifies into the sclerotium which remains covered by the integument of dead insect. Cordycepin, an antibiotic produced by *Ophiocordyceps*, does not allow the corpse of the insect to decay.

The sexual reproductive organs (ascus and ascospores) are present inside the perithecia. These filiform ascospores normally divide into the small fragments, which spread over the Earth surface by the wind. The infection of the insect larvae is initiated by the ascospores. The germ tube produced by the ascospores penetrates the integuments of larvae and produces hyphae or mycelium within the body of the

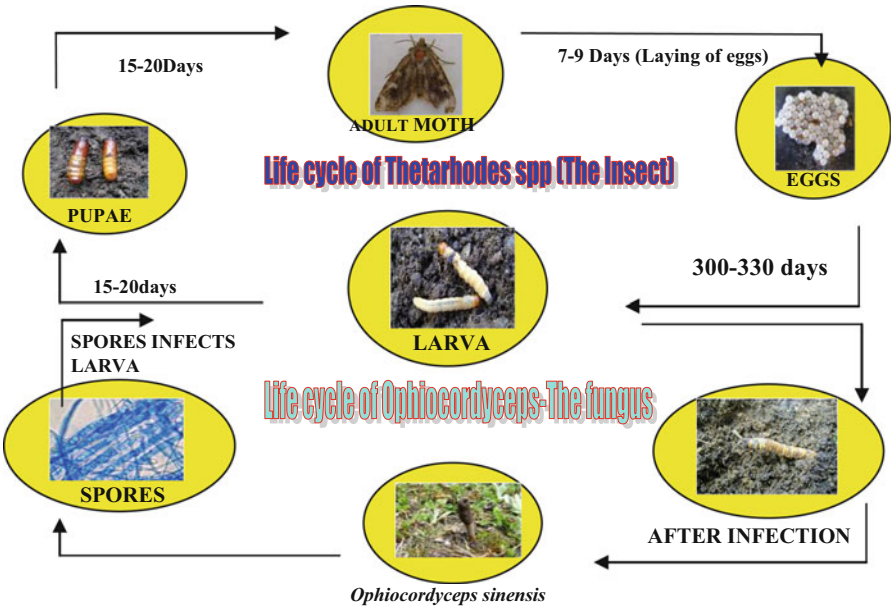


Fig. 2 Life cycle of *Ophiocordyceps and Thitarodes*

caterpillar. The fungus produces enzymes called cutinase, proteinase and lipase which help in the penetration and digestion of larval body tissue (Sung et al. 2007a). The mummy of the insect having endosclerotium lies buried underground until the favourable climatic conditions for the germination of sclerotium commence. As soon as the snow melts, the sclerotium starts germinating in the form of a structure resembling the blade of grass, which comes out of the insect body from the head region. The club-shaped mature stromata bear a stalk and a head above the ground. The head region of this mushroom contains numerous peripheral perithecia. The asci and ascospores are present within the perithecia. These filiform ascospores may divide into small fragments, the spores, and then fragments were dispersed by wind. The complete life cycle of the *Ophiocordyceps* and its associated insect moth is given in Fig. 2.

Ophiocordyceps Habitat in India

Ophiocordyceps sinensis occur rarely in higher reaches of hills from an altitude of 10,000 to 14,000 ft. above mean sea level in the Himalayas. Places of occurrence of this valuable fungus are Chhipalakedar, Brahamkot, Ultapara, Ghwardhap, Chhipalakot and Najari, which are spread in about 8–10 km of area in Dharchula region of Uttarakhand and were initially identified as a place of occurrence of *O. sinensis* (Arif and Kumar 2003; Garbyal et al. 2004; Das et al. 2005), but looking to its possibility of occurrence in several other places having the similar type of

Fig. 3 Local people exploring O*phiocordyceps* habitat for collection

climatic conditions in the Indo-Tibet Himalayan ridges, local people started searching the neighbouring places for its occurrence (Fig. 3). Till date many more places in the areas, namely, Lashpa, Darti, Milam, Burfu, Mapa, Tola and Ralam in Johar Valley and Nagindhura, Golfa, Bona and Chhipalakedar in the basement of Panchachuli in Munsiyari, were also identified as places of its occurrence (Sharma 2004). The main biodiversity-rich regions identified are situated within the four valleys, namely, Johar, Darma, Vyas and Chaudas of the Central Himalayan hills (Negi 2006). In Garhwal hills of Uttarakhand, this fungus is reported from Niti and Mana valleys of Chamoli district (Singh et al. 2010). Besides Uttarakhand, this fungus has also been reported from Arunachal Pradesh, Sikkim and Himachal Pradesh regions of India (Negi et al. 2010).

Ethnomycology

The specimens of *Ophiocordyceps sinensis* occur in slopes and plateaus in far reaches of higher hills at an altitude between 3500 m and 5000 m above mean sea level. The specimens become visible only after the snow melts during the months of May to June. Since it is a remunerating fungus, majority of the families residing in these difficult and remote localities are engaged in the collection of this fungus throughout its period of occurrence. Before proceeding for collection, people generally worship the local deities and pray for good harvest of the fruiting bodies. An average family of five to six members is usually able to collect around 1 kg of dried fungus in one season, i.e. between 34 and 45 days. The specimens after collection are properly washed to remove the soil adherent to the surface of caterpillar portion. The caterpillar is usually enclosed in a netlike cocoon, and the collectors normally use a toothbrush to remove it. The washed and cleaned specimens are spread in a plain surface usually on stones for drying. Because of the cold and humid climate at these

places, the specimens get dried completely in approximately a week time. Even traces of moisture make the specimens vulnerable to infection and spoilage by other fungi. Hence, people with prior harvesting experience now ensure complete drying of the specimens. The specimens are normally stored in locally available cotton bags wrapped in polythene sheet.

Ophiocordyceps sinensis is having huge morphological variation in nature. Variations have been observed in size and number of stroma. Specimens were generally having stromae at one end, but specimens with stromae at both the ends were also observed. Specimen with 2 and 4 stromae and sometimes bifurcated stromae at the middle were also observed.

Natural populations of *O. sinensis* are found having variation in colour, shape, size, number of stroma, in having single or multiple stromata at one or both the ends, etc. The biochemical analysis of in vitro-cultured mycelium vis-à-vis natural specimens of *O. sinensis* contains almost same amount of cordycepin, an important bioactive ingredient.

Medicinal Uses

Ophiocordyceps sinensis is considered as a miraculous medicinal mushroom because of its multiple utility in a wide range of human disorders. Cordycepin is one of the most valuable bioactive molecules which are highly effective against all strains of bacteria which have developed resistance against most of the antibiotics. Cordycepin is highly effective in curing disorders like leprosy and tuberculosis. It is also found useful in lung and respiratory problems. *Ophiocordyceps* increases the stamina of the body. Few important medicinal properties of *Ophiocordyceps* are discussed briefly as under.

Ophiocordyceps Improves the Respiratory Functions

Regular intake of *Ophiocordyceps* shows significant improvement in patients with respiratory ailments like chronic bronchitis and asthma. It significantly improves the maximum amount of oxygen assimilation.

Ophiocordyceps Improves the Functioning of the Heart

Regular use of this fungus improves functioning of the heart which is validated by numerous studies. It was found helpful in heart rhythm disturbances, heart failure and induced acute pulmonary oedema (pneumonia). *Ophiocordyceps* consumption significantly lowers the mortality rate by 20% from 80% mortality within 30 min of its consumption (Wang et al. 2000).

Ophiocordyceps Helps to Maintain Cholesterol and Protects the Liver

This fungus was found useful in lowering the total cholesterol of up to 21% and triglycerides up to 26% and helpful in increasing HDL cholesterol up to 30%. It was found useful in improving blood cell viability enhancement and promotes DNA repair (Wang et al. 1998a, b). The liver purifies the blood of impurities and *Ophiocordyceps* improves liver health and is helpful in the control of hepatitis and other liver ailments. Proper functioning of the liver is essential for healthy living (Manabe et al. 1996). Research clinical trials on hepatitis "B" patients and cirrhosis patients with *Ophiocordyceps* supplement in their diet showed remarkable improvement (Zhu 1990).

Ophiocordyceps Reduces Tumour Size in Cancer Patients

Ophiocordyceps mycelium-supplemented diet (6 g per day) intake by cancer patients resulted in significant reduction in tumour size (Chiou et al. 2000).

Immune System

Increased NK cell activity was observed in patients consuming *Ophiocordyceps* regularly. One study by Chung-Kuo Chung showed that natural *Ophiocordyceps* enhanced the NK cell activity of leukaemia patients by 400% (Kuo et al. 1996; Guo and Zhang 1995). Effect of *Ophiocordyceps sinensis* on NK cell activity and colony formation of B16 melanoma was also observed (Xie et al. 1988).

In Treatment of Leukaemia

Ophiocordyceps is highly effective in the treatment of leukaemia. Studies have shown that it improves the patients suffering from leukaemia. Proliferation and differentiation of human leukaemic cells was enhanced by its regular consumption. It has antitumour activity by suppressing the growth of sarcoma (Wasser 2002). Its use in diet stimulates blood mononuclear cells and inhibits the proliferation of leukaemic cells up to 78–83% (Kuo et al. 1996).

Anti-ageing

Ophiocordyceps is considered as one of the few natural anti-ageing supplements for human consumption. Clinical trials on old-age patients suffering from senility and other related symptoms showed improvements in the reduction of fatigue and dizziness after consumption of *Ophiocordyceps sinensis* for a quarter. Patients with respiratory problems also showed significant improvement after regular consumption (Guo and Zhang 1995).

Ophiocordyceps Protects Against Free Radical Damage and Reduces Fatigue

Ophiocordyceps sinensis, due to strong antioxidant properties, was found to protect against the damages caused by free radicals and as such has powerful antioxidant properties (Bao et al. 1988). *Ophiocordyceps sinensis* increases the cellular energy production and oxygen supply. Regular intake of 3 g of *Ophiocordyceps* daily showed improved breathing efficiency and reduced fatigue in patients suffering from chronic heart failure (Guo and Zhang 1995).

Endurance, Stamina, Fitness and Athletic Performance

Ophiocordyceps sinensis was found helpful in building body muscles and hence improves body endurance, stamina and fitness in athletes by increasing ATP synthesis (Blackwell and Gilbertson 1984). *Ophiocordyceps sinensis*-supplemented diet showed improvement in swimming ability in athletes after 6 weeks of regular consumption as compared to normal diet-fed athletes (Bok et al. 1999).

Ophiocordyceps Combats Sexual Dysfunction

Regular intake of *Ophiocordyceps* in daily diet showed significant improvement in sex hormone production in men and women and fights infertility. It increases sperm count and sperm survival. Research studies on animal models show *Ophiocordyceps* increases natural sex hormones (Wang et al. 1998a, b).

Field Collection of Natural Samples for In Vitro Culture Establishment

In order to establish the pure culture of *Ophiocordyceps sinensis*, live samples were collected from its natural habitat, i.e. Laspa region of Munsiyari, 13,500 ft. above mean sea level from Central Himalayan hills in Uttarakhand (India). Collection work was carried out during the month of May to June when snow starts melting. The specimens were surface sterilized with 0.1% $HgCl_2$ followed by washing with double-distilled water three to four times and then stored in low-temperature conditions. For pure culture establishment, the stroma portion was excised and resterized with ethanol and mercuric chloride followed by washing with sterile distilled water and surface dried by sterile filter paper. For in vitro mycelium multiplication, tissues were excised from the different parts of the fungus body like stalk and tissue from stroma region. These tissues were excised from the fungus body and were cultured on various combinations of culture media and standardized the protocol of pure culture establishment of the fungus. The Defence Institute of Bio-Energy Research, Haldwani, has succeeded in the establishment of the pure

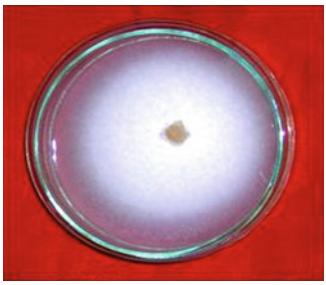


Fig. 4 Starter culture of *O. sinensis*

mycelium culture of *O. sinensis* in the laboratory conditions. Using the standardized protocol, a thick mat of mycelium was obtained in the laboratory culture which is further lyophilized and converted into the powder form. It should be stored under cold conditions at about 4 °C or below.

Starter Culture Establishment

Pure starter culture (Fig. 4) of *Ophiocordyceps sinensis* has been established at the Defence Institute of Bio-Energy Research, Haldwani (Uttarakhand), India, and for this purpose, tissue from stroma region of the live specimens were utilized. Optimum culture medium was standardized by the establishment of pure culture of *Ophiocordyceps* on different types of culture media, and it was observed that potato dextrose agar, beef extract dextrose agar, casein hydrolysate dextrose agar, soybean extract dextrose agar and rice extract dextrose agar were found suitable for its mycelium multiplication, but out of all the tried medium, casein hydrolysate dextrose agar media was found optimum for mycelial growth. This fungus generally prefers acidic pH (5–5.5) and low temperature (10–15 °C) for optimum mycelial growth. Fruiting body formation in *O. sinensis* on synthetic culture medium has yet not been achieved. However, mycelial growth of this fungus was also achieved in larvae of Silkworm (*Bombyx mori*).

Technology for Laboratory-Level Broth Culture

The broth culture is a method of fast multiplication of fungus mycelium; for this purpose, inoculum from starter culture is used for the fast multiplication of mycelium on liquid medium. Mycelium multiplies faster than solid starter culture and yields a thick mat of mycelium which may be converted into powder form after freeze-drying (Fig. 5). The powder of mycelial mat acts as the main raw material for the production of nutriceuticals in the form of food supplements, tablets and capsules. This powder of *Ophiocordyceps* may also be used in the development of various types of dietary supplements for the sports persons. The mycelium production of *Ophiocordyceps* on commercial scale can be grown on silkworm larvae and protein-rich various cereals.

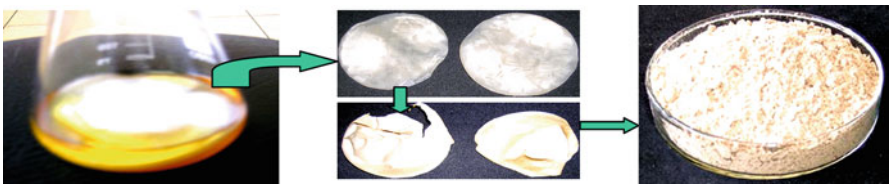


Fig. 5 *Ophiocordyceps* broth culture for mycelium mat and its powder production

Fig. 6 *O. sinensis* mycelium culture in bioreactor

Fruiting body formation in fungus is not essential to achieve a quality medicinal product (Huang 1999).

Laboratory-multiplied *Ophiocordyceps sinensis* mycelium production technology has little impact on the prevailing market of wild *Ophiocordyceps* in Asia, but in European countries, mycelium-based products are more popular rather than made up of natural specimens because western countries do not prefer products made from insect-based fungus. In China, the *Thitarodes* (*Hepialus*) *armoricanus* host insect larvae are bred on commercial scale which is placed in culture vials with lids; before harbouring the insect larvae, the culture vials were filled with the soil from natural habitat of the insect.

Technology for Mass-Scale Production

The Defence Institute of Bio-Energy Research (DIBER) has standardized large-scale mycelium production protocol using bioreactor (2.0 L capacity); with the help of this bioreactor, 500 g lyophilized mycelium powder can be produced in 60 days under in vitro conditions (Fig. 6).

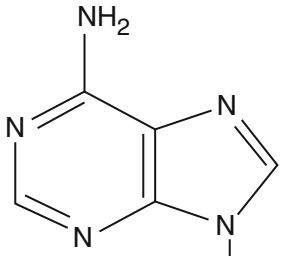
In vitro micropropagation of *Ophiocordyceps sinensis* in the laboratory is quite important because in vitro culture-produced mycelium eliminates the chances of contamination which is quite common in natural specimens with glass sticks, wooden sticks, some heavy metals, etc. The local inhabitants of the villages in the vicinity of

Ophiocordyceps habitat used to insert wooden sticks, etc. in the natural specimens just to increase the weight for getting higher price for their harvested *Ophiocordyceps* produce. Some incidents of lead poisoning were earlier reported in Taiwan (Ying et al. 1987). The population of the natural specimens in their habitat is very less, and its collection is labour and cost intensive. Keeping above conditions in view, culture of this fungus under laboratory conditions provides a solution to fulfil the demand of this high-value medicinal fungus for the development of commercial value products.

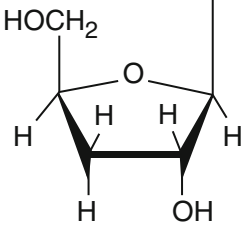
Standardization of laboratory culture techniques of broth culture on small scale and fast multiplication of mycelium in bioreactor on large scale of this fungus by DIBER (DRDO) have opened new *vistas* in India for the pharmaceutical and nutraceutical industries.

Biochemical Analysis of *Ophiocordyceps sinensis*

Ophiocordyceps sinensis (syn. *Cordyceps sinensis*) is a very important medicinal fungus because of its unique biochemical ingredients, one of which is cordycepin (3-deoxy adenosine) a nucleoside; besides this it is also found having good amount of cordycepic acid and essential amino acids like glutamic acid, phenylalanine, histidine, valine, oxyvaline and arginine. It is also found to be rich in oleic acid, linoleic acid, d-mannitol, vitamin B_{12}, etc. Cordycepin content was estimated in both natural and laboratory-cultured samples, and both samples were found quantitatively at par (Negi et al. 2010).



Cordycepin



Cordycepin

Antioxidant Properties of *Ophiocordyceps sinensis*

Antioxidant activity (Fig. 7) of natural and lab-cultured *Ophiocordyceps sinensis* was measured in terms of IC_{50} values using DPPH and ABTS free radicals. Laboratory-cultured mycelium powder was found having more antioxidant property in comparison with natural one (Anon 2015).

Antibacterial Activity of *Ophiocordyceps sinensis*

The antibacterial activity of natural and laboratory-cultured mycelium of *Ophiocordyceps* was studied against two bacterial strains *Escherichia coli* (MTCC 40) and *Pseudomonas aeruginosa* (MTCC 424). Methanolic extracts of both wild and in vitro cultured *O. sinensis* showed significantly higher antibacterial activity than that of aqueous extract for both samples (Anon 2015).

Testing of Immunomodulatory Property of *Ophiocordyceps sinensis*

Immunomodulation property of both the natural and laboratory-cultured samples was assessed by observing the response of hot water and alcoholic extracts towards cytokine production in mice. The findings revealed that alcoholic extract of laboratory-cultured mycelium is having better response towards the cytokine production in mice. Extracts of natural and laboratory-produced *Ophiocordyceps* were tested in terms of cytokine production from the splenocytes of mice. All the extracts

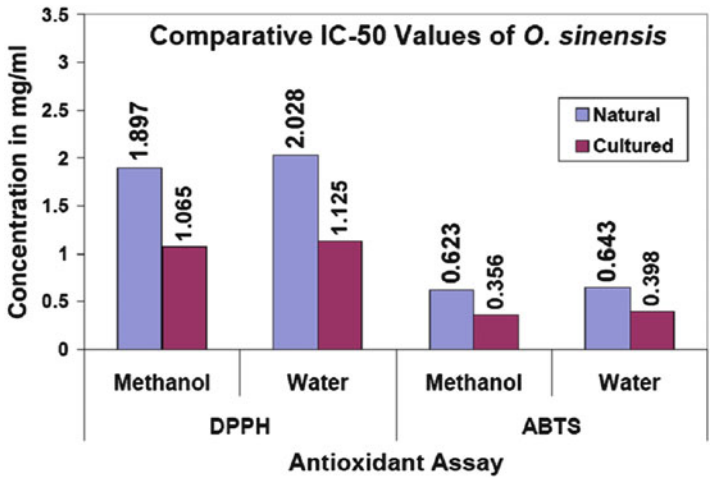


Fig. 7 Antioxidant assay activity of *Ophiocordyceps sinensis*

showed stimulation for IL-4, IL-10, IL-12 and IFNγ production from splenocytes of mice. Alcoholic extract of laboratory-cultured mycelium gave maximum response to stimulation of all four types of cytokines.

Development of Nutraceutical and Pharmaceutical Products

Ophiocordyceps has been used for the treatment of respiratory, cardiovascular, renal and pulmonary disorders since ages. Regular uses of *Ophiocordyceps* strengthen immune system of the body and build resistance to infections and treat cold, flu, cough, asthma, etc. and as an overall rejuvenator for treating fatigue and weakness. *Ophiocordyceps* has been found a potent drug for the treatment of hyposexuality, hyperlipidemia and immune disorders.

Important information about the medicinal effects of *Ophiocordyceps sinensis* has been published by various workers (Wang 1999; Hobbs 1995; Winkler 2003; UNW 1996). *Ophiocordyceps sinensis* contains altered nucleoside cordycepin, an antibiotic (3′- deoxyadenosine) (C_{10} $H_{13}0_3N_6$), glutamic acid, oleic acid, linoleic acid and *d*-mannitol. Other important ingredients are polysaccharides, nucleosides and nucleotide and most of the amino acids and micronutrients. In China, *Ophiocordyceps* is an important drug for the treatment of numerous disorders, i.e. chronic bronchitis, insomnia, hypertension, pneumonia, tuberculosis, pulmonary emphysema, anaemia, night sweats and cough and for treating physiological disorders (Wang 1999; Garbyal et al. 2004). *Ophiocordyceps* is effective against hepatic, renal and cardiovascular diseases and also shows anticancer properties (Evans and Samson 1982; Garbyal 2001; Halpern 1999). *Ophiocordyceps sinensis* is having immunomodulatory properties (Hobbs 1995; Hodge 2003; Huang et al. 2005). Cellular immunological function was found restored in patients suffering from advanced stage of cancer after regular consumption of *Ophiocordyceps* (Huelsenbeck and Ronquist 2001). The various products of *Ophiocordyceps* regulate the normal functioning of different body parts, strengthen the immune system and promote the overall vitality and longevity. It increases IL-1, interferon and TNF production under in vitro Kupffer cells of rats and elevates serum level of these substances. *Ophiocordyceps* also exhibits antitumour and antiviral activities (Liang 1991). *Ophiocordyceps sinensis* have diuretic effects and hence is found good for the prevention of nephralgia (Liang et al. 1991). *Ophiocordyceps* is also useful in the prevention of arteriosclerosis and coronary heart diseases. Intake of *Ophiocordyceps sinensis* was found to have the stimulatory effect in the production of corticosteroids (Liu et al. 2001). *Ophiocordyceps* has also shown activity against the human leukaemia. It was found beneficial for the patients of hyperglycaemia and hypertension because it has the potential to reduce the blood sugar as well as the blood cholesterol level (Massee 1895).

Today in the western countries, *Ophiocordyceps* is frequently consumed by old-age people and athletes to stall the ageing process in the former and for building stamina in the later. *Ophiocordyceps* has the potential as a source of new anticancer drugs. It was found effective in reducing the harmful effects of chemotherapy and

radiation in cancer patients. It inhibits the growth of certain tumours and also elicits the immune system (Petch 1924). A large number of cancer patients in China, Japan and Korea take *Ophiocordyceps* and other mushroom-derived polysaccharide immunomodulators along with chemo- and radiotherapy, and reduction in severity and duration of side effects has been reported (Spatafora et al. 2007). The *Ophiocordyceps* and other mushrooms sometimes improve the patient's immune system, thereby increasing the effectiveness of the treatment.

Ophiocordyceps in traditional medicinal system occasionally proved quite helpful in treating heart diseases and stroke patients (Holliday et al. 2004). It is useful in normalizing heartbeat and correcting heart arrhythmias, though the exact mechanism is only partly understood. Biochemicals like adenosine and deoxyadenosine have been found to have a positive effect on circulatory system. *Ophiocordyceps* is a non-toxic herbal substance, with little or no side effects except in some people who may experience dry mouth, nausea or diarrhoea. Local people of Munsiyari and Dharchula regions of Pithoragarh district of Uttarakhand (India) consume this fungus in fresh or dried form. They take it for maintenance of stamina in the body and relief from many human ailments. Normally one person takes two to three natural specimens per day. The dried powder of this fungus is boiled in milk or added to vegetables or mutton soup.

The Defence Institute of Bio-Energy Research (DIBER), Haldwani, is a pioneer research institute working on in vitro culture technology of *Ophiocordyceps sinensis*. Lyophilized mycelium powder has been utilized to develop products which are having a unique energy-rich metabolite cordycepin, at par with natural specimens. Some of our products are given below:

Cordyvit

It is a unique nutraceutical food supplement formulated using lab-cultured mycelium of *Ophiocordyceps sinensis* as base material which is supplemented with mineral mixture, vitamins and antioxidant-rich herbs (Fig. 8a). World over, such products have been formulated, and an attractive market exists in India as well; this product can potentially fill the void. Toxicity studies have already been carried out on this product and are found to be safe. This product is having cordycepin as a compound, which is a performance enhancer and has anti-ageing properties. Two grams of product per day with milk is sufficient for maintaining the vitality of an average human being.

Cordypower Capsules

Cordypower capsules (Fig. 8b) are formulated using lab-cultured mycelium of *Ophiocordyceps* as base material which is supplemented with other vitamins and antioxidants. These capsules are highly valued due to energy-rich metabolites which ensure vitality and performance. Toxicity studies have already been carried out of

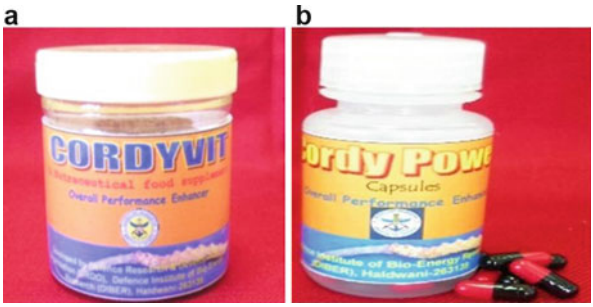


Fig. 8 *Ophiocordyceps*-based nutraceutical products (**a**–**b**). (**a**) Cordyvit, (**b**) Cordypower capsules

this product and are found to be safe for human consumption. World over, such products have been formulated, but there is no such indigenous product exists in India. One 100 mg capsule twice a day maintains the strength and stamina of the body daily.

Future Research Prospects

The natural habitat cover of this valuable fungus in the world as well in India is shrinking every year due to the excessive exploitation of natural specimens from its habitat because its high market price. Now it is the need of the hour to stop the excessive exploitation/collection of the natural samples from the habitat. Although researchers from China claim that it has produced the fruiting bodies of this fungus in culture vials, however, it is not a proven technology for other countries of the world. In India, DIBER (DRDO) has done pioneering efforts for in vitro multiplication of its mycelium, but efforts need to be done to produce the fruiting bodies of this valuable fungus in in vitro cultures. Following are the future areas of research on *Ophiocordyceps sinensis*:

Sustainable Utilization of Natural Specimens

Natural habitat of *Ophiocordyceps sinensis* is limited only to a few particular microclimatic regions. In India, it is limited to Uttarakhand, the Himalayas and in some parts of Sikkim. Local tribal population living in the vicinity of these habitats used to collect the natural specimens of this valuable fungus, so as to cater for their livelihood, but due to increase in the awareness and skyrocketing prices of the natural specimens in the market, exploitation of the natural habitat is increasing a lot every year, hence putting a pressure on its habitat.

Awareness Creation Amongst Local Population

Over-exploitation of any natural resource will result in its extinction sooner or later. In order to save the natural habitat of this fungus and for the sustainable utilization for the years to come, a check on the collection of natural specimens is required to be imposed strictly. The natural habitat of this fungus comes under restricted forest region under the control of forest department and local Van Panchayats (Kumar and Negi 2003). Nowadays, local people used to take out all the visible specimens, whether they are mature or immature, to earn more and more money by selling more specimens. Therefore, restriction should be imposed on the explorers as well as on local population for a limited number per person, and some areas should be identified as out of bound for everybody so that the natural multiplication of this fungus takes place undisturbed. Availability of *Ophiocordyceps* from the wild is limited, and increase in demand has resulted deterioration in quality of different *Ophiocordyceps*-based products.

In international market, lots of products formulated from natural as well as in vitro-cultured *Ophiocordyceps sinensis* are available, but their cost is very high, and common people in India cannot afford these costly products of foreign origin. Therefore, product formulated from indigenously developed in vitro culture technology-produced mycelium can fill up this gap, and these indigenized and cost-effective products will be very helpful in coming up to the expectations of health-conscious people in India.

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Indian Hawthorn (*Pyracantha crenulata*)

Ranjit Singh, P. S. Negi, and Sanjai K. Dwivedi

Introduction

Indian hawthorn (*Pyracantha crenulata* (D. Don) M. Roem. Syn., *Crataegus crenulata* Roxb. Fam. Rosaceae) is endemic to the Himalayan hills. In the Kumaon region of Uttarakhand (India), it is known locally by the name "Ghingharu". *Pyracantha* is a bushy and profusely branched very thorny shrub, with dark green leaves, that grows wild in barren, rocky, and dry grasslands. It is generally 2.4–3.0 m in height and the plant is laden with orange-red to dark red fruits during fruiting season (Fig. 1). In the Himalayan hills of Uttarakhand, it grows in areas from 900 m to 2500 m above mean sea level in Pine and Quercus forests (Osmastan 1926). The stem bark is dark brown and becomes glabrous when old. The white inflorescence is a compound corymb having many flowers. Flowering takes place in April to May and the fruits ripen in June to September. *Pyracantha* flowers are hermaphrodite (bisexual) having 20 stamens and one ovary. This perennial, deciduous, and thorny shrub is commonly known as Indian hawthorn or *Ghingharu.*

Indian hawthorn grows wild in hills in India and in neighboring Himalayan countries (Brandis 1921). *Pyracantha crenulata* is native to the temperate Himalayas (Weber 2003). Its fruits have a high total soluble solid content and are consumable; the leaves are used to make herbal tea (Kunkel 1984). The fruits are a good source of food for wild animals, including birds and langurs (*Presbytis* spp.) in the rainy season. The wood is used for making walking sticks and for fuel. The fruits have cardio-tonic properties. *Pyracantha* is quite useful in reducing the risks of heart failure, paroxysmal tachycardia, hypertension, arteriosclerosis, and Buerger's disease (Chauhan 1999). The ripe fruits are eaten fresh and a mixture of the dried fruit powder with yoghurt is used in the treatment of bloody dysentery. The plant is used in Ayurvedic medicines and is commercially harvested from forest areas for different medicinal preparations, such as infusions of the dried fruits, liquid extracts, and

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Fig. 1 (**a**) *Pyracantha* plant. (**b**) Fruit-bearing branch of *Pyracantha*

tinctures. So it appears to be important to standardize its propagation techniques and create awareness of its value among the people, besides creating awareness about its conservation (Singh et al. 2012).

Pyracantha also makes an excellent hedge and it can be used as a good soil binder for stabilizing degraded areas and slopes that are prone to landslides. The plant generally prefers sun-facing hill slopes and grows in moist and fertile land on hill slopes (Gamble 1972). It increases plantation cover by sending out suckers and spreading its seeds (Barnes et al. 1998). Conventionally this plant is exploited by the local inhabitants for the fencing of agricultural fields and for making tool handles for agricultural implements. However, conventional knowledge of the medicinal uses of this plant is not widespread, although several reports on the medicinal properties of other species of *Crataegus* are available in the literature. The bioflavonoids in most *Pyracantha* species are quite useful in the treatment of malfunctioning of the heart and blood circulatory system (Peschel et al. 2008). *Pyracantha* fruits also have antispasmodic, diuretic, sedative, and vasodilatory properties. The fruits and flowers of *Pyracantha* both have hypotensive properties, hence they are useful in cases of high blood pressure (Anonymous 2011).

Indian hawthorn fruits are storehouses of vitamins A, C, E, and B_{12}, as well as protein, calcium, magnesium, and potassium. Research and development activities for this plant have shown that *Pyracantha* has bioactive compounds in its fruits that are highly beneficial in curing hypertension. Clinical trials on heart-related ailments concluded that treatment with hawthorn bioflavonoids resulted in reductions of cholesterol levels in patients with heart disease (Negi et al. 2009). Its leaves show strong anti-inflammatory and immunomodulatory properties. *Pyracantha* is a natural source of medicinally important biological ingredients. It is also an environmentally

beneficial plant because it helps in soil conservation in hills and controls desertification. This shrub develops an extensive root system, which binds the soil and helps in reducing soil erosion and landslides. For this reason local people in the hills plant it on fragile hill slopes and on field boundaries.

Antioxidants present in hawthorn berries are helpful in reducing the damage caused by free radicals. The medicinal properties of this plant for the treatment of circulatory system disorders and respiratory illnesses were already established by the eighteenth century. In Ayurvedic medicine it is considered to increase blood flow to the heart muscles and to restore a normal heartbeat. This effect results from the presence of bioflavonoids in the fruit. The antioxidant-rich fruits strengthen the blood vessels. In other medicinal systems also, the flowers and fruits of *Pyracantha* are considered to act as a mild heart tonic.

Taxonomy and Morphology

Pyracantha crenulata (D.Don) M.Roem, syn., *Crataegus crenulata* Roxb. fam. *Rosaceae,* commonly known as Himalayan firethorn, Indian hawthorn, and Nepalese firethorn. The systematic classification of this plant is as shown below:-

Kingdom	Plantae
Phylum	Anthophyta
Class	Magnoliopsida
Order	Rosales
Family	Rosaceae
Genus	*Pyracantha*
Species	*crenulata*

Morphology

Pyracantha crenulata is a thorny, hard woody shrub of up to 2–5 m in height; it is found in the foothills of the Himalayas. It is found along streams, on the banks of tributaries of rivers, on roadsides, and in Pine and Quercus forests (Fig. 2), as well as in mixed forests (Osmaston 1926). It is an important plant in the foothills of the Himalayas and is found in Uttarakhand, Himachal Pradesh, and the North Eastern states of India and Nepal at elevations of 1600 to 2500 m (Brandis 1921).

Leaves

The leaves are dark green in color (Fig. 3a) with a smooth surface, 2.5–4.0 cm in length, and 1.0–2.2 cm in width with tapering ends. The leaves are rich in

Fig. 2 Naturally growing *Crataegus crenulata* (*Pyracantha crenulata*) in Pine forest

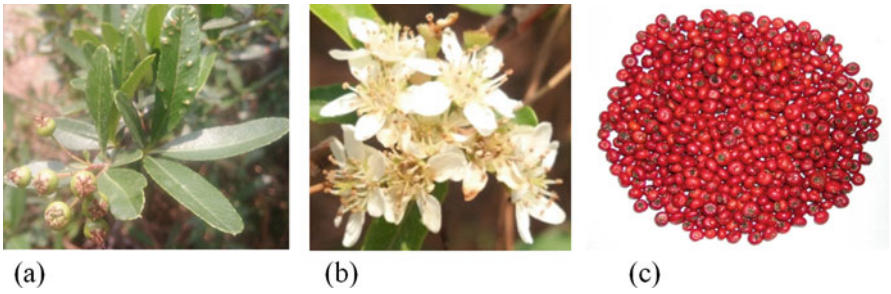

Fig. 3 *Pyracantha crenulata*. (**a**) Leaves, (**b**) flowers, and (**c**) fruits

antioxidants and phenolics and are one of the ingredients used to make herbal tea by the local people.

Flowers

Flowering occurs during the months of April to May. The white-colored inflorescence is a compound corymb with many flowers in it (Fig. 3b). The flowers are hermaphrodite (bisexual) having 20 stamens and one ovary in the center. Each flower also contains five sepals and five petals.

Table 1 Physical parameters of mature *Pyracantha* fruits

Length (mm)	Width (mm)	Weight of 1000 fruits (g)	Color	pH of fruit juice
15.9 ± 1.3	16.4 ± 1.9	260 ± 8.2	Orange-red	2.82 ± 0.4

All data are means ±SE

Table 2 Biochemical composition of fresh *Pyracantha* fruits (g/100 g)

Carbohydrate	Protein	Lipids	Moisture	Total ash
5.90 ± 0.96	0.60 ± 0.29	0.270 ± 0.19	82.6 ± 1.9	0.27 ± 0.06

All data are means ±SE

Fruit

The fruit is a pome type, consisting of pulpy berries, and fruiting occurs during the months of July to September in Uttarakhand conditions. The berries are small (Tables 1 and 2) and each berry weighs 250 mg; thus 1 kg weight of fruits contains about 4000 berries. The pome fruit is orange-red (Fig. 3c) and provides food for various birds. *Pyracantha crenulata* is native to the temperate Himalayas (Weber 2003). The fruits are edible and rich in sugar, and the leaves are used to make herbal tea (Kunkel 1984).

Seeds

Each berry generally contains five triangular brown-colored seeds; sometimes three or four seeds are observed, and the seeds are covered with a hard seed coat.

Biochemical Composition of Fruits

The Defence Institute of Bio-Energy Research, Haldwani (Uttarakhand), India, has analyzed the biochemical composition of *Pyracantha* fruits; the moisture content is 75% and the percentage of flavonoids is 2–3%, and vitamins A, B_{12}, C, and E; as well as protein, calcium, magnesium, and potassium, are present. Oligomeric proanthocyaninidins are also found in the fruits. A few heavy metals are also present, but levels are below the permissible limit. The quantitative biochemical content of the fruit is given in Table 3.

Traditional Uses

Most hawthorn species (*Pyracantha spp*) are traditionally used for their demonstrated cardio-protective benefits. It is also listed in the pharmacopoeias of some European and Asian countries. Flavonoids and oligomeric proanthocyaninidins have been reported as the bioactive constituents of standardized preparations from different species of *Pyracantha* used for the treatment of heart

Table 3 Biochemical composition of fresh fruits of *Pyracantha crenulata*

Serial No.	Biochemical constituent	Quantity
1.	Protein	1.8 ± 0.2%
2.	Flavonoids	2.9 ± 0.4%
3.	Calcium	3.08 ± 0.021 mg/100 g
4.	Magnesium	1.4 ± 0.02%
5.	Sodium	1.00 ± 0.2%
6.	Potassium	1.43 ± 0.05%
7.	Vitamin A	289 ± 0.07 IU/100 g
8.	Vitamin B_1	0.50 ± 0.04 mg/100 g
9.	Vitamin B_2	17.7 ± 1.10 mg/100 g
10.	Vitamin C	55 ± 3.03 mg/100 g
11.	Vitamin E	272 ± 5.0 mg/100 g

All data are means ± SE (Standard Error)

ailments. Conventionally it is exploited by the local Himalayan inhabitants for the fencing of agricultural fields, and making sticks and tool handles. It is also used as firewood. Local people chew its berries sometimes just for fun and for their taste. No edible product from this plant has been developed by the local people so far. Decomposed leaf litter, spread under the shrubs, is used by local farmers for mulching their fields. *Ghingharu* sticks are sold in the local market. In the hills of Kumaon, the villagers use the sticks for playing a hockey-like local game called '*Gira*'. However, no conventional knowledge on the medicinal uses of *P. crenulata* is available in the area's traditional knowledge.

Medicinal Uses

Pyracantha is a multipurpose medicinal plant, every part of the plant being useful in some ailments. At the beginning of the eighteenth century Western doctors started using hawthorn fruits for the treatment of circulatory disorders and respiratory illnesses. Bioflavonoids present in its fruits are useful for the treatment of cardiac ailments. Its fruits also have antispasmodic, diuretic, sedative, and vasodilatory properties. The fruits and flowers of this plant have hypotensive properties (Walker et al. 2006); hence they are useful in treating high blood pressure. Important medicinal uses of this plant are described below.

Reduction of Free Radical Damage

Antioxidants present in hawthorn berries are helpful in reducing the damage caused by free radicals (Anna et al. 2007). Western herbalists consider the berries as a potent heart tonic that is very useful in normalizing heart rhythm in hypertensive patients (Chang et al. 2002).

Tonic for Cardiac Ailments

Both the fruits and the flowers of *Pyracantha* are utilized in the preparation of a heart tonic that is considered to be a potent drug for all heart ailments. Its regular use was found to be useful for strengthening the heart and its muscles and normalizing high blood pressure. The flowers and berries of the hawthorn plant are reported to be useful in the treatment of irregular heartbeat, high blood pressure, chest pain, hardening of the arteries, and heart failure (Tassell et al. 2010). It is commonly used to strengthen the heart, and its berries are especially indicated for the treatment of a weak heart, combined with high blood pressure, manifestations of old age, inflammation, arteriosclerosis, and nervous heart problems.

Memory Enhancer

A mixture of *Pyracantha* and Ginkgo (*Ginkgo biloba*) leaves was found to enhance brain cell activity and hence to enhance memory (Guven et al. 2006).

Other Medicinal Uses

A decoction of the shoots and bark of *Pyracantha* stops excess menstrual flow, while a stem extract is useful in fevers. Extract of its bark applied topically protects skin from sunburn and all body organs from frostbite. The juice of hawthorn fruit has a hypoglycemic effect in the treatment of diabetes (Andrade-Cetto and Heinrich 2005).

Anti-hypertensive Activity

It is a well established fact in medical science that its fruits are effective in curing hypertension (Wagner and Grevel 1982 and Tassell et al. 2010). Its leaves also have antioxidant, immunomodulatory and anti-inflammatory properties. Undoubtedly it is quite effective in curing various diseases. Cardiovascular disease is a major disease accounting for the death of about 17 million people per year globally. Changing food habits and modern lifestyles have further increased the numbers of antihypertensive patients in the modern world with its hectic lifestyles. Various synthetic drugs for the treatment of hypertension are available in the market, but they have their own side effects. Plant-based drugs have additional advantages as they have a lower rate of side effects and are less costly. Hence there is always a high demand for plant-based antihypertensive agents; this can be met by *Pyracantha*-based products.

A study was conducted at the Indian Defence Institute of Bio-Energy Research (DIBER) a constituent Institute of the Defence Research and Development Organization (DRDO), to observe the effect of a *Pyracantha* herbal formulation on

hypertensive rats (Fig. 5). The results of the study confirmed the positive antihypertensive effect of this beverage (Anonymous 2011).

Antihypertensive Activity in Normotensive Rats (Noninvasive Model)

All the animals in the above study by DIBER were trained for 1 week to remain in a restrainer for a period of 4–6 h per day. The mean blood pressure was measured using an LE-5002 Storage Pressure Meter (UGO, Basile, Italy), an indirect blood pressure measuring system. Measurements were taken from the tail in pre-warmed unanesthetized rats by the tail-cuff technique, for which all animals were pre-trained. An average of three readings were recorded for each animal. After a baseline period of 7 days, the experimental groups received different doses of the test material p.o. (0.25, 5.0, 1.0, and 2.0 ml/25 g rat) for 2 weeks. The control group received only the vehicle, in a volume corresponding to that of the test material.

Antihypertensive Activity in Normotensive Rats (Invasive Model)

Rats were anesthetized with an i.p. injection of urethane (25%, 0.6 ml/100 g body weight). A tracheostomy was performed and a polyethylene cannula was inserted to allow the rat to breathe spontaneously. The carotid artery was dissected, the vagus nerve was separated, and the carotid artery was cannulated and connected to the pressure transducer of a multichannel polygraph (Richter Gedeon, Budapest, Hungary) for recording the blood pressure and heart rate. The jugular vein was also cannulated, for drug administration. After the surgery, 30–40 min was allowed for the cardiovascular indices to stabilize. The test doses (25, 50, and 100 μl/rat) of the formulation were intravenously administered and the effects on blood pressure and heart rate were recorded.

Antihypertensive Activity in Cadmium Chloride-Induced Hypertension

Hypertension was induced by the chronic administration of $CdCl_2$ (1 mg/kg, i.p. for 2 weeks). The rats were anesthetized by an i.p. injection of urethane (25%, 0.6 ml/100 g body weight). The surgical procedure described earlier was followed. The formulation, at test doses of 25, 50, and 100 μl/rat, was administered i.v. The effects on blood pressure and heart rate were monitored (Anonymous 2009).

In the antihypertensive activity studies (normotensive rats, noninvasive model), the formulation administered at doses of 1, 1.5, and 2.0 ml/rat, to conscious rats lowered the blood pressure in a biphasic manner. However, no significant effect on heart rate was observed (Fig. 4). In the normotensive rats (noninvasive model), at all the test doses, the formulation lowered the mean blood pressure for up to 5 h. No significant effect on heart rate was observed (Table 4). Blood pressure was lowered for up to 4 h at the dose of 100 μl/rat in the cadmium chloride-induced hypertensive rats (Tables 4, 5, and 6). This study suggested that the formulation prepared from the fruit of *P. crenulata* possessed significant antihypertensive properties.

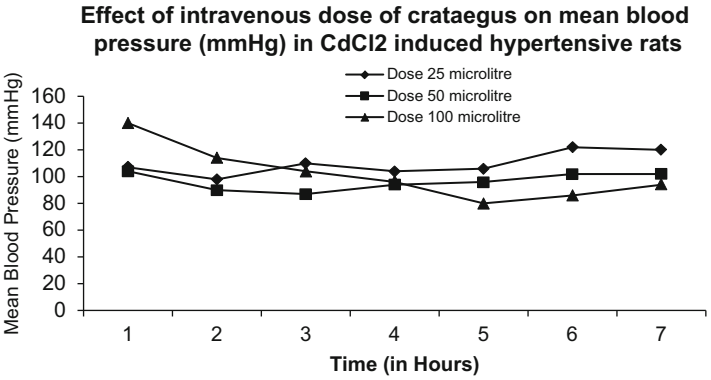

Fig. 4 Effect of *Hridayamrit* herbal beverage (prepared from fruits of *Pyracantha*) on hypertensive rats

Table 4 Mean blood pressure (MBP; mm Hg) and heart rate (HR; beats/min) measured at different time intervals after different doses of *Pyracantha* fruit juice formulation (Anonymous 2009)

Dose, µl per rat/i.v.	Parameter	0 h	0.5 h	1 h	2 h	3 h	4 h	5 h
25	MBP	123	60	67	56	54	62	73
	HR/min	356	306	306	317	347	356	383
50	MBP	128	115	95	51	54	63	68
	HR/min	406	390	375	370	340	350	353
100	MBP	125	101	93	64	57	62	85
	HR/min	363	352	340	324	314	308	307

Standardization of Propagation Techniques

There is a lack of knowledge of agronomic practices for the propagation of *P. crenulata*. Further, the plant is being exploited by the local inhabitants for fencing and fuel purposes, leading to considerable challenges to the existence of this plant species in the near future. Therefore, a scientific method of propagation was the need of the hour. The Defence Institute of Bio-Energy Research, Haldwani, has standardized the propagation techniques (Singh et al. 2012). For such a multipurpose plant species in the hills, techniques for mass propagation through seeds and cuttings were found to be most suitable according to the experiments conducted at the Institute.

Propagation Through Cuttings

Pyracantha can be propagated by cuttings (Fig. 5a–c). Pencil-thick uniform cuttings (30 cm long and 0.5–0.8 cm thick) with at least two nodes were taken from 2-year-old

Table 5 Effects of oral doses of hawthorn on mean blood pressure (mmHg) in normotensive conscious rats (Anonymous 2009)

	Time (h)						
Dose (ml)	0	1	2	3	4	5	6
1.0	114	94	90	82	80	104	108
1.5	94	93	87	91	95	98	97
2.0	108	95	86	90	93	104	107

Table 6 Effects of intravenous doses of *Pyracantha* on heart rate/min in $CdCl_2$-induced hypertensive rats (Anonymous 2009)

	Time (h)						
Dose (μl)	0	1	2	3	4	5	6
25	377	380	370	352	355	353	352
50	355	348	340	352	335	338	340
100	310	326	306	310	312	326	335

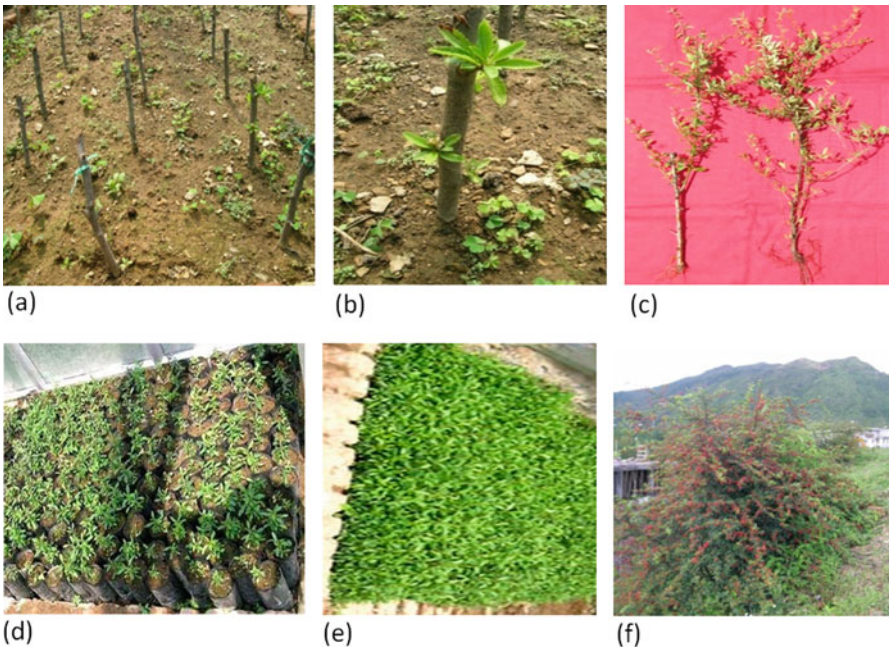

Fig. 5 (**a**) Newly planted *Pyracantha* cuttings. (**b**) Sprouted cutting. (**c**) Rooted cuttings. (**d**) Seedlings in polybags. (**e**) Seed-raised seedlings. (**f**) Plant

plants during the first week of February, before the emergence of new leaves. The upper ends of the cuttings were cut on the slant and the lower ends were blunt cut. The cuttings were kept in a stream of running water overnight and subsequently transferred to solutions of different compounds that act as Plant Bio-Regulators (PBRs),

Table 7 Effects of different concentrations of PBRs on *Pyracantha* cuttings (Singh et al. 2012)

	Concentration (ppm)														
	IBA					IAA				NAA					
Parameters	100	150	200	250	500	100	150	200	250	500	100	150	200	250	500
Rooted (%)	23	31	40	72	54	12	16	21	32	30	19	30	42	72	43
Days to rooting	36	35	35	32	31	36	35	35	32	30	36	35	35	32	30
Survivability (%)	23	31	40	72	54	12	16	21	32	30	19	30	42	72	43

IBA Indole butyric acid, *IAA* indole acetic acid, *NAA* naphthalene acetic acid

i.e., indole butyric acid (IBA), indole acetic acid (IAA), and naphthalene acetic acid (NAA), at different concentrations (100, 150, 200, 250, and 500 ppm) for about 24 h (Table 7). These cuttings were planted in nursery beds, keeping line-to-line and plant-to-plant distances of 30 cm apart. Watering was done on alternate days. Three replicates of cuttings were maintained for each treatment. The data for sprouting percentage was recorded 30 days after planting, and the rooting percentage and root length were recorded 3 months after planting. The experiment was repeated in February 2009 to confirm the results obtained in the preceding year.

Sprouting and rooting percentages were recorded by observing all cuttings of each replicate. Adventitious roots from five cuttings of each replicate were randomly collected and their root length was measured. The data collected was analyzed with Systat 12 software (Chicago, Illinois, USA), using analysis of variance (ANOVA). Least significant values were calculated at $p = 0.05$ for comparing the mean values of the treatment results (Gomez and Gomez 1984).

Propagation Through Seeds

Seeds were soaked in lukewarm water for 24 h before being sown. The seeds were sown in nursery beds, keeping a row-to-row distance of 30 cm. Watering was done on alternate days. The seed germination percentage was recorded 30 days after sowing. Intercultural operations were carried out at regular intervals to facilitate better growth of the seedlings. A field trial of the seed-raised seedlings (Table 8) was conducted at the DIBER field station, Pithoragarh, Uttarkhand. Twelve seedlings, each with one shoot of approximately 30 cm length, were selected and planted in three replications in a randomized block design (RBD) and were irrigated twice a week. One-year-old seeds were found to be the most suitable for propagation because they had the maximum germination percentage (Fig. 5d–f). Stored seeds require warm and cold stratification for germination (Bird 1990; Sheat 1948; McMillan 1985).

Pyracantha orchards can be established in fallow land and on the hill slopes that lie vacant. The ideal distances for Hawthorn orchard plantation are 2 m for plant to plant and 4 m for row to row. Both moist and well-drained soils are ideal for its cultivation.

Table 8 Growth performance of *P. crenulata* treated with different compounds and seed-raised seedlings 6 months after transfer to the field (Singh et al. 2012)

Treatment	Average plant height (cm)	Average number of branches per plant
IBA (250 ppm)	189.32	6.45
NAA (250 ppm)	164.45	5.34
IAA (250 ppm)	156.87	5.12
Seed-raised seedlings	187.78	5.23
LSD $P = 0.05$	9.15	NS

ppm parts per million, *LSD* least significant difference

Prospects for Product Development

Pyracantha is a plant with potential for the development of various nutraceutical and pharmaceutical products. Its fruits are a rich repository of bioactive compounds and, hence, various food products, such as herbal beverages, jams, energy bars, fruit juice powder, and many more items may be formulated. DIBER (DRDO) has developed a herbal beverage, "*Hridayamrit*", from its fruit juice (Fig. 6). The fresh fruits were washed thoroughly with tap water to remove the adhering soil particles. The washed fruits were dipped in sterile distilled water for 30 min before juice extraction. An amount of water equal to the amount of juice was placed in a separate container and sugar solution was added; this solution was boiled. Then the cold sugar solution was added to the fruit juice. Sodium benzoate solution was added as a preservative. The *Hridayamrit* herbal beverage formulated using its fruits is highly nutritious, energizing, and refreshing. It is rich in vitamins, minerals, proteins, and antioxidants. Besides being effective for hypertensive patients, this beverage is an instant energizer and has refreshing properties that are liked by the general public. No artificial colors or flavors were used because the fruit pulp has its own natural color and flavor.

Future Prospects

Pyracantha is a multipurpose and highly important plant of the foothills of the Himalayas. The plant has environmental importance owing to its extensive root system, which prevents soil erosion on fragile hill slopes. Owing to the array of bioactive molecules present in its fruits, hawthorn is also highly important for the pharmaceutical industry, being potentially useful for antihypertensive drug formulations. The leaves can be utilized for herbal tea formulations, and the plant also has immense potential for the formulation of nutraceutical products of general

Fig. 6 *Pyracantha* herbal beverage

public importance. Because of its thorny nature *Pyracantha* can be planted as an excellent bio-fence for agricultural fields to protect the fields from stray and wild animals. It also has aesthetic importance in the moist land of the hills. Most of the plant population to date is natural, but its large-scale planned propagation and successive plantation in the hills will not only help in environmental improvement of the area but will also provide economic benefits for the local people, who will be able to sell the fruits and leaves of this plant to the pharmaceutical and nutraceutical industries as raw material for product formulation. Thus, the main emphasis must be on the mass-scale propagation of the plant and the development of scientific plantations, as well as its conservation and studies on genetic diversity.

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Advancement in Medicinal Mushroom Research

V. P. Sharma and Sudheer Kumar Annepu

For thousands of years, mushrooms have been prized as highly nutritious foods by many civilizations in the world. In addition to their nutritional properties, the people of orient region are using a large number of edible and nonedible mushrooms for curing various ailments. Mushrooms fall somewhere in between the true plants and animals and have been mentioned, reported and researched upon to possess unique and potent pharmacological properties. Many such claims have also been validated, and new therapeutic applications have been developed as result of extensive scientific studies. These are being produced and traded in significant quantities – the annual trade in the medicinal mushrooms and their products is roughly estimated around $24 bn. Though more than 20 species of the medicinal mushrooms are currently being produced and commercially traded, the value-wise most important ones are the species of *Ganoderma*, *Grifola*, *Cordyceps*, *Lentinula*, *Hericium* and *Schizophyllum* of which *Ganoderma* is the unquestioned "king of medicinal mushrooms". Many of these mushrooms are known for its anticancer properties, but they also possess other potentially important immunological and curative properties such as free radical scavenging, antiviral, antihypercholesterolaemia, antimicrobial, detoxification, hepatoprotective and antidiabetic effects (Wasser 2011).

Current Status

In the past three decades, there has been an upsurge in research on the medicinal value of traditionally used fungi and their products, particularly in China, Korea and Japan. Consequently, the traditional uses of many fungi have been confirmed and new wider usages discovered. Several classes of biomolecules such as lipopolysaccharides, glycoproteins and polysaccharides were identified as mushroomceuticals with the potent effects on the human immune system. The

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beneficial role of these compounds extracted from medicinal mushrooms has drawn the attention of various research and pharmaceutical industries to understand their bioactivity. Some of these compounds are noted as biological response modifiers (BRMs) and became an integral part in the cancer treatments together with the chemotherapy, radiotherapy and surgical treatments. Several mushroom products developed with clinical and commercial applications are D-fraction from *Grifola frondosa*, schizophyllan/sonifilan from *Schizophyllum commune*, lentinan from *Lentinula edodes*, polysaccharides from *Ganoderma lucidum*, polysaccharide-K (PSK) and polysaccharopeptide (PSP) from *Trametes versicolor*, etc (Fig. 1). Many other secondary metabolites such as terpenoids, lactones, lectins, antibiotics, alkaloids and metal chelating agents were extracted from medicinal mushrooms which are showing the positive responses in improving the human immune system. Mushrooms secrete extracellular ligninolytic enzymes such as laccase, glucose oxidase, superoxide dismutase and peroxidase to degrade the lignocellulosic substrates. Many reports are showing that these enzymes are playing a great role in preventing the oxidative stress and inhibiting the tumour growth in various cancer treatments.

Along with the stimulatory effects on the human immune system, it has also been documented the role of mushroom bioactive compounds in modulating the specific cellular responses by interfering in transduction pathways. For example, caffeic acid phenethyl ester (CAPE) isolated from *Agaricus bisporus* and *L. edodes* has shown positive results in curing breast cancer. The CAPE specifically alters the cellular responses in MCF-7 cells by inhibiting the DNA-binding activity of NF-κB proteins. Panepoxydone is a novel compound extracted from *Panus conchatus*, and *Lentinus crinitus* was also found very effective in inhibiting the expression of several NF-κB-dependent pro-inflammatory genes by preventing the phosphorylation of IκB protein and thereby interrupts the signalling pathway. These reports are indicating that such novel mushroom metabolites can be effectively used in malignant cells to combat the cancer and other tumour growths. Unlike the synthetic compounds, the mushroom metabolites can penetrate into the target cell membrane easily and increase its bioefficacy due to the low molecular size. There have been new discoveries and developments in the field of medicinal fungi which proved the beneficial role of mushroom compounds not only as crude drugs but also as functional foods and dietary supplements that can improve the overall human immune system (Wasser and Akavia 2008) (Fig. 1).

Mushroom Polysaccharides in Cancer Treatment

In cancer treatments it is well recognized that the usual radiotherapy and chemotherapy damage the immunological defence system of the patient under treatment. Hence, in the contemporary oncology treatments, the chemotherapy including the orally formulated functional foods is gaining much importance. In the recent clinical studies on testing of new antitumour drugs, much emphasis is given on the compounds that stimulate the apoptosis to induce programmed cell death in

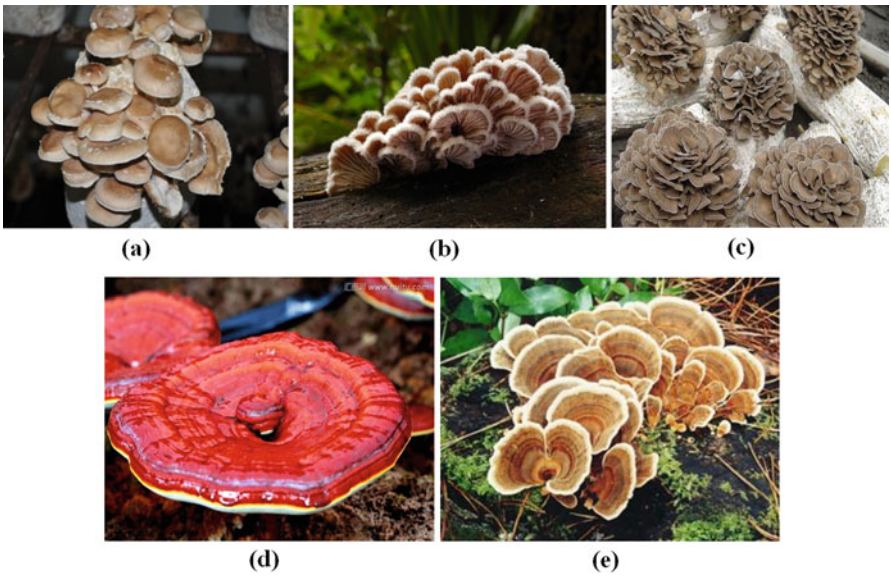

Fig. 1 Important medicinal mushrooms. (**a**) *Lentinula edodes*, (**b**) *Schizophyllum commune*, (**c**) *Grifola frondosa*, (**d**) *Ganoderma lucidum*, (**e**) *Trametes versicolor*

malignant cells (Zhang et al. 2007). Mushroom polysaccharides when administered through i.v. or i.p. along with standard chemotherapeutic agents will act as immunostimulants with no adverse effects. Furthermore, many of these medicinal mushroom compounds in crude and processed form have undergone phase I and II clinical trials and shown promising results to stimulate programmed cell death in tumour cells (Fullerton et al. 2000).

Lentinula edodes

L. edodes (Berk.) commonly known as shiitake has been under cultivation for hundreds of years in many Southeast Asian countries. Shiitake is considered as the world's most popular edible mushroom with high medicinal value. As foodstuff, its flavour and taste is highly appetizing and nourishing. As a pharmacological material, it contains a most important polysaccharide, lentinan, a β-1, 3-glucan with intravenous antitumour activity (Bisen et al. 2010). Lentinan has been approved as an adjuvant in many cancer treatments, especially in Japan and China. The purified form of lentinan from the fruit bodies of shiitake was first isolated and studied its antitumour effects by Chihara et al. 1970.

The anticancer activity of mushroom polysaccharides was observed for first time in farmers who were engaged in cultivation of medicinal mushrooms. The death rate caused by cancer of those farmers was exceptionally low than the general population

(Ikekawa 2001). Several studies were conducted to understand the reasons for this phenomenon.

The xenobiotic compounds such as polycyclic aromatic hydrocarbons (PAHs) will induce the activity of cytochrome P450 (CYP) and metabolically activate the procarcinogens. The cytochrome P450 (CYP) is a xenobiotic metabolizing enzyme which expresses mainly in the liver. Hence, it was hypothesized that suppression of the activity of CYP by the mushroom compounds will reduce the risk of procarcinogenesis. Accordingly, a laboratory study was conducted by injecting lentinan intraperitoneally to female mice. The liver cells were examined to investigate the effect of lentinan on expression of CYPs (Hashimoto et al. 2002). The shiitake polysaccharide reduced the level of CYP1A activity induced by 3-methylcholanthrene, a PAH accompanied by the TNF-α (tumour necrosis factor) production through the suppression of DNA-binding activity of aryl hydrocarbon receptor and an increase in the DNA-binding activity of nuclear factor-κB (Hashimoto et al. 2002; Okamoto et al. 2004). These results clearly indicated the anticarcinogenic activity of *L. edodes* polysaccharides as it has downregulated the activity of CYP and further to preclude the metabolic activation of procarcinogenesis. In addition to this, inhibition of telomerase activity by lentinan also attributed the anticarcinogenic activity (Sreenivasulu et al. 2011).

Schizophyllum commune

S. commune is a shell-shaped greyish fungus with leathery texture and grows throughout the year on deciduous wood. It is probably the most common fungus with widespread in existence. This mushroom has very little culinary importance. The polysaccharides derived from this mushroom are known as schizophyllan or sonifilan which is chemically a β-1, 3-glucan with β-1, 6 side branching. The polysaccharides of this mushroom act also as a biological response modifier and a nonspecific stimulator of the immune system. At present, schizophyllan is available in commercial pharmaceutical formulation in many Asian countries for patients undergoing antitumour therapy. The host-mediated antitumour activity of schizophyllan against sarcoma 180 was first discovered by Komatsu et al. (1969). Schizophyllan was also found effective against different allogeneic and syngeneic tumours in mice and rats, such as MM-46 and MH-134 carcinomas, BC-47 bladder tumour, AMC-60 fibrosarcoma and A-755 mammary carcinoma. Schizophyllan also inhibited L1210 leukaemia, B-16 melanoma and Meth-A fibrosarcoma in association with other chemotherapeutic agents. When SPG was administered at the time of radiation or shortly after radiation, best results were found against the cell damage by radiotherapy, and also mitosis of bone marrow cells was restored which was suppressed by antitumour drugs. Moreover, cellular immunity was reported to be increased in mice with SPG administration by restoring the killer-cell activity to

normal levels (Borchers et al. 1999). When SPG administered in combination with chemotherapy drugs such as tegafur or mitomycin C and 5-fluorouracil to 367 patients suffering with inoperable gastric cancer, significant increase in median survival was observed in controlled clinical trial. Similarly, the survival time in stage II was significantly prolonged with the combination of SPG and radiotherapy in cervical cancer patients.

Grifola frondosa

G. frondosa popularly known as maitake mushroom is famous for its delicious culinary taste and excellent aroma. Maitake is a key component in traditional oriental medicine. *G. frondosa* has been reported to have significant blood pressure-lowering effects and some cholesterol-lowering and hepatoprotective activities. Recent studies are showing that the polysaccharide-protein complexes extracted from maitake are having strong activities against breast, lung, liver and prostate cancers (Kurashiga et al. 1997). The dried maitake is extensively being marketed in the USA and Europe in the form of capsules, teas and maitake drinks. A highly purified β-glucan extracted from maitake fruit bodies has become available in the commercial formulation as Grifron-D. This purified maitake D-fraction was found effective compared to the raw extracts in curing the hepatic metastases in an immunocompetent mouse model (Nanba 1995). Fullerton et al. (2000) under in vitro conditions reported that Grifron-D have cytotoxic effect on human prostate cancer cells (PC9), by creating oxidative stress-induced apoptosis resulting in more than 95% cell death.

Hiroaki and Keiko (1997) conducted a trial on mice to test the cancer-preventive potential of maitake polysaccharides. Methylcholanthrene, a carcinogenic substance, was injected to mice when they were at the age of 5 weeks. After 15 days of injecting the carcinogenic substance, the mice were fed with maitake D-fraction at 0.2 gm per day for 15 consecutive days. The mice kept as control treatment were fed with saline solution. After 30 days of observations, 30.7% of the mice in the group fed with maitake D-fraction and 93.2% in control group were found with cancer cells. Similar effect was found with lentinan, Polysaccharides extracted from shiitake on reducing the procarcinogenesis induced by PAHs. But lentinan was found effective only under i.v. administration because of less bioavailability. Maitake extracts were found even effective with oral administration and made much easier in practical use. In a similar manner to lentinan, maitake D-fraction and maitake crude extracts are exhibiting the synergism in conventional chemotherapy. Maitake extracts promote the natural killer cells and cytotoxic T-cells which can attack the malignant cells and raise its efficiency to defend against cancer and AIDS. In another animal-based clinical study, the mice were exposed to a urinary bladder carcinogen, n-butyl-N-butylnitrosoamine, for 8 weeks. After the exposure period, the mice were started feeding with maitake, shiitake and oyster mushrooms. The urinary bladder cancers in mice fed with the medicinal mushrooms were found significantly low, with maitake being the most effective.

Ganoderma lucidum

G. lucidum is an important and leading medicinal mushroom that has been treasured in China for over three thousand years. It is commonly known as "Ling zhi" in China and "reishi" in Japan. In countries like China and Korea, reishi mushroom is considered as Mushroom of Immortality. Ancient Taoist literature confers that Ling zhi can promote immortality and allow one to rise above the weight of the physical realm. In the modern world, Ling zhi has a long list of beneficial properties, antiviral, antibacterial, anti-allergy, anti-inflammatory, antioxidant, and antitumour, pain release, reducing blood pressure and reducing high cholesterol, because it contains various compounds. The fungus grows naturally on dead wood and is found in many parts of the world. The standardization of its cultivation technology under artificial conditions makes it more accessible and affordable.

The fruiting body of *G. lucidum* contains the bitter components, highly oxidized triterpenoids, which have been shown to have anti-allergy, hepatoprotective and antihypertensive action. Because of its perceived health benefits, it has gained wide popularity as a health food. This mushroom is nonedible because of its bitter taste and indigestible structure. But the commercial products are available worldwide, in the form of hot water extracts, tablets and liquid products. Over the last three decades, many reports on preclinical antitumour activity of reishi mushroom extracts on different tumour cells were published. When these extracts were administered alone or in combination of chemotherapy, it effectively inhibited the metastasis in animal models and has shown the biological response-modifying effects. Gao 2000 reported that aqueous extracts of *G. lucidum* showed immunomodulating effects in in vitro systems through the activation of T-lymphocytes, macrophages and natural killer cells. To study the efficacy and safety of aqueous extracts of *G. lucidum* (ganopoly) against advanced cancers, a clinical trial was conducted on 143 patients with advanced lung, breast and liver cancers. The patients were given with the aqueous extracts of reishi orally at 1800 mg three times a day. Haematologic, biochemical and immune function observations were taken after 6 and 12 weeks after oral therapy. The natural killer-cell activity was increased up to 75% by ganopoly therapy. Lymphocyte mitogenic reactivity to concanavalin A and phytohaemagglutinin was significantly increased by 48–52% and increased the patient's quality of life (Green and Weiss 1992).

Trametes versicolor

T. versicolor is a nonedible mushroom that has a long history of use as medicinal mushroom in traditional oriental medicine. In traditional Chinese medicine, it was considered important for improving one's spirit and vital energy. Two polysaccharides with medicinal properties were found in the tough cell wall of both fruit bodies and vegetative mycelium. These polysaccharides were purified from the hot water extracts and termed as polysaccharide-K (PSK) and polysaccharopeptide (PSP). Both compounds were shown remarkable anticancer

properties with less or no side effects. This is the only mushroom to have gone through double-blind clinical trials in humans. Tested both singly and in combination with conventional therapies, it doubled or tripled survival rates and immune function. In Japan, the extract known as PSK, or Krestin, is the top cancer medicine. It even combats the disabling side effects of surgery, chemotherapy, radiation and compromised cellular immunity attributed to conventional cancer drugs. Preclinical animal studies and phase I clinical studies indicate that *Tv* derivatives may be beneficial in treatment of oestrogen receptor cancers by overcoming the tumour antigen tolerance (Morimoto et al. 1996).

PSK prepared by water extraction and salting out from *T. versicolor* is composed of polysaccharide and protein composition in 62:38%. PSK consists of a ß1-4 main chain with ß1-3 and ß1-6 side chains. In many studies it was found PSK is bio-available by the oral administration and made it much easier practical use. The oral LD50 value of PSK was found very low in subacute and chronic toxicity tests. These toxicological studies indicate that PSK is nontoxic and has no adverse side effects while treating the cancer patients (Ikuzawa et al. 1988). In a clinical study, 185 patients suffering from epidermoid carcinoma and adenocarcinoma were given with PSK followed by radiotherapy. The disease-free survival rate was found significantly highest in PSK group than the control group (Hayakawa et al. 1993). Polysaccharopeptide (PSP) is another compound purified and isolated from *T. versicolor* in 1983 by Chinese oncologists. Even though PSP contains arabinose and rhamnose instead of fucose in its polypeptide structure, there is a close similarity between PSP and PSK in their immunomodulation activities. In a phase I clinical trials conducted by Xu (1993), an oral dose of 6 g/day stabilized the haematopoietic parameters and showed improvement in appetite and general condition. In another phase II clinical studies, PSP was given to the patients with stomach, lung and oesophagus cancers at 3 g per day in three intervals for 60 days. Immunological status of the patients was increased significantly after the conventional oncological treatments (Liu and Zhou 1993). Moreover, observations showed increase in the percentage of apoptotic cells after 24 hours of the administration of PSP in combination with radiotherapy compared to the radiotherapy alone. These results summarized that the antitumour mechanism of PSP action may also involve the induction of DNA damage by apoptosis in the target cancer cells (Stephens et al. 1991).

Antioxidant Properties of Mushrooms

Supply of oxygen is absolutely essential for the existence of higher organisms. During the metabolic processes for the release of energy, molecular oxygen is completely reduced and converted to water. If the reduction of oxygen is incomplete, a series of reactive radicals are formed which are called ROS/free radicals. External factors like drug metabolism, ionizing radiations and internal cellular metabolic activities such as phagocytoses and membrane lipid peroxidation due to the activity of oxidase enzymes of free radicals will be generated inside the human body and trigger the ageing process by creating oxidative stress. Besides the above O2-, H2O2

and OH-, the other free radicals and ROS of biological importance include hydroperoxyl radical (HOO-), lipid peroxide radical (ROO-), nitric oxide (NO-) and peroxynitrite (ONOO-) which causes the damage to all kinds of biomolecules, cells and tissues. Many nutritional dietary sources were tested for antioxidant properties that can reduce the damage caused by these ROS. Medicinal mushroom extracts and purified compounds tested for antioxidant activities showed promising results to reverse the oxidative damage caused by the reactive species.

Hyperoxide radical, which is believed to be a main cause of ageing process in human beings, can affectively be removed from the biological system by the extracts of reishi mushroom. A clinical study in this regard was conducted with 30 people and was given with extracts of *G. lucidum*. The extracts were administered orally @ 1.5 g, three times daily for 30 days, and interleukin-2 and interferon (IFN) production by peripheral mononuclear cells was measured under in vitro conditions. The results proved that interleukin-2 and interferon (IFN) production was significantly increased after oral application of *G. lucidum* extract.

Kobayashi and Kariya (1994) reported that polysaccharide-K extracted from *T. versicolor* inhibited the mimetic activity of superoxide dismutase (SOD) enzyme and relieved the oxidative stress in cancer-bearing hosts. The extract of *T. versicolor* (PSK) was reported to trigger the gene expression of natural antioxidant enzymes and protects the macrophages from lipoperoxide accumulation and foam cell formation (Chen and Zhou 1997). Similarly, many mushroom polysaccharides were reported to have the ability to scavenge superoxide and hydroxyl radical activities.

Hepatoprotective Effects

Medicinal mushrooms (especially *G. lucidum*) were repeatedly reported to possess strong antihepatotoxic properties. Ganoderic acids R and S extracted from the vegetative mycelium showed hepatoprotective activity in cytotoxic tests conducted with cultured rat hepatocytes. Ganosporeric acid A, a new ether soluble fraction of *G. Lucidum* spores, was found with strong liver-protecting properties. The hot water extracts of reishi mushroom showed beneficial results in curing the active hepatitis B and improved the patients' quality of life particularly in cases without severe liver impairment. Polysaccharides of *L. edodes* also reported to improve the liver function in animal-based studies by enhancing the production of antibodies to hepatitis B (Mizuno 1995). The polysaccharide extracts from fruit bodies (lentinan) and vegetative mycelium of shiitake (LEM) showed promising results in treating the patients with viral hepatitis and chronic persistent hepatitis. Extracts of many other interesting medicinal mushrooms such as *S. commune*, *Dendropolyprus umbellatus*, and *Poria cocos* have been found in medical reports relating to their curing ability against liver cirrhosis and chronic hepatitis B.

Cardiovascular and Hypercholesterolaemia Effects

Coronary artery disease (CAD) is a highly significant problem causing higher death rate in most of the developed countries. Disturbance in blood platelet augmentation, higher blood pressure levels, diabetes and hypercholesterolaemia are the main risk factors associated with CAD. Along with these factors, the low-density lipoprotein (LDO) and very-low-density lipoprotein (VLDL) cholesterol levels in the blood will increase in patients suffering from CAD. It is estimated that the source of 60–65% of the blood cholesterol is originated from endogenous dietary sources. Hence, many clinical studies clearly indicate the need of therapeutic measures than drug therapy to correct the hypercholesterolaemia by modification of diet pattern. The diet with a nutritional regime including foods with low saturated fatty acids and high crude fibres is gaining popularity for treating the patients with hypercholesterolaemia. Because of the low calorific value, high protein and crude fibre contents, mushrooms are considered as ideal diet for treating cardiovascular diseases (Hobbs 1995).

Apart from the diet modifications, attempts were made to identify the biomolecules that can inhibit the cholesterol synthesis in the biosynthetic pathway. HMG-CoA reductase (microsomal specific enzyme 3-hydroxy-3-methylglutaryl coenzyme-A reductase) plays a major role in the biosynthetic pathway of cholesterol formation. Bioactive compound, viz., mevinolin, (lovastatin) extracted from the *Aspergillus terreus* was found to be the specific inhibitor of HMG-CoA reductase enzyme, and this drug got the approval for the treatment of hypocholesterolaemia. The same compound was found in the genus *Pleurotus* which is the most widely cultivated edible mushroom in the world. Genus *Pleurotus* comprises of several species, out of which *P. ostreatus* was found with the production of highest amount of lovastatin in their reproductive structures.

Several clinical trials were conducted in order to study the effect of lovastatin extracted from *Pleurotus* spp. in reducing the cholesterol levels. When 4% of the dried *Pleurotus* was supplemented in the diet of experimental rats, it resulted in reduced accumulation of cholesterol in the serum and liver. Addition of 15–20 g of dried *Pleurotus* supplemented in the diet of patients suffering from hypocholesterolaemia effectively reduced the production of LDL and VLDL cholesterol levels over a period of 1 month (Bobek et al. 1998). Based on the experimental findings, diet with *Pleurotus* mushrooms could be recommended as an effective natural source for lowering the blood cholesterol levels. Other mushrooms such as *Grifola frondosa*, *Auricularia auricula*, *Tremella fuciformis* and *T. aurantia* have been reported to lower the plasma cholesterol levels (Kiho et al. 2000). Sugiyama and Yamakawa (1996) reported that a specific bioactive compound, viz. eritadenine, extracted from shiitake mushroom is able to reduce the blood serum cholesterol by accelerating the excretion of ingested cholesterol and its metabolic decomposition. It was also reported that nucleic acids from shiitake mushroom also have platelet antithrombotic activity.

Future Scope

Many of these new bioactive compounds extracted and purified from medicinal mushrooms have undergone the basic clinical trials and showed considerable effectiveness in curing many ailments, especially different kinds of cancer tumours. In traditional chemotherapy and radiotherapy, these mushroom-based compounds are used as adjuncts to lessen the negative effectives of the conventional treatments. In addition, the ability of these nontoxic compounds to improve the overall immune function system by lessening the debilitating effects of synthetic drugs is notable. Many research reports also show the possibilities that these mushroom-based compounds allow the reduction in dosage required for toxic chemotherapeutic compounds, without affecting its efficacy. It is undoubtedly accepted that the immune function of the patients under chemotherapy can be severely impaired. Limiting this suppression of immunity by supplementing with mushroom extracts and mushroom polysaccharides will be a great benefit to the cancer patients. In animal-based experimental models when mushroom extracts and whole mushroom powders incorporated into the diet, it clearly exerted cancer chemoprevention. Even though there is no valid information available on the dosage and frequency of mushroom compounds for cancer treatments with approved clinical results, many human epidemiological studies suggest that regular consumption of certain medicinal mushrooms for considerably longer periods reduces the levels of cancer incidence. Many medicinal mushrooms were accepted as dietary supplements but not as drugs in clinics. Therefore there is an urgent need to study more widely and deeply the medicinal effect and chemical structure of active compound of various wild medicinal mushrooms which had been used as medicine in Asia. After finding some active compound, it needs to resolve the problem how to obtain abundant mushroom. Because many mushrooms are rare in the wild, this problem makes plenty of exciting results only lay in paper. For resolving this problem, two ways are used, one is to cultivate medicinal mushroom and another is to ferment medicinal mushroom and obtain the active substance from mycelia.

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Biotechnological Strategies for Improvement of *Withania somnifera* (L.) Dunal

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Abbreviations

2,4-D	2,4-Dichlorophenoxyacetic acid
BAP	6-Benzylaminopurine
IAA	Indole-3-acetic acid
IBA	Indole-3-butyric acid
KN	Kinetin
MeJ	Methyl jasmonate
NAA	1-Naphthalene acetic acid
PGRs	Plant growth regulators
SA	Salicylic acid

Introduction

Withania somnifera (L.) Dunal is a reputed medicinal plant of the family Solanaceae. It appears in the monographs of the World Health Organization (WHO) as a high-value medicinal plant (Mirjalili et al. 2009). *W. somnifera* is also known as winter cherry, Indian ginseng, asgandh, or ashwagandha. Due to its immense medicinal value, *W. somnifera* is included in top 32 important medicinal plants by the National Medicinal Plants Board of India (www.nmpb.nic.in). The genus *Withania* comprises

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more than 23 species, out of which, only two, viz., *W. somnifera* and *W. coagulans*, are economically important and hence have been widely investigated. The other less explored species are *W. adpressa*, *W. frutescens*, *W. obtusifolia*, and *W. aristata* (Verma and Srivastava 2014). *W. somnifera* have wide geographical distribution. It is spread in East Africa, Palestine, Israel, Egypt, India, Sudan, Jordan, Afghanistan, Baluchistan, Pakistan, and Iran (Kumar et al. 2007, 2011). In India, *W. somnifera* grows wild mainly in Himachal Pradesh, Punjab, and Jammu (Singh and Kumar 1998), whereas it is cultivated in Andhra Pradesh, Madhya Pradesh, Rajasthan, and Uttar Pradesh covering 5000 ha of land (Kothari et al. 2003; Kumar et al. 2007). *W. coagulans* is also known as "vegetable rennet" or "Indian cheese maker." It is commonly found in Iran, Pakistan, Afghanistan, and India. In India, it is distributed in drier parts of Rajasthan and Punjab (Mirjalili et al. 2009).

Economic Importance

Recently, there is a global resurgence in consumer choice toward herbal medicines owing to the absence of or minimal adverse effects. Around 80% population of developing countries depends upon tradition medicinal systems for their health care needs. According to WHO, the international market of herbal products is about $6.2 billion, and it is expected to reach $5 trillion by the year 2050 (Manivel et al. 2017). *W. somnifera* is an important medicinal plant and is reported to be used in Indian, African, and Unani traditional medicine systems. Conventionally, its leaves and roots are used in herbal medicines for a large number of ailments including cancer, arthritis, asthma, hypertension, and rheumatism (Jayaprakasam et al. 2003). *W. somnifera* is also known for its immunomodulatory, neuroprotective, adaptogenic, antiaging, cardioprotective, anti-oxidant, anti-depression, and anti-inflammatory properties (Singh et al. 2017; Kaur et al. 2017). The pharmaceutical properties of *W. somnifera* are due to the presence of numerous secondary metabolites, including alkaloids (pseudotropine, tropine, cuscohygrine, hygrine, anahygrine, anaferine, 3-trigloyloxytropine, choline, withanosomine, and dl-isopelletierine), steroidal lactones (withaferin A, withanones, withanolide A, and withanolide D), polyphenols (caffeic acid, catechin, coumaric acid, chlorogenic acid, gallic acid, and ferulic acid), flavanol glycosides, glycowithanolides, and sterols (Singh et al. 2016a; Chaurasiya et al. 2009; Alam et al. 2011; Chaurasiya et al. 2012; Sangwan et al. 2008). Among these, the chief chemical constituents are steroidal lactones (Mirjalili et al. 2009). Withanone and withaferin A constitute the main steroidal lactones present in leaves, whereas in roots, withanolide A and withanolide D are the major ones (Singh et al. 2015a). The presence of withanone in the methanolic leaf extract of *W. somnifera* has been reported to selectively kill the tumor cells by restoring the function of P53, a tumor suppressor protein (Widodo et al. 2007, 2008). The water extract of *W. somnifera* leaves also exhibits anticancer properties that may be linked to the presence of triethylene glycol (TEG). TEG

activates two tumor suppressor proteins, viz., pRB and p53 (Wadhwa et al. 2013). Withanolide A in methanolic extract from *W. somnifera* roots confers neuroprotection. Withanolide A caused a significant extension of axons and dendrites after 4 days of treatment of cortical neurons with amyloid-β fragments (Kuboyama et al. 2005). It was further shown to improve neurocognitive impairment associated with HIV-1 and drugs of abuse (Kurapati et al. 2014).

Genotypic and Chemotypic Variations in *W. somnifera*

The existence of genetic and chemotypic diversity is an indication of adaptation of a population against adverse environmental conditions and plays an important role in the breeding programs for the development of improved varieties. Considerable genetic and morphological variations have been recorded in different accessions of *W. somnifera* from different geographical locations (Kumar et al. 2007). The morphological variations include plant height, branches per plant, number of seeds per berry, root length, diameter, and yield. The morphological variability was later correlated with genetic variability by using molecular markers such as RAPD and AFLP. This analysis pointed to the existence of two different clusters corresponding to cultivated and wild accessions (Mir et al. 2011). Furthermore, genetic variability was also recorded in the accessions obtained from same geographical location (Dharmar and De-Britto 2011).

Depending upon the composition of major bioactives, *W. somnifera* is classified into various chemotypes. Three distinct chemotypes have been recorded from Israel, whereas one each was identified from South Africa and India (Abraham et al. 1968, 1975; Kumar et al. 2007). However, the studies have pointed toward the existence of more than one chemotype in India (Kumar et al. 2007). Chemotypes I, II, and III from Israel possess withaferin A, withanolide D, and withanolide E, respectively, as major steroidal lactones. South African chemotype is rich in withaferin A and withaferin D, whereas withanone and withaferin A are the main withanolides of Indian chemotype (Kirson et al. 1970; Anjaneyulyu and Satyanarayana Rao 1997).

Challenges in Cultivation and Improvement of *W. somnifera*

W. somnifera is used in many herbal formulations leading to its high demand in national and international market (Jayaprakasam et al. 2003; Kumar et al. 2011; Singh et al. 2015a). The estimated annual production of *W. somnifera* is 1500 tonnes, whereas its annual demand is 7000 tonnes, creating a huge gap between demand and supply (Shinde et al. 2015). The cultivation of *W. somnifera* is limited mainly due to poor seed viability, low percentage of seed germination, and seedling survival (Vakeswaran and Krishnasamy 2003). Further, the susceptibility to various pests and pathogens and its suitability to specific agroclimatic conditions hamper its cultivation (Sharma and Pati 2011a, b, 2012a, b; Singh et al. 2016a). The improvement of *W. somnifera* is also compromised due to its narrow genetic base, presence

of chemotypic variations, low yield of pharmaceutically important secondary metabolites under field conditions, involvement of complex processes in chemical synthesis of withanolides, non-availability of information regarding genome sequence, and meager information on biosynthesis, accumulation, and transport of important secondary metabolites (Bhatia et al. 2013; Grover et al. 2013). Hence, in this background it is much warranted to resort into new biotechnological tools to produce superior varieties and mass propagate them. The present chapter discusses some efforts undertaken for in vitro mass propagation and regeneration and in understanding the production and accumulation of pharmaceutically important secondary metabolites in *W. somnifera.*

In Vitro Mass Propagation and Regeneration in *W. somnifera*

Micropropagation offers the advantage of rapid multiplication and provides uniform quality clones without seasonal constraints. In *W. somnifera*, various explants such as seeds, nodal segments, and shoot tips have been used for micropropagation (Table 1; Fig. 1; Singh et al. 2017). The roles of nutrient medium, plant growth regulators (PGRs), temperature, light, and carbon source on seed germination and proliferation of microshoots have been investigated (Khanna et al. 2013; Sivanesan and Murugesan 2008). In most of the studies on in vitro micropropagation in *W. somnifera*, Murashige and Skoog's (MS) medium has been used. However, other media like B5, Nitch and Nitch, Schenk and Hildebrandt, and WPM have also been employed (Singh et al. 2017). It has been observed that shoot proliferation depends largely on exogenous supply of PGRs. BAP (2–9 μM) is most commonly used PGR for multiple shoot formation and its proliferation. BAP in combination with KN, IAA, or IBA are also frequently used for proliferation of shoots (Sen and Sharma 1991; Ray and Jha 2001; Furmanowa et al. 2001; Sivanesan 2007; Sivanesan and Murugesan 2008; Sabir et al. 2008; Soni et al. 2011; Nayak et al. 2013). The type and concentration of carbon source affect the multiplication of microshoots. Among different sources of carbon, sucrose (2–3%) is generally employed for proliferation of shoots. Enhanced sucrose concentration (4–10%) led to decline in shoot growth. Furthermore, addition of glucose in the nutrient medium did not improve shoot multiplication, whereas, fructose (2%) resulted in reduced shoot growth (Ray and Jha 2001).

An enhanced shoot multiplication has been recorded in liquid medium supplemented with BAP (4.44 μM) (Ray and Jha 2001). However, hyperhydricity of shoots restrict the use of liquid culture in *W. somnifera* (Singh et al. 2017). The rooting of the microshoots is achieved in both half-strength MS medium and full-strength MS medium alone or in the presence of IBA (Udayakumar et al. 2013). The rooted microshoots are hardened under field conditions using 1:1 mixture of sand and soil. Manure and vermiculite can also be used for hardening. The genetic fidelity of in vitro propagated plants can be tested using molecular markers such as RAPD and ISSR (Rana et al. 2012).

Table 1 Micropropagation in *W. somnifera*

Explant	Medium	Response	Reference
Nodes	MS + 2.5 μM BAP	Multiple shoot	Fatima et al. (2016)
	Half-strength MS + 0.5 μM NAA	Rooting	
	MS + 2.2 μM BAP	Multiple shoot	Jain et al. (2016)
	Half-strength MS + 71.7 μM Choline chloride + 3.9 μM Phloroglucinol	Rooting	
	MS + 2.5 μM BAP (solid and liquid medium)	Shoot proliferation	Singh et al. (2016c)
	MS + 10 μM IBA	Rooting	
	MS + 4.4 μM BAP + 4.65 μM KN	Multiple shoots	Sabir et al. (2008)
	MS + 9.8 μM IBA	Rooting	
	MS + 4.44 μM BAP	Multiple shoots	Soni et al. (2011)
	Full-strength and half-strength MS	Rooting	
	MS + 2.5 μM BAP + 0.5 μM NAA + $CuSO_4$ (100 μM);	Multiple shoots	Fatima et al. (2011)
	MS + 2.5 μM BAP + 0.5 μM NAA + $ZnSO_4$ (300 μM)		
	Half-strength MS + 0.5 μM NAA	Rooting	
	MS + 6.66 μM BAP + 1.71 μM IAA + 20 mg/l spermidine	Multiple shoots	Sivanandhan et al. (2011)
	MS + putrescine (20 mg L^{-1})	Rooting	
Shoot tips	MS + 2.2 μM BAP	Multiple shoot	Jain et al. (2016)
	Half-strength MS + 71.7 μM Choline chloride + 3.9 μM Phloroglucinol	Rooting	
	MS + 4.44 μM BAP	Multiple shoots	Ray and Jha (2001)
	NN + 4.44 μM BAP + 4.95 μM IBA	Multiple shoots	Furmanowa et al. (2001)
	NN medium + 0.46 μM Kinetin + 2.46 μM IBA + 10 mg L^{-1} adenine sulfate	Rooting	
	MS + 8.88 μM BAP + 11.4 μM IAA	Multiple shoots	Sivanesan (2007)
	Half-strength MS + 9.8 μM IBA	Rooting	
	MS + 13.2 μM BAP + 40% coconut water	Multiple shoots	Ahmad Baba et al. (2013)
	MS + 4.4 μM BAP + 2.3 μM 2,4-D; MS +	Multiple shoots	Sen and Sharma (1991)
	4.4 μM BAP + 2.5 μM IBA		
	MS (liquid)	Rooting	
Alginate encapsulated shoot tips	MS; MS + 2.46 μM IBA	Shoot formation	Singh et al. (2006)

(continued)

Table 1 (continued)

Explant	Medium	Response	Reference
In vitro elongated shoot	MS + 6.66 μM BAP + 1.7 μM IAA + 4% sucrose	In vitro flowering and fruiting	Sivanandhan et al. (2015)
Cotyledonary nodes	MS + 4.44 μM BAP	Multiple shoots	Nayak et al. (2013)
	Half-strength MS + 4.9 μM IBA	Rooting	
Apical buds	Revised Tobacco medium (RT) + 4.5 μM 2,4-D	Multiple shoots and rooting	Kanungo and Sahoo (2011)
Axillary buds	MS + 8.8 μM BAP + 0.54 μM NAA	Multiple shoots	Saritha and Naidu (2007)
	MS + 2.32 – 18.6 μM KN + 0.57 μM IAA	In vitro flowering	
	MS + 9.29 μM KN + 0.57 μM IAA	In vitro fruiting	
Seeds	MS + 2.66 μM BAP + 2.28 μM IAA	Multiple shoots	Supe et al. (2006)
	MS + 1.97 μM IBA + 2.28 μM IAA	Rooting	
	MS + 4.4 μM BAP + 2.3 μM 2,4-D	Multiple shoots	Sen and Sharma (1991)
	MS	Rooting	
	MS + 2.5 μM BAP (solid and liquid medium)	Multiple shoot	Singh et al. (2016c)
	MS + 10 μM IBA	Rooting	

An efficient and reproducible regeneration system is a prerequisite for genetic transformation studies. Selection of suitable explants is important for shoot bud regeneration. Various explants such as node, internode, leaf, stem, axillary shoot tip, epicotyl, hypocotyl, cotyledonary leaf, petiole, embryo, and root have been used for regeneration in *W. somnifera* (Table 2; Singh et al. 2017). It was reported that very young leaves or very old leaves did not show regeneration (Kulkarni et al. 1996). Similarly, petiole exhibited lower regeneration potential than leaves (Ghimire et al. 2010), mainly due to differential distribution of auxin in leaf and petiole. Further, as compared to in vitro leaves, in vivo leaves showed better frequency of callus induction (Rout et al. 2011). A comparison of regeneration frequency from the callus of different explants showed that leaf-induced callus possesses the highest regeneration frequency followed by cotyledon, hypocotyl, and epicotyl (Arumugam and Gopinath 2013).

Cell differentiation and organogenesis are governed by the interaction of phytohormones: auxins and cytokinins. BAP is the most commonly used PGR for shoot bud regeneration. However, BAP has also been used in conjunction with IAA,

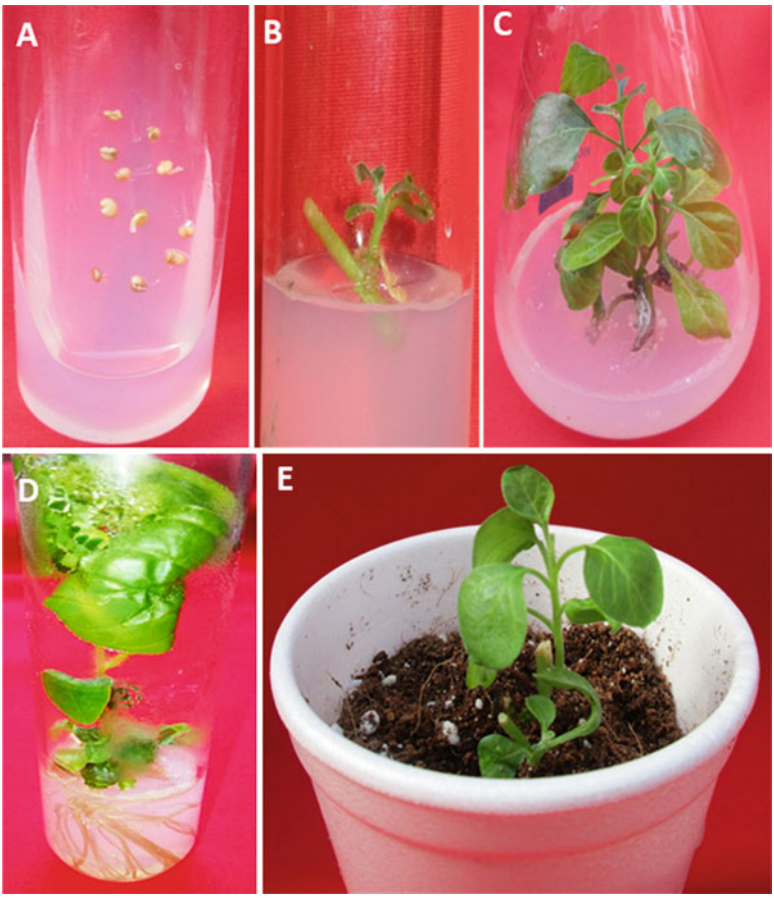

Fig. 1 Micropropagation in *W. somnifera*. (**a**) Initiation of aseptic culture from seed explants, (**b**) initiation of aseptic culture from nodal explant, (**c**) proliferation of microshoot, (**d**) rooting of microshoot, (**e**) hardening of microshoot

KN, and NAA (Ghimire et al. 2010; Udayakumar et al. 2013; Kulkarni et al. 1996; Joshi and Padhya 2010; Fatima and Anis 2012). For indirect regeneration, 2,4-D and KN were most frequently used for callus induction (Rani and Grover 1999; Manickam et al. 2000; Rani et al. 2003; Rout et al. 2011; Udayakumar et al. 2013; Chakraborty et al. 2013). Some workers have also reported the use of BAP along with IAA and 2,4-D (Dewir et al. 2010; Rout et al. 2011). BAP alone or in combination with IAA and NAA was documented to be effective for maintenance of calli and regeneration of shoot (Rani and Grover 1999; Kulkarni et al. 2000; Rani et al. 2003; Sivanesan and Murugesan 2008; Rout et al. 2011).

Table 2 In vitro shoot regeneration in *W. somnifera*

Explant	Medium	Response	Reference
Direct shoot regeneration			
Leaf	MS + 4.4 μM BAP + 7.99 μM IAA; MS + 8.8 μM BAP + 7.99 μM IAA	Shoot buds	Kulkarni et al. (1996)
	MS + 4.0 μM BAP + 4.0 μM KN		Joshi and Padhya (2010)
	MS + 8.88 μM BAP; 8.88 μM BAP + 0.53 μM NAA		Ghimire et al. (2010)
	MS + 6.66 μM BAP + 8.55 μM IAA		Kumar et al. (2011)
Node	MS + 2.22 μM BAP	Shoot buds	Kulkarni et al. (2000)
	MS + 22.2 μM BAP; MS + 0.91 μM TDZ		
	MS + 1.36 μM TDZ		
	MS + 2.5 μM BAP + 0.5 μM NAA		Fatima and Anis (2012)
	MS + 6.66 μM BAP + 1.71 μM IAA		Udayakumar et al. (2014)
Internodes	MS + 4.44 μM BAP	Shoot buds	Kulkarni et al. (2000)
	MS + 22.2 μM BAP		
Epicotyl	MS + 8.88 μM BAP + 1.14 μM IAA	Shoot buds	Udayakumar et al. (2013)
Hypocotyl	MS + 2.22 μM BAP	Shoot buds	Kulkarni et al. (2000)
Embryo	MS + 0.91 μM and 1.36 μM TDZ	Shoot buds	Kulkarni et al. (2000)
Shoot tip	MS + 2.5 μM BAP + 0.5 μM NAA	Shoot buds	Fatima and Anis (2012)
Petiole	MS + 8.88 μM BAP; 8.88 μM BAP + 0.53 μM NAA	Shoot bud	Ghimire et al. (2010)
Indirect shoot regeneration			
Leaf	MS + 9.05 μM 2,4-D + 0.93 μM KN	Callus induction and shoot regeneration	Rani and Grover (1999)
	MS + 56 μM IAA + 56 μM KN	Callus induction	Teli et al. (1999)
	MS + 8.88 μM BAP + 2.85 μM IAA	Callus induction and shoot regeneration	Dewir et al. (2010)
	MS + 4.44 μM BAP + 4.5 μM 2,4-D	Callus induction	Rout et al. (2011)
	MS + 8.88 μM BAP + 5.37 μM NAA	Shoot regeneration	
	MS + 13.5 μM 2,4-D	Callus induction	Arumugam and Gopinath (2011)
	MS + 17.6 μM BAP	Shoot regeneration	
	MS + 2.26 μM 2,4-D + 0.93 μM KN	Callus induction	Chakraborty et al. (2013)
	MS + 8.88 μM BAP	Shoot regeneration	
Shoot	MS + 8.87 μM BAP	Callus induction and shoot regeneration	Rani and Grover (1999)
	MS + 56 μM IAA + 56 μM KN	Callus induction and shoot regeneration	Teli et al. (1999)

(continued)

Table 2 (continued)

Explant	Medium	Response	Reference
Node	MS + 4.44 μM BAP + 9.2 μM KN	Callus induction	Siddique et al. (2004)
	MS + 4.44 μM BAP + 11.6 μM KN	Shoot regeneration	
Internode	MS + 4.44 μM BAP + 4.5 μM 2,4-D	Callus induction	Rout et al. (2011)
	MS + 8.88 μM BAP + 5.37 μM NAA	Shoot regeneration	
Cotyledon	MS + 9.05 μM 2,4-D + 0.93 μM KN	Callus induction	Rani et al. (2003)
Epicotyl	MS + 9.05 μM 2,4-D + 2.79 μM KN	Callus induction	Udayakumar et al. (2013)
	MS + 4.44 μM BAP + 20 mg/L adenine sulfate	Shoot regeneration	
Hypocotyl	MS + 9.05 μM 2,4-D + 0.93 μM KN	Callus induction and shoot regeneration	Rani and Grover (1999)
	MS + 9.05 μM 2,4-D + 0.93 μM KN	Callus induction and shoot regeneration	Rani et al. (2003)
Stem	MS + 2.26 μM 2,4-D	Callus induction	Manickam et al. (2000)
	MS + 4.44 μM BAP + 0.57 μM IAA	Shoot regeneration	
Root	MS + 9.05 μM 2,4-D + 0.93 μM KN	Callus induction and shoot regeneration	Rani and Grover (1999)
	MS + 9.05 μM 2,4-D + 0.93 μM KN	Callus induction	Rani et al. (2003)
Apical bud	MS + 2.26 μM 2,4-D + 0.93 μM KN	Callus induction	Gupta and Sahu (2015)
	MS + 8.88 μM BAP	Shoot regeneration	

Biosynthesis of Withanolides in *W. somnifera* and Deciphering Strategies for Its Modulation

The medicinal values of *W. somnifera* have been realized recently, and therefore various laboratories around the world are trying to increase the production of withanolides. The existing knowledge of withanolide biosynthetic pathway and regulation of critical genes provide impetus for laying the foundation for industrial production of withanolides. The present section discusses withanolide biosynthesis and various strategies to increase secondary metabolite production in *W. somnifera*.

Withanolide Biosynthesis in *W. somnifera*

Withanolides, the chief secondary metabolites of *W. somnifera*, have their genesis from 24-methylene cholesterol, which in turn is derived from isoprenoid pathway (Fig. 2). The biosynthesis of isoprenoid in plants proceeds through two independent pathways, viz., mevalonate (MVA) pathway and 2-C-methyl-D-erythritol-4-phosphate (MEP) pathway, that operate in cytosol and plastids, respectively. MVA pathway contributes to 75%

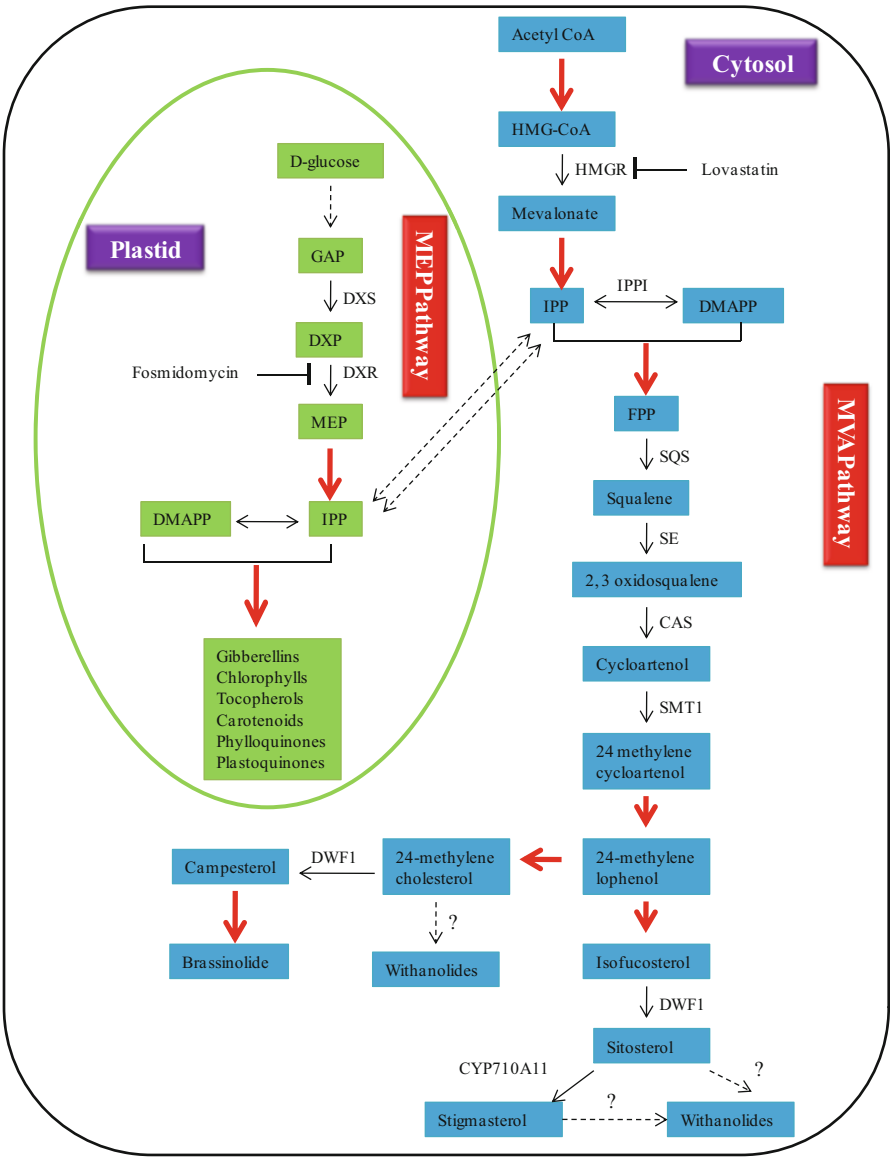

Fig. 2 Biosynthetic pathway of withanolides showing the site of action of important inhibitors (lovastatin and fosmidomycin). *HMG-CoA* 3-hydroxy-3-methylglutaryl-CoA, *HMGR* 3-hydroxy-3-methylglutaryl-CoA reductase, *IPP* 3-isopentenyl pyrophosphate, *IPPI* isopentenyl pyrophosphate isomerase, *DMAPP* 3,3-dimethyl allyl pyrophosphate, *FPP* farnesyl diphosphate, *SQS* squalene synthase, *SE* squalene epoxidase, *CAS* Cycloartenol synthase, *SMTI* sterol methyltransferaseI, *DWFI* delta-24 sterol reductase, *GAP* D-glyceraldehyde-3-phosphate, *DXP* 1-deoxy-D-xylulose-5-phosphate, *DXS* DXP synthase, *DXR* DXP reductoisomerase, *MEP* 2-methyl-D-erythritol 4-phosphate. Red arrows represent involvement of more than one biosynthetic step, which are not depicted here. One headed dashed arrows with question mark indicates the routes that have not been elucidated yet. Double headed dashed arrows represent exchange of intermediates between cytosol and plastid

carbon pool for biosynthesis of withanolides, whereas, MEP pathway accounts for 25% carbon pool (Sangwan et al. 2008; Chaurasiya et al. 2012).

MVA pathway starts the formation of acetoacetyl-CoA through the activation of acetyl-CoA. Acetyl-CoA condenses with acetoacetyl-CoA to yield 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA), and this reaction is catalyzed by the enzyme HMG-CoA synthase (HMGS). In the subsequent step, HMG-CoA is converted into mevalonic acid in an irreversible reaction catalyzed by 3-hydroxy-3-methylglutaryl-CoA reductase (HMGR). Mevalonate kinase and phosphomevalonate kinase catalyze the next two phosphorylation reactions, leading to the production of 5-phosphomevalonate and 5-pyrophosphomevalonate, respectively. The enzyme mevalonate-5-pyrophosphate decarboxylase converts 5-pyrophosphomevalonate into 3-isopentenyl pyrophosphate (IPP). In the presence of enzyme farnesyl diphosphate synthase (FPPS), IPP condenses with 3,3-dimethyl allyl pyrophosphate (DMAPP, an isomer of IPP) to form geranyl pyrophosphate (GPP). The subsequent reactions include formation of squalene from farnesyl diphosphate in a reaction catalyzed by enzyme squalene synthase (SQS). Squalene is a linear 30-carbon compound, which is converted into 2,3-oxidosqualene by squalene epoxidase. The latter yields different triterpenoidal skeletons upon ring closure.

In case of MEP pathway, pyruvate condenses with D-glyceraldehyde-3-phosphate (GA-3P) and forms 1-deoxy-D-xylulose-5-phosphate (DXP) in the presence of enzyme DXP synthase (DXS). DXP reductoisomerase (DXR) catalyzes the conversion of DXP into 2-methyl-D-erythritol 4-phosphate (MEP). In the subsequent reactions, 4-diphospho-cytidyl-2-methyl-D-erythritol (CDP-ME) forms 2-C-methyl-D-erythritol-2,4-cyclodiphosphate (ME-cPP) leading to a mixture of IPP and DMAPP (Hunter 2007., Singh et al. 2015a, Rodriguez-Concepcion and Boronat 2002).

Organ and Cell Culture Systems and Its Implication in *W. somnifera*

In vitro culture systems such as organ and cell culture are excellent systems to increase the production of withanolides under in vitro conditions mainly due to its active growth and high metabolic rate (Singh et al. 2017). As compared to leaves of field-grown plants, leaves of in vitro raised plants accumulated 1.14-fold and 1.20-fold higher withaferin A and withanones, respectively, whereas 1.10-fold increased accumulation of withanolide A is recorded in the roots of in vitro raised plants (Sivanandhan et al. 2011). These studies emphasize the use of in vitro propagation system to increase the production of withanolides which are otherwise severely hampered under natural conditions. The formulation of nutrient medium is one of the most important requirements of in vitro culture system. The effect of type of medium, type and concentration of PGRs, and carbon source has been studied in various in vitro culture systems. Among these, PGRs are the most critical factor. MS medium amended with BAP is most suitable for production of withaferin A and withanolide D in shoot culture. BAP in combination with KN favor enhanced accumulation of withanolide A (Ray and Jha 2001; Sangwan et al. 2007; Mir et al.

2014). Half-strength MS liquid medium fortified with BAP resulted in higher content of withaferin A and biomass accumulation within 5 weeks of shoot culture (Mir et al. 2014). Exogenous application of elicitors like polyamines, SA, and MeJ in the shoot culture medium significantly improved the production of secondary metabolites (Sivanandhan et al. 2011, 2012).

Cell suspension culture provides a platform to investigate cellular and molecular events leading to enhanced production of secondary metabolites in medicinal plants (Singh et al. 2017). In *W. somnifera,* cell suspension culture offers great potential to increase withanolides. Though a less yield of withanolide A and withaferin A has been reported in cell suspension culture, the yield can be enhanced considerably by optimizing the culture medium, composition of micro- and macronutrients, PGRs, source of carbon, and inoculum mass. MS medium is best suitable for the accumulation of cell biomass and production of withanolide A (Nagella and Murthy 2010, 2011). Among the different sources of carbon (sucrose, glucose, fructose, and maltose), sucrose (2–4%) favors maximum accumulation of biomass and withanolide A (Nagella and Murthy 2011; Sivanandhan et al. 2013b). Further, manipulation of nitrogen nutrient in culture medium is an excellent option to enhance pharmaceutically important secondary metabolites. In cell suspension culture of *W. somnifera*, MS medium having 2X KNO_3 resulted in highest accumulation of withanolide A. The roles of various PGRs have also been studied, and it was reported that MS medium supplemented with 2,4-D and KN resulted in enhanced cell biomass accumulation and withanolide A production (Nagella and Murthy 2011). Additionally, elicitation is a promising strategy to increase the production of pharmaceutically active constituents. Cell suspension culture of *W. somnifera* supplemented with elicitors such as $CuSO_4$ and cell extract of *Verticillium dahliae* produced maximum withaferin A (Sivanandhan et al. 2013b).

Gene Transfer Technology and Its Role in Improvement of Withanolide Production in *W. somnifera*

Development of an efficient and reproducible genetic transformation system is one of the most important requirements to understand the role and regulation of genes involved in withanolide biosynthetic pathway and also to increase the production of secondary metabolites through metabolic engineering. For metabolic engineering in *W. somnifera* and increasing the carbon flux toward a specific metabolite, major emphases have been laid on identification and overexpression of the rate-limiting enzyme(s) or key gene(s) associated with withanolide biosynthetic pathway. *Agrobacterium*-mediated transformation system has been widely used for genetic manipulation in plants. In *W. somnifera,* leaf, node, apical segment, and seeds have been successfully used for transformation with varying efficiency (Ray and Jha 1999; Pandey et al. 2010). Various factors, viz., choice of explants, strain of *Agrobacterium*, time of co-cultivation, selection conditions, and plant germplasm, influence the efficiency of transformation (Herrera-Estrella et al. 2004). Nevertheless, the transformation efficiency can be enhanced significantly by employing

vacuum infiltration, sonication, and addition of thiol compounds in co-cultivation medium (Sivanandhan et al. 2015).

The first successful transformation of *W. somnifera* was performed using leaf explants and *A. tumifaciens* strain LBA4404 having pIG121Hm vector (Pandey et al. 2010). The vector carried three expression cassettes, viz. a cassette expressing nptII gene (Pnos:nptII:nospA), another a cassette expressing gusA reporter gene (P35S: Intron gusA:nospA), and a third cassette driving the expression of hptII gene (P35S: hptII: nospA). The transformants were selected on kanamycin (50 mg/l^{-1}). Fertile transgenic plants of *W. somnifera* were obtained upon successful acclimatization. In addition, various critical genes of withanolide biosynthetic pathway have been overexpressed or downregulated by *Agrobacterium*-mediated transformation in *W. somnifera*, leading to enhanced production of withanolides (Grover et al. 2013; Patel et al. 2015). In some reports, heterologous systems have been used to study withanolide biosynthetic pathway genes, providing important clues for regulation of the concerned genes (Singh et al. 2014).

Constitutive expression of *WsHMGR2* in heterologous system, *Nicotiana tabacum*, resulted in drastic increase in the accumulation of cycloartenol (200%), sitosterol (160%), campesterol (546%), and stigmasterol (123%). There was also an enhancement of cholesterol content by 38% in *WsHMGR2* overexpression lines (Singh et al. 2014). Furthermore, RNAi-mediated transient suppression of *WsHMGR2* in *W. somnifera* leaves led to a remarkable reduction in the content of cycloartenol (25%), sitosterol (16%), campesterol (59%), stigmasterol (12%), cholesterol (11%), withaferin A (87.2%), withanolide A (58.7%), and 12-deoxywithastramonolide (86.4%).

Similar to *WsHMGR2*, complementary strategy has been followed to characterize *WsDXR2*. An increase of 88, 25, 28, 66, and 41% has been recorded for cycloartenol, sitosterol, stigmasterol, campesterol, and cholesterol, respectively, in lines of *N. tabacum* overexpressing *WSDXR2*. However, the level of cycloartenol, sitosterol, stigmasterol, campesterol, cholesterol, 12-deoxywithastramonolide, withaferin A, and withanolide A showed a decrease of 9, 0, 3, 24, 35,56.2, 56.8, and 54.5%, respectively, in transiently suppressed leaves of *W. somnifera* (Singh et al. 2014). The complementary approach involving overexpression and knockdown studies suggest that as compared to *DXR2*, *HMGR2* play more significant role in the biosynthesis of all the major sterols and withanolides. This information is considered as critical for enhancement of the production of withanolides through metabolic engineering in *W. somnifera*.

Squalene synthase (*WsSQS*) is an important rate-limiting enzyme in withanolide biosynthesis. Overexpression of *WsSQS* in cell lines of *W. somnifera* resulted in 2.5-fold and 4-fold respective enhancement in the level of withanolide A and squalene synthase activity (Grover et al. 2013). However, its overexpression in transgenic *W. somnifera* plant showed 1.5–2-fold enhancement in the content of total withanolides and 2–5-fold increase in its protein expression (Patel et al. 2015). Additionally, virus-induced gene silencing (VIGS) of *WsSQS* resulted in considerable reduction in the content of total withanolides (54%), stigmasterol (26%), sitosterol (15%), and campesterol (16%) (Singh et al. 2015b).

Cycloartenol synthase (CAS) catalyzes the cyclic conversion of 2,3-oxidosqualene into cycloartenol, a key precursor of withanolide biosynthesis. Transgenic lines of *W. somnifera* were raised with RNAi and overexpression vectors of *WsCAS* (Mishra et al. 2016). As compared to control lines, silenced lines showed suppression of *WsCAS* transcript up to 96.7%, whereas overexpression lines displayed 1.2–7-fold increased accumulation of *WsCAS* transcript. A corresponding variation was seen in the content of withanolides. A reduction of 33–91% and enhancement of 1.06–1.66-fold were recorded in silenced lines and overexpression lines, respectively.

Sterol glycosyltransferase (SGT) functions to attach the glycon moieties to sterols and their derivatives to yield steryl glycosides that have been implicated to play an important role in defense response of plants against biotic and abiotic stress. *WsSGTLs* belongs to *WsSTG*s family. Artificial miRNA and VIGS system has been used to functionally characterize *WsSGTL1* and *WsSGTL2* (Singh et al. 2016b). The silenced lines showed increased accumulation of withaferin A, withanolide A, sitosterol, and stigmasterol. This is added by a decline in the level of withanolide V. The key genes of withanolide biosynthesis such as *WsDXR*, *WsHMGR*, *WsFPPS*, *WsCYP710A1*, *WsSTE1*, and *WsDWF5* were upregulated in silenced lines, implicating a positive feedback regulation of MVA pathway genes by *SGTL*s silencing. Further, silenced lines were found to be more susceptible to *Alternaria alternata* (Singh et al. 2016b). After infection with *A. alternata*, the level of SA increased, and this resulted in higher expression of defense-related genes such as *WsPR1*, *WsPR10*, *WsSP1*, and *WsDFS* in *SGTLs* silenced lines.

These studies provided a glimpse of plausible options to modulate withanolide biosynthetic pathway that may remarkably enhance the production of pharmaceutically important secondary metabolites.

Hairy Root Culture Offers Exciting Prospects for Enhancement of Withanolide Production in *W. somnifera*

Hairy root culture holds immense potential to revolutionize the production of secondary metabolites. The hairy roots are raised by infection of susceptible explant with *Agrobacterium rhizogenes* and are characterized by absence of geotropism, high genetic stability and growth rate, profuse branching, and increased accumulation of secondary metabolites (Fig. 3) (Shanks and Morgan 1999; Cai et al. 2012).

Manipulation of nutrient medium affects the potential of hairy root system. MS medium is most suitable for hairy root growth (Murthy et al. 2008; Saravanakumar et al. 2012). In hairy root culture of *W. somnifera*, differential accumulation of withanolides can be achieved by manipulation of type and concentration of carbon source. At low concentration (3%) sucrose is suitable for withaferin A and withanolide A production, whereas high concentration of sucrose (4%) led to higher accumulation of withaferin A (Praveen and Murthy 2012). Furthermore, nutrient medium supplemented with 14.38 mM NH_4 and 37.60 mM NO_3 resulted in higher biomass accumulation and withanolide A production (Praveen and Murthy 2013).

Fig. 3 Hairy root culture of *W. somnifera*

Treatment of hairy roots with elicitors such as SA and MeJ increases the production of pharmaceutically important secondary metabolites (Sivanandhan et al. 2013a).

Transgenic hairy roots can be easily raised by using *A. rhizogenes* carrying binary vector containing gene of interest, thereby, providing another powerful tool for root metabolic engineering. Furthermore, development of an efficient trap system for withanolides and harnessing the ability of hairy roots to exudate the metabolites in culture medium would prove to be a boom for large-scale production of withanolides.

Future Strategies to Modulate Withanolide Production in *W. somnifera*

In vitro propagation using meristematic tissues and culture of cells have resulted in limited success in enhancing secondary metabolite production in *W. somnifera*. Hence, gradually the major focus shifted toward the mapping of secondary metabolite biosynthetic pathway for higher accumulation of pharmaceutically important secondary metabolites in this valuable medicinal plant. This effort resulted in identification and characterization of critical genes involved in withanolide biosynthetic pathway to facilitate effective metabolic engineering. However, addition of knowledge in production and accumulation of secondary metabolites in medicinal plants clearly suggests that any single approach may not be adequate for systemic and effective metabolic engineering (Oksman-Caldentey and Inzé 2004). In this context, the present chapter discusses various other strategies which could play crucial role in modulation of important secondary metabolites in *W. somnifera* (Fig. 4).

A. Redirection of metabolic flux toward desirable secondary metabolites by suppressing the competitive branch pathway. This can be done by silencing

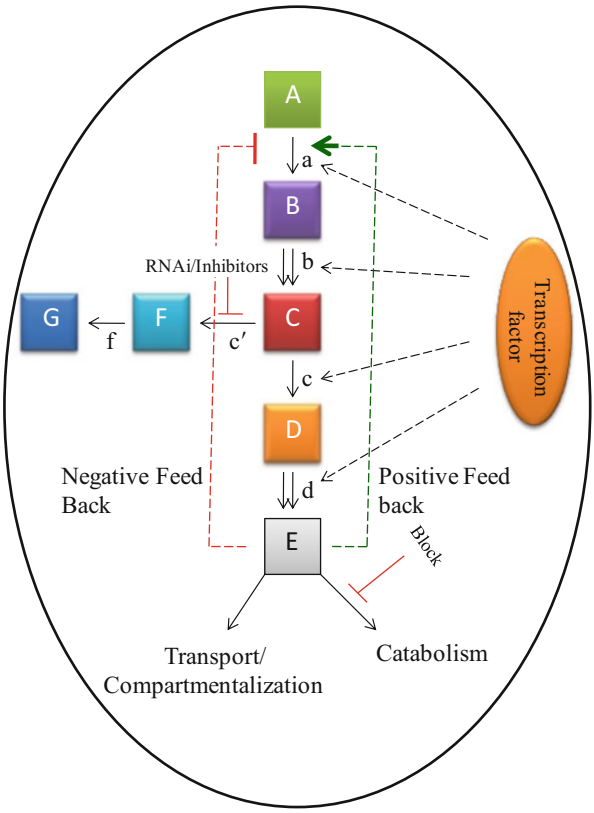

Fig. 4 A model diagram depicting metabolic engineering to enhance the production and accumulation of E (a secondary metabolite). b and d are rate-limiting enzymes. Double arrows indicate overexpression of b and d. A to E Substrate, intermediates, and final desirable product of secondary metabolite biosynthetic pathway. F–G A competitive branch. a to f Enzymes involved in biosynthetic pathway

(using RNAi approach) or by utilizing enzyme-specific inhibitors (Singh et al. 2014). A similar approach was adapted in *Artemisia annua*, where metabolic flux was intensively channeled toward artemisinin production by antisense fragment-mediated downregulation of β-caryophyllene synthase (CPS), a gene involved in competitive branch of artemisinin (Tang et al. 2014). Extrapolating this information, experimental strategies can be developed to redirect withanolide biosynthetic pathway to different routes in order to harness various inputs in secondary metabolite synthesis in *W. somnifera* in large amounts.

B. A more rational approach is to modulate a master regulator(s)/transcription factor (s). The strategy of increasing the production of withanolides using overexpression of *a* single rate-limiting gene is overpowered by utilizing the transcription factor that regulates a large number of genes in the pathway. Recently, in *W. somnifera*, nitrogen fertilizers mediated increase in sterol and withaferin A, and the corresponding increase in the gene expression of withanolide biosynthetic pathway is linked to WRKY transcription factor (Pal et al. 2016). A similar study in *Catharanthus roseus* employing overexpression of CrWRKY1 transcription factor in its hairy roots caused significant upregulation of several key genes of terpenoid indole alkaloid biosynthetic

pathway (Suttipanta et al. 2011), suggesting that exploration of this approach would be instrumental in enhancing the yield of withanolides.

C. Decreasing the catabolism/conversion of the concerned secondary metabolite (Verpoorte et al. 1999). In *W. somnifera*, several *CYP* genes have been identified and characterized. The functional annotation of one such *CYP* genes, *WsCYP93Id* in *Escherichia coli*, revealed its role in converting withaferin A into its hydroxylated derivative (Srivastava et al. 2015). However, this work is limited to bacterial system only. Such studies are much warranted at plant level in order to design suitable strategies to prevent modification/inactivation of withanolides. Further, once the entire pathway of withanolide biosynthesis is elucidated, efforts can be focused to decrease the conversion/breakdown of medicinally important secondary metabolites. This approach may give dual advantage if the concerned metabolite is responsible for positive feedback regulation of its biosynthesis.

D. Compartmentalization of the desirable secondary metabolite. For instance, redirection of biosynthetic pathway of sesquiterpene from its cytosolic location to chloroplast resulted in 100–1000-fold higher accumulation of sesquiterpenes in *N. tabacum* as compared to expression of same genes in cytosol (Wu et al. 2006). This tremendous increase in the sesquiterpene content was achieved by targeting the patchoulol synthase (PTS) activity alone or along with *FPP synthase* (FPS) to the plastids either as a protein fusion or as independent of each other. Enzymes were targeted to plastids by fusing a sequence of plastid-targeting signal peptide to the 5′ end of both the genes. However, while targeting a particular subcellular compartment for metabolic engineering, substrate availability in the compartment should be taken into account. The latter impose a major setback for exploiting compartmentalization-based metabolic engineering in *W. somnifera*, as no information is available regarding transport of metabolites between subcellular compartments and their transporters. An additional advantage of compartmentalization of secondary metabolites is in overcoming negative feedback regulation of the secondary metabolite biosynthetic pathway.

Conclusion

W. somnifera is a reputed medicinal plant owing to its various health benefits. In recent past, serious research efforts are undertaken to identify elite chemotypes of *W. somnifera* and bring them into rational scientific use. Various in vitro approaches such as organ and cell culture, genetic manipulations, and hairy root cultures have been adapted to increase the production of withanolides. Further, addition of knowledge on withanolide accumulation and characterization of key genes involved in its biosynthetic pathway has provided suitable impetus for its appropriate modulation. Development of suitable future strategies and its integration to modern experimental

approaches will help in the improvement of this important medicinal plant which would be better suited for human welfare.

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Part II

Phytomedicine and Raw Herb Quality

Quality Control of Herbal Drugs: Advancements and Challenges

Sharad Srivastava and Ankita Misra

Introduction

The majority of population inhabiting in developing countries are still dependent on herbal medicine to meet out their health needs. In the last years, there is global revival in use of herbal drugs/phytochemicals for various purposes, i.e. in medicine, nutraceuticals and cosmeceuticals. As the advanced medical aids are not easily available around the globe, the World Health Organization (WHO) also advocates the use of herbal medicine and other traditional remedies having proven safe and effective.

According to a report, worldwide turnover of herbal medicines is nearly around US$ 90 billion and is constantly expanding at an annual rate of 10–15%. However it is estimated that by the year 2030 the value will reach to approximately US$ 6 trillion. The Indian share to global herbal market is merely less than 2%; one of the reasons responsible for low export of Indian herbal medicine is lack of scientific validation, inconsistency in quality and safety data as per requirement of other countries. The deep correlation between plants and human health supported with modern analytical tools and techniques has opened a new avenue for introducing a new class of plant-derived therapeutics, viz. phyto-pharmaceuticals, polyherbal formulation, dietary supplements, functional foods and recombinant proteins of plant origin. The dietary consumption of such phyto-products accolade the traditional therapies for holistic prevention and treatment of diseases. The original system of disease treatment in India (AYUSH) and the Chinese system of medicine will constitute the health preventive, promotive, protective, correction and curative strategies. These traditional medicine systems are gaining worldwide recognition, and a so-called green wave is sweeping the whole market of food stores/health centres all over the world with herbal drugs and plant-derived products (Fig. 1).

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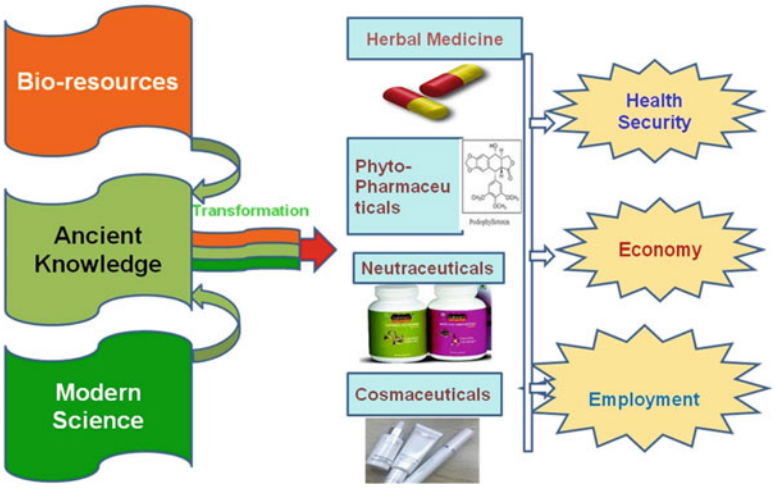

Fig. 1 Herbal drug: opportunities in the twenty-first century

It is to mention that only few scientifically validated herbal medicines are available in herbal market, and the other locally available natural resources are used for traditional claims by inhabitants to protect, restore or improve health. Thus, most of the herbal medicines used in the different indigenous systems of medicine still need to be studied scientifically, and their validation are urgently required, in addition to the knowledge acquired from their traditional literature. It had been noted that a range of modern drugs owe their origin from ethnobotanical claims, through modern approach of reverse pharmacology. Mostly plants synthesize a large variety of active constituents/secondary metabolites or phytochemicals in response to various ecophysiological stimuli; most of these compounds originate from a relatively few known biosynthetic pathways that include the pathways for alkaloids, terpenes/terpenoids/steroids, shikimic acid/aromatics and polyketides. The presence of active constituents and its quantity in plant parts are needed to be understood for the development of herbal drugs.

The Uniqueness of Herbal Products

The herbal product constitutes either a single herb or is polyherbal in nature, having a combination of different herbs which are believed to exhibit synergistic effects. Majority of products in indigenous systems are polyherbal in nature and have more than five single plant species or more. One of the oldest and most reputed formulations of Ayurveda 'Chyavanprash' even contains around 40 single herbs. Further, some traditional formulations also contain animal- and mineral-derived products. Herbal products are marketed either in the form of raw plant powder or extract of whole plant/plant part. The extraction involves treating (hot/cold method)

the plant part with suitable solvent, i.e. water, alcohol, ether, etc., depending upon the polarity of active constituents which is targeted to be extracted. The extracted liquid will then treated further at extremely low temperature to more concentrated liquids, paste or completely lyophilized powder. However, the constituent of raw herbs as well as their extract contain complex mixture of secondary metabolites like alkaloids and nitrogen containing compounds, terpenes, sterols, fatty acids, hydroxyl group containing compounds as flavonoids and phenolics, glycosides, saponins, tannins etc. (Rotblatt and Ziment 2002). Thus it became difficult to identify the single component which is responsible for particular therapeutic activity in humans. Apart from this, various processes required for formulation and storage of herbs like heating, boiling, etc. will also affect the pharmacological response of active constituents. Similarly, the effect of environmental conditions like precipitation, sunlight, temperature, humidity, altitude, etc. are one of the other major contributing factors for chemical variation in raw material and which finally leads to batch variation during product development. In addition to this, other miscellaneous factors including pest and insect attack, habitat competition with other species, species density, sowing time, harvesting stage and genetic factors are playing a crucial role in producing uniform herbal products (Wijesekera 1991).

Challenges for Developing Standardized Herbal Drugs/ Formulation

Three basic principles which needs to be addressed for the development and/or clinical use of herbal medicine:

1. There must be uniform regulation and standardization protocol for the product being studied or being used clinically.
2. There must be enormous/bulk scientific data produced and/or established by rigorous clinical research for claimed therapeutic effect to the patients.
3. There must be adequate scientific data to claim the safety (accepted toxicity) of product intended for human use by rigorous clinical research.

The guidelines on quality evaluation and development of herbal medicine is utmost important in setting the pace for global acceptability. This will lead to the increase in acceptability of the herbal drugs in developed countries. These should be:

(i) The drug should meet the standard of consistency, such as loss on drying, ash and extractive values as per respective pharmacopoeias of the country for each raw herb.
(ii) The parameters for identifying the herbal substance/compounds, using botanical parameters as well chemical investigations such as HPTLC or HPLC fingerprints.
(iii) The drug/formulation should address the prescribed limits of permissible heavy metals content, aflatoxins, microbial load and specific microorganisms.

(iv) The drug should not have any added toxic phytochemicals like cardiac and cyanogenic glycosides.
(v) Finally the herbal formulation should be safe as evident in laboratory animals.

In India the guidelines and regulations are described under the Drug and Cosmetic Act (1940), laid down by the Ministry of Health and Family Welfare and ICMR, New Delhi.

Need of Quality Assurance

Regulation of quality and standardization of herbal medicine are essential for the monitoring of the entire process of formation of herbal drugs, bioactivity-guided fractionation and bioprospection utilizing technical standards as per authorized gazette/pharmacopoeias (Folashade et al. 2012). The standardization, as briefed, is the process of describing a whole set of inherent characteristics, fixed parameters and definitive qualitative and quantitative values which assures the quality, efficacy, safety and reproducibility of herbal medicines (Bhutani 2003; Kokate et al. 2005).

The various factors affecting the quality of herbal drugs are:

(a) Complex nature of phytochemicals, which are responsible for holistic pharmacological response
(b) Active principle(s) is generally unknown/uncertainty of active principle(s)
(c) Absence of a wide range of major-minor compounds
(d) Shortage of standard fingerprints of phytochemicals to record/analyse efficacy within different batches
(e) Associated natural variability inherited with plants in wild and non-wild varieties
(f) Variability in spectrum of pharmacological potential of species grown naturally vs. chemo varieties/cultivars
(g) Variability in quality of the raw material, its source of origin, etc.

Sophisticated analytical techniques are constantly developed and upgraded day by day to isolate, identify and quantify the phytoconstituents responsible for the therapeutic potential of herbs/plants. It is also evident that these metabolites vary qualitatively and quantitatively. There are several biotic and abiotic factors like physiological and environmental stresses, geographical locations, climates, physical and chemical stimuli which are often known as elicitors and alter the content of bioactive secondary metabolites either qualitatively or quantitatively. Enzyme-mediated biosynthetic pathways are highly susceptible and inducible, often leading to variability in the content of metabolite (Ebel and Cosio 1994). This is evident in case of phytochemicals which are well studied/investigated for their pharmacological activities, e.g. phenylpropanoids containing compounds (Dixon and Paiva 1995), alkaloids and related compounds (Facchini 1994) and terpenoids (Trapp and Croteau 2001). The quantity of metabolites in such cases often increases in magnitude (two-

Fig. 2 Three pillars of herbal drug development

or threefold) following the elicitation response or stress (Darvill and Albersheim 1984). Standardization and optimization protocols of growing conditions should be designed in such a way that it produces a quality-borne and cost-effective production of plant-derived products. This will be useful in improving the safety and consumer acceptability for wide usage.

The herbal drug used in developing countries of the world lacks official quality control regulations/standards, and therefore the consistency in the quality of different batches is not fixed. Often there is no well-defined/characterized chemical composition. It is needful to mention that the three pillars for rational use of herbal drug are quality, safety and efficacy (Fig. 2). However traditionally it is not rationale because the treatment regime is designed for each individual, and hand-picked plant/raw herbs are used for treatment.

Collection of medicinal plants also needs expertise. In the ancient time, traditional physicians were skilled to collect the right plant/part from specific habitat. The time and season of collection is also very crucial for potential therapeutic activity. The traditional, medicinal plant collectors had immense knowledge of species/herb identification and are able to collect the promising species among the population of several plants. Such unique expertise is very beneficial for them to maintain a standard level of quality in therapeutic activity of raw material/herbal drugs. There has been severe shortage of such skilled plant collector by the turn of the twentieth century. One of the main reasons for such crises is due to the transformation of traditional medicine system of individual-/patient-based strategic treatment to bulk and commercial manufacturing approach. This leads to huge decline in process and procedure of traditional medicine system; moreover the quality of drug was completely compromised during the whole transformation.

The raw material required for traditional medicines/herbal medicines preparation is majorly collected from wild resources and is about more than 80%. The day-by-day increasing demand of medicinal plants for commercial use will lead to inadequate, non-holistic and non-scientific collection, without having any consideration about the quality of the material. Such unauthentic practices had caused extensive drainage in traditional wisdom, knowledge and expertise skills of medicinal plant collector. The greed for monetary benefit encourages many to fulfil the commercial demand by adulteration and addition of spurious material in genuine material. It further complicates the quality standard of herbal drugs and phyto-pharmaceuticals (Fig. 3).

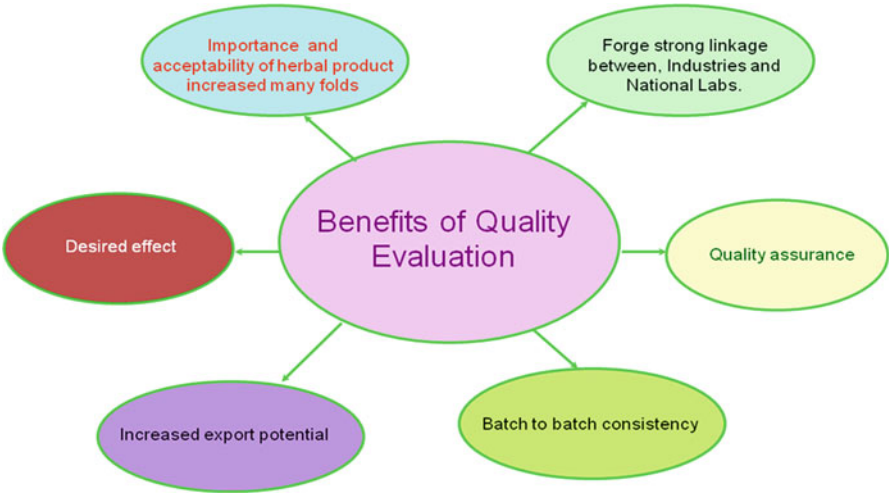

Fig. 3 Benefits of quality evaluation of herbal drugs

Issues and Challenges in Collection

As evident, the pharmacological potential of medicinal plants is due to the presence of biologically active chemical compound, viz. primary or secondary metabolites. The production of majority of phytochemicals, particularly secondary metabolites, is invariably governed by various biotic and abiotic factors, viz., genetic makeup, climatic conditions of plant habitat, season, stage of growth and its development, etc. The traditional AYUSH and other Indian systems of medicine provide standardized collection practices for the collection of medicinal plants, precisely by indicating species location, geographical conditions, ecology and habitat, effect of seasonal variation and sometimes even the stage of plant growth and development. Scientific literature now provides ample data to support the fact that there is variation in quantity of chemical constituents, especially secondary metabolites, subjected to change in the above-mentioned variable factor. Thus it became essential to define a reference standard and to establish the quality standards of medicinal species/plants by thorough studies of our traditional treaties of Indian classical system of medicine. This information when amalgamated with modern scientific knowledge and methods using sophisticated analytical tools will put forward a more pronounced and sound system.

Chemical Variation in MAP Germplasms

Different chemotypes of various medicinal and aromatic plants (MAPs) like *Ocimum*, *Hedychium*, *Thymus*, *Acorus*, *Piper betle* and *Valeriana* species exist in natural condition, and variation in their phytochemicals resulted in variable

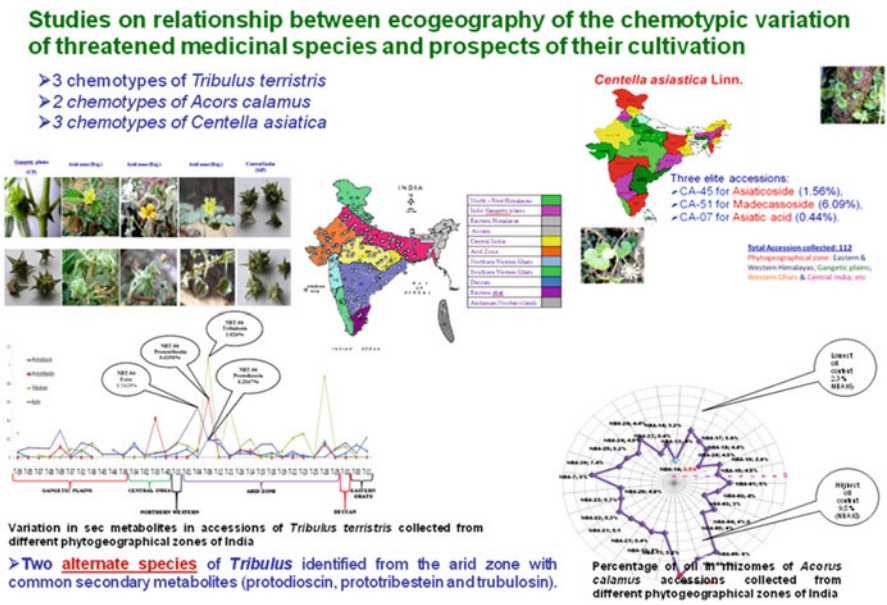

Fig. 4 Chemotypic variations among medicinal plants

pharmacological activities. For example, chemoprofiling studies on *Acorus calamus* have shown that out of 37 accessions collected from various phytogeographical zones of India, significant variation was observed in the essential oil content of rhizome. Maximum content (9.5% on dry weight basis) was reported in germplasm collected from North-West Himalayan zone at an altitude of 1097 metre with average temperature (23 °C to 2 °C), rainfall (13–428 mm) and high-altitude clay soil, whereas minimum oil content (2.3% on dry weight basis) was reported in germplasm collected from Lucknow. This will clearly reflect the effect/role of ecological conditions on variation of metabolites (Rana et al. 2013).

Interestingly, similar kind of chemotypic variations (Fig. 4) was observed in many more industrially important medicinal plants, viz., *Gloriosa superba*, *Coleus forskohlii*, *Ageratum conyzoides, Centella asiatica*, *Tribulus terrestris*, etc. (Misra et al. 2016; Srivastava et al. 2014, 2017).

Issues of Adulteration/Substitution in Herbal Drug Industries

The herbal drug industries in India usually encounter the problem of procurement of good-quality raw material from the markets due to common practice of adulteration and substitution which resulted in inferior-quality of the finished products.

It is a common practice in herbal market of the country to sell the various species of particular genera under the same vernacular name. Sometimes species of entirely different taxa, under the vernacular name of Ayurvedic drug 'Talispatra'—leaves of

Taxus wallichiana, *Abies spectabilis* and *Rhododendron anthopogon*—are being sold. Similarly different parts of various species, viz. *Fumaria parviflora*, *Peristrophe bicalyculata*, *Oldenlandia corymbesa* and *Rungia*, are sold under the name of 'Pittapapra' in various crude drug markets (Rawat et al. 1996).

This practice was very common in cases of 'Daruharidra', 'Sankpushpi', 'Kuth', 'Vidarikand', 'Sariva' and 'Ratanjot', where two or more different plant species are being sold in the markets under the same name. The adulteration and substitution of raw material by inferior-quality material may be deliberate or sometimes unintentional too. But this results in deterioration of raw material(s) quality because of low therapeutic potential and variation in batch-to-batch consistency (Srivastava and Rawat 2008; Pandey et al. 2007).

Adulteration and substitution is of two categories:

(a) *Undeliberate/unintentional*
(b) *Deliberate/intentional*

Undeliberate/Unintentional

This is considered to be substitution of raw materials which may be reasoned due to confusion in local/common/vernacular name of herbal drugs sold in local markets in different parts of India (Table 1, Fig. 5).

Deliberate Adulteration

It is intentionally using admixture of different plant materials by the traders to earn more profit or sometimes in conditions of scarcity of right material (Table 2).

Table 1 Few unintentional adulterants

Vernacular name	Plant species
Kuth	*Saussurea lappa*, *Atrium lappa*, *Inula racemosa*
Brahmi	*Bacopa monnieri*, *Centella asiatica*
Shankhpushpi	*Evolvulus alsinoides*, *Convolvulus microphyllus*
Talispatra	*Taxus wallichiana*, *Abies spectabilis*,
	Rhododendron spp.
Ratanjot	*Arnebia benthamii*, *Arnebia nobilis*,
	Arnebia ecuroma, *Onosma hispidum*

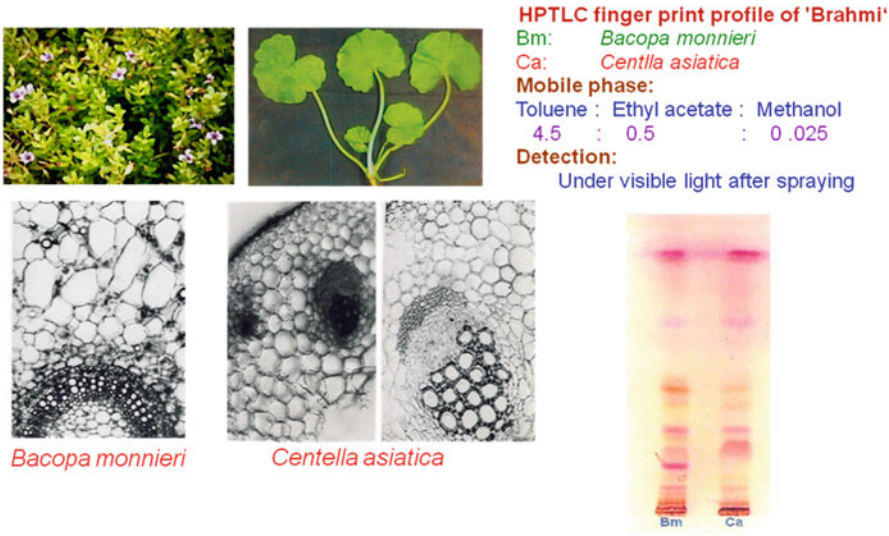

Fig. 5 Two different herbs, being sold with the same common name 'Brahmi'

Table 2 List of deliberate adulterants to important medicinal plants

Drug	Genuine	Adulterate/substitute
Ashoka	*Saraca indica*	*Polyalthia longifolia*, *Shorea robusta*
Kurchi	*Holarrhena antidysenterica*	*Wrightia tinctoria*
Senna	*Cassia acutifolia/Cassia angustifolia*	*Cassia auriculata*

Sometime traders also use exhausted raw material in place of authentic crude samples (Fig. 6).

A detailed list of adulterant/substitute to some of the common industrially important herbs has been worked out as mentioned in Table 3.

Basic Tools for Quality Evaluation of Herbal Drugs

Quality evaluation starts from the proper identification of desired plants, collection of right plant material, season of collection, extraction and purification processes. The regulatory authorities like the Ministry of AYUSH, Indian Council of Medical Research and National Medicinal Plants Board have provided guidelines and important tests which are required for the development of quality assurance parameters for herbal drugs as mentioned in Fig. 7.

Fig. 6 Substitution of exhausted products

Table 3 List of substitutes/adulterants to important medicinal plants

1.	Aakashbalii	a- *Cuscuta chinensis* Lam.
		b- *Cuscuta reflexa* Roxb.
2.	Apamarg	a- *Achyranthes aspera* Linn.
		b- *Achyranthes bidentata*
3.	Ashoka	a- *Saraca indica* Linn.
		b- *Polyalthia longifolia* (Sonn.) Thw.
		c- *Saraca declinata* Linn.
		d- *Bauhinia variegata* Linn.
		e- *Shorea robusta* Gaertn. F.
4.	Bala or Atibala	a- *Sida acuta* Burm. F. emend. K. schum.
		b- *Sida alba* Linn.
		c- *Sida cordata* (Burm. F.) Borssum.
		d- *Sida rhombifolia* Linn. Emend. Mast.
5.	Banafsha	a- *Viola odorata* L.
		b- *Viola pilosa* Blume
		c- *Viola betonicifolia*
6.	Bhavya	a- *Dillenia indica* Linn.
7.	Bhumyamalaki/Bhuiamla	a- *Phyllanthus amarus*
		b- *Phyllanthus fraternus* Webster.
		c- *Phyllanthus maderaspatensis* Linn. Adu.
8.	Brahmi	a- *Centella asiatica*
		b- *Bacopa monnieri*

(continued)

Table 3 (continued)

9.	Daruharidra	a- *Berberis aristata* DC.
		b- *Berberis asiatica* Roxb. Ex DC.
		c- *Berberis chitria*
		d- *Berberis lycium* Royle
		e- *Coscinium fenestratum* (Gaertn.) Colebr.
10.	Deodar	a- *Cedrus deodara* D.Don (Deodar)
		b- *Pinus roxburghii* Sargent (Chir)
11.	Dronpushpi	a- *Leucas aspera* (Willd.) Link.
		b- *Leucas cephalotes* (Roth) Spreng.
12.	Dugdhika	a- *Euphorbia prostrate*
		b- *Euphorbia reticulate* Forsk.
		c- *Euphorbia microphylla* Heyne.
		d- *Euphorbia thymifolia*
13.	Gandeer	a- *Coleus forskohlii*
		b- *Ranunculus sceleratus* Linn.
14.	Hansraj	a- *Adiantum capillus-veneris*
		b- *Adiantum caudatum* Linn.
		c- *Adiantum venustum* Linn.
15.	Jatamansi	a- *Nardostachys grandiflora* DC.
		b- *Selinum varinatum* CB Clarke
16.	Jivanti	a- *Leptadenia reticulata* (Retz.) Wt. Arn.
		b- *Dregea volubilis* (Linn. F.) Benth.
		c- *Sarcostemma brevistigma* W. And A
		d- *Ephemerantha macraei* (Lindl.) Hunt Summerh
17.	Kantakari	a- *Solanum surattense* Burm. F.
		b- *S. indicum* Linn.
18.	Kapikachhu	a- *Mucuna pruriens* (Linn.) DC.
		b- *Mucuna utilis*
		c- *Mucuna nivea*
19.	Mustak	a- *Cyperus rotundus* Linn.
		b- *Cyperus scariosus* R.Br.
20.	Nagkesar	a- *Calophyllum inophyllum* Linn. Syn. O*chrocarpus* spp.
		b- *Mammea longifolia* Planch. Ex Triana
		c- *Mesua ferrea* Linn.
21.	Parpat/Pitpapra	a- *Fumaria parviflora* Lam.
		b- *Oldenlandia corymbosa* Linn.
22.	Ratanjot	a- *Arnebia nobilis* (Royle) Johnston
		b- *Arnebia benthamii* (Wall. Ex G. Don) Jhonston
		c- *Arnebia euchroma* (Royle) Johnston
		d- *Maharanga emodi* (Wall.) DC.
		e- *Onosma hispidum* Wall. Ex G.Don
23.	Renuka	a- *Vitex negundo* Linn.
		b- *Vitex agnus-castus* Linn.
24.	Resha Khatmi	a- *Althea officinalis* Linn. (root)
		b- *Althaea rosea* Linn. (root)

(continued)

Table 3 (continued)

25.	Sappan	a- *Caesalpinia sappan* Linn.
		b- *Pterocarpus dalbergioides* Roxb.
		c- *Pterocarpus marsupium* Roxb.
		d- *Pterocarpus santalinus* Linn.
		e- *Toona ciliata* M.J. Roem.
		f- *Gluta travancorica* Bedd.
26.	Shatavari	a- *Asparagus adscendens* Roxb.
		b- *Asparagus racemosus* Willd.
		c- *Asparagus sprengeri*
27.	Talispatra	a- *Taxus wallichiana* Zucc.
		b- *Abies spectabilis* (D.Don) Spach.
		c- *Rhododendron anthopogon*
28.	Tukhm-e-Khatmi	a- *Althaea officinalis* Linn. (seed)
		b- *Althaea rosea* Linn. (seed)
29.	Tulsi	a- *Ocimum americanum* Sirns.
		b- *Ocimum basilicum* Linn.
		c- *Ocimum gratissimum* Linn.
		d- *Ocimum sanctum* Linn.
30.	Vidarikand	a- *Pueraria tuberosa* (Roxb. Ex Willd.) DC.
		b- *Ipomoea digitata*
31.	Pattharchoor	a- *Bergenia ciliata*
		b- *Bergenia stracheyi*
		c- *Bergenia ligulata*

Fig. 7 Authentication and standardization of herbal raw material

Botanical Tools

Microscopic Identification of Crude Herbal Drugs

Botanical descriptors provide supportive aid in identification of raw materials, and depending upon the morphological group of drugs, the method adopted for the preparation of specimen varies for microscopic studies; it should be studied entirely, cut or in powdered form.

Study of Stomata

The type of stomata present in plant material is characterized by the arrangement of guard cells and its associated subsidiary cells. The five types of stomata are *anomocytic* (irregular-celled), *anisocytic* (unequal-celled), *diacytic* (cross-celled), *paracytic* (parallel-celled) and *actinocytic*, and on the basis of which, two similar looking plants can be differentiated.

Macroscopic Studies

For microscopic studies of leaf, aerial part and flower (whole or section), the following methods of clarification are adopted:

Whole Plant Material For examination, the studied specimens, viz. complete leaf (margin and vein of leaves only), aerial part (only leaf) and flowers (only calyx and corolla), were taken in the test tube. A solution of caustic alkali or nitric acid is added to the test tube and boiled for 1–2 min. The content was poured into porcelain dish to drain off the solution. The treated specimen must be washed with water and left idle for some time. Remove the specimen from the water with a spatula, and put on the slide and mount with solution of *glycerol or chloral hydrate*. Before examination the material is washed and covered with slip.

Sectioned Material The same protocol is repeated for the examination of cut material also with several pieces of specimen in a test tube.

Fruit and Seeds

Whole Seed/Fruit Materials

The macroscopic examination of whole fruit and seed are done with specimen or outer coat as per the method given below:

Outer Coat Three or four seeds of fruits were boiled in caustic alkali solution for 1–2 min (outer coat specimens with intensive pigmentation are boiled for longer

period) in a test tube. The specimen under study was then kept on slide; layers of coat were then removed and were examined after mounting in glycerol solution.

Section In case of hard seed and fruits, the specimen is allowed to boil in water for 15–30 min or more as required to soften them. Alternatively, soak them in water and chloroform solution, then sectioned for examination. The small flat seed which are difficult to hold is sectioned by placing between pith of potato/paraffin block before slicing. In the method, a block of paraffin ($0.6 \times 0.5 \times 1.5$ cm) is used onto which the seed is embedded by making a cavity through hot needle. Slice the section with sharp razor/blade together with paraffin and place on the slide. Paraffin adhered to the section is removed either by needle or washed off with xylene, and examine the section after washing with chloral hydrate solution.

Barks

Lignified Elements Testing of lignified substance is done by addition of few drops of phloroglucinol followed by hydrochloric acid (concentrated) to the section, pat dry the excess liquid and mount the specimen in glycerine for study. The lignified element turns pink in colour which can then be easily detected.

Starch Iodine solution is used as staining agent for detection of starch.

Tannin Tannin is analysed by staining with *ferric ammonium sulphate* solution or with *potassium-dichromate solution*; presence of blue-black or green-black and brown colour indicates tannin content in cell.

Anthraquinone Derivatives Treatment with alkali solution stains anthraquinone derivative to blood red colour.

Roots and Rhizomes

Entire Materials

The microscopic examination of whole roots and rhizomes is done on its transverse and/or longitudinal sections. The specimen is kept soft within 2–3 days without any heating; the roots/rhizomes were then sectioned with sharp razor into thin slices. The sliced sample was kept in water to prevent the rupturing of cells. The section was then stained with *phloroglucinol* and *concentrated hydrochloric acid* or with *safranin*. Specimen was examined under a dissecting microscope.

The histochemical tests on small and thin sections were performed as per the procedure given below:

Starch Starch in sample is identified with iodine solution as staining reagent; the stained granules of starch were then measured with ocular micrometre.

Inulin Molisch's reagent is used to identify inulin. For test of inulin, the sample was treated with naphthol (1–2 drops) followed by a few drops of sulphuric acid (concentrated), and the test was confirmed if powder turns reddish-violet coloured.

Lignified Elements Specimen when treated with *phloroglucinol* and concentrated *hydrochloric acid* turns (fibrovascular bundles, mechanical tissue, etc.) into reddish pink; colour confirms the presence of lignified elements.

Fixed Oil Sudan IV is used for testing of fixed oils, as mentioned above for fruits and seeds.

Quantitative Microscopy

This is adopted for leaf-containing herbs and is essential for identification within the two similar species (*Quality Control Methods for Medicinal Plant Materials*, 1998).

Stomatal Index

The stomatal index is the percentage ratio of the number of stomata formed by the total number of epidermal cells, including the stomata, each stoma being counted as one cell.

Palisade Ratio

Palisade ratio is the average number of palisade cells beneath one epidermal cell.

Vein-Islet Number

The mesophyll of a leaf is divided into small portions of photosynthetic tissue by anastomosis of the veins and veinlets; such small portions or areas are termed 'vein-islets'. The number of vein-islets per square millimetre is termed the 'vein-islet number'. This value has been shown to be constant for any given species and, for full-grown leaves, to be unaffected by the age of the plant or the size of the leaves. The vein-islet number has proved useful for the critical distinction of certain nearly related species.

Vein-Termination Number

Vein-termination number is the number of veinlet terminations per mm of the leaf surface.

Physicochemical Parameters for the Standardization of Crude Drugs/ Products

Foreign Organic Matter

The genuine crude drug must be free from any foreign organic material like pests, moulds, insects, animal faecal matter and inorganic material/other contaminations like soil and extraneous material.

Determination of Moisture Content (Loss on Drying)

This is used to determine the volatile constituents of drug which is evaporated on exposure to high temperature. It is specifically used to examine the content of water/ moisture in the drug under the defined protocols. This helps in the prevention of decaying of sample with excess inhabitant moisture and must be regulated during storage of sample.

Determination of Ash Value (Total Ash, Acid Insoluble Ash, Water Soluble Ash)

The data obtained from preliminary physicochemical screening is helpful in establishing the authenticity of the drug. Ash content of herbal drug gives the account of total earthy material like silica or inorganic and other organic impurity present along with authentic drug.

Determination of Extractive Values

The extractive values are essential to evaluate the nature of phytoconstituents present in crude drug. The estimation of phytoconstituents is solvent specific and is useful for estimation of exhaustive and adulterated drug. The extractive values can be calculated with water, alcohol, hexane or any other desired solvent.

Chromatographic Techniques

Chromatography is a physical method of separation that segregates the various components (chemical constituents) of herbal drugs between the two phases, one of these is a stationary phase and the other is mobile phase. The graded separation of component is based on its affinity for the above-mentioned two phases.

Based on the stationary and mobile phase, the chromatographic technique can be of the following types:

1. *Thin-layer chromatography (TLC)*: A liquid mobile phase moves through a layer of sorbent by the action of capillary forces. TLC is an open bed technique as pressure is not required for the movement of the mobile phase.
2. *Liquid chromatography*: The mobile phase is a liquid of low viscosity that is caused to flow through a bed of sorbent. The sorbent may be immiscible liquid coated onto a porous support or an inert sorbent of controlled pore size.
3. *Gas chromatography*: The mobile phase is an inert gas, and the stationary phase is either an adsorbent or a liquid distributed over the surface of a porous, inert support.

The common chromatographic techniques used are column chromatography, paper chromatography, HPTLC, GC, OPLC and HPLC.

HPTLC: An Efficient Analytical Tool for Qualitative and Quantitative Evaluation of Phytoconstituents in Herbal Drugs and Formulations

This is the easiest tool for qualitative and quantitative estimation of metabolite content in any raw material. It also supports in identifying adulterants and substitutes (Fig. 8).

HPLC As a Tool for Quantitative Evaluation of Herbal Drugs and Formulations

High-performance liquid chromatography is the suitable method of choice for the quantification of a number of groups of secondary metabolites like phenols especially due to its extremely high versatility and precision. This technique has been

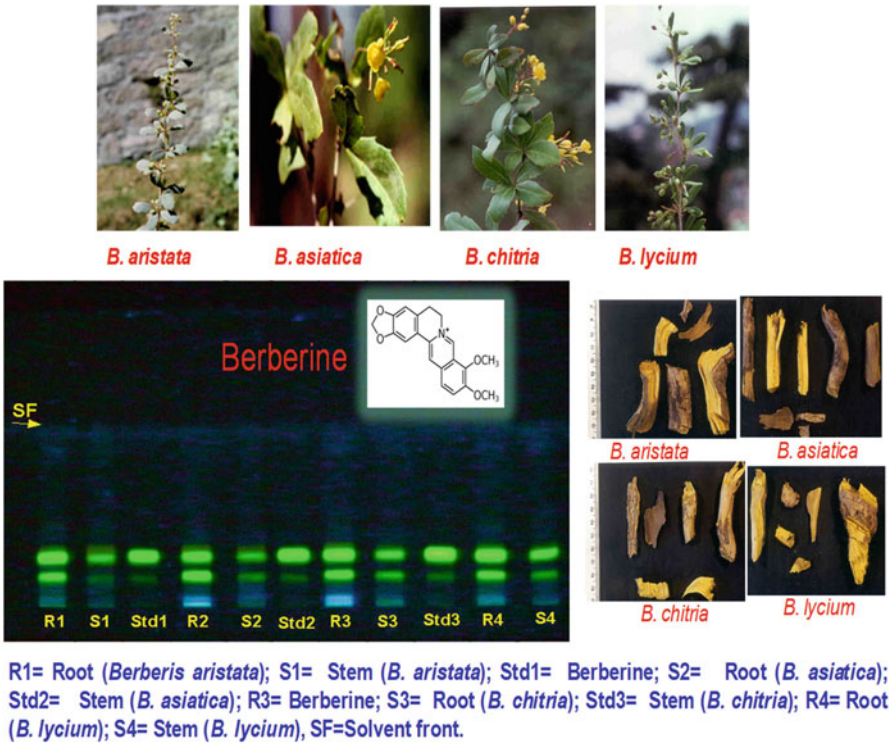

Fig. 8 HPTLC profile of *Berberis* species

used successfully in the identification of different *Bergenia* species. Figure 9 shows a simple, highly precise RP-HPLC profile of gallic acid (1) and bergenin (2) in three species of *Bergenia*, viz., *B. ligulata* (Wall) Eng., *B. ciliata* (Royle) Raizada and

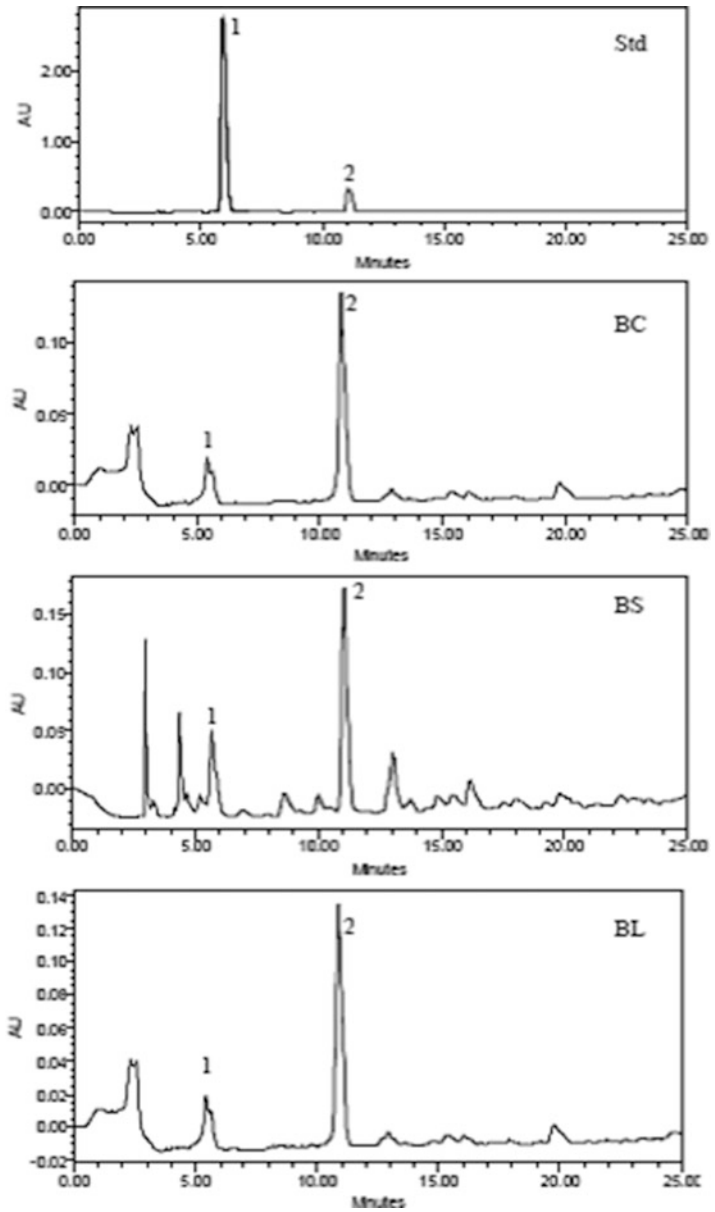

Fig. 9 HPLC profile of bergenin in different *Bergenia* species
Abbreviations: *BC Bergenia ciliata*, *BS Bergenia stracheyi*, *BL*, *Bergenia ligulata*

B. stracheyi Engl. Study showed that bergenin was abundantly found in *B. ciliata* (3.275%) and *B. stracheyi* (3.277%), whereas *B. ligulata* contained 2.419% bergenin. Thus the latter can be used as substitute in scarcity of former species (Singh et al. 2007).

Safety/Toxicological Studies

The studies on toxicity reflect the level of pesticide residue content which is potentially toxic element. LD_{50} and other preclinical toxicity parameters are required as per norms of drug regulation. Analytical tools, viz. HPTLC and HPLC, are frequently used in herbal industry for detection and identification of adulterants as well as pesticide residue, and mycotoxins were also quantified for quality control of herbs and health food (Di et al. 2003).

Microbial Load/Assay

The presence or absence of potentially harmful organism and their traces are essential to ascertain the quality of material. To limit this, certain regulatory standards are laid by various monitoring agencies like USFDA, WHO etc. (Bhanu et al. 2005).

Authentication of Raw Herbal Drug and Formulations

The Govt. of India (Ministry of AYUSH, New Delhi) has developed the quality assurance parameters of single and classical herbal formulations and published different volumes of *The Ayurvedic Pharmacopoeia of India* covering 518 single raw drugs and 200 Ayurvedic formulations. Therefore, it is essential for researcher/industries that before production of any herbal formulation, each and every single raw material of the formulation should be evaluated for particular quality parameter whether the test values of all the ingredients compliance with the pharmacopoeial standards.

Not only that, quality assurance parameters of the final product should also be worked out so that batch-to-batch consistency can be maintained. A set of quality parameters are mentioned in different categories of single and compound formulations and dosage forms of Ayurvedic formulations like Churnas, Kvatha, Avaleha, Bati, etc. (Lohar 2006).

Conclusion

There is an urgent need for developing pharmacognostical parameters for the identification of substitutes or adulterants for proper differentiation of official plant species mentioned in Ayurvedic formulary. The Govt. of India (Department of AYUSH, New Delhi) CSIR, ICMR and other agencies are working in this direction. The Department of AYUSH has already published eight volumes of Ayurvedic herbal pharmacopoeia wherein detailed botanical, chemical and physicochemical standards of different crude drugs used in Ayurveda, Siddha and Unani have been described (Anonymous 2001–2012).

Similarly, the Indian Council of Medical Research (ICMR), New Delhi, has initiated a programme on evolving standards for medicinal plants, and under this programme, the council has published 11 volumes of *Quality Standards of Indian Medicinal Plants*. However, the source of the herbal raw material is a crucial factor to be taken into account for batch-to-batch consistency of herbal products/drugs. Extensive work in the area of identification of elite chemotypes of medicinal plants and their conservation is urgently required. Development of suitable agrotechnologies and micropropagation techniques for high-value medicinal plants as well as threatened medicinal plants is required for continuous supply of superior-quality raw material to the industry. Further, application of advanced tools for functional characterization of medicinal plants for identification of novel phytomolecules for drug standardization purposes is also needed.

Looking towards the problem of adulteration and substitution existing in market of herbal drugs in India, the Pharmacognosy & Ethnopharmacology Division of National Botanical Research Institute, Lucknow, is extensively involved from the last 20 years in developing the quality control parameters of crude drug and standardization of polyherbal formulations. Under the various programme running in the Division, over 150 single herbal drugs (crude) and approximately about 20 polyherbal formulations had been standardized till date. Some of the medicinally important single raw drugs which were already standardized include species like Amraharidra, Ativisha, Bhuimala, Chiriata, Daruharidra, Kali Musli, Kalmegh, Pitpapra, Safed Musli, Salam Panja, Satawar, Resha Khatmi, Talispatra, Vidarikand, etc. (Singh et al. 2008; Khatoon et al. 2008).

To meet out the industrial demand of raw herbs for production of standardized and quality herbal products and to promote their export, it is essential to maintain the quality of raw materials. However, such steps will keep unwanted foreign matter and other contaminants like pesticide, heavy metal, etc. at bay. Therefore, identification of authentic species and its chemotypes, harvesting time of species, its drying and proper storage and packing are some of the important steps to restore batch-to-batch consistency, desirable therapeutic efficacy and safety of crude drug and also of final product.

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Part III

Pharmacognosy

Cultivation and Bioprospecting of Medicinal Plants

Kewalanand and Brajkishor Prajapati

Introduction

Plant-based drugs have been used in India since the Vedic period, with an extensive literature on these drugs since that time. During these ancient periods the herbal medicines used were of high quality and there were large quantities of naturally growing herbal plants. With time, the utilization of herbal medicines has greatly increased and natural sources are becoming depleted at a rapid rate. This has necessitated the cultivation of these plants to fulfill the requirement of industries for raw material. Until recent times, most medicinal plants were collected from their natural habitats and this has led to the extinction or near-extinction of most of these valuable plant species, as well as diminished genetic diversity. However, wild collected plants are mostly not true to type or quality and their refined products are often contaminated or unstable. Cultivation solves these problems, providing uniformity and enhanced yields with high-quality products. The medicinal properties of the plants have led to the evolution of various therapies. Indeed, the bioactive compounds found in different medicinal plants are the basis of most of the medicines used in modern therapy. Over the past few years 10–18% of total medicinal plant biodiversity (50000 plants) has gained wide recognition in pharmaceutical industries due to high efficacy and it has been revealed that about 80–85% of the world population depends on herbal products, because their side effects are less severe than those of synthetic products. India is well known for its diversity, and certain ethnic groups (totaling more than 84.4 million people), mainly the Tharu, Buksa, Gonds, Santhals, Khasis, Angamis, Bhutias, and Great Andamanese, who have age-old cultures, traditions, languages, and lifestyles, have healthcare systems that depend on herbal products.

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Options for Cultivation of Medicinal Plants

Some medicinal plants can be protected by appropriate laws and policies and in-situ conservation methods, but the best way to protect these plants is by improvements in cultivation technology. Producers and other related stakeholders have to focus on the cultivation of high-market-value crops such as *Mentha* spp, *Ginkgo biloba* Linn, *Hypericum perforatum* L. (St Johns Wort), *Artemisia annua* L., *Papaver somniferum* L., *Plantago ovata* Forsk, *Ocimum* spp., and *Eucalyptus citriodora* Hook, which are under large-scale cultivation and are least affected by collection in the wild. Most lesser known medicinal plants are perennial and their production is considerably at risk owing to their longer harvesting period, and thus their future market is highly uncertain.

Active Compounds

The aim of cultivating medicinal plants is to increase the content of bioactive compounds, reduce the concentration of toxic compounds, and enhance the uniformity of the bioactive constituents. In cultivated plants, phenotypic variations can provide the required content of bioactive compounds, which are secondary metabolites that are helpful in the survival of plants under adverse conditions. For example, the plants are protected by antioxidants during variations in light and temperature, by proline during stress conditions, and by flavonoids against infections, while alkaloids protect the plants from herbivory. Significant locational variations in the constituents of the same plants are seen. It has been noted that warmer conditions lead to a higher alkaloid content in *Atropa belladonna* L. (1.3%) compared with that in a relatively cool climate (0.3%). *Mentha piperata* L. grown under shade and partial shade conditions contains less oil and menthol than plants grown in full sunlight. Opium poppy crops grown in a cool climate have a higher morphine content than those grown under hot conditions. Moisture and nutrient availability, stress, the activity of soil microbes, and pH variations similarly influence the content of secondary metabolites.

Selection of Medicinal Plants

The quality of medicinal plants (species/varieties) is very sensitive to the ecological conditions of the area in which they are grown. Also, the cultivation of medicinal plants may lead to variations in biodiversity and in the agro-ecological environment. Introducing non-indigenous medicinal plants into an area also adversely affects the biodiversity and ecology of the environment and their impact has to be monitored. The cultivation of medicinal plants has social impacts on wages and employment opportunities, and fair prices and capital reinvestment must be taken into consideration in order to save both small- and large-scale cultivators. To overcome these impacts the selection of species/varieties must be based on appropriate

authentication according to national pharmacopoeias. Exotic species/varieties of medicinal plants for cultivation must be specialized according to their source and authenticated according to the pharmacopoeia of the country in which they will be cultivated. These plants should be botanically identified. Information on the names (botanical, local, and English), ecotypes, chemotypes, and phenotypes of the medicinal plants must be provided before cultivation is commenced. Varieties that are commercially available for cultivation must be identified with the name of the supplier. For varieties that are being developed, records must be maintained, including items such as the local name; original sources of seeds, plants, or propagation materials of the collected landraces; and time of collection .

Selection of Site

Biotic and abiotic factors at the cultivation site have great influence on the quality of medicinal plants, and these factors vary at different sites. The physical appearance of the cultivated plants and variations in their constituents and their biosynthesis are affected by variations in the environment, ecology, and geography of the region. Therefore the site selected should have features identical to those of the source material. The soil and water should be free of hazardous chemical pollution. The land use history of the field under cultivation must be known to avoid any deficiency in soil. The site should be free of weed flora (annual and perennial) as well as soil-borne pathogens. All efforts should be made to make the soil pH near neutral.

Crop Improvements

For improving the yield and quality of medicinally important plants under cultivation, techniques such as traditional plant breeding and those involving biotechnology are used at the genetic level. Both the agronomic and biochemical characteristics of plants can be improved by conventional breeding and molecular marker-assisted selection methods. Techniques such as tissue culture for propagation material and genetic transformation for the required biomolecules in plants can be successfully employed. Important crop improvement methods are detailed below:

Traditional Breeding

Traditional breeding is done simply by selecting fertile lines of the required varieties with the aim of stabilizing bioactive compounds and reducing levels of toxins. Scientific validation of *Ginkgo biloba* Linn. extract has been done and the extract has been standardized to contain flavonoid glycosides (25–27%), terpene lactones (6–7%), and ginkgolic acid (<5 ppm). Such standard extracts can be achieved only if selection is done for true-to-type plant species. Therefore the plants must be identified at an early growth stage.

Selection Assisted by Genetic Markers

Selection of genotypes assisted by genetic markers is based on identifying specific DNA sequences (gene alleles) of the required trait. Alhough this is an expensive and lengthy technique it can facilitate association with the whole genome sequence of the ideal species. Work on this aspect is meager in medicinal plants. For example, *Cannabis sativa* L. selection assisted by genetic markers is only implemented for fiber crops and for forensic use (Gilmore and Peakall 2003; Miller et al. 2003). For breeding pharmaceutically useful lines of *Cannabis* spp., cannabinoid biosynthesis has been sequenced though codominant alleles (Canter et al. 2005).

Genetic Transformation Systems

For better crop improvement, not only targeted biochemical traits, but also other traits, such as resistance to biotic and abiotic factors, stability, and uniformity in growth and development need to be considered (Dubey and Guerra 2002). The literature shows that considerable work has been done for enhancing secondary metabolite precursors in medicinal plants. Through genetic transformation, essential oil synthesis has been increased in the trichomes of *Mentha* spp. and increased resistance to fungal infections and abiotic stresses has been developed (Veronese et al. 2001). Tissue culture is another method for the commercial production of phytochemicals. In some important medicinal plants, such as *Papaver somniferum* L., *Artemisia* spp. (Chen et al. 1999), and *Taraxacum platycarpum* Dahlst. (Lee et al. 2004), their bioactive compounds have been improved through genetic transformation, using bacterial genes transferred into the DNA of cultured plants. *Agrobacterium tumefaciens*-mediated transformation at the gene level is well established for higher productivity in Taxus (Han et al. 1994), Echinacea (Wang and To 2004), Scrophularia (Park et al. 2003), Digitalis (Sales et al. 2003), Thalictrum (Samanani et al. 2002), and Artemisia (Chen et al. 2000). It has now been made easy to regenerate whole plants from culture, except for *G. biloba* Linn., as it remains recalcitrant (Balz et al. 1999).

Pathway Engineering

Through transgenesis, new transgenic medicinal plants can be successfully developed for higher alkaloid production. In medicinal plants, active biochemical metabolism pathways are rarely understood and only a few genes have been isolated for the main enzymatic steps. Progress in pathway engineering has been made for higher alkaloid production in *P. somniferum* L. (Facchini et al. 2001). The development of transgenic Artemisia plants has led to three times more artemisinin alkaloid in the plant than in normal Artemisia plants (Chen et al. 1999, 2000). Thus, transgenic approaches, coupled with well organized gene isolation techniques, can certainly clarify the complicated metabolic pathways in medicinal plants needed for enhancing secondary metabolite production.

Improving Agronomic Traits

For improving the agronomic traits of medicinal plants, characteristics such as resistance to herbicides, pests, and diseases are taken to be the first step. For example, transgenic Atropa plants show resistance to bialaphos and glufosinate (Saito et al. 1992) and *Panax ginseng* shows resistance to Basta herbicides (Bayer crop science Pvt Ltd, Australia). This has been made possible by the induction of the phosphinothricin acetyltransferase enzyme (Choi et al. 2003). Transgenic medicinal plants like *Panax quinquefolium* L. (American ginseng) have been developed by transferring the chitinase/thaumatin antifungal gene into American ginseng, and the resultant plant shows considerable resistance to fungal disease (Punja and Chen 2003). The application of biotechnology has great potential for the development of medicinal plants that are resistant to abiotic stresses (Dubey and Guerra 2002). Morphological variations can also be introduced successfully in medicinal plants. It has been observed that when genes from *A. rhizogenes* were transferred to *Taraxacum platycarpum* Dahlst., morphological variations were shown when *T. platycarpum* Dahlst. was regenerated from root culture (Lee et al. 2004).

Planting Materials

It must be ensured that the identity of seed/vegetative parts should be specific to the true-to-type plant. All necessary information, such as breeding history, collection area, quality, identity, and product performance must be provided for the germ plasm of the raw material. Healthy, vigorous planting materials that have resistance/tolerance to pests (weeds/diseases/insects) and environmental conditions should be selected. Preference must be given to certified seeds. All planting materials must be well labeled and documented in terms of the methods of genetic/traditional improvement. Genetically modified planting materials must meet regional/national regulation criteria and the requirements of the production systems. During the production process, care must be taken for the safely and purity of the planting materials and the lack of adulteration.

Cultivation

Medicinal plants are very sensitive to cultivation management factors, such as the appropriate use of input resources and the duration of cultivation, which are the major considerations in maintaining the quality of medicinal plants. The principles of best cultivation practices, including good agricultural practice standards and organic farming norms, should be followed for safe cultivation. Practices that enhance the physical, chemical, and biological properties of the medicinal plants should be followed to ensure their best possible growth and quality. All conservation

practices that lead to better soil organic matter and nutrient build-up, and reduced pests (weeds, diseases, and insects) should be adopted. The following points must be considered.

Climate and Soil

The phenology and biochemical properties of medicinal plants are greatly influenced by the length of the day, water supply, rainfall, temperature, duration of sunlight, and relative humidity. Soil factors such as the type of soil, use of tillage, drainage conditions, capacity for moisture retention, productiveness, and pH, should be met before the sowing/planting of the crop.

Seeds and Sowing

Germination of the seeds and ecological requirements are critical for some medicinal plants (Vines 2004). Fungal infection or mechanical damage to seeds generally leads to low seed germination rates. Seed treatment before sowing, stratification of the hard seed coat, and controlled conditions to provide a good environment for the seeds are some important factors that improve seed germination. For example, in f *Rauvolfia serpentina* (L.) Kurz. and *Panax quinquefolium* L. seeds, a controlled environment significantly shortened the time to stratification and increased seed viability, germination, and readiness of the seed for germination (Li et al. 2000). Suitable conditions for natural pollinators and artificial pollination for better seed development and germination are also essential. Protected cultivation techniques and soil-less cultivation systems can be used for raising a nursery of medicinal plants that are difficult to propagate. Planting time and method, spacing, depth, and seed rate must be performed in a timely and accurate manner. Planting time and spacing have a great influence on plant growth, yield, and bioactive constituents.

Cropping Systems

Cropping systems are some of the best ways to utilize land, as well as to build up soil health. These systems also control most pest/pathogen problems. Cover cropping with green manure should be done with legumes before planting/sowing medicinal plants. Green manure is the best option for enhancing the soil biological, chemical, physical, and ecological environment. Medicinal plants or other crop-based cropping systems should be adopted to meet the requirement of the farmer economy. At the same time, proper utilization of land and the co-existence of main and associated crops should be ecofriendly to manage the agro ecosystem. The following cropping systems can be used.

(i) *Crop rotation*: Rotating crops that can build up soil biological and nutritional properties should be used. For example, rainy season pulses should be followed by plants such as *Plantago ovata* Forsk. and *Anethum graveolens* L./*Ammi*

majus L. in the winter season, followed by cowpea fodder in summer. If a farmer is growing kharif grain crops like maize, sorghum, and pearl millet, these should be followed by short-duration leguminous medicinal crops like fenugreek, opium poppy, and rabi season seed species (e.g., coriander, dill), followed by summer legumes like mugdaparni (*Phaseolus trilobus*), or if the farmer is interested in growing fodder, then sorghum+cowpea (summer) can be grown.

(ii) *Intercropping*: Shade-loving medicinal crops like *Centella asiatica* L. can be intercropped with arhar *(Cajanus cajan)*. Also onion/garlic can be intercropped with *Mentha arvesis* L. Aromatic grasses (lemon grass, citronella, palmarosa) can be successfully grown under *Eucalyptus citriodora* trees in plantations.. *Rauvolfia serpentina* L. can be grown successfully with mung bean and mugdaparni (*Phaseolus trilobus*) in both kharif and summer seasons. In addition to pulses, the planting of onion/garlic with *Rauvolfia serpentina* L. can be more remunerative.

Fertilizer Use

Fertilizer use must be site-specific and of sufficient quantity. Well decomposed organic manure needs to be used in a timely manner. A safe standard of acceptable microbial limits is essential. To prevent weed seed germination it is essential to use pure seeds and follow sanitation standards for soil health. Fertilizers need to be applied as needed for the crop and with consideration of minimum leaching. Activities such as soil conservation and minimization of tillage should be included in the fertilizer plan. Crops requiring low fertilizer use, e.g., *Ocimum* spp., should be grown in soils with low fertility.

Irrigation

The precise use of irrigation water is required according to the need of the medicinal crop, considering important growth stages of the crop plants. The irrigation water should meet quality standards. Excess moisture and stress conditions should be avoided. Irrigation water used must be checked regularly for contamination. Proper drainage facilities should be provided, as most medicinal crops, such as isabgol, mints, ocimum, and root crops are sensitive to waterlogging.

Intercultural Operations

Intercultural operations such as earthing, mulching, detopping, debudding, pruning, and providing shade where required are essential for better quality, growth, and development of the crop under cultivation. To control weed problems, mulching (organic mulch, crop residues etc.) should be done. Perennial weeds should be removed and, when required, appropriate intercropping should be performed.

Agrochemicals

Agrochemical use should be in accordance with the recommendations on the product labels and should be at minimal levels. Integrated techniques should be applied to

reduce the use of chemicals. Regulatory requirements applicable to both the producer and user countries should be followed. No agrochemicals containing chlorine molecules should be used in terpene-containing medicinal plants, because when chloride is conjugated with the alcoholic group, as in menthol mint, menthol is conjugated with the chlorine molecule and the plant quality is degraded. The person applying the agrochemicals should be well trained in all operations. The use of all these chemicals, including intervals between applications, should be documented. The interval between the use of the chemical and harvest should be in accordance with the product label, and such treatments should be carried out with the agreement of the buyer of the produce. International standards/norms on pesticide use, such as the Codex Alimentarius, good agricultural practice (GAP) standards, and organic standards, must be followed. The best way to avoid chemical use is to use resistant/tolerant varieties of medicinal crops.

Harvesting

Climatic conditions and the time of harvesting are the most important factors for obtaining higher production of medicinal plants and their quality products. Decision on time of harvesting depends on the plant part used. The best time for harvesting is at the crop growth stage when the quality and quantity of biologically active constituents are highest. Clean harvesting procedures should be followed. Harvesting of the crop should be avoided during rainy or humid conditions. Material harvested under wet conditions needs to be immediately transported to an indoor drying facility to protect the material from fermentation and mold. All cutting equipment/machinery must be well sanitized and stored in places free from contamination by insects, animals, birds, and chemicals. Contact of the harvested material with soil must be avoided. Underground plant parts (tubers and roots) must be cleansed of adhering soil immediately after harvest.

Transportation

Clean and dry conditions are essential for the transportation of the harvested materials to the processing units. The containers used need to be well decontaminated before use. To avoid the growth of mold, there should be no moisture in plastic containers. During transport, the harvested materials should not be compacted . Before transportation, decaying and undesirable material should be removed from the harvested materials to prevent quality losses due to microbial contamination.

Harvesting from Natural Sources

The majority of medicinal plants in the trade are harvested and collected from natural sources. The long-term viability of wild collected medicinal plants must be ensured

by following conservation techniques. Good agricultural practices standards for the wild collection of medicinal plants must be followed. The person harvesting medicinal plants from natural sources should be trained in the best harvesting and collecting techniques, and in the proper transportation and handling of equipment and plant materials, as well as in removing undesirable material from the harvested material, and proper drying and contamination-free dry storage methods. Wild collection of medicinal plants has certain social and ecological impacts on local communities, and therefore the sustainability of the target species must be ensured. When collecting roots, only a few lateral roots should be collected. For bark collection, only a longitudinal bark strip on one side of the trunk/thick branches of the tree is harvested.

Processing

Processing of the plant material depends on the state in which the material is to be used. If it is to be used in the fresh state, it must be transported to the processing unit as soon as possible. If the material needs to be stored for some time, refrigerated storage must be used. The use of preservatives should be avoided. Some processing techniques are detailed hereunder.

Primary Processing

Processing is done in different ways according to requirements.

Drying

To increase shelf life and protect the harvested plants from microbial contamination, moisture reduction is performed so that the moisture content is less than 10%. For different crops/plant parts, standards are given in various pharmacopoeias/authoritative monographs, which should be consulted for standard moisture content. In general, 6–8% moisture content is safe for storing dry products. If drying is done under field conditions, a clean muslin cloth should be placed between the harvested plants and the soil. For shade- and sun-drying, the plant materials should be spread on a cement surface and covered with a tarpaulin sheet. Pests, livestock, and domestic animals should not have any access to drying sites. Drying can be done using the following techniques.

Shade Drying

In the open air, the harvested material is spread in thin layers on a shaded floor. The material can also be spread on drying frames or in wire-screened rooms in buildings. A thin cloth at the interface of floor and over the plant material is used to avoid direct contact of the plant material with the floor. For volatile oil-containing medicinal plants, a lower temperature is preferred for drying. Some plants, e.g., mints, ocimum,

and aromatic grasses, are essentially dried to the wilting stage in the field or semidried under shade before further processing.

Natural Drying (Sun-Drying)

For sun-drying, open areas are preferred. A thin cloth is spread on the drying floor and the harvested material is spread in a thin layer on this cloth. For uniform drying, the plant material is frequently turned over. This helps in reducing the chance of mold contamination.

Artificial Drying

The most commonly used artificial drying method is a hot air dryer. Other methods, such as baking, lyophilization, or the use of microwave ovens or infrared devices may also be utilized, depending on availability and need. Temperature and humidity are important factors to be considered during artificial drying. High temperature and humidity lead to the destruction of biochemicals in the plants, and the quality of the final products, as well as the raw material, thus deteriorates. Hot conditions are needed for drying and natural gas or butane or propane should be used to generate heat . Fire drying should be avoided or it should be done under specific conditions as an alternative. A suitable temperature in dryers is 65 ± 2 °C. In artificial drying, direct contact between the source of heat, the medicinal plant raw material, and smoke must be prevented.

Specific Processing

After drying/semidrying, the plant materials must meet specific requirements to maintain purity. To reduce processing time and prevent damage, detoxification and the enhancement of therapeutic efficacy are some of the processing factors required. Common processing practices include the selection of uniform material, peeling of underground plant parts, cooking the plant material in boiling water or in steam for some time, soaking it in water, hydro-steam distillation, roasting, fermentation, and altering larger parts according to requirements. After these steps the raw material is subjected to secondary processing for various products.

Packaging

Packaging is done in clean and dry containers according to standard operating procedures and regulations. Hygienic, dehydrated, eco-friendly, and untouched materials should be used for packaging. The packaging materials should be free from contaminants and stored in a clean, safe, and dry place and thoroughly dried

prior to use. The packaging material must conform to the quality requirements for medicinal plant materials. Delicate plant materials need to be packed in hard containers.

Labeling

The labeling shows the source of origin and date of processing. The label on the packaging should contain the common/scientific name of the material, the plant part and origin, name of the cultivator/collector/processor, date of collection/processing, quality approval, and batch number. A certificate indicating production details and quality characteristics of the packed material should also be added.

Storage and transportation

Clean transportation should be used for the packed materials. All transport modes, i.e., truck, ship, or rail, must be clean, well ventilated, and sanitized. Low temperatures, 2–8 °C for fresh medicinal plant materials and <−20 °C for frozen products, should be maintained in storage areas. Drying material should be stored in dry and dark rooms over wooden frames. If the material is stored in containers, these should be filled to the brim and closed with corks to make them airtight. Essential oils should be kept in colored bottles filled to the brim and the bottle mouth should be closed to make them airtight; the bottles should be stored in a cool, dark room.

Bioprospecting

Bioprospecting of medicinal plants is the study, collection, and viewing of their habitat and distribution, as well as traditional knowledge about medicinal plants that are commercially valuable. During the early stages of the bioprospecting of medicinal plants, this activity was largely centered on the forest ecosystem only for the direct utilization of plants. At present, secondary metabolites, insect extracts, decoctions, and other living organisms are also subject to bioprospecting. Further prospecting of medicinal plants has been undertaken in the area of nutraceuticals and aromatherapy. Coffee, tea, and prune juice are examples of foods that are actually used as drugs. The bioprospecting of medicinal plants for pharmaceutical purposes is useful for pharmaceutical firms as well as for the country and the people who use and live in the vicinity of these plants, confirming the rights of natural possession. Ancient Indian records of the period between 4000 and 3000 BC, as well as ancient Chinese and Egyptian records, indicate that plants were used therapeutically. Herbs

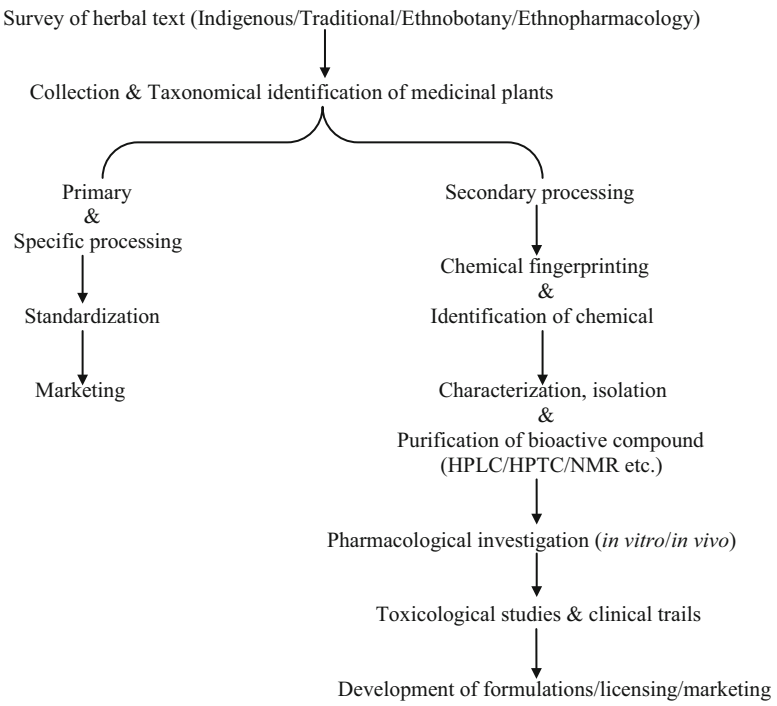
were also used indigenously as medicines and in healing rituals. The rich knowledge of medical herbalism in Siddha medicine and Ayurvedic medicine (India) and Kampo (Japan) is still used by a large majority of people. In both developing and developed countries, a majority of people still use traditional Chinese medicine (TCM), and Unani medicine (Middle East and South Asia). In the past two centuries medicinal practices in India have mainly been plant-based. Reports from different systems indicate that in Ayurvedic medicine, a maximum of 2000 plants are used, followed by 1300 in Siddha medicine, 1000 in Unani medicine, 500 in Tibetan medicine, and 800 in homeopathy. In the folk tradition 4500 plants are used for the treatment of different ailments. The Tibetan and modern systems of medicine use about 500 and 200 different medicinal plants, respectively. Worldwide, 52,885 (10%) out of about 297,000 native species of plants have medicinal value. In India, 8000 species of higher plants (44%), out of 17,000 species, are known to have medicinal value. A significant percentage of the population in developed countries such as Belgium (31%), United States (42%), Australia (48%), France (49%), and Canada (70%) rely on traditional and alternative remedies using plant-based drugs. Europe, North America, Asia, Japan, and other countries account for 33, 20, 26, 11, and 10%, respectively. The historical use of medicinal plants can be divided into at least three different but overlapping philosophies and forms of application, as described below.

(i) *Popular or folk medicine*: This is a non-institutional medical system. The oldest form of medicinal therapy, folk medicine has survived in most countries to the present day. Traditional knowledge of the use of medicinal plants for therapeutics has been transferred from one generation to another in indigenous tribes as well as individual families.

(ii) *Alternative medicine*: This is a worldwide institutionalized system of medicine. It forms a link between folk medicine and modern medicine. Most of the therapeutic concepts in alternative medicine developed long before the origin of modern medicine. A global resurgence of various alternative medicinal systems has been noted for some time; the most viable of these systems are homeopathy, Ayurvedic medicine, and TCM.

(iii) *Modern Western medicine*: This therapeutic system has existed for the last 300 years. Most of the molecules used for manufacturing medicines used in this system originate from the active constituents of medicinal plants. In this system, disease symptoms are treated with a precise amount of active chemical. These chemicals are mostly synthesized in pharmaceuticals laboratories and used as single or in combination of different chemicals. Recently, a consensus has developed that no medical system can claim exclusive healing capacity, and that the folk/traditional, alternative, and modern systems of medicine can complement one another if they are mutually open to each other's methods and principles. This holds true for medicinal plants, where it is common to see one and the same species being used for the treatment of symptoms in the alternative as well as modern Western medicinal systems.

Methodology for Bio-prospecting of Medicinal Plants



Herbal Medicinal Trade

India

India's share of the global herbal medicinal market is 0.5%, valued at $US358.60 million, exporting to around 150 countries in 2015–2016, while the export of value-added products of medicinal plants amounted to $US234.15 million in 2014–2015. The Indian nutraceutical industry is expected to grow by 20% to $US6.1 billion by 2019–2020. India is next only to China in herbal medicinal exports, accounting for about 13% of global exports. The United States is the principal market for Indian medicinal plants. India is exploiting less than 50% of its potential. In 2015–2016, India mainly exported opium alkaloids, senna derivatives, isabgol, ipecac root alkaloids, vinca extract, cinchona alkaloids, diosgenine/16DPA, solasodine, gurmar herb, menthol, mehndi leaves, sarpagandha, papain, guar gum, agarwood oil, sandalwood oil, and jasmine oil. It is estimated that approximately 25,000 licensed pharmacies operate in the Indian system of medicine.

At present about 1000 single drugs and 3000 compound formulations are registered in this system, but fewer than 25 pharmacies are classified as large-scale manufacturers. Although the importance of medicinal plants and the natural product industries to the national economy is being stressed at all governmental levels, there are too few large-scale manufacturers in India. Thus, prospects are great to develop these industries. To achieve higher quality of the desired biochemicals, the standardization of medicinal plant raw materials is now of growing interest . Such standardization will lead to the identification of the total biochemical content in plants and this could be correlated with the biological activity of the plants in treating ailments.

Global

The global herbal industry is expected to be worth about $ US107 billion, with good growth potential, by the end of 2017. According to World Health Organization (WHO) estimates, the international market for herbal products is expected to grow to $US5 trillion by the year 2050 (Agarwal et al. 2013). The high prices and the side effects of synthetic drugs have led people worldwide to depend on herbal drugs. The global nutraceutical market is expected to reach US$278.96 billion by the end of 2021, with a growth rate of 7.3% per annum. The six nations of the Gulf Cooperation Council—Saudi Arabia, Kuwait, United Arab Emirates, Qatar, Bahrain, and Oman—represented the biggest nutraceutical market, of about €3.25 billion in 2014, and this market is set to hit €4.86 billion by the year 2020. The increasing demand for botanical remedies is both a national and an international trend. Worldwide, the most widely used and best-selling botanicals are ginseng, santalum, ginger, garlic powder, isabgol, herbal tea, and red clover extract, and these are demanded owing to the increasing population of senior citizens and the relative lack of side effects of botanicals. A United Nations Department of Economic and Social Affairs survey (2017) estimated that there would be over 2 billion people aged 60 and above worldwide by the end of 2050. This increasing number of older people has led to the importation of herbal drugs by several countries.

Bioprospecting of Medicinal Plants in Regard to Various Ailments

Medicinal plants specifically used to treat major ailments are grouped in this section, with a brief outline of the ailments.

Asthma

Asthma is a common disease with a high prevalence in industrialized countries. Bousquet et al. (2005) have suggested that 400 million people worldwide will be affected by this disease by 2025. Asthma is a complicated multifactorial pulmonary disease that is diagnosed through reversible blockage in the bronchial tract, increased external bronchial responses, and chronic inflammation of the bronchial system. Asthma is defined as a chronic inflammatory disorder with intermittent symptoms of

Table 1 Plants used to treat asthmatic problems (Common name in Hindi/English)

Serial No.	Botanical, common name	Part used	Active principles
1	*Achyranthes aspera* L, Apamarg/prikly chaff flower	Roots, leaves	Ecdysterone, saponins
2	*Ageratum conyzoides* L, Jangli Podina/Goat weed	Leaves	Friedelin, sterol, ß-sitosterol
3	*Bacopa monnieri* L. Thyme Leaved Gratiola/ Brahmi	Whole plant	Bacosides, stigmasterol
4	*Bryophyllum pinnatum* (Lam.), Patharchur/ Cathedral Bells	Leaves	Astragalin
5	*Cassia sophera* L., Kasaundi/Senna	Root bark	β-Sitosterol, chrysophenol, physcion
6	*Casuarina equisetifolia* L., Vilayati Saru/ Bull-oak	Leaves	Saponins
7	*Euphorbia hirta* Linn., *Bari* Dudhi/common spurge	Whole plant	Afzelin, quercitrin
8	*Hedychium spicatum* Buch.-Ham. ex Sm., Kapoor Kachri/Kaempferia	Rhizome	Tocopherol, terpinene, linalool
9	*Menthe spicata* L., Spear Mint	Leaves	Limonene, dihydrocarvone, menthone
10	*Piper betel* L. Paan/betel leaf	Leaves	Arakene, glutathione peroxidases
11	*Saussurea lappa* C.B.Clarke, Kuth/saw-wort	Roots	Phenolics, flavonoids
12	*Adhatoda vasica* Nees, Vasaca/Malabar nut	Leaves	Vasicine

cough, dyspnea, wheezing, and chest pain. When an asthmatic patient comes into contact with an allergen, histamine and other substances are released, causing the bronchial muscles to be constricted, reducing the diameter of the bronchi; the bronchial mucous membranes become swollen, and secretions from the swollen mucous lining fill the constricted lumen of the bronchi, leading to difficulty in breathing. Medicinal plants with immunomodulatory, smooth-muscle relaxant, anti-allergic, anti-inflammatory, and antihistaminic properties are most suitable to control this disease. The major relevant plants are listed in Table 1.

Oral Health

Oral health is related to the functioning of the complex craniofacial area. Most of the oral cavity hosts microbiota. Of the total of 750 bacterial species inhabiting the oral cavity, causing oral diseases, only 50% have been identified. The most important oral health problems are oral/pharyngeal cancers, oral tissue lesions, dental caries, and periodontal problems. In India, most adults and about 90% of school-aged children are affected by dental problems. Traditional systems of medicine together with folk medicine systems continue to be used to treat oral diseases in a large proportion of the population, particularly in rural areas. Important medicinal plants used for enhancing oral health are listed in Table 2.

Table 2 Medicinal plants used for oral health (Common name in Hindi/English)

Serial No.	Botanical/common name	Part used	Active principles
1	*Azadirachta indica* A. Juss., Neem/Indian lilac	Leaves, twigs, flowers	β-Elemene, pentacosane
2	*Acacia arabica* (lamk) Willd, Babool/Indian gum	Bark	Tannins
3	*Acacia catechu* Willd., Khair/acacia tree	Bark	Tannins
4	*Albizia lebbeck* (L.) Willd., Siris/lebbek tree	Bark	Tannin, *d*-catechin
5	*Achyranthes aspera* Linn., Apamarg/ prikly chaff flower	Root	Saponins
6	*Argemone mexicana* Linn., Satyanashi/ Prickly Poppy	Seed	Berberine
7	*Agave americana* L., Kamal Cactus/ American aloe	Whole plant	Steroidal saponins, hecogenin
8	*Bombax ceiba* L., Semal/'Malabar Silk-cotton Tree of India	Bark	Lupeol, saponins
9	*Bauhinia variegata* L. Kachnar/Orchid Tree	Tender twigs	Tannins
10	*Caram carvi* Linn., Kala Jira/Caraway	Dried ripe fruit	(R)-Carvone
11	*Camellia sinensis* (L.) Kuntze/Green tea	Leaves	Polyphenols
12	*Commiphora molmol* Engl., Hirabol/ Myrrh	Stem	Resin, gums, volatile oil
13	*Curcuma longa* Linn., Haldi/Turmeric	Dried roots	Tannins
14	*Echinacea purpurea* (L.) Moench, Indrajao/Purple Coneflower	Roots	Alkylamides
15	*Eucalyptus globosus* Labill, Neelgiri eucalyptus/Blue Gum	Leaves	1,8-Cineol
16	*Emblica officinalis* Gaertn. Amla/Indian Gooseberry	Fruit	Vitamin C
17	*Ficus bengalensis* L. Indian banyan/ banyan fig	Prop roots	Flavonoids, alpha-D glucose
18	*Ferula asafoetida* Linn. ferula/Hing	Root extract	Foetisulphide A, foetisulphide C
19	*Glycyrrhiza glabra* Linn., mulathi/ Liquorice	Roots	Glycyrrhizin, flavonoids
20	*Hibiscus rosa sinensis* Linn., Gurhal/ changing rose	Young stems	Glycosides, diterpenes
21	*Indigofera tinctoria* L., True indigo/ Neell/true indigo	Young branches	Glycoside, alkaloids
22	*Jatropha curcas* L., Jangli arandi/ Purging nut	Fresh stem	Flavonoids, saponins
23	*Krameria triandra* Ruiz & Pav., Ratanya/Peruvian rhatany	Root bark	Tannic acid
24	*Mangifera indica* L., Aam/Mango	Leaves	Pentacyclic triterpene alcohol

(continued)

Table 2 (continued)

Serial No.	Botanical/common name	Part used	Active principles
25	*Menthe piperita* L./Peppermint	Leaves	Menthol
26	*Moringa oleifera* Lam., Sahajan/Moringa	Leaves, stem, roots	Carotenoids, vitamin C
27	*Morus alba* Linn., sahtut/Mulberry	Fruits	Anthocyanosides
28	*Matricaria chamomilla* L., *Baboosa*/ Chamomile	Dried flowers	Volatile oils, flavonoids, biflavonoids
29	*Ocimum sanctum* Linn. Ram tulsi/Basili	Leaves	Ursolic acid, apigenin, luteolin
30	*Potentilla erecta* L., Septfoil/Tormentil	Dried roots	Tannins
31	*Piper betle* L., Paan/betel leaf	Leaves	Glutathione peroxidases
32	*Qurcus alba* L., Bargad tree/White oak	Bark	Tannins
33	*Rosa canina* L. Gulab/Rose	Hips, leaves, flowers	Tannins, vitamin C
34	*Rubus fructicosus* L., Bramble/ Blackberry	Leaves, roots	Tannins
35	*Syzgium aromaticum* L., Lawang/Clove	Flower buds	Eugenol
36	*Salvadora persica* Linn., Miswak/ Mustard tree	Bark, leaves	Eucalyptol
37	*Salvia officinalis* Linn., Salvia/Sage	Leaves	α-Thujone, α-humulene
38	*Vaccinium myrtillus* Linn., Bilberry/ Blueberry	Ripe berries	Anthocyanosides

Diarrhea and Dysentery

Diarrhea and dysentery are said to be endemic in many regions of Asia and are the leading causes of high rates of morbidity and mortality. Diarrhea alone causes about 5 million deaths annually worldwide, of which 2.5 million are in children aged less than 5 years. The WHO defines diarrhea as the "passage of loose or watery stools at least three times in a 24-h period", but emphasizes the importance of change in stool consistency rather than frequency, and the usefulness of parental insight in deciding whether or not children have diarrhea. Blood in the stool is indicative of an acute diarrheal illness. Diarrhea in humans is mainly due to infection with *Escherichia coli* Castellani & Chalmers, *Shigella flexneri* Castellani & Chalmers, *Salmonella typhi, Candida albicans* Berkhout, and *Staphylococcus aureus* Rosenbach. *Shigella* spp. are also responsible for acute bloody diarrhea that accounts for about 15% of all diarrheal deaths. The WHO also advocates the use of traditional medicines as practiced by indigenous people, since a large proportion of rural people in developing countries still use these medicines as the first safety measure. Table 3 indicates some major plants used to treat diarrhea and dysentery.

Malaria

Malaria is one of the oldest known diseases in humans, having been described in ancient times. The disease is caused by the malaria parasite, which is transmitted by

Table 3 Medicinal plants used for the treatment of diarrhea and dysentery (Common name in Hindi/English)

Serial No.	Botanical/common name	Part used	Active principles
1	*Acacia catechu* (L.f.) Willd., Khair/Acasia tree	Leaves	Kaempferol
2	*Acorus calamus* L. Bach/Sweet flag	Rhizome	Acorenone
3	*Asparagus racemosus* Willd., Shatavari/Asparagus	Whole plant	Shatavarin (roots) quercetin, rutin, hyperoside (flowers/fruits), glucuronide (leaves)
4	*Azadirachta indica* A. Juss, Neem/Indian Lilak	Leaves, bark	Terpenoids
6	*Boerhavia diffusa* L., Punarnava/spreading hogweed	Roots	Rotenoids
7	*Bryophyllum pinnatum* (Lam.), Patharchur/Cathedrel bells	Leaves	Glycosides
8	*Coriandrum sativum* L., Dhaniya/Coriender	Seeds	Linalool
9	*Cynodon dactylon* (L.) Pers., Doob/Bermuda grass	Plant extract	Hexadecanoic acid
10	*Cyperus rotundus* L., Motha/ Purple nutsedge	Rhizomes	α-Cyperone
11	*Dactylorhiza hatagirea* (D. Don), Hattazari/Himalayan Marsh Orchid	Tubers	Starch, mucilage
12	*Dalbergia sissoo* DC., Shisam/ North Indian Rosewood	Leaves	Isoflavone, sissotrin
13	*Euphorbia hirta* L., Lal dudhi/ Common spurge	Whole plant	Terpenes, anthocyanins
14	*Ficus racemosa* L., Gular/Indian fig tree	Stem bark	Steroids, lupeol
15	*Madhuca longifolia* Roxb., Mahua/Honey tree	Flowers	Carotene, vitamin C
16	*Murraya koenigii* L., *Bishahari/Curry leaf-tree*	Green tender leaves	Carbazole, alkaloids
17	*Musa balbisiana* Colla., Kela/ Banana	Half-ripened fruit	Tannin, alkaloids, saponin
18	*Oxalis corniculata* L., Amrul/ Creeping woodsorrel	Leaves	Vanillic acid, tartaric acid
19	*Punica granatum* L., Anar/ Pomegranate	Fruit rind (dried)	Ellagitannin
20	*Sesbania grandifolia* L., Agathi/Hummingbird tree	Leaves	3,4,5-Trimethoxyphenol
21	*Syzygium cumini* L., Jamun/ Black plum	Roots	Isorhamnetin 3-O-rutinoside

(continued)

Table 3 (continued)

Serial No.	Botanical/common name	Part used	Active principles
22	*Saraca indica* L., Sita Ashok/ Ashoka	Stem bark decoction	Leucopelargonidin, leucocyanidin
23	*Terminalia arjuna* (Roxb.ex DC.), Arjun/Arjun tree	Bark	Arjunolic acid, terminic acid
24	*Tamarindus indica* Linn., Imli/ Tamarind	Fruit pulp	Serine, beta-alanine
25	*Ziziphus mauritiana* Lam., Ber/Indian plum	Roots	Tannin, cyclopeptide alkaloids

an infected mosquito. Global estimates indicate that 3.4 billion people are still at risk of malaria. According to WHO (2016) in 2015, there were roughly 212 million malaria cases and an estimated 429 000 malaria deaths. In 2012, 207 million people globally were reported to be infected with malaria, and 90% of deaths due to malaria occurred in Africa, of which 77% occurred in children under the age of five. Ayurveda also mentions malaria and its management under the heading of irregular fever (*Vishhamjar*), especially tertian (*Trutiyaka*) and quartan (*Chaturtak*). Various plants have good prospects for the treatment of malaria, as used by Indian communities; the main relevant plants are listed in Table 4.

SnakeBite

Snakebite is a major health hazard worldwide, with 600,000 cases of snakebite and more than 20,000 deaths per year. Most reports of snakebite are from agricultural and tropical regions. Only 4 of the 216 species of snakes in India are highly poisonous; namely, the cobra, krait, Russell's viper, and saw scaled viper. In India alone more than 200,000 snakebite cases are reported per year, with deaths due to snakebite estimated to be 35,000 to 50,000 per year. Traditional herbal healers know about herbs to treat snakebite. They use single plants or combinations as antidotes for snakebite. Important plants with the parts used and active constituents are listed in Table 5.

Gynecological Disorders

Ethno-gynecology is a traditional approach of rural people for dealing with female health issues. Medicinal knowledge incorporating knowledge of plants, or any other natural/spiritual therapies used to treat such gynecological problems such as abortion, menstrual pain, menopause, morning sickness, leucorrhea, infertility, and delivery problems are taken into consideration in this approach. Nowadays, gynecological disorders are mostly treated according to the modern Western system of medicine. Though the efficacy of these treatments is great, there are potential side effects related to the skin; sexual problems; digestive problems; and liver, kidney, and heart injury. Moreover, during pregnancy, the embryo is at risk of harm by some drugs used. A number of traditional herbal medicines are used as abortifacients and

Table 4 Medicinal plants used for the treatment of malaria (Common name in Hindi/English)

Serial No.	Botanical/common name	Part used	Active principles
1	*Ailanthus glandulosa* Desf., Mahanimb/Tree of Heaven	Bark	Ailanthone
2	*Artabotrys hexapetalus* Linn./Hara champaa/ Climbing lang-lang	Roots	Artabotrine
3	*Cinchona calisaya* Wedd./Kunain	Bark	Quinine
4	*Enicostemma hyssopifolium* Willd., Chota Chirayata/Indian gentian	Roots	Gentianine
5	*Gloriosa superba* Linn., Kalihari/Aloriosa lily	Rhizome	Colchicine
6	*Hedychium spicatum* Ham. Kapur kachri/spiked ginger lily	Rhizome oil	Cineole
7	*Jatropha gossypifolia* Linn., Ratanjoti/black physicnut	Whole plant	Triterpenes, diterpenes
8	*Lantana camara* Linn., Raimuniya/Lantana	Whole plant	Lantanine
9	*Leonotis nepetaefolia* L., Lal guma/Lion's Ear	Seeds	Oleic acid
10	*Parthenium hysterophorus* Linn., Congress grass/ Santa Maria feverfew	Whole plant	Parthenin
11	*Spathodea campanulata* Beauv., Ruugatuuraa/ Fountaintree	Stem bark	Oleanolic acid
12	*Toddalia asiatica* Linn., Jangali Kaali-mirch/Orange Climber	Root bark	8-Formyllimettin

anti-abortifacients, to regulate the menstrual cycle and enhance fertility. Some major medicinal plants used in these herbal drug preparations are listed in Table 6.

Heart and Cardiovascular Diseases

Cardiovascular diseases creating heart problems have become a major concern worldwide. This is mainly due to our inappropriate eating habits and stressful living, which leads to thickening or hardening of the arteries, causing obstruction of blood flow to the heart. Associated problems are ischemic heart disease and hypertension. According to a WHO (2017) survey, an estimated 17.7 million people died from CVDs in 2015, representing 31% of all global deaths. Of these deaths, an estimated 7.4 million were due to coronary heart disease and 6.7 million were due to stroke. The number is expected to reach 23.3 million by 2030. It is well recognized that secondary plant metabolites such as triterpenes and flavonoids are mostly effective in controlling various heart diseases. These two primary groups of biochemicals are found in various herbs of medicinal value in the Indian system of medicine. Lowering of blood pressure is generally achieved by the use of ginseng, owing to its high content of triterpenes. *Crataegus* spp. and *Ginkgo biloba* Linn. leaves are rich sources of flavonoids, the largest group of polyphenols, used to treat heart disease. The most effective flavonoids are flavonols. Bark extract of *Terminalia*

Table 5 Plants used for the treatment of snakebite (Common name in Hindi/English)

Serial No.	Botanical/common name	Part used	Active principles
1	*Achyranthes aspera* L., Apamarg/prikly chaff flower	Roots, leaves	Glycosides, oleanolic acid
2	*Acacia catechu* (L.f.) Willd., Khair/Acasia tree	Bark	Tannins, catechin
3	*Aegle marmelos* Linn., Bael/ Apple wood	Root bark	Marmelosin
4	*Albizia procera* Roxb., Shveta Shirisha/Tall albizia	Pods	Oleanolic acid, saponin
5	*Allium cepa* Linn. Pyaj/Onion	Bulbs	Alliin, allicin
6	*Ageratum conyzoides* L., Jangali tulsi/Goat weed	Leaves	Friedelin, sterol, ß-sitosterol
7	*Amaranthus spinosus* Linn., Katili-chaulai/Amaranth	Leaves	α-Spinosterol
8	*Azadirachta indica* A. Juss, Neem/Indian Lilak	Flowers	Pentacosane
9	*Bacopa monnieri* L., Brahmi/ Water hyssop	Stems, leaves	Bacosides, brahmine, stigmasterol; the moleaves contain herpestine and monnierine
10	*Bryophyllum pinnatum* (Lam.) Kurz., Zakham-e-hyat/ Cathedral Bells	Seeds	Alkaloids, glycosides
11	*Cassia tora* L., Chakavad/ Foetid cassia	Seeds	Anthraquinone, glycosides
12	*Cyperus rotundus* L., Motha/ Purple nutsedg	Rhizomes	α-Cyperone
17	*Ocimum sanctum* Sims., Tulsi/ Holy Basil	Leaves	Ursolic acid, apigenin, luteolin
18	*Piper nigrum* L., Kalimirch/ Black Pepper	Flower buds	Piperine
19	*Punica granatum* L., Anar/ Pome granate	Fruit, seeds	Ellagitannin, malvidin pentose glyglycoside
21	*Rauvolfia serpentina* (L.) Kurz., Sarpagandha/ Serpentroot	Roots	Reserpine, yohimbine
22	*Salix alba* L., Salix/White willow	Bark	Salicylic acid
23	*Withania somnifera* Linn., Asgandh/Indian ginseng	Roots	Withanine

arjuna Wight & Arn. is commonly used to treat heart problems in the Indian system of medicine. These natural herbal bioactive products are very useful in heart-related problems and are also found in abundance in nature. The major plants used for the treatment of heart and cardiovascular diseases are listed in Table 7.

Table 6 Medicinal plants used for the treatment of gynecological problemss (Common name in Hindi/English)

Serial No.	Botanical/common name	Part used	Active principles
Abortion (to induce)			
1	*Averrhoa carambola* Linn., Carambola/ Country gooseberry	Seeds	Galactogenic acid
2	*Asparagus racemosus* Willd., Satavari/ Asparagus	Roots	Antioxytocic saponins
3	*Eclipta prostrata* Linn., Bhringraj/False daisy	Roots	Thiophenes
4	*Plumbago zeylanica* L., Chitrak/Wild leadwort	Roots	Plumbagin
5	*Tectona grandis* Linn., Teak/Teak tree	Heartwood	Anthraquinones
Childbirth (to aid in delivery)			
1	*Achyranthes aspera* L., Apamarg/prikly chaff flower	Roots	Glycosides, oleanolic acid
2	*Anogeissus latifolia* Wall.ex Bedd., Bakli/ Axlewood	Gum	Ghattic acid
3	*Bombax ceiba* Linn., Semal/cotton tree	Stem bark	Mangiferin
4	*Ocimum sanctum* Sims., Tulsi/Holy basil	Leaves	Ursolic acid
5	*Rauvolfia serpentina* (L.), Sarpagandha/ Serpent root	Roots	Ajmaline
6	*Uvaria narum Blume*., Neelavalli/Indian Uvaria	Root bark	Stereoisomers
Gonorrhea			
1	*Bacopa monnieri* L., Brahmi/Water hyssop	Stem, leaves	Bacosides
2	*Corchorus depressus* L., Bhauphali/ Corchgorus	Dried plant parts	Mucilage
3	*Hemidesmus indicus* (L.) R. Br., Anantmool/Indian sarsaparilla	Roots	Coumarinolignoids
4	*Ocimum gratissimum* Linn., Jangali tulsi/ African basil	Leaves	Myrcene
5	*Phyllanthus simplex* Retz., Bhumyaamalaki/ Sand spurge	Fruit	Oxalic acid
Leucorrhea			
1	*Acanthus ilicifolius* Linn., Hargozaa/holy mangrove	Roots	Acanthicifoline
2	*Amaranthus tricolor* Linn., Laal Shaak/Edible amaranth	Whole plant	Amarantin
3	*Acacia catechu* (L.f.) Willd., Khair/Katechu tree	Seeds	Arecaidine
4	*Butea monosperma* (Lam.) Taub., Paalasha/ Butea	Bark	Kinotannic acid
5	*Cocculus hirsutus* (Linn.) Diels., Vasan vel/Broom Creeper	Leaves	Coclaurine
6	*Ficus lacor* Buch.-Ham., Pakar/White fig	Stem bark	Methylricinolate
7	*Ficus religiosa* Linn., Peepal/Sacred fig	Stem bark	Beta-sitosteryl-D-glucoside
8	*Nymphaea alba* Linn., Kumuda/White water lily	Flowers	Quercetin

Table 7 Plants used for the treatment of heart and cardiovascular disease (Common name in Hindi/English)

Serial No.	Botanical/common name	Part used	Active principles
1	*Aesculus hippocastanum* Linn., Buckeye/Horse chestnu	Seeds	Aescin
2	*Ayapana triplinervis* (Vahl) R. M.King & H.R., Ayapan/White snakeroot	Leaves	Ayapanin, ayapin
3	*Bryophyllum pinnatum* (Lam.) Zakham-e-hyat/cathedral bells	Leaves	Astragalin, bryotoxin A
4	*Camellia sinensis* (L.), Chaay/Tea	Leaves	Catechins
5	*Cinnamomum tamala* Nees & Eberm., Tejapatra/Indian bay leaf	Leaves	Furanogermenone
6	*Commiphora wightii* (Arnott), Guggal/Indian bdellium tree	Gum resin	Guggulipid, guggulsterone
7	*Crataeva nurvala* Buch.-Hum., Varuna/Three leaved caper	Stem, root bark	Lupeol
8	*Digitalis purpurea* Linn., Tilpushpi/Foxglove	Leaves	Digitoxin, digoxin
9	*Emblica officinalis* Gaertn., Amla/Indian gooseberry	Fruit	Vitamin C, gallic acid
10	*Erythroxylum coca* Lam., Sivadari/Coca plant	Leaves	Cocaine, tropacocaine, cinnamoylcocaine
11	*Ginkgo biloba* Linn., Ginkgo/Maidenhair tree	Leaves	Ginkgolides A and B, bilobalide
12	*Glycine max* Merrill., Soya bean/Soybean	Seeds	Protein, lecithin, saponins
13	*Hordeum vulgare* L., Jau/Barley	Seeds	Vitamin C, β-glucan
14	*Nelumbo nucifera* Gaertn., Lotus/Indian lotus	Leaves	Quercetin, luteolin
15	*Psidium guajava* Linn. Amrood/Guava	Roots, leaves, flowers, fruit	Quercetin
16	*Raphanus sativus* Linn., Muli/Radish	Roots	Caffeic acid
17	*Tamarindus indica* Linn., Imli/Tamarind	Fruit pulp	Serine, beta-alanine
18	*Vitis vinifera* Linn., Angoor/Grapevine	Ripe fruit	Procyanidin

Skin Diseases

In the developing world, skin diseases are mostly caused by adverse climatic conditions and various microbes contaminating food, water, and skin, coupled with unawareness of good hygiene. Bacteria, mainly *Staphylococcus aureus* and *Streptococcus*, viruses (Poxvirus, human papilloma virus, and herpes simplex virus) and fungi (yeast) are the major infectious agents causing skin diseases. The most common skin diseases in the developing world are wounds, conditions that cause itching, ringworm, dermatitis, eczema, leprosy, skin allergy, scabies, psoriasis, and conditions that cause swelling. Psoriasis affects about 2% of the population worldwide. Various plants used for the treatment of skin diseases are listed in Table 8.

Table 8 Plants used for the treatment of skin diseases (Common name in Hindi/English)

Serial No.	Botanical/common name	Part used	Active principles
1	*Acacia catechu* Linn., Khair/Catachu	Wood	Catechin
2	*Achillea millefolium* Linn., Gandana/ Common yarrow	Leaves	Achilleine
3	*Acacia farnesiana* L., Vilaayati Kikar/ Sweet acacia	Bark	Tannins, polyphenolic compounds
4	*Alpinia officinarum* Hance, Kulanjan/ Chinese ginger	Rhizome	Galangin
5	*Argemone mexicana* Linn., Satyanashi/ Prickly poppy	Roots, leaves, oil	Isoquinoline
6	*Artocarpus integrifolia* Linn., Kathal/ Jackfruit	Roots	Artocarpesin
7	*Bauhinia variegata* Linn., Kachanar/ Orchid tree	Bark	Hentriacontane, octacosanol
8	*Carthamus tinctorius* Linn., Safflower/ Dyer's Saffron	Seed oil	Polyunsaturated fat
9	*Cassia occidentalis* Linn., Kasamarda/ Coffee Senna	Leaves	Chrysophenol
10	*Ficus religiosa* Linn., Peepal/Sacred fig	Bark	β-Sitosteryl-D-glucoside
11	*Indigofera tinctoria* Linn., Neel/True indigo	Leaves	Indicine
12	*Lawsonia inermis* Linn., Mehndi/Henna	Leaves	Naphthoquinones
13	*Terminalia tomentosa* W., Sain/Indian laurel	Bark	β-Sitosterol, ellagic acids

Ulcers

Ulcers are a common disorder, showing inflamed dead tissue, with lesions on the surface skin or in mucous membranes. Gastrointestinal ulcers are most common in the lower extremity of the gastrointestinal tract, although they may occur at any site. Common ulcers are genital, oral, esophageal, and peptic. Worldwide, peptic ulcer is the most common, affecting about 10% of the population. The majority of peptic ulcers are duodenal. It has been estimated that about 15,000 deaths per year worldwide occur due to peptic ulcer. The main cause of gastrointestinal ulcers was said to be the high use of spicy foods, but these only exacerbate the problem. The actual cause is infection by bacteria (*Helicobacter pylori*), as well as reactions to nonsteroidal anti-inflammatory drugs. Factors such as the prolonged use of nonsteroidal medicines (such as pain killers), loss of beneficial bacteria, and a high multiplication rate of *Helicobacter pylori* bacteria, as well as the use of alcohol and smoking, together with emotional stress, are the most common causes of the disease. The major plants used to treat ulcers are listed in Table 9.

Table 9 Plants used for the treatment of ulcers (Common name in Hindi/English)

Serial No.	Botanical/common name	Part used	Active principles
1	*Acacia arabica* Willd., Babul/ Gum Arabic	Leaves	Phenolic compounds, tannins
2	*Adansonia digitata* Linn., Gorakh imli/Boabab	Leaves	Albuminoids
3	*Aegle marmelos* L., Bael/Apple wood	Fruit	Coumarins
4	*Allium sativum* Linn., Lahsun/ Garlic	Whole part	Alliin, allicin
5	*Aloe vera* L., Ghritkumaari/Aloe	Leaves	Barbalin, isobarbolin, saponins
6	*Annona squamosa* Linn., Sitaphal/Custard apple	Leaves	Saponins, tannins, flavonoids
7	*Azadirachta indica* A. Juss/Neem/ Margosa tree	Leaves	Nimbidin
8	*Bauhinia variegata* L., Kachnar/ Orchid tree	Bark	5, 7- dihydroxy flavanone-4-O-α-L rhamnopyrosyl-β-D-glycopyranosides
9	*Berberis aristata* DC., Dar Hald/Indian barberry	Roots, bark	Berberine
10	*Beta vulgaris* Linn., Chukandar/ Beetroot	Roots	Betin
11	*Carica papaya* Linn., Papita/ Papaya	Fruit	Chymopapain, papain
12	*Euphorbia neriifolia* auct. non Linn., Sehund/Common milk hedge	Whole plant	Euphorbon, euphol
13	*Ficus religiosa* Linn., Peepal tree/ Sacred fig	Bark	β-Sitosteryl D-glucoside
15	*Hibiscus rosa sinensis* Linn., Gurhal/changing rose	Roots	Quercetin
16	*Indigofera tinctoria* Linn., Nili/Indian indigo	Leaves	Indicine
17	*Lawsonia alba* L., Mehndi/Henna	Leaves	Lawsone
18	*Mangifera indica* Linn., Aam/Mango	Leaves	Pentacyclic triterpene alcohol
19	*Mimosa pudica* Linn., Chui-mui/ Touch me not	Leaves, seeds	Quercetin, naringin
20	*Momordica charantia* Linn., Karela/Bitter gourd	Whole plant	Momordicoside A
21	*Ocimum sanctum* L., Tulsi/Holi basil	Leaves	-Sitosterol
22	*Phyllanthus niruri* Linn., Bhuiamla/Stonebreaker	Whole plant	β-Sitosterol, gallic acid
23	*Psidium guyava* Linn., Amrud/ Gwava	Leaves	Quercetin, guaijaverin
24	*Shorea robusta* Gaertn. f., Sal/Sal Tree	Bark	Ursolic acid, amyrin
25	*Tamarindus indica* W., Imali/ Tamarind	Leaves	Tartaric acid

Table 10 Plants used for the treatment of digestive disorders (Common name in Hindi/English)

Serial No.	Botanical/common name	Part used	Active principles
1	*Aegle marmelos* Linn., Bael/Apple wood	Fruit	Scoparone, xanthotoxol
2	*Andrographis paniculata* (Burm.f.) Wall, Kalmegh/King of bitters	Whole plant	Kalmeghin, triterpenoids
3	*Baliospermum montanum* Willd., Danti/Red physic nut	Roots	Diterpenes, baliospermin
4	*Bacopa monnieri* Linn., Barami/Water hyssop	Leaves	Brahmine
5	*Carica papaya* Linn., Papita/Papaya	Fruit	Chymopapain, papain
6	*Commiphora mukul* Hook., Guggal/Indian bedellium	Gum	Guggulsterones Z and E
7	*Cinchona calisaya* Wedd., Kunain/Peruvian bark	Bark	Quinine, quinidine
8	*Coleus amboinicus* Lour., Patharchoor/Indian Borage	Leaves	Cirsimaritin
9	*Emblica officinalis* Gaertn., Amla/Indian Gooseberry	Fruit	Vitamin C
10	*Gmelina arborea* Roxb., Gamhar/White teak	Roots	Gmelinol
11	*Piper longum* Linn., Pipli/Indian long pepper	Roots	Piperine, piplartine
12	*Plumbago rosea* Linn., Chitrak/Plumbago	Roots	Plumbagin
13	*Pongamia pinnata* Linn., Karanja/Indian beech	Leaves	Triterpenoids
14	*Robinia pseudoacacia* Linn., Kikar/False acacia	Leaves	Toxalbumin

Digestive Disorders

With people changing to modern lifestyles, various digestive disorders are seen, such as excessive gas; constipation; burping; feelings of burning and acidity; vomiting; indigestion; bloating; and pain. Worldwide, significant numbers of people die due to digestive disorders. Lack of cleanliness and lack of awareness about avoidance of disease have led to the occurrence of various types of digestive diseases. The major plants used in India to treat digestive disorders are listed in Table 10.

Liver Disorders

Jaundice is the most common liver disorder. It is caused by hepatitis A, B, C, D, and E viruses; other liver diseases; anemia; typhoid fever; yellow fever; malaria; obstruction of bile ducts; pancreatic cancer; and alcohol abuse. WHO (2016) estimation shows that hepatitis C infection is the major cause of death. Most of the morbidity and mortality is caused by hepatitis B and C virus infections because these two viruses cause chronic, life-long infections, resulting in progressive liver damage that leads to cirrhosis and hepatocellular carcinoma. Hepatitis-related mortality was highest ($\geq$33.50 deaths per 100 000 population per year) in Oceania, western sub-Saharan Africa, and central Asia. However, East Asia and South Asia have the greatest number of hepatitis deaths (52% of the total number of deaths).

Every year, there are an estimated 20 million hepatitis E virus infections worldwide, leading to an estimated 3.3 million symptomatic cases of hepatitis E and approximately 399 000 people die each year from hepatitis C, mostly from cirrhosis and hepatocellular carcinoma. In India, people in remote areas mostly treat jaundice with local herbs such as *Eclipta alba* Hassk. and *Phyllanthus* spp. whole plant extract. Several pharmaceutical manufacturers in India make products based only on these plants. In the Indian system of medicine, and worldwide, another wild plant, an alternative drug for treating liver disorders, is the milk thistle, whose seeds are used. Various plants used for the treatment of liver disorders are detailed in Table 11.

Diabetes

Diabetes, a disease in which blood glucose levels are elevated (hyperglycemia), has become a major health problem worldwide. There are three types of diabetes: insulin-dependent or juvenile diabetes (type I or IDDM, which results from the body's failure to produce insulin), non-insulin-dependent or adult onset diabetes (type II or NIDDM, which results from the failure of cells to use insulin properly or sometimes an absolute insulin deficiency), and gestational diabetes (which occurs in pregnant women because of the action of placental hormones). According to WHO (2017), almost half of all deaths are attributed to high blood glucose occurring before the age of 70 years. It is projected that diabetes will be the seventh leading cause of death in 2030. According to HPPI (2017) diabetes is a potential epidemic to India with more than 62 million individual currently diagnosed with diabetes. Major plants used for the treatment of diabetes in the Indian system of medicine are detailed in Table 12.

Cancer

Cancer prevention with natural phytochemical compounds is an emerging strategy to prevent, impede, delay, or cure cancer, as the disease is a global problem. A WHO report (2014) indicates that the number of cancer patients will increase to 22 million worldwide in the next two decades. That report also stated that in 2012, of 14 million cases, 8.2 million patients died of cancer. Phytochemicals from plants such as *Catharanthus* spp. yield vinca alkaloids (0.03%), which are now synthesized on a large scale for treating various cancers. The Indian system of medicine uses the whole plant (Panchang) to treat cancer effectively. Several other alkaloids derived from plants (podophyllotoxin analogues and taxol) are now being used as chemotherapy for cancer. Several medicininal plants are now being used as alternative medicines for the treatment of cancer (Sharma et al. 2011). Organically grown medicinal plants and their ingredient-based treatment are safe compared with chemotherapy. Treating cancer patients with medicinal plants is a natural alternative because these plants have properties that reduce the risk of developing cancer. Some important plants for the treatment of cancer are detailed in Table 13.

Ocular Diseases

The tears work as defence mechanisms against various eye infections and the damage they cause. The disruption of this defence mechanism may result in inflamed

Table 11 Plants used for the treatment of jaundice (Common name in Hindi/English)

Serial No.	Botanical/common name	Part used	Active principle
1	*Adhatoda beddomei* C.B.Clarke., Adusa/ Malabar nut tree	Leaves, juice	Vasicine
2	*Aloe vera* L., Ghritkumaari/Aloe	Leaves	Barbalin, isobarbolin
3	*Argemone mexicana* L., Satyanashi/Prickly poppy	Latex	Berberine
4	*Andrographis paniculata* (Burm. f.), Kalamegh/King of bitters	Leaves	Kalmeghin
5	*Boerhavia diffusa* L., Puraniama/Spreading Hog-weed	Roots	Rotenoids
6	*Benincasa hispida* Thunb., Petha/Ash Gourd	Fruit	Lupeol
8	*Centella asiatica* L., Khulakudi/IndianPennywort	Leaves	Asiatic acid, madecassic acid
9	*Cassia fistula* L., Amaltas/Indian Laburnum	Fruit	Glycerides, galactomannan
10	*Cynodon dactylon* L., Doob/Bermuda Grass	Leaves	Triticin, β-sitostero
11	*Eclipta prostrata* L., Bhringaraj/False Daisy	Leaves	Wedelolactone
12	*Euphorbia nivulia* Buch.-Ham., Katathohar/Holy Milk Hedge	Latex	Cycloart-25-en-3 beta-ol, cyclolaudenol
13	*Ficus religiosa* Linn., peepal/Sacred fig	Stem bark	Bergapten, bergaptol
14	*Glycosmis pentaphylla* (Retz.) DC, Ban-nimbu/Orangeberry	Leaves	Quinolone, glycolone
15	*Indigofera tinctoria* Linn., Nili/Indian indigo	Leaves	Indicine
16	*Mangifera indica* L., Aam/Mango	Bark	Protocatechic acid, mangiferin
17	*Momordica charantia* L., Karela/Bitter gourd	Fruit	Alkaloids, glycosides
18	*Phyllanthus fraternus* Webster, Jar amla/ Gulf leaf-flower	Whole plant	Quercetin, quercitrin
19	*Rumex acetosa* Linn., Chukram/Common Sorrel	Roots	Anthraquinones
20	*Solanum americanum* Mill., Makoi/ American black nightshade	Whole plant	Glycoalkaloids
21	*Vitex negundo* L., Sambhalu/Chaste Tree	Leaves	Nishindine, flavones
22	*Silybum marianum* (L.)., Bhat-kataiya/Milk thistle	Seeds	Silymarin

eyes. Eyes can be infected by bacteria, fungi, and viruses. In the eye, more than 95% of herpes infections are due to *Herpes simplex virus*-1 infection. *Chlamydia trachomatis* Busacca is the causal agent of trachoma and infection with this microbe leads to ocular morbidity and blindness worldwide. About 146 million people globally suffer with trachoma. Another common eye disease is glaucoma, which

Table 12 Plants used for the treatment of diabetes (Common name in Hindi/English)

Serial No.	Botanical/common name	Part used	Active principles
1	*Allium sativum* L., Lahsun/Garlic	Bulb	Allicin
2	*Actinodaphne hookeri* Meissn., Pisaa/ Actinodap	Leaves	β-Sitosterol, rutin
3	*Azadirachta indica* A.Juss, Neem/Margosa tree	Leaves	Nimbidin
4	*Balanites aegyptiaca* Linn., Hingol/Soap berry tree	Fruit pulp	Saponins
5	*Catharanthus roseus* Linn., Sadabahar/ Periwinkle	Whole plant	Alkaloids
6	*Casearia esculenta* Roxb., Saptrangi/Wild coryfruit	Roots	Lucopelargonidin
7	*Ceiba pentandra* Linn., Safed semal/White silk cotton	Roots	Linarin
8	*Cinnamomum zeylanicum* L., Daalchini/ Cinnamon	Leaves	Eugenol
9	*Emblica officinalis* Gaertn., Amla/Indian Gooseberry	Fruit	Vitamin C
10	*Ficus benghalensis* Linn., Bargad/ Banyan tree	Root bark	Leucopelargonidin glycoside
11	*Glycyrrhiza glabra* Linn., Mulethi/Licorice	Whole plant	Glycyrrhizin
12	*Gymnema sylvestre* Retz., Gudmar/Australian cowplant	Leaves	Nonacosane
13	*Madhuca indica* J. F. Gmel., Mahua/Indian Butter Tree	Bark	Lupeolacetate
14	*Mangifera indica* Linn., Aam/Mango	Leaves	Pentacyclic triterpene alcohol
15	*Tinospora cordifolia* Willd., Giloe/Heart leaved Mooseed	Whole plant	Cordifol

occurs due to retinal cell degeneration and increased intraocular pressure. The flower extract of *Hedychium coronarium* J. Koen. has potential to control glaucoma and cataracts. Thus, herbal medicines are a good alternative to costly modern medicines. Various plants used for the treatment of ocular diseases are detailed in Table 14.

Future Prospects

Global

Bioprospecting of medicinal plants at the global level is widespread, as is evident from a recent survey made by the WHO, indicating that 80% of the world's population is dependent on traditional healthcare systems. Of note, 25% of modern

Table 13 Plants used for the treatment of cancer (Common name in Hindi/English)

Serial No.	Botanical/common name	Part used	Active principles
1	*Aglaia roxburghiana* Miq. Hiern., Priyangu/ Droopy Leaf. Aglaia	Leaves	Bisamide
2	*Ajuga bracteosa* Wall. ex Benth., Ratapaati/ Bracted bugleweed	Leaves	Bracteosin A
3	*Asparagus racemosus* Willd., Satavari/ Asparagus	Roots	Asparagin
4	*Bauhinia racemosa* Lamk., Bidi Leaf Tree/ Burmese Silk Orchid	Bark	Beta-amyrin
5	*Caesalpinia pulcherrima* Sw., Guletura/ Barbados pride	Leaves	Hydrocyanic acid
6	*Cannabis sativa* Linn., Bhang/Marijuana	Leaves	Delta-9-tetrahydrocannabinol
7	*Catharanthus roseus* Linn., Sadabahar/ Periwinkle	Leaves	Vinblastine, vincristine
8	*Cocculus hirsutus* Linn., Farid buti/Broom creeper	Stems	Cyclopeptides
9	*Coix lacryma-jobi* Linn., Samkru/Job's tears	Seeds	Coixenolides
10	*Curcuma longa* Linn., Haldi/Turmeric	Rhizome	Curcumin
11	*Datura metel* Linn., Dhatura/Thorn apple	Fruit	Daturaolone
12	*Heliotropium indicum* Linn., Hathajori/Scorpion tail	Aerial parts	Indicine
13	*Emblica officinalis* Gaertn., Amla/Indian Gooseberry	Fruit	Ellagic acid
14	*Heliotropium indicum* Linn., Vrischikaali/Indian heliotrope	Roots	Indicine N-oxide
15	*Hibiscus populneus* Linn., Paras pipal/Indian tulip tree	Fruit	Thespesin
16	*Podophyllum emodi* Wall., Podophylum/ Himalayan mayapple	Rhizome	Podophyllin
17	*Terminalia bellirica* Craertn., Baheda/Belleric myrobalan	Fruit	β–Sitosterol

medicines are based on the phytochemicals obtained from plants used widely in traditional medicines. Several synthetic analogues have been built on phytochemical compounds isolated from medicinal plants. A drug-use survey indicates that 50% of prescription drugs worldwide are based on herbal drugs. It has been reported that about 75% of the herbal drugs used worldwide are obtained from local medicinal plants. The use of natural products derived from plants has been on the increase, as is evident from the increased popularity and acceptance of plant-derived healthcare products, as well as cosmetics/perfumery items for personal health maintenance, even though the cost is of these natural products is high. This dual role of medicinal plants as a source of income and in healthcare makes the production of medicinal plants ever-demanding. Although herbal products require quality control and

Table 14 Plants used for the treatment of ocular diseases (Common name in Hindi/English)

Serial No.	Botanical/common name	Part used	Active principles
1	*Acalypha indica* L., Kuppaimeni/Indian Acalypha	Leaves	Acalyphine
2	*Adhatoda vasica* L., Adusa/Malabar nut tree	Flowers	Vasicine
3	*Ageratum conyzoides* L., Jungali pudina/Goat weed	Leaves	Coumarin
4	*Allium sativum* L., Lahsun/Garlic	Bulb	S-Alk(en)yl-l-cysteine sulfoxides
5	*Azadirachta indica* A. Juss., Neem/Margosa tree	Seed oil	Nimbidin
6	*Beta vulgaris* Linn., Chukandar/Beet root	Rhizome	Betanine
7	*Berberis asiatica* L., Dar Hald/Indian barberry	Roots	Berberine
8	*Boerhavia diffusa* L., Puraniama/Spreading Hog-weed	Roots	Punarnavine
9	*Camellia sinensis* (L.), Chaay/Green tea	Leaves	Epigallocatechin
10	*Curcuma longa* Linn., Haldi/Turmeric	Rhizome	Curcumin
11	*Hedychium coronarium* J. Koen., Bakawali/Butterfly Ginger Lily	Flowers	β-Trans-ocimenone
12	*Ginkgo biloba* Linn., Balkuwari/Ginkgo	Leaves	Flavonoids
13	*Vaccinium myrtillus* Linn., Neelabadari/Blueberry	Ripe berries	Anthocyanosides
14	*Vitis vinifera* Linn., Angoor/Grapevine	Ripe fruit	Resveratrol

evidence-based validation of their efficacy, the production and supply of medicinal/nutraceutical/perfumery herbs is becoming a booming agro-business. Recent observations worldwide indicate that developed countries are also including these plants in their healthcare systems.

India

India has natural plant resources in abundance because of the clear climate variations throughout the year. In India the wealth gained from medicinal plants and products is about 50% higher than that for flowering plant species. Medicinal plant-based industries have great potential for the economic development of India. As a source of safe food, medicines, and cosmetics, the trend in the use of medicinal plants is continuously increasing. Nutraceuticals act both nutritionally and medicinally, with health benefits. Current medicinal science in India has developed from the ancient practices of using medicinal plants. The oldest literature of the Vedic period is the basis of modern pharmaceutical practice, which uses various traditional medicinal plants, including digitalis for digitoxin, cinchona for quinine, and opium poppy for opium alkaloids. Almost 70% of modern medicines in India are derived from natural

herbs/botanicals, which provide effective treatments for most common disease conditions, such as constipation and hyperacidity, paralysis, skin diseases, renal stones, fistula, piles, and diabetes; thus the raw material of medicinal plants is in high demand. Various medicinal plants species of different families are highly traded in India. Considering the global and national use of and demand for medicinal plants, the cultivation and bioprospecting of these plants has great future promise.

Conclusion

Changing trends in agribusiness indicate that non-food market high-value low-volume crops (medicinal plants) are more valuable than low-value high-volume commodities. Medicinal plants are becoming important for bio-energy crops, the renewal of industrial feed stock, and bio-remedial sectors of the market, and are benefiting from technological advances that were originally developed for the food chain. The cultivation of medicinal plants deserves top priority for sustaining rural communities and supplying healthy products. The cultivation of medicinal plants will also help to solve world energy security problems and land management issues. The major challenge in the cultivation of medicinal plants after their domestication is the lack of synergy between producers, herbalists, and industry. Finally, we note that for increased efficacy in the treatment of ailments, the use of herbal drugs needs to be based on the content of the major bioactive compound/s in the drug/botanical extracts.

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High-Yielding Improved Varieties of Medicinal and Aromatic Crops for Enhanced Income

J. R. Bahl, A. K. Singh, R. K. Lal, and A. K. Gupta

Medicinal and aromatic crops are now being considered as important commercial items for sustainable economic development of the country. To meet the demand of prominent industries producing herbal drugs, pharmaceuticals, cosmetics, nutraceuticals, other confectionary items, etc., it has become imperative to produce the quality raw materials in significant quantities by evolving improved varieties through application of various breeding tools and developing improved agro-technologies and processing technological of the harvested produces. Presently, the medicinal plants and their various products/derivatives are looked upon not only as a source of affordable health care but also as an important commodity item of international trade and commerce. The medicinal plants-related trade is growing rapidly every year, but India's share in the global market is not very impressive (about 2%). This dismal situation warranted to further gear up research and development activities in the area of medicinal plants followed by dissemination of improved plant varieties and agro-technologies among the growers and entrepreneurs.

Wide variations prevailing in agroclimatic conditions in our country provide enough opportunity to grow and harvest such medicinal and aromatic crops in one or the other parts. Availability of improved varieties and agro-technologies developed by various R&D institutions, especially CSIR-CIMAP, has further enabled a large number of farmers to adopt these crops in the existing farming system for additional economic gains (Rahman et al. 2015; Anonymous 2016a). Being comparatively resistant in nature, many of these crops have also emerged as saviour of farmers in present times when production of food and vegetable crops are adversely affected by the change in climate and also due to natural calamities like drought, floods, etc. Several such medicinal and aromatic crops having potential for

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commercial cultivation in different parts of the country and their current status in terms of cultivated area, profitability and marketing are summarized below.

Ashwagandha (*Withania somnifera*)

Roots of Asgandh or Ashwagandha *(Withania somnifera*) are used in traditional system of medicines variously. This shrubby bush plant grows well in dry and subtropical regions, is a potential cash crop for the dry land zones and is also grown for beautifying the wasteland (Fig. 1a). It is cultivated in about 5000 ha area on marginal land in north-western region of Madhya Pradesh. It is also grown in Kota, Jaipur, Jodhpur districts of Rajasthan and Jammu forest. One hectare plantation of Ashwagandha yields on an average 8–10 q of dried roots which are sold at about Rs 10,000 per quintal giving a net return of Rs 75,000 from a 6- to 8-month crop. CIMAP's initiatives in popularizing this important crop in Anantapur district of Andhra Pradesh have shown a new hope of agri-economic development in otherwise dry land area. An annual requirement of dried roots is estimated to be around 7000 tonnes annually, whereas 1500 tonnes are being produced in India.

Poshita The variety Poshita of Ashwagandha developed by CSIR-CIMAP has the potential of producing dry root yield of 14 q/ha with total alkaloids and withanolide content (steroidal lactones) which are the major group of secondary metabolites of medicinal interest containing 1.292 and 3.469 kg/ha, respectively. The fresh and dry leaf yields are also high up to 2.83 and 0.50 q/ha with high withaferin content in dry leaves 0.528%. The variety developed by CSIR-CIMAP is presently grown in about 3000 ha of land due to the continued efforts made by CIMAP Research Centre, Hyderabad, under the institute's rural development programme.

NMITLI-118 The variety NMITLI-118 was developed jointly by CSIR-CIMAP and NBRI and was released in September 2009. The variety has uniform crop canopy, non-spreading plant architecture (more plant/unit area), high root yield and high withanolide yield per unit biomass, and phytochemically uniform and is the first pharmacologically validated variety. It has withanolide A and withanone in roots and high content of withaferin A (up to 2%) and no withanone in leaves. The variety is reported to give dry root yield of about 15 q/ha. Another variety of Ashwagandha named NMITLI-101 was also released in 2015 which has potential to yield up to 23 q dry roots under optimal agronomic conditions (Anonymous 2016b).

Sarpagandha (*Rauvolfia serpentina*)

More than 70 compounds are known in Sarpagandha, *Rauvolfia serpentina*, roots among which the reserpine and rescinnamine are used for control of high blood pressure, whereas ajmaline and ajmalicine are used for cardiac disease under modern

Fig. 1 (**a**) Ashwagandha, *Withania somnifera*; (**b**) Sarpgandha, *Rauvolfia serpentina*; (**c**) Kalmegh, *Andrographis paniculata*; (**d**) Satavar, *Asparagus racemosus*; (**e**) Quinghao, *Artemisia annua*; (**f**) Bhumyamalaki, *Phyllanthus amarus*

system of medicine (allopathy). It is an erect, evergreen 0.60–1.0-m high shrub found growing in the Himalayan region, Meghalaya, Assam, East UP, Bihar, Shimla, Uttaranchal and Southern India (Fig. 1b). Generally, it is being collected from wild, and its uncontrolled collection from wild has led to the inclusion of Sarpagandha in the list of threatened plant species. Its cultivation is on the limited scale and is currently being promoted by different organizations/institutes as the

plant has become rare in most of the accessible areas of its natural occurrence due to over exploitation.

CIM-Sheel To facilitate the quality production of this plant, CSIR-CIMAP has developed an improved variety 'CIM-Sheel' using directed breeding efforts having high root and alkaloid yields with defined reserpine content. CIM-Sheel plants grow up to the height of about 50–100 cm having green erect stem with soft branches and white-pink flowers. The roots go up to the depth of 40–60 cm, which are brittle in nature and bitter in taste with a peculiar smell. The plants raised from seeds give better yield of roots ranging from 100 to 400 gm per plant. Average root yield/ha under irrigated condition from 2-year-old plantation is about 1200 kg, and the roots are sold at about Rs 150/kg, so a grower can get a net return of Rs 150,000 from 1 ha land area.

Kalmegh (*Andrographis paniculata*)

Kalmegh is an important medicinal plant which is employed in Indian traditional system of medicine mostly to cure liver disorders. It is widely distributed throughout the plains of India (Fig. 1c). The plant has astringent, anodyne, antipyretic, anti-inflammatory, immunosuppressive and anthelmintic properties. Recently, its cultivation has been started in India due to efforts made by CSIR-CIMAP and other organizations. Considering its pharmaceutical potential, there is a need to increase its large-scale systematic cultivation in India.

CIM-Megha The variety CIM-Megha developed as seed progeny selection by CSIR-CIMAP consistently produces high herbage with major bitter principles diterpenoids – andrographolide and neo-andrographolides. The average dry herb, andrographolide and neo-andrographolide contents in the variety are 32 q/ha, 2.23%, 0.76%, respectively. A well-maintained crop grown in 1 ha area yields 2.5–3.0 tonnes of dried herb giving a net income of about Rs 45,000 from a 3-month crop.

Satavar (*Asparagus racemosus*)

Tuberous roots or rhizomes of satavar containing saponins and vitamins are highly valued in traditional system of medicine. These are also used as galactagogue and improve body weight and general health. It is estimated that in India, more than 500 tonnes of satavar roots are required every year for medicinal preparations. The cultivation of satavar by the farmers is increasing due to its rising demand in the country (Fig. 1d).

CIM-Shakti CSIR-CIMAP is promoting cultivation of satavar by providing seeds/saplings of the improved variety of 'CIM-Shakti', developed recently. It takes about 18–20 months for the crop to mature. However, better yield can be obtained after

2 years of planting. The crop should be harvested during October and December (dormancy period). On an average, 5–6 tonnes of dried root may be obtained from 1 ha and a net profit of about Rs 350,000 from a 2-year crop.

CIM-Sunehari Recently a new variety of yellow satavar named as CIM-Sunehari has been developed and released by CSIR-CIMAP (Anonymous 2016b). The average dry root yield was found to be 9 tonnes/ha containing 11% saponins in this variety.

Quinghao (*Artemisia annua*)

Artemisia annua, commonly known as quinghao, contains an active compound artemisinin used as antimalarial drug. The demand of this drug is on the rise due to World Health Organization (WHO) approving artemisinin combination therapy (ACT) for the treatment of cerebral malaria.

The manufactures of artemisinin derivative of the drug have been importing artemisinin from international sources, but the non-availability of sufficient raw material has forced Indian industry to depend on cultivation of this crop indigenously for self-reliance. It is estimated that to sustain >100 million doses requiring to meet global need of malaria affected people, the crop may need to cover an area of about 25,000 ha only, and with the current production levels, farmers may earn a profit of about Rs 60,000 per ha in a span of 6 months for single harvest. The pharma companies are coming forward to go for contract farming for the planned cultivation and sustained production of herbage for isolation of active compound artemisinin to meet the global demand and also the national need to combat this disease. The agrotechnology for cultivation of improved variety of *Artemisia annua* was licensed by CSIR-CIMAP to seven leading companies in India who promoted the cultivation of this crop involving farming community (Fig. 1e).

CIM-Arogya The variety of *Artemisia annua* 'CIM-Arogya', was developed by CSIR-CIMAP with characters like 280–305 cm plant height, oval growth habit, 0.9–1.0% artemisinin content and about 50 q/ha dry leaf yield.

CIM-Sanjeevani CSIR-CIMAP scientists have also developed genetically improved variety CIM-Sanjeevani of *Artemisia annua* with 1.2% artemisinin content (Anonymous 2016b). This variety has been developed using classical breeding method of polycrossing between two existing varieties, i.e. Jeevan Raksha and CIM-Arogya, followed by population enrichment with desirable genes. The plants of variety CIM-Sanjeevani have a yield potential of producing an average 50–55 kg of artemisinin from an average dry herb yield of 43–45 q/ha in a single harvest. The plants of this variety have intermediate morphology between Jeevan Raksha and CIM-Arogya. This variety is about 10 days late in flowering as compared to two earlier varieties and is, therefore, also suited for three harvests. This variety will benefit both farmers and industries involved in *Artemisia* cultivation/business.

Farmers can have an extra income of Rs 10,000–15,000 from 1 ha crop (giving 10–12% higher yield of dry leaves compared to CIM-Arogya).

Bhumyamalaki (*Phyllanthus amarus*)

The plant is valued for its antiviral properties used extensively in traditional formulations effective against liver infections such as hepatitis, diarrhoea, dysentery and urinogenital problems. The plant is found growing all over India and is being collected from wild to be used in medicinal preparation in which the chemical components vary leading to variation in quality (Fig. 1f). So the need was felt to develop a high-yielding cultivar for widespread cultivation on one hand and to conserve the wild germplasm on the other. The crop of *Phyllanthus* harvested after 60 days of planting gives 15–16 q of dried herb and a net profit of Rs 30,000 per ha.

CIM-Jeevan A high-yielding variety called CIM-Jeevan with defined marker chemicals like phyllanthin and hypophyllanthin (0.70–0.77% and 0.32–0.37%, respectively) was developed following planned breeding programme. The dry herb yield of the variety CIM-Jeevan was found to be 15–20 q/ha.

Isabgol (*Plantago ovata*)

Plantago ovata, commonly known as isabgol or Psyllium, is valued for its seeds and husk which have been used in medicine long since as laxative. It is particularly beneficial in habitual constipation, chronic diarrhoea and dysentery for centuries all over the world. India continues to rank first in its production and trade in the world market. It is also the sole exporter of isabgol to the world market, and about 80–90% produce is being exported. The export of husk and seed was valued at Rs 4650 and 6120 million. Isabgol can be grown on variety of well-drained soils having pH ranging from 7.2 to 7.9 and thrives well in warm temperate regions (Fig. 2a).

Niharika The variety Niharika was developed and released by CSIR-CIMAP, especially for north Indian conditions. One hectares crop may yield about 10–12 q of seeds giving a net profit of about Rs 40,000 from a crop of 4–5 months.

Mayuri The variety Mayuri was developed by mutation breeding and is propagated by seeds for commercial cultivation with yielding potential of about 13 q/ha seeds. It is an early maturing, higher seed and husk yielding variety of psyllium with distinct pigment marker of the panicles relatable to the maturing, thereby indicating the harvesting stage.

Fig. 2 (**a**) Isabgol, *Plantago ovata*; (**b**) Senna, *Cassia angustifolia*; (**c**) Aloe, *Aloe vera*; (**d**) Haldi, *Curcuma longa*; (**e**) Menthol mint, *Mentha arvensis*; (**f**) Peppermint, *Mentha piperita*

Senna (*Cassia angustifolia*)

Cassia angustifolia, a small leguminous shrub, is exclusively grown for its foliage and pods which contains glycosides having usefulness in a variety of ailments, such as liver complications and abdominal troubles (Fig. 2b). It is also used in modern medicine as a laxative because of its glycosides-sennoside A and B. Leaves and pods

from *Cassia angustifolia* Vahl and *Cassia acutifolia* Del are the commercial senna drug of the Unani system of medicine. Both species, *Cassia angustifolia* (native of South Arabia, West Asia) and *Cassia acutifolia* (Sudan, East Africa), are exotic to India. In their native lands, these species grow on arid tracts as perennial bushes. However, these are maintained as annual herb when cultivated. Now both the species are commonly known as *Cassia senna*. Although most plant parts contain sennosides (glycosides), but leaves and pod shells contain highest concentration described as sennosides A, B, C and D. The pods contain higher amount of total sennosides (3–5%) as compared to foliage (2.5–4%). Indian pharmaceutical industry uses about 100 tonnes of leaves and pods. The total world requirement is about 10,000 tonnes of leaves and pods. India is the major exporter and exports up to 5000 tonnes worth Rs 20 crores every year. Senna grows well on sandy loam and laterite soils with low to moderate fertility and pH ranging from 7.0 to 8.5. Dry summer with moderate temperature is the actual requirement of the crop. Fall in temperature, rain and water logging conditions are injurious to the plant. It is a 130–150 days summer crop in Northern India whereas winter crop in southern India.

Sona CSIR-CIMAP's variety Sona has become popular among the farmers of Rajasthan which covers huge area under cultivation. It has been observed that younger leaves and pods contain high sennoside content. To obtain desired level of biomass, first picking should be done between 70 and 90 days when sennoside content is optimum. The picking is done by hand so that most of the growing tops are removed to induce further better leafy growth and delay the flowering. Second picking can be done between 90 and 110 days and third between 130 and 150 days when entire plants are harvested to include both leaves and pods together producing dry leaves up to 8–10 q/ha and that of dry pods as 4–5 q/ha. Hence, 1 ha plantation of senna can give a net profit of about Rs 27,000–30,000 in a year.

Aloe (*Aloe vera*)

Aloe (Ghrit Kumari) is found in abundance throughout the world. The aloin group of glycosides are the major constituents of *Aloe* juice or pulp which are variously used in cosmetics and medicines for treatment of chronic constipation, burns and skin diseases. The plant can be grown easily in poor degraded soils with minimum irrigation in hot and dry climate (Fig. 2c).

CIM-Sheetal CSIR-CIMAP has developed CIM-Sheetal variety to provide a genetically defined planting material to the growers and has been released to farmers for cultivation. On an average about 50 tonnes of leaves can be obtained from 1 ha crop of aloe giving a net return of about Rs 1,25,000. The cultivation should be promoted only when processing units for aloe are located in the vicinity so that the fresh leaves can be sold for making sap, juice or gel.

Haldi (*Curcuma longa*)

Turmeric is not only one of the most popular spices for Indian cuisines; it is also one of the most valuable medicinal plants of traditional systems of Indian medicine due to its large repertoire of preventive and curative effects (Fig. 2d). The pharmaceutical importance of turmeric is due to its curcuminoids which are credited with anti-inflammatory, hypo-cholesterolemic, anti-oxidant, antiparasitic, antispasmodic, antimicrobial, antirheumatic, anti-ageing and anticancer properties. India is producing more than 80% of the total global turmeric production, and it is being cultivated over an area of 150,000 ha, Andhra Pradesh (70%) being the leader in turmeric production followed by Tamil Nadu, Odisha, Karnataka, West Bengal, Gujarat and Kerala.

CIM-Pitamber Extensive R&D efforts made at CSIR-CIMAP have resulted in the development of superior variety CIM-Pitamber, which can produce 60–65 tonnes fresh rhizomes/ha containing 12.5% curcuminoids in a relatively short span of 180–190 days (Anonymous 2016c). This variety has been developed using the method of clonal selection breeding method. The variety is also tolerant to leaf blotch disease of turmeric, and a farmer can get a net profit of Rs 1.25–1.50 lakhs/ha.

Menthol Mint (*Mentha arvensis*)

Menthol mint, *Mentha arvensis*, is grown for menthol used in pharmaceutical and flavour industry. India is a leading supplier of menthol mint oil to the world, and a large number of farmers in India are being benefitted by its cultivation. Generally, the crop is cultivated during January to July, either by suckers or transplanting plantlets for production of mint oil (Fig. 2e). Among different essential oils produced in India today, *Mentha arvensis* (menthol mint) oil holds prominent position in terms of acreage under the crop production and domestic consumption and export to the world market. Today India is the largest producer and exporter of natural menthol in the world. The annual turnover of the menthol industry has been in the range of Rs 3500–4000 crores during the past one decade. This has been possible due to the improved agro-technologies and plants varieties developed by CSIR-CIMAP coupled with extension of the crops in the farmers' fields with an active involvement of user industries, government departments, nongovernment organizations and traders and manufactures mainly from UP (Singh and Khanuja 2007).

Menthol mint is presently cultivated in more than 2.50 lakh hectares land of North India. It is believed that over 5 lakh farming families grow menthol mint crop contributing 75–80% global menthol mint oil produce. Pradesh contributes about 70–75% of the total national production of menthol mint oil. Taking the lesson of success of menthol mint cultivation from the farmers of UP, the area under mint is now spreading to other states in the country including Bihar, parts of Punjab,

Madhya Pradesh, etc. Menthol mint yields 130–150 kg mint oil/ha (single harvest) giving a net profit in the range of Rs 60–70,000 in about 3 and a half months.

Kosi The high-yielding variety Kosi developed through half-sib progeny selection is tall with robust growth and wider adaptability in different parts of the country. The variety is early maturing by about 10 days, and the essential oil is containing 75–78% menthol.

CIM-Saryu Another high-yielding variety developed with large canopy and huge biomass. The leaf fall is less as compared to other varieties and is also tolerant to sudden rainfall at maturity. The variety yields 140–150 kg essential oil per hectare containing 78–80% menthol.

CIM-Kranti The improved variety 'CIM-Kranti' of menthol mint has been developed through half-sib progeny selection (Bahl et al. 2013). The variety is cold and frost tolerant and has the potential to produce higher oil (≅100 kg/ha oil having 80% menthol) when grown in winter compared to all popular commercial varieties. However, during winter season (September to January) when all other varieties suffer senescence by the cold and frost conditions, 'CIM-Kranti' remain green in the field. During this period, the variety CIM-Kranti growing vigorously yields two to three times higher essential oil, compared to the popular commercial varieties 'Kosi' and 'CIM-Saryu'. The oil yield during the main summer crop from this variety is 10–12% higher compared to the best check varieties. Hence, this variety is suitable for commercial cultivation to generate additional income without any extra input during both winter and summer seasons.

CIM-Vishisht The variety 'CIM-Vishisht' rich in pulegone was developed through a half-sib progeny selection in menthol mint cultivar, 'Shivalik'. The new variety has the potential of yielding 60 kg/ha of essential oil rich in pulegone in the range of 65–68%. The pulegone has wide usage in aromatherapy, flavouring agents, perfumery, etc. and also can be chemically converted into some other important compounds like menthone, carvone or thymol and into high value commercially important menthofuran through biotransformation. Therefore, this new variety 'CIM-Vishisht' will be helpful in opening new avenues for industry and research**.**

Peppermint (*Mentha piperita*)

Peppermint, *Mentha piperita*, yields an essential oil which is known for its freshness and sweetness and finds its extensive use in food and flavour industries (Fig. 2f). The major use of the oil is for flavouring toothpastes and oral care products, candies and chewing gums, chocolates, ice creams, beverages, baked products, confectionary and pharmaceutical syrups. The crop can be profitably grown in hilly and/or plain areas by yielding essential oil ranging between 80 and 100 kg with a profit of about Rs 90,000–1,00,000/ha.

Kukrail This variety of peppermint was developed through mutation breeding and has erect plant habit with vigorous growth. It proved superior in oil yield over the local cultivars and has wider adaptability with low disease incidence.

Tushar A higher oil yielding variety called Tushar of *Mentha piperita* (peppermint) has been developed through mutation breeding which can produce 85–90 kg/ha oil having 27% menthone, 33% menthol, 2% menthofuran and 9% methyl acetate. The variety can also be grown in partial shading areas without affecting the yield.

CIM-Indus The variety CIM-Indus was developed among open-pollinated seed raised progeny of var. Kukrail of *Mentha piperita.* The essential oil obtained from var. CIM-Indus is rich in menthofuran (27%) and is an important compound in the formulation of certain synthesized essential oils. The variety has the potential of producing higher oil yield in comparison to var. Kukrail.

CIM-Madhuras CIM-Madhuras was selected among open-pollinated seed raised progeny of var. Kukrail of *Mentha piperita.* CIM-Madhuras has the potential of producing 20% higher oil yield in comparison to var. Kukrail, and the oil is sweet smelling which is useful for flavouring industry.

Indian Basil (*Ocimum basilicum*)

Indian basil (*Ocimum basilicum*) is a short-duration crop and is cultivated in India for its essential oil used extensively in flavour, fragrance, food, oral health, etc. (Fig. 3a). The crop thrives well on moderate fertile and well-drained sandy loam oil. It can be grown in subtropical and tropical climate conditions. Temperate climate is not suitable for this crop. CSIR-CIMAP has developed high-yielding varieties with chemical variability, namely, CIM-Saumya, CIM-Snigdha and CIM-Surabhi. The crop is propagated by seeds/seedlings during months of June and July, and it can yield about 80 kg oil giving a net profit of Rs 35,000–40,000 per ha in about 3 months.

CIM-Saumya The variety was developed through selection for uniformity of selected traits and their stability among seed raised progeny. It is a short-duration crop of 3 months and has the potential to produce about 80–100 kg/ha oil rich in methyl chavicol (62%) and linalool (25%).

CIM-Snigdha This variety developed by CSIR-CIMAP is distinct in leaf morphology and has unique aroma. The variety matures in 80–90 days yielding essential oil rich in methyl cinnamate content (78.7%).

CIM-Surabhi The essential oil of sweet basil with linalool, linalool acetate in desired combinations is used in various cosmetic and perfumery products. Intensive breeding techniques and selection process were undertaken at CSIR-CIMAP to

Fig. 3 (**a**) Indian basil, *Ocimum basilicum*; (**b**) Tulsi, *Ocimum sanctum*; (**c**) Chamomile, *Chamomilla recutita*; (**d**) Geranium, *Pelargonium graveolens*; (**e**) Rose, *Rosa damascena*; (**f**) Patchouli, *Pogostemon cablin*

develop this high oil-yielding variety (100–120 kg/ha) with a unique chemical composition having 70–75% (−) linalool with 99% purity. The (−) linalool obtained from this variety is superior to that obtained from lavender and will be a cheaper source of linalool for the industry.

Tulsi (*Ocimum sanctum*)

Tulsi is well known for its traditional medicinal values due to its anti-oxidant and anti-ageing properties. The decoction of leaves is effective for relief from seasonal cold and cough and stomach disorders. The crop is cultivated by a large number of farmers which can give a net profit of about Rs 70–80,000 per ha (Fig. 3b).

CIM-Ayu The variety CIM-Ayu developed by CSIR-CIMAP has the potential to produce 16 q dry leaf yield or 110 kg/ha oil rich in eugenol (83%) even in rainy season. The variety is being cultivated as annual crop in around 4000 ha in Mathura, Uttar Pradesh, Gujarat, Karnataka and Maharashtra states of India for its leaf oil and dry leaves for use in herbal tea.

CIM-Angna A new and distinct genotype was developed through half-sib progeny selection between the isolated single-plant progenies from the diverse germplasm. The plant morphology is distinct by having greyish purple stem with green leaves, which turn purplish in winter season. The variety is producing dry leaf herb yield (14 q/ha) or 90 kg/ha essential oil yield containing eugenol (40%) and germacrene-D (16%).

Chamomile (*Chamomilla recutita*)

The blue oil obtained from flowers of chamomile finds extensive applications in cosmetic and foods, and the active constituents α-bisabolol and chamazulene are reported to possess anti-inflammatory and antispasmodic activities (Fig. 3c). Its flowers are also in great demand owing to their extensive use in herbal tea and mouthwash, and a crop of chamomile from 1 ha land area can give a net profit of about Rs 40–50,000.

CIM-Sammohak The variety CIM-Sammohak has been developed through intensive mutation breeding with higher yield of dry flowers (7.5 q/ha) and oil yield of 6–7 kg/ha containing 12% chamazulene, 20% bisabolol oxide A and 11% bisabolol oxide B.

Geranium (*Pelargonium graveolens*)

The oil of geranium possesses a strong heavy rose-like odour with minty top note, which is as such used in high-grade perfumes and cosmetics. Geranium oil is produced by steam/hydrodistillation of fresh herb of *Pelargonium graveolens* belonging to the family Geraniaceae (Fig. 3d). Geranium oil is rich source of rose alcohols and is processed to isolate 1 citronellol (rhodinol) which is used very extensively in fine fragrances for its sweetness and delightful freshness. Current international demand of geranium oil is 600 tonnes and is largely met by China,

Egypt, Morocco, Reunion Island and South Africa. Presently, India's production is not more than 5 tonnes in a year against its annual requirement of around 150 tonnes. Considering the heavy demand of the cosmetic and perfumery industry, export promotion/import substitution, yield potential, quality standards and economics of its cultivation in India, this is high time to produce geranium oil. With good soil and better management, a yield of 20–25 tonnes of fresh herb is obtained. The herb is distilled fresh, and the 0.1% oil on commercial scale is obtained from the herb. Second harvest of 5–10 tonnes of herb is also available. Thus, a total of 30–35 kg oil/ha is obtained giving a net profit of Rs 60,000 per ha.

CIM-Pawan It is the result of selections from the somaclones of cv. Bourbon for higher oil content and subsequent yield evaluation. The variety CIM-Pawan possesses higher oil content (0.23%) and has the potential to produce about 30% higher essential oil yield as compared to cv. Bourbon. The major active constituents in the oil like citronellol and geranial are present with 25–33% and 21–26% contents, respectively.

Rose (*Rosa damascena*)

Rose oil is obtained from the flowers of *R. damascena* and is the costliest oil used in high-grade perfumes (Fig. 3e). The institute has developed improved technology for rose oil extraction. The recovery of oil varies from 0.025% to 0.03% as compared to 0.01% being obtained using country-made stills. A low-cost, directly fired field distillation unit has also been developed, and technology has been transferred to perfumery industry for commercialization. The area under rose cultivation in UP alone is estimated at 1500 ha where about 100 kg oil valued at about Rs 6 crores is produced annually. A Bulgarian strain of rose was introduced in Kashmir valley, which gave higher yields than those obtained in Bulgaria.

Noorjahan CIMAP has also developed a superior variety of Damask rose named 'Noorjahan' which is suitable for cultivation in the subtropical areas of Uttar Pradesh and the adjoining states. About 600 g rose oil valued at Rs 3 lakhs is obtained from 1 ha of rose plantation based on 0.025–0.030% oil content in subtropical regions and 0.035–0.040% in temperate regions. The oil of Noorjahan variety contains nearly 30% geraniol, 24% citronellol, 12% nerol and 1.3% rose oxide.

Ranisahiba This variety developed through half-sib selection with higher flower biomass up to 40 q/ha, which is available for longer period of about 3 months and is suitable for making rose water. The oil is reported to contain 35% geraniol, 7% geranyl acetate, 5% citronellol and 10% trans-rose oxide.

Patchouli (*Pogostemon cablin*)

Patchouli, *Pogostemon cablin* Benth, is an aromatic undershrub. The plant is cultivated on commercial scale for its essential oil which has traditionally been one of the most important natural base materials used in perfumery industry and as flavour ingredient in major food products (Fig. 3f). Further, patchouli oil is known to possess bacteriostatic properties. Patchouli oil is obtained from leaves of the plant and is used practically in most of the perfumes as base because of its fixative property. Indonesia and China are the major exporters of the oil to the world market. Indigenous agro-technology for cultivation has been developed at CIMAP Field Station, Bangalore, and it has been found that the crop could be grown in coastal areas as an intercrop in coconut plantation. India imports substantial quantities of patchouli oil to meet its requirement, and there is a good scope to produce patchouli oil in order to save valuable foreign exchange. Patchouli plantation raised in 1 ha gives generally about 40 kg oil valued at about Rs 80,000 − and a net profit of about Rs 60,000 can be expected.

CIM-Samarth CSIR-CIMAP has developed an improved variety called CIM-Samarth for cultivation in northern plains of India, which can produce 10–-15 tonnes/ha fresh herb giving 60–70 kg oil in two harvests. The variety is tolerant to all common diseases and insects and has better regeneration ability.

Lemongrass (*Cymbopogon flexuosus*)

The lemongrass oil as such is widely used in perfumery, soaps and cosmetics to obtain typical lemon note. Besides, it is an important source of citral, which is used in perfumery and medicine (Fig. 4a). While citral forms a significant raw material for confectionary and beverages, about 320 tonnes of the oil valued at about Rs 12 crores are being produced annually for internal consumption as well as for export. CSIR-CIMAP has developed improved agro-technology for the cultivation of this plant. A superior variety named 'Krishna' has the potential to yield up to three times more oil than the existing varieties. This variety is being popularized among the farmers for large-scale cultivation in different parts of the country. Lemongrass oil has very good demand in the world market. Guatemala, China, Sri Lanka and Brazil are the other major producing countries. The production of lemongrass oil in the country has declined in recent years, and there are immense possibilities for production of good quality lemongrass for the domestic market as well as for export. The slips of the lemongrass can be planted in February, March and June to September. The crop remains economically productive up till 5 years. One hectare plantation of lemongrass yields about 100–125 kg oil under unirrigated conditions and 200–250 kg under irrigated conditions giving a net profit of about Rs 60,000 and 1,00,000, respectively, per annum.

Fig. 4 (**a**) Lemongrass, *Cymbopogon flexuosus*; (**b**) Java grass, *Cymbopogon winterianus*; (**c**) Palmarosa, *Cymbopogon martinii*; (**d**) Khus, *Vetiveria zizanioides*

Krishna A high oil-yielding variety has been developed by CSIR-CIMAP suitable for South Indian to North Indian plains and hilly areas. The variety Krishna is a perennial crop and is very popular among farmers. The variety can produce up to 300 q/ha herb yield with 0.8–0.9% oil content and thus yielding 230–250 kg oil/ha containing 80–82% citral.

Nima This novel variety 'Nima' of lemongrass is a selection from the open-pollinated seed raised progenies obtained from *C. flexuosus* cv. OD-19. Nima is distinct having essential oil yield (250–260 kg/ha) rich in citral content (85–90%). The variety is salt tolerant and is suitable for usar land prevailing in Uttar Pradesh and other parts of northern India.

CIM-Shikhar This new variety is able to produce 20–25% more essential oil in irrigated conditions with around 80% citral content. The oil yield of this variety is more than 280 kg/ha, and an average oil content is 1.6% in herbage (Anonymous 2016b).

Java Grass (*Cymbopogon winterianus*)

Citronella oil of Java quality is obtained by steam distillation of leaves. It an important source of perfumery chemicals, such as citronellal, geraniol and hydroxyl-citronellal, which finds use in soap, perfumery, cosmetic and flavouring industries (Fig. 4b). The oil is also used as insect repellent. The oil is produced in Indonesia, China and Sri Lanka, which are the major suppliers of the oil in the world market. As a result of transfer of technology by CIMAP and RRL (Jorhat), more than 300 tonnes of citronella Java oil worth about Rs 7.5 crore is produced annually. The plant is grown in north-eastern states and in south India. However, it has been observed that slightly acidic soils are much more favourable for this crop. Newer areas are coming under citronella crop in the north Indian plains owing to popularization of the improved technologies and development of varieties by CIMAP. It has great potential for Uttar Pradesh, Madhya Pradesh and Maharashtra where good irrigation facilities are available. CIMAP has also developed several high-yielding varieties in citronella Java. These varieties, Manjusha, Mandakini, Manjari, Medini, Bio-13 and CIM-Jeeva, are becoming popular among citronella growers of the country. One hectare crop of citronella yields about 250 kg oil per year giving about Rs 185,000 net return.

BIO-13 The variety is suitable for North Indian plains and can produce an average of 250 kg/ha oil per year in four harvestings over the year under normal conditions. The oil contains 32–45% citronellal, 12–18% geraniol, 11–15% citronellol and 3–8% geranyl acetate. It is a perennial crop and is maintained for 6–7 years, and later it can be replaced with new plantation for better higher yields.

CIM-Jeeva The variety is high oil yielding and can produce oil yield up to 285 kg/ha with citronellal content of 41%. A better adaptability and establishment after plantation is observed in this variety and is also found tolerant to yellowing disease.

Palmarosa (*Cymbopogon martinii*)

Palmarosa oil is obtained from the flowering tops, leaves and above ground parts of the plant *C. martinii* variety *motia*. The oil is used in perfumery, cosmetic and flavour industries. Currently, it is cultivated to a great extent in Madhya Pradesh, Uttar Pradesh, Rajasthan, Maharashtra and Karnataka states of India (Fig. 4c). Indonesia and Guatemala are the major competitors to India in the world market. CSIR-CIMAP developed agro-technology and provides quality palmarosa seeds to the farmers in various parts of the country. It is estimated that 50–60 tonnes of palmarosa oil worth about Rs 5 crores is being produced annually in the country. Palmarosa oil is exported to France, Germany, the Netherlands, USA, etc., and ample opportunities are available for extending cultivation of the crop to areas where salt-affected, cultivable wastelands are available.

PRC-1 The seeds of palmarosa are sown in the nursery during May/June, and seedlings are planted in the month of July. One hectare crop of palmarosa gives about 80 kg oil under unirrigated conditions and 125–150 kg oil under irrigated conditions resulting into a net return of about Rs 60,000 and 1,00,000, respectively, per annum.

CIM-Harsh Recently, another high oil-yielding variety named CIM-Harsh has been released for commercial cultivation, which has the potential of producing up to 175–200 kg/ha oil containing 85% geraniol. The variety CIMAP-Harsh is fast growing and is tall with light yellow stem, higher number of tillers/plant and longer inflorescence.

Khus (*Vetiveria zizanioides*)

Vetiver (Khus) is cultivated for the production of essential oil derived from roots and is being used in high-grade perfumes, soaps, sherbets and cosmetic preparations. The total world production is estimated to be 600–700 tonnes per annum as compared to 20–25 tonnes produced annually in India. Therefore, khus cultivation in Indian context seems to be profit-driven with increasing oil demand for perfume and soap industries (Fig. 4d). Currently, about 20 tonnes of oil, valued at about Rs 30 crores, is produced annually. The crop offers immense possibilities for expansion, especially along the river beds. Whereas, CSIR-CIMAP has developed agro-technology and improved processing technology for vetiver oil production and several high-yielding varieties.

KS-1 The slips of this variety can be planted during February to March or July to August, and roots can be harvested after 12–18 months during December to January. The variety is tall and produces oil yield in the range of 18–20 kg/ha with traditional vetiver oil aroma.

CIM-Vridhi It is a short-duration variety having potential of producing 20–25 kg oil per ha in a span of 10–12 months and thus, giving a net profit of Rs 1,50,000/ha, has become very popular among a large number of farmers of UP, Bihar, Chhattisgarh, Jharkhand, Karnataka and Odisha states in recent years. In addition, cocultivation of khus with wheat, lentil, peas, mint, basil, etc. brings an additional profit of about Rs 30,000/ha.

CIM-Samriddhi A new variety is developed called CIM-Samriddhi with unique aroma of its essential oil containing major aroma ingredients as >30% Khusilal (*nor*-sesquiterpene (C_{14}) aldehydes) and >19% Khusol. This variety has the potential of producing 20% higher oil yield. Moreover, this variety can be grown in the unutilized or underutilized lands (Anonymous 2016c).

Opportunities Available and Challenges Ahead

Medicinal and aromatic crops offer several opportunities for entrepreneurship development and job creation especially in rural sector. These include cultivation, processing, value addition, product formulation, marketing, supply of seeds and propagules of high-yielding varieties and testing services for quality evaluation of the produce. Industries requiring quality raw material in bulk are approaching R&D institutions for cultivation technology(s) and farmers for contractual cultivation. In this way public-private partnership model has started taking shape which has been steered by CSIR-CIMAP adopting 'biovillage' approach based on antimalarial drug plant *Artemisia annua* (Khanuja and Singh 2007; Kumar et al. 2015). Similar approach can be explored for other medicinal and aromatic crops.

Though several high-yielding improved varieties were developed and popularized for cultivation of medicinal and aromatic crops during the last three and a half decades, there appears to be a continued need for such efforts due to frequent changes in the climate resulting into alarming decline in available resources such as water, land, manpower, etc. for growing such crops. R&D institutions are expected to formulate strategy for developing varieties which can be grown with comparatively lesser inputs under adverse climatic conditions and in stressed soils without affecting yield and quality of the produce. Integration of medicinal and aromatic crops with traditional food crops is another area requiring urgent attention. Though challenges on the R&D front are enormous, the future may hold great promise looking at the available gene pool of MAPs, scientifically trained manpower and research facilities available in the country.

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Indian Plant Genetic Resources of Medicinal Value

Veena Gupta

Introduction

The modern allopathic system of medicine is well developed around the globe; still today many sections of rural population rely on herbal medicines. During the last few decades, nature therapy has gained a great momentum resulting in the expanding use of herbal or plant-based medicines. This can be attributed to the realization of the self-concern of the human being for better health. The herbal tradition is well spread across the world and for many of the rural populations; plants are still the integral part of their life, religious or cultural. Today many pharmaceutical industries are aiming on the human genome as a source of the many unsolved mysteries as to how diseases can be diagnosed, prevented or treated; the scope of developing plant-based drugs has greater significance. Medicinal plants not only support the health problems of rural people but also play a significant role in alleviating their socio-economic status especially through the sale of wild harvested material to the local market. In many parts of India, it is observed that woman folklore, children and landless people are engaged in collection of medicinal plants from wild forest areas, and these are traded directly to urban markets from where they are marketed to national and international markets, thus accounting for about 30–40% of the total household income.

Technology-rich developed countries are spending exuberantly on the medicinal plant research as is evident from the mushroom growth of pharmaceutical companies globally. The research laboratories are engaged in analysing a large number of botanicals as probable remedies to upcoming diseases. Most of the raw material for this screening originates from wild populations, thereby posing a threat to the existence of wild medicinal plants. Additional threat to the existence of forest resources including medicinal plants is posed by activities like deforestation for

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the sake of developing roads or establishing industries in or near forest areas which are the rich repositories of medicinal plants. Such activities have long-lasting negative effects on wild medicinal plant populations. This reservoir of medicinal plant diversity needs immediate attention of scientists, researchers, environmentalists and government so that our future generations are not deprived of this natural wealth.

Herbal Heritage of India

India has a strong base for Ayurveda, "the science of life", which dates back to 5000 years. About 700 plants are described in Charaka Samhita and Sushruta Samhita dating back to 1st millennium BC. The World Health Organization enlists approximately 21,000 plants of medicinal value, of which approximately 7500 species are used by approximately 4365 ethnic communities in India in human and veterinary healthcare system and about 2500 plant species in indigenous systems of medicine. This vast knowledge about the usage of these plant species is available in coded (ISM) and non-coded forms (folklore medicinal system). The folklore system of medicine is entirely empirical as the information is passed orally through generations and is well sustained in the indigenous tribal communities. The Indian system of medicine with all five components, viz. Ayurveda, Siddha, Unani, homeopathy, yoga and naturopathy, stands unique in the world with well-documented information in the form of Vedas, nighuntas, pharmacopoeias of homeopathy and Unani and Siddha system, so while "Rig Veda" and "Atharva Veda" describe the meticulous produces and usage of medicinal plants popular among northern region of India, Siddha system of medicine is rooted in Dravidian culture of the prevedic period predominant in Tamil Nadu and popular in other states also. The Unani system of medicine was introduced by the Arabian during the Mughal Empire. The Unani pharmacopoeia is a treasure book enlisting the medicinal use of plants mixed with some animal parts, minerals and marine products. The Unani, "materia medica", describes over 2000 plant species. It is estimated that more than 4.00 lakh registered medical practitioners are engaged in these systems of Ayurveda, Siddha and Unani for the primary healthcare problems.

Medicinal Diversity in India

India by virtue of being situated at the tri-junction of global biogeographical zones, namely, agro-tropical, Indo-Malayan and Paleo-Arctic, offers great diversity both in terms of ecological and biological components. By virtue of this, India harbours a rich flora and fauna and is considered as one of the 12 mega centres of biodiversity with three hotspots. The Indian flora represents nearly 12% of the global floral diversity distributed in various agro-climatic zones of the country encompassing the medicinal flora. Table 1 enlists the representative medicinal flora of India distributed in 21 agrobiodiversity heritage sites as described by Singh (2015). Of

Table 1 Distribution of medicinal plant genetic resources in India[a]

Name of the agrobiodiversity heritage site	Diversity in representative medicinal crop species
The Cold-Arid Region of Ladakh and Adjacent Areas	*Echinops cornigerus* DC., *Viola biflora L.*, *Artemisia laciniata* Willd., *Nepeta floccose* Benth., *Delphinium* Tourn. ex L., *Ephedra gerardiana*, *chhmumasseo (Lactuca lessertiana* C.B. Clarke), *Clematis (Clematis tibetana* Kuntze), *Arnebia euchroma* I.M. Johnst.; *A. guttata* Bunge, *Podophyllum hexandrum* Royle, *Biebersteinia odora* Royle, *Bergenia stracheyi* (H.K.) Engl., *Gentianella paludosa* (Hook.) Harry Sm., *Mentha longifolia*, *Capparis spinosa*, *Cirsium wallichii* DC., *Artemisia scoparia* Waldst. & Kit., *Cousinia falconeri* Hook. f., *Carum carvi* L., *Gentiana decumbens* L.f., *Picrorhiza kurroa* Royle ex Benth., *Aster tibeticus* Hook.f., *Artemisia biennis* Willd., *Inula obtusifolia* A. Kern.; I. royleana DC.; *Cremanthodium reniforme* (DC.) Benth., *Juniperus macropoda* Boiss.; J. excels Webb ex Parl., *Hyoscyamus niger* L., *Saussurea bracteata* Decne, *Corydalis govaniana* Wall., *Astragalus rhizanthus* Royle, *Astragalus strictus* Benth., *Berberis ilicinia* Hook., *Allium carolinianum* DC., *Saussurea bracteata* Decne., *Artemisia dracunculus* L., *Plantago depressa* Willd., *Potentilla anserina* L. *Hippophae rhamnoides*: and *Myricaria squamosal* Desv.
The Western Himalayan Region	*Achillea millefolium* L., *Aconitum heterophyllum*, *Allium-stracheyi*, *Angelica glauca*, *Arnebia benthamii* (Wall. ex G. Don) I.M.Johnst., *A. euchroma* I.M.Johnst., *Asparagus (Asparagus adscendens* Roxb.), *Atropa acuminate* Royle, *Berberis asiatica* Roxb. ex DC., *Dactylorhiza hatagirea*, *Delphinium denudatum* Wall, *Dioscorea deltoidea* Wall. ex Griseb, *Ephedra gerardiana* Wall, *Nardostachys jatamansi*, *Picrorhiza kurroa*, *Podophyllum hexandrum*, *Potentilla fulgens* Wall. ex Hook., *Rheum australe* D.Don, *Rubia manjith* Roxb. ex Flem., *Saussurea costus* (Falc.) Lipsch, *S. lappa* (Decne.) C.B. Clarke, *Senna sophera* (L.) Roxb., *Swertia chirayita* (Roxb.) Buch.-Ham. ex C.B. Clarke, *Taxus baccata* ssp. wallichiana Zucc. and *Valeriana jatamansi*
The Eastern Himalayan Region	*Berberis asiatica* Roxb. ex DC., *Eurya arunachalensis* AS Chauhan, *Centella asiatica* (L.) Urb., *Terminalia chebula* Retz., *Swertia chirayita* (Roxb.) Buch.-Ham. ex C.B. Clarke, *Taxus baccata* L., *Costus speciosus* Sm., *Curculigo orchioides* Gaertn., *Berberis aristata* DC., *Coptis teeta* N. Wallich, *Podophyllum hexandrum* Royle, *P. sikkimense* R. Chatterjee & Mukerjee, *Aconitum lethale* Griff., *Panax sikkimensis* R.N. Banerjee,

(continued)

Table 1 (continued)

Name of the agrobiodiversity heritage site	Diversity in representative medicinal crop species
	Picrorhiza kurroa Royle ex. Benth., *Cinchona officinalis* L., several *Saussurea* DC. spp.
The Brahmaputra Valley Region	*Aquilaria malaccensis* Lamk; syn. *A. agallocha*, *Emblica officinalis*, *Saraca indica* L., *Curcuma aromatica* Salisb., *Canarium strictum* L., *Hydnocarpus kurzii* Warb., *Costus speciosus* Sm., *Holarrhena antidysenterica* (L.) Wall., *Alpinia galangal* (L.) Sw., *Gynocardia odorata* R. Br., *Terminalia* spp., *Cymbopogon jwarancusa* (Jones) Schult. var. *assamensis* BK Gupta, *Eugenia jambolana* Lam. (*Wedelia calendulacea*), *Centella asiatica* (L.) Urb., *Litsea cubeba* Pers., *Mucuna pruriens* (L.) DC., *Strychnos nux-vomica* L., *Piper longum*, *Asparagus racemosus* Willd., *Solanum khasianum* C.B. Clarke; syn. *S. aculeatissimum* (Jacq.), *Acorus calamus* L., *Terminalia* spp., *Garcinia* spp., *Phlogacanthus thyrsiflorus* (Roxb.) Nees and *Ocimum* L. spp.
The Garo, Khasi and Jaintia Hills Region	*Achyranthes aspera* L., *Acorus calamus* L., *Aegle marmelos* (L.) Correa ex Roxb., *Arisaema jacquemontii* Blume, *Aristolochia tagala* Cham., *Asparagus racemosus* Willd., *Baliospermum micranthum* Muell., *Catharanthus roseus* (L) G. Don, *Centella asiatica* (L) Urb., *Cinnamomum bejolghota* (Buch.-Ham.) Sweet, *C. tomala*, *Clerodendron serratum* (L) Moon, *Costus speciosus* Sm., *Curcuma longa* L., *C. zedoaria* Rose., *Cuscuta reflexa* Roxb., *Dioscorea alata*, *Eryngium foetidum* Walter, *Fagopyrum dibotrys* (D.Don) Hara., *Gloriosa superba* L., *Gynocardia odorata* R. Br., *Houttuynia cordata* Thunb., *Hedychium spicatum* Sm., *kayaphal* (*Myrica esculenta* Buch.-Ham. ex D.Don), *Lycopodium clavatum* L., *Nepenthes khasiana* Hook.f., *Ocimum sanctum* L., *Plantago ovata* Forssk, *Plumbago zeylanica* L., *Scoparia dulcis* L., *Solanum khasianum* C.B. Clarke, *Swertia chirayita* (Roxb. ex Flem.) Karst., *Terminalia chebula* Retz., *Viburnum foetidum* Wall., *yew* (*Taxus baccata* L.), *Zanthoxylum armatum* Druce, etc.
The Northeastern Hills of Nagaland, Manipur, Mizoram and Tripura Region	*Aquilaria agallocha* Roxb., *Eryngium foetidum* L., *Piper brachystachyum* Wall., *Swertia paniculata* Wall., *S.macrosperma* C.B.Clarke, *Taxus baccata* L. var. *wallichiana*, *Costus speciosus* Sm., *Curculigo orchioides* Gaertn., *Butea monosperma* Kuntze, *Alpinia galangal* Willd., *Tinospora cordifolia* (Willd.) Hook.f. & Thomson, *Hedychium coronarium* J. Koenig, *Panax ginseng* C. A. Mey., *Terminalia chebula* Retz., *Rhus semialata* Murr.,

(continued)

Table 1 (continued)

Name of the agrobiodiversity heritage site	Diversity in representative medicinal crop species
	Hodgsonia macrocarpa Cogn., *Berberis lycium* Royle, *B. feddii* Ahrendt., *B.micropetala*, *B. wardii*, *Zanthoxylum rhetsa* D.C., *Clerodendrum colebrookianum* Walp., *Curcuma amada* Roxb., *Clerodendrum serratum* (Spreng), *Morinda angustifolia* Roxb., *Panax pseudoginseng* (Burkill) Hoo & Tseng, *Strychnos nux-vomica* L., *Rhus javanica* L., *Rauvolfia serpentina* L., *Smilax lanceifolia* Roxb., *Hedychium spicatum* Sm., *Acorus calamus* L., *Curcuma aromatica* Salisb., *Vitis quadrangularis* (L.) Wall. ex Wight & Arn., *Curcuma caesia* Roxb. and *Curcuma zedoaria* (Christm.) Rosc.
The Arid Western Region	*Calotropis gigantea* (L.) R. Br., *C. procera*, *Abutilon Indicum* (Linn.) Sweet, *Justicia adhatoda* L., *Alhagi maurorum* Medik., *Aloe barbadensis* Miller.; syn. *A. vera* (L.) Burm.f., *Anticharis glandulosa* var. *caerulea* Blatt. & Hallb. Ex Santapau, *Withania somnifera* (L.) Dunal, *Pedalium murex* L., *Barleria prionitis* L. var. *dicantha* blatt. & Hallb., *Leonotis nepetifolia* L., *Blepharis sindica* T. Anders, *Eclipta prostrata* L., *Cassia auriculata* L., *Barleria acanthoides* Vahl., *Boerhavia diffusa* L., *Tribulus terrestris* L., *Commiphora wightii*; syn. C. mukul and wild *C. caudate* (Wight & Arn.) Engl., *Cordia gharaf*, *Peganum harmala*, *Cissus quadrangulars* L., *Balanites aegyptiaca* L., *Gloriosa superba* L., *Clitoria ternatea* L., *Zygophyllum simplex*, *Cleome vahliana* Fresen., *Tribulus rajasthanensis* Bhandari & Sharma, *Senna auriculata* L., *Withania coagulans* (Stocks) Dunal, *Coleus amboinicus* Lour., *Calligonum polygonoides* L., *Salvadora persica*, *Convolvulus auricomus* (A. Rich.) var. volubilis (C.B. Clarke) Bhandari, *Convolvulus auricomus* (A. Rich.) var. ferruginosus Bhandari, *Cressa cretica*, *Boswellia serrata* Roxb. ex Colebr, *Argemone mexicana* L., *Cassia angustifolia* M. Vahl, *Convolvulus microphyllus* Choisy, *Achyranthes aspera* L., *Psoralea corylifolia* Linn., *Fagonia cretica* L., *Guaiacum officinale* L., *Plantago ovata* Forssk., *Cadaba fruticosa* (L.) Druce and *Pedalium murex* L.
The Malwa Plateau Region	*Aloe barbadensis* Miller; syn. *A. vera* (L.), *Alstonia scholaris* (L.) R.Br., *Cassia fistula*, *Emblica officinalis*, *Argemone mexicana* Linn., *Terminalia arjuna*, *Withania somnifera* (L.) Dunal, *Acacia arabica*, *Aegle marmelos*, *Terminalia bellirica*, *Balanites aegyptiaca* (L.) Del., *Baliospermum montanum* (Willd.) Mull. Arg., *Bixa orellana* L.,

(continued)

Table 1 (continued)

Name of the agrobiodiversity heritage site	Diversity in representative medicinal crop species
	Butea monosperma, *Cassia tora*, *Celastrus paniculatus* Willd., *Clitoria ternatea*, *Curculigo orchids* Gaetrn, *Curcuma angustifolia* Roxb., *Cyperus rotundus* L., *Embilia ribes* Burm., *E. tsjeriam-cottam* (Roem. & Schult.) A. DC., *Evolvulus alsinoides* (Linn.) Linn., *Glycyrrhiza glabra* L., *Helicteres isora* Linn., *Tribulus terrestris* L., *Tinospora cordifolia* (Thunb.) Miers, *Commiphora wightii* (Arn.) Bhandari; syn. C. *mukul*, *Terminalia chebula*, *Holarrhena antidysenterica* (Roxb. ex. Fleming) Wall. ex. DC., *H. pubescens* (Buch.-Ham.) Wall. ex G. Don, *Acorus calamus* L., *Madhuca indica*, *Mucuna pruriens* (L.) DC., *Strychnos nux-vomica* L., *Ocimum americanum* L., *Fumaria indica*, *Plumbago zeylanica* L., *Pongamia pinnata*, *Psoralea corylifolia*, *Pterocarpus marsupium* Roxb., *Chlorophytum borivilianum* (Santapu & Fernandes), *Rauvolfia serpentina* (Benth. Ex Kurz.), *Asparagus racemosus* Willd., *Swertia chirayita* (Roxb. ex Flem.) Karst., *Vitex negundo* L., *Woodfordia fruticosa* (L.) Kurz
The Kathiawar Peninsula Region	*Terminalia arjuna* (Roxb. ex DC.) [Wight & Arn.], *Terminalia bellirica* (Gaertn.) Roxb., *Centella asiatica* (L.) Urb., *Crataeva nurvala* Buch.-Ham., *Curculigo orchioides* Gaertn., *Garuga pinnata* Roxb., *Aloe barbadensis* Mill., syn. *A.vera* L., *Chlorophytum borivilianum* Santapau & R.R. Fernandes, *Asparagus racemosus* Willd.), *Cassia italica* (Mill) Spreng; syn *Senna italica* Mill. subsp. italica, *Terminalia chebula* Retz., *Calotropis procera* (Ait.) R. Br., *Balanites aegyptiaca* (L.) Delile, *Emblica officinalis*, *Adhatoda vasica* (L.) Nees, *Acacia nilotica*, *Plumbago zeylanica* L., *Clitoria ternatea* Linn. var. pilosus Wall. ex Baker, *Myristica fragrans* L., *Pongamia pinnata* (L.) Pierre, *Capparis decidua*, *Vetiveria zizanioides* L. Nash, *Cymbopogon citratus* Stapf, *Cymbopogon martinii* (Roxb.) Wats, *Kalanchoe pinnata* (Lam.) Pers., *Ficus religiosa* L., *Boswellia serrata*, *Bombax ceiba* L. and *Solanum purpureilineatum* Sabnis & Bhatt
The Bundelkhand Region	*Alstonia scholaris* (L.) R. Br., *Terminalia arjuna*, *Terminalia bellirica* (Gaertn.) Roxb., *Cannabis sativa*, *Balanites aegyptiaca* (L.) Del., *Baliospermum montanum* (Willd.) Mull. Arg., *Celastrus paniculatus* Willd., *Embelia tsjeriam-cottam* (Roem. & Schult.) A. DC., *Terminalia chebula* Willd. Ex Flem., *Helicteres isora* Linn.,

(continued)

Table 1 (continued)

Name of the agrobiodiversity heritage site	Diversity in representative medicinal crop species
	Holarrhena antidysenterica (Roxb. ex. Fleming) Wall. ex. DC., *Acorus calamus* L., *Plumbago zeylanica* L., *Psoralea corylifolia* L., *Strychnos nux-vomica* L., *Vitex negundo* L., *Woodfordia fruticosa* (L.) Kurz., *Commiphora* wightii (Am.) Bhandari, *Withania somnifera* (L.) Dunal, *Chlorophytum borivilianum* Santapau & Fernandes, *Andrographis paniculata* (Burm.f.) Wall. ex Nees, *Martynia annua* Linn. and *Kirganelia reticulata*
The Upper Gangetic Plains Region	*Adhatoda zeylanica* Medik., *Aloe barbadensis* Mill., *Ricinus communis* L., *Withania somnifera* (L.) Dunal, *Psoralea corylifolia* L.; *Terminalia bellirica*, *Plumbago zeylanica* L., *Datura stramonium* L., *Digera muricata* Mart., *Tinospora cordifolia* (Willd.) Hook.f. & Thomson, *Rubia manjith* Roxb. ex. Flem., syn. *R. cordifolia* L., *Sesbania cannabina* (Retz.) [Pers.], *Andrographis paniculata* Nees, *Gloriosa superba* L., *Cymbopogon martinii* (Roxb.) Wats., *Mentha piperita* L., *Lactuca remotiflora* DC., *Papaver somniferum* L., *Rosa damascena* L., *Asparagus curillus* Buch.-Ham. ex Roxb., *A. racemosus* L., *A. sarmentosus* L., *Desmodium gangeticum* (L.) DC., *Ocimum sanctum* L., *Acorus calamus* L.
The Middle Gangetic Plains Region	*Alangium salviifolium* (L.F.) Wangerin, *Terminalia arjuna*, *Psoralea corylifolia* L.; syn. *Cullen corylifolium*, *Acorus calamus* L., *Plumbago zeylanica* L., *Clausena excavata* Burm.f., *Celastrus paniculatus* Willd., *Gloriosa superba* L., *Andrographis paniculata* (Burm. F.) Wall, *Mucuna pruriens* (L.) DC. var. pruriens; syn *M. cochinensis*, *Lactuca remotiflora* DC., *L. runcinata* DC., *Soymida febrifuga* A. Juss., *Oroxylum indicum* (L.) Benth. Ex Kurz., *Piper longum* L., *Catharanthus roseus* (L) G. Don, *Chlorophytum borivilianum* Santapau & Fernandes, *Rauvolfia serpentina* Benth. Ex Kurz., *Asparagus racemosus* L., *Trichosanthes tricuspidata* Lour., *Mucuna deeringiana* (Bort) Merr., *Urginea indica* (Kunth.), *Cymbopogon winterianus* Jowitt; *C. flexuosus* (Steud.) Wats., *Mentha arvensis* L., *Cymbopogon nardus* (L.) Rendle, *Pandaus fascicularis* Lam., *Mentha piperita* L., *Cymbopogon* martinii (Roxb.) Wats., *Pogostemon cablin* Benth.; syn. *P. patchouli* L. and *Ocimum sanctum* L.
The Lower Gangetic Plains or Delta Region	*Curcuma amada* Roxb., *Acanthus ilicifolius* L. (*Sundarbans*), *helencha* (*Enhydra fluctuans* Lour.), *Nardostachys grandiflora* DC., *Curcuma zedoaria* Rosc., *Strychnos potatorum* L.; syn.

(continued)

Table 1 (continued)

Name of the agrobiodiversity heritage site	Diversity in representative medicinal crop species
	S. nux-vomica L., *Pterocarpus marsupium* Roxb., *Trigonella corniculata* (L.), *Curcuma angustifolia* Roxb., *Terminalia* spp., *Curcuma aromatica* (Salisb.)., *Hemidesmus indicus* (L.) R. Br. Ex Schult., *Tylophora indica* Merr., *Withania somnifera* (L.) Dunal., *Eupatorium ayapana* Vent., *Swertia chirata* C.B.Clarke, *Aloe vera* (L.) Burm. f., *Rauvolfia serpentina* Benth. Ex Kurz., *Cymbopogon flexuosus* (Steud.) Wats. and citronella (*Cymbopogon spreng.* spp.)
The Chota Nagpur Plateau Region	*Hemidesmus indicus* R. Br., *Alangium salvifolium* (L.F.) Wang., *Terminalia arjuna*, *Withania somnifera* (L.) Dunal., *Terminalia bellirica* (Gaertn.) Roxb., *Centella asiatica* (L.) Urb., *Caesalpinia bonduc* (Linn.) Roxb., *Plumbago zeylanica* Linn., *Urginea indica* Kunth., *Cissus quadrangularis* Linn. (CQ), *Flemingia strobilifera* (L.) W.T. Aiton., *Boerhavia diffusa* L., *Aloe barbadensis* Mill., *Celastrus paniculatus* Willd., *Andrographis paniculata* Wall. ex Nees, *Mucuna pruriens* (L.) DC., *Rubia cordifolia* L., *Cissus repanda* Vahl; syn. *C. rosea* Royle, *Vitis repanda* Wight & Arn., *V. rosea* Royle, *Pueraria tuberosa* (Roxb. ex Willd.) DC., *Boswellia serrata*, *Rauvolfia serpentina* Benth. Ex Kurz., *Asparagus racemosus* L., *Oroxylum indicum* (L.) Benth. Ex Kurz; syn. *Bignonia indica* L., *Soymida febrifuga* (Roxb.) A. Juss., *Symplocos racemosa*, *Berberis asiatica* Roxb., *Terminalia alata*, *Vigna sublobata* (Roxb.)
The Bastar Region	*Achyranthes aspera*, *Tylophora indica* (Burn.f.) Merr., *Emblica officinalis*, *Terminalia arjuna*, *Withania somnifera* (L.) Dunal., *Psoralea corylifolia* L., *Acorus calamus*, *Aegle marmelos*, *Terminalia bellirica*, *Baliospermum montanum* (Willd.) Muell., *Clerodendron serratum* (L.) Moon, *Pterocarpus marsupium*, *Bacopa monnieri* (L.) Pennell, *Plumbago zeylanica* L., *Cissus quadrangularis* L., *Leptadenia reticulata* Wight & Arn., *Tinospora cordifolia* (Willd.) Hook.f. & Thomson, *Aloe vera* (L.) Burn.f., *Gloriosa superba* L., *Gymnema sylvestre* R.Br., *Terminalia chebula*, *Helicteres isora* L., *Hemidesmus indicus* (L.) R. Br., *Balanites aegyptiaca* (L.) Del., *Curculigo orchioides* Gaertn., *Andrographis paniculata*, *Costus speciosus* (Koen ex. Retz.) Sm., *Strychnos nux-vomica* L., *Butea monosperma*, *Phyllanthus niruri* L., *Piper longum*, *Hibiscus sabdariffa*, *Smilax macrophylla* L., *Chlorophytum borivilianum* Santapau & Fernandes and *Chlorophytum* spp.,

(continued)

Table 1 (continued)

Name of the agrobiodiversity heritage site	Diversity in representative medicinal crop species
	Rauvolfia serpentina, *Evolvulus alsinoides*, *Alstonia scholaris* (L.) Br., *Asparagus racemosus*, *Semecarpus anacardium* Linn.f., *Bryonia laciniosa* Linn., *Spilanthes acmella* L., *Curcuma amada*, *Adhatoda vasica*, *Ventilago maderaspatana* Garten., *Vitex negundo*, *Allium porrum*, *Woodfordia* fruticosa Kurz., *Wrightia tinctoria* R.Br.
The Koraput Region	*Tylophora indica* Merr., *Aegle marmelos*, *Caesalpinia bonducella* (L.) Flem., *Centella asiatica* (L.) Urb., *Plumbago zeylanica* L., *Costus igneus*, *Curcuma pseudomontana*, *Tinospora cordifolia* (Willd.) Hook.f. & Thomson, *Boswellia serrata*, *Gymnema sylvestris* R. Br., *Helicteres isora* L., *Hypericum* L. spp., *Garuga pinnata* (Roxb.), *Mucuna monosperma* DC. Ex Wight, *Strychnos nux-vomica* Linn., *Pogostemon* Desf. spp., *Psychotria rubra* (Lour.) Poir., *Rauvolfia serpentina* (L.) Benth. Ex Kurtz, *Sarcostemma acidum* Voigt, *Solanum* L. spp., *Terminalia alata* Herb. Madr. Ex Wall, *T. arjuna* (Roxb. ex DC.) Wight & Arn., *T. bellirica* (Gaertn.) Roxb., *T. chebula* Willd. Ex Flem. and *Cissus quadrangularis* L.
The South-Central Region of Eastern Ghats	*Terminalia bellirica* (Gaertn.) Roxb., *Centella asiatica* (L.) Urban, *Costus speciosus* (Koen ex Retz.) Sm., *Cassia fistula*, *Tinospora cordifolia* (Willd.) Hook.f. & Thomson, *Gloriosa superba* L., *Terminalia chebula*, *Andrographis paniculata* (Burm. f.) Wall., *Cymbopogon flexuosus* (Steud.) Wats., C. *martinii* (Roxb.) Wats., *Morinda citrifolia*, *Phyllanthus amarus* Schum., *Piper longum* L., *Plumbago zeylanica* L., *Strychnos nux-vomica* L. and *Tacca leontopetaloides* (L.) Kuntze
The Cauvery Region	*Acalypha indica* Linn., *Adhatoda vasica* Nees, *Alangium salviifolium* (L.f.) Wangerin, *Cassia auriculata* L., *Eclipta prostrata* L.; syn. *E. alba* (L.) Hassk., *Bacopa monnieri* L. Pennell, *Centella asiatica* L., *Gloriosa superba* Linn., *Hemidesmus indicus* L. R.Br., *Hedyotis* L. spp., *Phyllanthus niruri* L., *Sida cordifolia* L., *Piper longum* L., *Rauvolfia serpentina* Benth., *Senna alexandrina* Mill.; syn. *Cassia senna* Linn., *Strychnos nux-vomica*, *Terminalia arjuna* (Roxb. ex DC.) Wight & Arn., *T. bellirica*, *Tylophora indica* (Burm. f.) Merrill., *Pergularia daemia* Forssk., *Abelmoschus manihot* (L.) Medik. and *Acorus calamus* L.
The Northwestern Deccan Plateau Region	*Achyranthes aspera* Linn., *Hibiscus cannabinus*, *Blumea eriantha* DC., *B. lacera* L., *Boerhaavia diffusa*, *Chlorophytum tuberosum* (Roxb.) Baker,

(continued)

Table 1 (continued)

Name of the agrobiodiversity heritage site	Diversity in representative medicinal crop species
	Cissus quadrangularis Linn., *Corchorus depressus*, *Eriolaena hookeriana*, *Cynodon dactylon*, *Eclipta alba* (L.) Hassk., *Imperata cylindrica* (L.) Beauv., *Hibiscus sabdariffa*, *Cassia tora* L., *Leucas aspera*, *Wrightia tinctoria*, *Phaseolus trilobus*, *Cymbopogon martinii* (Roxb.) Wats, *Santalum album* L., *Hemidesmus indicus* (L.) R. Br., *Canthium parviflorum* Lam. and *Canavalia gladiata* (Jacq.) DC.
The Konkan Region	*Aloe barbadensis* Mill., *Ceropegia hirsute* Wight & Arn., *Cicer arietinum*, *Anisochilus carnosus*, *Citrus aurantifolia (*Cristm.) Swingle, *Zingiber officinale*, *Achyranthes aspera* L.), *Aloe vera* (L.) Burn. f.; syn. *A. barbadensis* Mill., *Andrographis echioides* (L.) Nees., *Anisochilus carnosus* (L.f.) Wall., *Bauhinia variegata* L., *Chlorophytum tuberosum* Bake, *Cissus quadrangularis* L., *Cissus repanda* Vahl, *Cymbopogon martinii* (Roxb.) W. Watson, *C. nardus* (L.) Rendle., *C. citratus* Stapf, *Terminalia chebula*, *Gymnema sylvestre* R. Br.), *Chlorophytum borivilianum* L. and *Ocimum tenuiflorum* L.
The Malabar Region	*Terminalia arjuna* (Roxb. ex DC.) Wight & Arn., *Boehmeria malabarica, Bacopa monnieri* (L.) Wettst., *Tinospora cordifolia* (Willd.) Miers, *Kaempferia galangal* Linn., *Vitex negundo* L., *Centella asiatica* (L.) Urban, *Cymbopogon flexuosus* (Steud.) Wats. var. coimbatorensis, *C. martinii* Roxb. var. tofia, *Hemidesmus indicus* L. R. Br., *Coleus amboinicus* Lour., *Desmodium gangeticum* L. DC., *Pergularia daemia* (Forsk.) Chiov., *Vetiveria zizanioides* (L.) Nash; syn. *Andropogon zizanioides* Linn., *Rauvolfia serpentina* (L.) Benth. ex Kurz., *Ruta graveolens* Linn., *Asparagus racemosus* var. javanicus (Kunth.) Baker. J. Linn., *Piper longum* Linn., *Ocimum sanctum* L.; syn. *O. tenuiflorum* L., *Tylophora indica* (Burm. f.) Merrill. and *Vateria indica* L.

[a]Source: Singh (2015)

this total diversity, only 2000 plants are used in the classical formulations of various systems of medicine (Fig. 1).

The rural population has its own diverse system of health management, and some remedies for common ailments include plants like tulsi, turmeric, cloves, ginger, cumin, fenugreek, amla, triphala, etc. This system although has no systematic inventory and documentation, but surveys conducted under the Global Environment Facility (GFF)-funded subproject "Harmonizing biodiversity conservation and

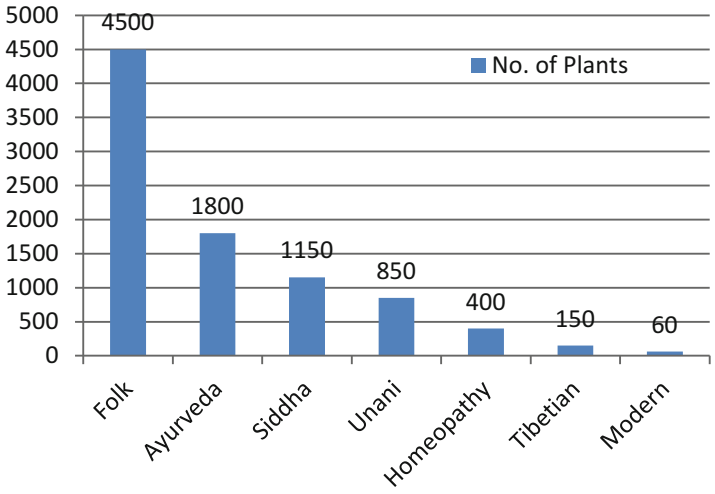


Fig. 1 Medicinal plants used in different systems of medicine

agricultural intensification through integration of plant, animal and fish genetic resources for livelihood security in fragile ecosystems" under component 3 (SRLS) in three states, viz. Rajasthan, Andhra Pradesh and Himachal Pradesh, have revealed approximately 4500–5000 plant species of medicinal value being used by the traditional village physicians/vaids/healers.

Conservation Strategies and Priorities

The increasing interest in plant-based medicines has resulted in an exponential increase in demand of raw medicinal plants for drug formulations. This has resulted in the over-exploitation, illegal harvesting, and extinction or threat to many medicinal plant species in the wild. In addition to these, habitat degradation due to human encroachment to wild habitats on protest of development activities has further accelerated the current rate of extinction of biodiversity in general and medicinal plants in particular, and further this also leads to loss of associated traditional knowledge. Moreover inadequate laws, weak legislations and ineffective control over the management and harvest of wild species outside the protected areas where rural people are lured for illegal collection and trade of medicinal plants, just for getting financial benefits, also add to threat to these valuable genetic resources.

The Convention on Biological Diversity (CBD) emphasizes the conservation, sustainable use and fair equitable sharing of benefits arising from access and commercialization of genetic resources; the Aichi Targets of the CBD also emphasize the nutritional and health security through proper management and conservation of plant genetic resources. Since conservation strategies are based on the reproductive behaviour and habit of the species in concern and due to the vast diversity from

annuals to perennial to tree species, no single strategy can be adopted for conservation of medicinal plants. Therefore a holistic approach involving all the various strategies is needed for conserving this immense wealth.

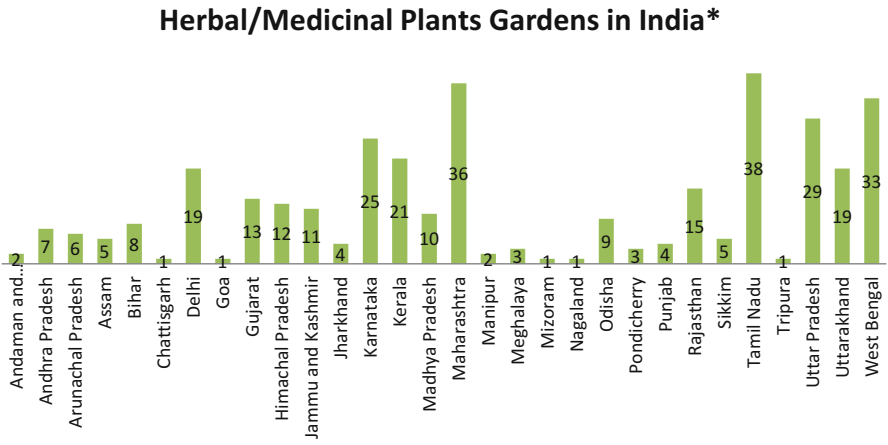
The various conservation strategies followed for these groups of plants include ex situ conservation through botanical gardens, herbal gardens, seed gene banks, herbarium, tissue culture in vitro gene banks or cryo banks, in situ conservation through forest managements, sacred grooves, protected areas, biosphere reserves, etc. The third recently adopted strategy for the conservation of medicinal plants is being a participatory approach of the communities, i.e. conservation through use by maintaining the local herbal plants in their kitchen gardens or backyard gardens.

A number of organizations, government as well as non-government, are working in the field of medicinal plants in India. Institutes working under AYUSH plus a large number of research centres of Central Council for Research in Ayurveda and Siddha under Ministry of Health and Family Welfare, CSIR under Department of Science and Technology, Department of Biotechnology, CSIR-Central Institute of Medicinal and Aromatic Plants (CIMAP), state forest departments under Ministry of Environment and Forests (minor forest products), Defence Research and Development Organisation and a large network of state agriculture universities are working independently on research and developmental activities of medicinal plants. The Ministry of Agriculture and Farmers Welfare under the aegis of Indian Council of Agriculture Research (ICAR), Directorate of Medicinal and Aromatic Plants Research (DMAPR) and National Bureau of Plant Genetic Resources (NBPGR), New Delhi, is actively engaged in the collection, conservation and characterization of medicinal and aromatic plants. Under G-15 project funded by Department of Biotechnology, three medium-term conservation facilities were created in India, viz. TBGRI in Thiruvananthapuram, NBPGR in New Delhi and CIMAP in Lucknow, especially for conservation of medicinal plant genetic resources. Besides the many in situ conservation programmes including declaration of gene sanctuaries, wildlife protected areas, biosphere reserves, national parks and herbal gardens established under Central Sector Scheme, botanical gardens almost at every institute and college are ensuring the onsite conservation of biodiversity including medicinal plants. Recently in 2000, the Government of India had established the National Medicinal Plants Board (NMPB) to regulate and co-ordinate the various activities regarding research and development on medicinal plants.

Looking into this enormous efforts made by all these organizations, still the medicinal plants have not attained their rightful position in the Indian economy. The NMPB through the establishment of State Medicinal Plant Boards is still trying to focus on these aspects, but the lack of co-ordination among various organizations, scarcity of trained manpower and absence of network between the research organization, farmers and traders have resulted in poor development of the medicinal plant industry.

Since conservation strategies are mainly based on the geographic distribution and reproductive biology of the species to be conserved, a holistic approach is needed to meet the requirement. The best-suited ideal conservation strategy for medicinal plants is in situ conservation, designating protected areas, biosphere reserves,

biome reserves, national parks or gene sanctuaries. But the success of this approach depends on the active participation of the local inhabitants. The Government of India through the Ministry of Environment and Forests has already declared 537 wildlife sanctuaries, 18 biosphere reserves, 103 national parks and one gene sanctuary to protect the biodiversity occurring in various biogeographical areas of the country including medicinal flora. The forest departments of the states of Karnataka, Kerala and Tamil Nadu in collaboration with FRLHT have established 32 medicinal plant protected areas across Western Ghats. A total of 108 MPCA has been established in India since 1993 through FRLHT, Bangalore (Website envis. Frlht. Org/mpca.php accessed on 6-1-17).



*Source NMPB website http://nmpb.nic.in/index1.php?level=2&sublinkid=598&lid=248

The most ideal and safe conservation strategy in additional to in situ approach is ex situ conservation through setting up gene banks or herbal gardens.

Status of Medicinal Plant Genetic Resources at NBPGR

The National Bureau of Plant Genetic Resources (NBPGR), New Delhi, under the aegis of the Indian Council of Agricultural Research (ICAR), is actively engaged in the collection, conservation and characterization of medicinal and aromatic plants and is leading the conservation programme with the National Gene Bank. The Indian National Gene Bank located at NBPGR has its germplasm conservation facilities created and maintained to the international standards. Presently the gene bank has 19 storage modules with a holding capacity of about 1.0 million accessions including 16 modules for long-term conservation of base collections of germplasm seed accessions (−20°C), 0.25 million accession holding capacity at cryobank (−150° to −196 °C) and a sufficient in vitro repository (4−25 °C).

The National Gene Bank is second largest in the world in its capacity and compares well with the international gene banks such as NSSL, Fort Collins, USA. The present holding in the National Gene Bank is the result of meticulous effort made over years at an average rate of 10,000–15,000 accessions per year. The source network for supply of base collections comprises:

- NBPGR headquarters
- NBPGR regional stations and base centres (in ten different zones)
- National active germplasm sites (59) at crop-specific institutes and SAUs
- International agricultural research centres duplicate base collections
- Non-governmental organizations, farmers and others

The Indian National Gene Bank (NGB) at ICAR-NBPGR is primarily responsible for conservation of unique accessions on long-term basis, as base collections for posterity, predominantly in the form of seed stored in refrigerated modules maintained at –18 °C. Presently 4,30,982 accessions belonging to 1547 species have been conserved at National Gene Bank including 5756 accessions of medicinal plant representing 412 genera and 578 species (Table 2, Fig. 2). A state wise representation of medicinal plants conservation is given in Fig. 3. ICAR-NBPGR is also maintaining a large number of germplasm at its various regional stations located in different agro-climatic regions of the country either in medium-term storage or in field gene bank (Table 3). The NGB also maintains 1891 accessions belonging to more than 158 vegetatively propagated plant species including 155 cultures of medicinal plants under in vitro conditions (Anonymous 2016). Alternately, around 12,015 accessions belonging to 720 species have been cryopreserved in vapour phase of liquid nitrogen at a temperature of −150 to –196 °C (Table 4, Figs. 4 and 5).

National Legislations for Protection of Medicinal Plants in India

Development of several negotiations at global level like Convention on Biological Diversity (CBD), Trade-Related Aspects of Intellectual Property Rights (TRIPS) resulting in emergence of World Trade Organization (WTO) and International Treaty on Plant Genetic Resources for Food and Agriculture (ITPGRFA) had created a great turmoil in the field of plant genetic resources in general and medicinal and aromatic plants in particular. Modernized technological tools in the field of molecular biotechnology and bioinformatics for utilization of genetic resources have arisen the necessity of adopting legal instruments for access to genetic resources (GRs) and sharing of benefits from their use thereafter. Though once considered as “common heritage of mankind”, it has now become the “sovereign rights of a country”.

India having around 8% of total world’s biodiversity including the medicinal plant genetic resources can emerge as a global leader in medicinal plant trading sector. Today 45% of all patents on herbs or herbal-based or related medicines are with China closely followed by Japan with 28% share. As on today, Patent Act gives protection only to the methodology developed for preparation of a product but not to

Table 2 Status of cultivated and wild species of medicinal value conserved in National Gene Bank, NBPGR (As of January 2017)

Crop name	Botanical name	Acc. No.
Cultivated species		
Indian liquorice	*Abrus precatorius*	132
Country mallow/Indian mallow	*Abutilon indicum*, *Abutilon persicum*	59
King of bitters	*Andrographis paniculata*, *Andrographis echioides*	112
Mexican prickly poppy	*Argemone mexicana*	26
Kemuk	*Costus speciosus*	30
Vetches	*Asparagus adscendens*, *Asparagus officinalis*, *Asparagus racemosus*	48
Safed musli	*Chlorophytum borivilianum*, *Chlorophytum glauca*, *Chlorophytum glaucoides*, *Chlorophytum nimmonii*	41
Datura/ Indian thorn apple/ tree datura	*Datura alba*, *Datura metel*, *Datura bernhardii*, *Datura discolor*, *Datura fastuosa*, *Datura ferox*, *Datura inoxia*, *Datura quercifolia*, *Datura stramonium*, *Datura suaveolens*	81
Makhana/fox nut	*Euryale ferox*	108
Mehndi/henna	*Lawsonia vasica*, *Lawsonia alba*, *Lawsonia inermis*	110
Lemon basil/sacred basil/ shrubby basil/sweet basil/basil	*Ocimum americanum*, *Ocimum x citriodorum*, *Ocimum sanctum*, *Ocimum tenuiflorum*, *Ocimum gratissimum*, *Ocimum basilicum*, *Ocimum canum*, *Ocimum kilimandscharicum* Gurke, *Ocimum* sp.	542
Opium poppy	*Papaver somniferum*	429
Psyllium/isabgol	*Plantago arenaria*, *Plantago hookeriana*, *Plantago lanceolata*, *Plantago major*, *Plantago coronopus*, *Plantago ovata*, *Plantago indica*	89
Babchi	*Psoralea corylifolia*	86
Pala indigo	*Wrightia tinctoria*, *Wrightia arborea*, *Wrightia tomentosa*	36
Asgand	*Withania somnifera*	295
Miscellaneous	57 genera and 78 species	315
Wild species		
Indian birthwort	*Aristolochia bracteata*, *Aristolochia grandiflora*, *Aristolochia indica*, *Aristolochia tagala*	32
Hogweed	*Boerhavia diffusa*	22
Milkweed	*Calotropis gigantea*, *Calotropis procera*	23
Balloon vine	*Cardiospermum halicacabum*	15
Cassia/Indian barberry/Indian laburnum/Indian senna/*Luvunga*	*Cassia alata*, *C. auriculata*, *C. frondosa*, *C. hirsuta*, *C. italica*, *C. mimosoides*, *C. obtusa*, *C. obtusifolia*, *C.occidentalis*, *C. rotundifolia*, *C. sophera*, *C.tomentosa*, *C. toria*, *C.* sp., *C. angustifolia*, *C. fistula*, *Cassia senna*, *Cassia renigera*, *Casssia pumila*	191
Malaysian apple	*Diplocyclos palmatus*	26

(continued)

Table 2 (continued)

Crop name	Botanical name	Acc. No.
Entada	*Entada monostachya*, *E. pursaetha*, *E. rheedii*, *E. scandens*	26
East Indian screw tree	*Helicteres isora*	30
Holarrhena	*Holarrhena antidysenterica*, *Holarrhena arnottiana*	26
Touch-me-not	*Mimosa pudica*	29
Morinda/noni	*Morinda angustifolia*, *Morinda pubescens*, *Morinda citrifolia*, *Morinda tinctoria*, *Morinda tomentosa*	166
Broken bones plant	*Oroxylum indicum*	29
Burra gokhru	*Pedalium murex*	20
Himalayan rhubarb	*Rheum australe*, *Rheum emodi*, *rheum spiciforme*	10
Bhootkesh/bhutkesh	*Selinum elatum*, *Selinum wallichianum*, *Selinum tenuifolium*, *Selinum vaginatum*	11
Chebulic myrobalan/Indian laurel/Arjun	*Terminalia chebula*, *T. alata*, *T. tomentosa*, *T. bellirica*, *T. catappa*, *T. muelleri*, *T. pallida*, *T. paniculata*, *T.* sp., *Terminalia arjuna*	144
Gokharu, puncture vine	*Tribulus pentandrus*, *Tribulus rajasthanensis*, *Tribulus terrestris*	44
Prickly ash/zanthoxylum	*Zanthoxylum armatum*, *Zanthoxylum rhetsa*, *Zanthoxylum alatum*	16
Desmodium	*Desmodium elegans*, *D. gangeticum*, *D. gyrans*, *D. heterocarpon*, *D. intortum*, *D. motorium*, *D. triquetrum*, *D.* sp.	27
Sea buckthorn	*Hippophae rhamnoides* L., *Hippophae rhamnoides* var. *turkestanica*, *Hippophae salicifolia*	180
Nux vomica	*Strychnos nux-vomica*	40
Oregano	*Origanum vulgare*	34
Periwinkle	*Catharanthus roseus*	22
Persimmon tree	*Diospyros chloroxylon*, *Diospyros melanoxylon*, *Diospyros oocarpa*, *Diospyros tomentosa*, *Diospyros* sp.	23
Rohida	*Tecomella undulata*	241
Sage/chia	*Salvia deserta*, *S. hians*, *S. hispanica*, *S. horminum*, *S. moorcroftiana*, *S. nemorosa*, *S. officinalis*, *S. sclarea*, *S. tesquicola*, *S. verbenaca*	25
Sarpagandha	*Rauvolfia beddomei*, *R. canescens*, *R. serpentina*, *R. tetraphylla*	33
Shivlingi	*Bryonopsis laciniosa*	21
Sida	*Sida cordata*, *Sida cordifolia*, *Sida ovata*, *Sida acuta*, *Sida rhombifolia*, *Sida rhombifolia* ssp., *Retusa*	28
Soapberry	*Sapindus emarginatus*, *Sapindus laurifolius*, *Sapindus mukorossi*, *Sapindus trifoliatus*, *Sapindus* sp.	17

(continued)

Table 2 (continued)

Crop name	Botanical name	Acc. No.
Spider flower	*Cleome icosandra*, *Cleome viscosa*	47
Spurge	*Euphorbia cyathophora*, *Euphorbia hirta*, *Euphorbia nivulia*, *Euphorbia thymifolia*	18
Tephrosia	*Tephrosia candida*, *Tephrosia purpurea*, *Tephrosia* sp.	21
Vidanga	*Embelia ribes* Burm, *Embelia tsjeriam-cottam*	16
Wormwood	*Artemisia annua*, *Artemisia maritima*, *Artemisia nilagirica*, *Artemisia scoparia*, *Artemisia vulgaris*, *Artemisia* sp.	26
Yarrow	*Achillea asiatica*, *Achillea biebersteinii*, *Achillea borealis*, *Achillea conferta*, *Achillea falcata*, *Achillea filipendulina*, *Achillea lanulosa*, *Achillea millefolium*, *Achillea nobilis*, *Achillea santolina*	19
Nirgundi	*Vitex negundo*	19
Hemp, bhang	*Cannabis sativa*	20
Hogweed	*Heracleum pinnatum*, *Heracleum sosnowskyi* Mandenova, *Heracleum* sp., *Heracleum grande*, *Heracleum lanatum*, *Heracleum candicans*	22
Indigofera	*Indigofera dielsiana*, *Indigofera glandulosa*, *Indigofera linnaei*, *Indigofera longeracemosa*, *Indigofera tinctoria*	32
Jyotismati	*Celastrus paniculatus*	30
Leadwort	*Plumbago zeylanica*	28
Miscellaneous	297 genera and 361 species	1368
	Total Accessions	5756

Fig. 2 National seed Genebank at ICAR-NBPGR, New Delhi

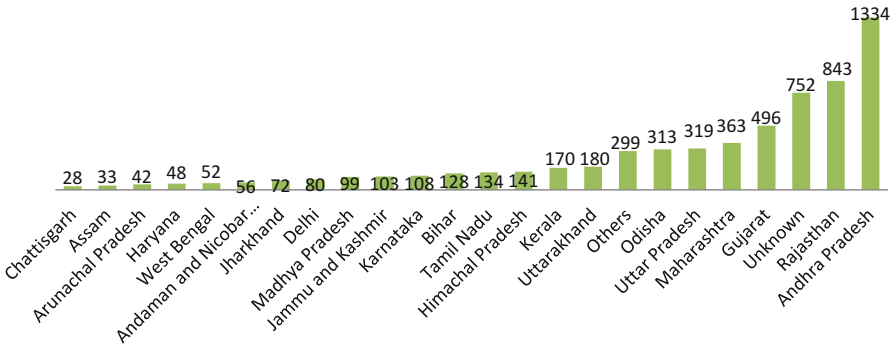


Fig. 3 Status of medicinal plants germplasm conserved at National Genebank from different states

Table 3 Status of germplasm accessions conserved at ICAR-NBPGR regional stations (no. of medicinal plants in parenthesis)

Name of the station	Accessions maintained at medium-term storage (MTS)	Field gene bank (FGB)
NBPGR New Delhi	28,367 (258 MAPs)	323
Akola regional station	20,262	Data not available
Bhowali regional station	11,565 (320 MAPs)	1285 (125 MAPs)
Cutttak regional station	254	579 (254 MAPs)
Jodhpur regional station	35,849 (581 MAPs)	Data not available
Shillong regional station	Data not available	187
Shimla regional station	12,565	1215 (52 MAPs)
Thrissur regional station	**10,886 (Total in MTS + FGB)**	

the product as such. The main reason of India for not leading the patent registration is because as per the Patent Act traditional knowledge, when not properly documented, cannot serve as a part of prior art. Illegal access and commercialization of this traditional knowledge by the industrially developed countries on the pretext that it will be for welfare of mankind are not actually benefiting the communities which possess it. Therefore this age-old traditional heritage should be properly documented and be made available to the International Patent Classification (IPC) committee of experts on priority. Setting up of Traditional Knowledge Digital Library (TKDL)

Table 4 Status of in vitro gene bank and cryobank of germplasm at ICAR-NBPGR (as of 31 December 2016)

Crop group	Present status
In vitro bank (Fig. 4)	
Tropical fruits	420
Temperate and minor tropical fruits	322
Tuber crops	597
Bulbous crops	171
Medicinal and aromatic plants	155
Spices and industrial crops	226
Total	*1891*
Cryobank (Fig. 5)	
Recalcitrant	
Intermediate	6398
Orthodox	3725
Dormant bud (mulberry)	387
Pollen	522
DNA	983
Total	*12,015*

Fig. 4 Tissue Culture bank at ICAR-NBPGR

through collaborative project of CSIR and AYUSH was done with an objective to avoid grant of patent on traditional knowledge of India. TKDL is an exclusive database containing information 500 Ayurvedic, 500 Unani and 200 Siddha formulations retrieved from more than 150 books published on these systems. Today the access to 2.5 lakh medicinal formulations reported in TDKL to various patent offices worldwide thoroughly safeguards our traditional knowledge from biopiracy.

For further strengthening of this vast reservoir of biological diversity including medicinal plants, India has developed a strong network of policy framework which

Fig. 5 Cryobank at ICAR-NBPGR

addresses the issues which pertain to the access of genetic resources for sustainable utilization and fair and equitable sharing of benefits arising from their commercialization. In compliance to CBD, India had notified the Biological Diversity Act 2002 (BDA) on the 5th of Feb. 2003 followed by the publication of Biological Diversity Rules 2004. The Government of India has envisaged the establishment of the National Biodiversity Authority (NBA) with its headquarters at Chennai with State Biodiversity Boards (SBBs) at state level and Biodiversity Management Committees (BMCs) at local level to regulate the access of these resources and the traditional knowledge associated with them. NBA recommends documenting the available diversity of each village as plant biodiversity register (PBR) which includes medicinal plants also along with the associated traditional knowledge. PBRs in future can serve as documentary proofs to prior art. The high-yielding varieties developed under crop improvement programmes are well protected through regular notifications issued from the Department of Agriculture and Co-operation (DAC), Central Varietal Release Committee (CVRC) on Crop Standards, Notification and Release of Varieties, State Varietal Release Committee and Protection of Plant Varieties and Farmers' Right Authority. During this process, a big chunk of germplasm lines which do not qualify the standards of varietal release procedure but are having promising traits to further serve as backbone material for future breeding programmes and having resistant or tolerant traits for various abiotic and biotic stresses and various quality and agronomic traits are identified. The Indian Council of Agricultural Research (ICAR) had developed a mechanism to recognize this trait-specific potentially valuable germplasm through the Plant Germplasm Registration Committee (PGRC) entrusting the National Bureau of Plant Genetic Resources (NBPGR) as the implementing authority. Till date 35 PGRC meetings have been held registering a total of 1286 germplasm including 61 germplasm accessions of medicinal and aromatic plants (http://www.nbpgr.ernet.in:8080/ircg/index.htm) which are well conserved in the National Gene Bank.

Future Line of Work

Medicinal and aromatic plants play a very pivotal role in the primary healthcare system of India as well as in the allopathic system by providing basic raw material for drug discovery. Medicinal plants not only promote the health security but also support the financial system, cultural identities and livelihood security of the rural population. Enormous research is being carried out in India in the medicinal plant sector, and many organizations are involved, but there is a strong need of cohesion among various stakeholders to avoid duplication of the work. The medicinal plant sector requires a renewed thrust and prioritization in all respects. Development of multidisciplinary network on collection, conservation, documentation, value addition, postharvest management, shelf-life monitoring, quality standards, safety measures and marketing strategies is the need of hour. There is a need for strong co-ordination among the various organizations involved in the research of collection or conservation aspects of these resources under the aegis of the newly created Ministry of Rural Development recognizing our traditional system of healing. The participation of private sector including pharmaceutical companies is very neglible; hence it is desirable to develop public-private partnership models (Gupta 2016) where the private sector can contribute through funding and guiding farmers for quality production of raw material for supply to pharmaceutical industries and ensuring economic security to the local health programmes. The persistent illiteracy regarding IPR regimes and rights among the rural population results in the biopiracy which can be erased through organization of grassroot-level IPR awareness programmes.

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Common Pests and Diseases of Medicinal Plants and Strategies to Manage Them

T. Marimuthu, M. Suganthy, and S. Nakkeeran

Introduction

Medicinal plants are known to Indian traditional healers since time immemorial. The plants were collected, by and large, from the wild and were used in many preparations of wellness products. India is endowed with diverse group of medicinal plants accounting for more than 8000 species which are being used in more than 10,000 herbal products. Ninety percent of herbal industry's requirement of raw materials is meted out from the natural ecosystem – forests – resulting in ruthless exploitation and destruction of its natural habitats (Mathivanan et al. 2016). As one of the measures to conserve the precious species, commercial cultivation began which inadvertently brought the problem of pests and diseases leading to crop loss of various magnitudes. This chapter will discuss on major pests and diseases of some important medicinal plants like noni (*Morinda citrifolia* L.), glory lily (*Gloriosa superba*), makoi (*Solanum nigrum*), medicinal coleus (*Coleus forskohlii*), senna (*Cassia angustifolia*) and ashwagandha (*Withania somnifera*). Medicinal plants being used in health-care system and chemical methods of management of pests and diseases are becoming obsolete. It is a must that non-chemical, eco-friendly safer methods of management are essential which are also being discussed in this chapter.

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Pests and Disease Scenario of Medicinal Plants

Noni (*Morinda citrifolia* L.)

Pests of Noni

Noni, *Morinda citrifolia* L., is one of the most valued medicinal tree plants whose medicinal properties are known to Indian since time immemorial. All parts of the plants – leaves, flowers, fruits, roots and stems – are being exploited for medicinal properties (Peter 2009). The crop is reported to be affected by various insect and noninsect pests (Jayakumar 2010; Malarvannan 2010; Sithanantham et al. 2010).

Lacewing Bug: *Dulinius conchatus* (Tingidae, Hemiptera)

Lacewing bug is one of the serious pests of noni, especially in nurseries. Nymphs and adults of lacewing bug feed on the sap resulting in brown spots on the upper surface of the leaves. In cases of severe attack, spots become brown and leaves shrivel and dry up. Adults have densely reticulate body and wings measuring 5–8 mm long which dwell on the underside of leaves.

Black Fly: *Aleurocanthus terminaliae* (Aleyrodidae, Hemiptera)

Leaves are mostly susceptible and often become blackish and sometimes curled upside due to desapping of leaves from lower surface of the leaves. Sooty mould develops on the sticky substance secreted by the insect pest. Adult female lays 15–20 eggs in a spiral pattern. A female can lay one to three spirals in its life time.

Green Coffee Scale: *Coccus viridis* (Green) (Coccidae, Hemiptera)

Adult green coffee scale is oval to elongate in shape with dorsoventrally flattened oval body. Insects feed from the phloem of the plant and are found on stems, leaves, fruits and green twigs. The sap feeding by green scales results in yellowing of leaves, in severe cases resulting in defoliation and reduction in fruit set besides overall reduction in plant growth and vigour.

Large populations of green scale could cause yellowing, defoliation, reduction in fruit set and loss in plant vigour. Scales feeding on older trees could cause dieback of twigs reducing the vigour of trees, besides reduction in fruit size. Adult females could be identified by the shiny pale green colour with U-shaped internal markings which are black and visible to the naked eye. The black internal marking is lost as the scales become dead which turn into buff or light brown. The eggs are normally laid singly which are whitish green with elongate-oval shape. Eggs hatch beneath the females and they are well protected. The incidence is more during summer than in other seasons. Infestations are accompanied by sooty mould due to honey secretion.

Southern Green Sting Bug: *Nezara gramineae* (Fabricius) (Pentatomidae, Hemiptera)

The pest is distributed in tropics and subtropics and is known for its polyphagous nature. Nymphs and adults feed on tender growing shoots and growing fruits. The sting bugs secrete a defensive characteristic scent, and the males elicit aggregation

pheromone which attracts the females. The affected shoots usually wither or die in extreme cases (Malarvannan 2010).

White Fly: *Bemisia tabaci* (Gennadius) (Aleyrodidae, Hemiptera)
White flies are minute plant bugs infesting the foliage of *M. citrifolia*. The nymphs and adults desap the plant, while honeydew secreted attracts sooty mould development on the plant. The incidence is severe during summer months (Malarvannan 2010).

Mealy Bug: *Maconellicoccus hirsutus* (Pseudococcidae, Hemiptera)
Mealy bugs often infest the young growing shoots which results in stunted growth with swollen growing points. In severe cases the leaves are crumpled which results in defoliation and even the death of plant. The mealy bugs migrate to the healthy tissues like twigs, branches and finally down the trunk. Adult females lay eggs and hatch in 6–9 days. Under a warm climate, the cycle is completed in about 5 weeks' time.

***Humming Bird* Moth: i. *Macroglossum gyrans* (Walker) (Sphingidae, Lepidoptera)**
Adult moths resemble humming birds when they hover to draw nectar from flowers. The larvae are green in colour with characteristic long horn at anal end. They feed voraciously on leaves of all stages. Young larvae normally feed on leaf buds and later move into the neighbouring leaves and feed on young as well as matured leaves. Female moth lays eggs singly (up to 300) on the undersurface of leaves.

Leaf-Folding Caterpillar: *Psara obscuralis* (Lezderer) (Pyralidae, Lepidoptera)
It is also called as leaf webworm. The adults are strictly nocturnal and take off erratically when disturbed. Female adult lays eggs along the midrib of leaves on the upper surface either singly or in mass. Well-grown larvae are translucent, creamy or green coloured. Very young larvae feed on upper layer of leaf leaving the lower epidermis intact, while the midsized larva chews the marginal notches of leaves. Fourth and fifth instar larvae devour the whole leaf blade forming web within and leaving green faecal pellets on the leaf. Infested leaves show transparent glossy patches.

Leaf Beetle: *Schizonycha ruficollis* (Melolonthidae, Coleoptera)
The grubs feed on roots of the plant but the damage may not be seen as significant. The adult beetles emerge in rainy season form the soil and congregate to feed on the leaves during night time. When the beetle populations are high, they could even completely destroy the plant as the leaves of all stages of the crop are susceptible.

Grasshoppers

(a) Wingless grasshopper: *Orthacris maindroni* (Pyrgomorphidae, Orthoptera)

The nymphs and adult grasshoppers feed on the leaves resulting in total defoliation. The female grasshoppers are distinctly larger than the male ones.

(b) Cotton grasshopper: *Cyrtacanthacris tatarica* (Acrididae, Orthoptera)

Nymphs are green in colour, with rudimentary wings. Adults are with contrasting shades of black, brown, cream yellow and white; the dorsal longitudinal stripe is extended from the vertex between the compound eyes across the length of the pronotum and the tegmina with well-developed wings. Males are distinctly smaller than females. The damage on leaves is visible by irregular feeding by either the nymphs or adults.

Stem Girdlers

(a) *Sthenias grisator* (Cerambycidae, Coleoptera)
(b) *Apriona swainsoni* (Cerambycidae, Coleoptera)

The beetles are nocturnal and feed on the main stem about 15 cm above the ground level leaving a ringlike marking around the affected stem. The beetles also attack the young green branches resulting in death of the affected branches above the point of feeding. Beetles hide beneath the leaves or new branches during daytime and become active at night.

Fruit Fly: *Bactrocera dorsalis* (Tephritid Fruit Flies) (Tephritidae, Diptera)

Fruit flies are potential constraint to the yield of noni fruit. The flies can cause dark spots in the fruits by oviposition. Maggots hatch within the fruit, develop inside and pave way for microbial infection which can cause extensive rotting and fruit drop, rendering the fruit unfit for use. Infested fruits fall prematurely and the maggots enter into soil for pupation. It has also been shown that internal fruit damage can affect the nutraceutical values of fruits, by causing significant reduction in the content of scopoletin and total flavonoids, besides protein and carbohydrate levels (Sithanantham et al. 2016). The fruit flies causing damage to noni elsewhere in Seychelles (Legal et al. 1992) belong to a different family (Drosphilidae).

Diseases of Noni

Anthracnose: *Colletotrichum gloeosporioides*

Small brown shaped spots of varying size (0.5–2.5 cm) appear on the leaves which gradually enlarge and coalesce. The centre of the coalesced lesion turns greyish white leaving a shot hole symptom. Under humid conditions acervuli with pink masses of spores emerged on the lesions. The occurrence of anthracnose is the first report in India (Manjunath Hubbali et al. 2012; Nakkeeran et al. 2013).

Dry Fruit Rot: *Colletotrichum gloeosporioides*

Colletotrichum gloeosporioides infect all parts of noni plant irrespective of stages of the crop growth. The symptoms were observed on twigs, flowers and fruits. Symptoms of the infection caused by *C. gloeosporioides* on the flowers appear as dull brown lesions. The infected flowers dried within 48 h after infection. Examination of the flowers under stereo zoom microscope revealed the presence of brown necrotic spots on the corolla tube adhering on the noni fruit. The twigs infected by *C. gloeosporioides* were characterised by the presence of necrotic brown lesions

with yellow halo. Later the lesions enlarge in size and were characterised with grey centre. On the grey centre, the number of minute pinhead-shaped fructifications of the acervuli was observed. The necrotic lesions spread gradually towards fruits through peduncle which leads to the development of infection on flowers and fruits.

Characteristic small circular reddish brown sunken necrotic spots appear on the fruits which later turn into dark brown lesion expanding both upward and downward which finally coalesced. As fruits enlarge, the lesions also expand, leading to splitting and drying of fruits. Subsequently, small pinhead-like acervuli appear on the infected tissues. Later, the infected fruits shrink, dry off and got mummified. After mummification the infected fruits were colonised by saprophytic moulds like *Aspergillus* and *Penicillium* (Nakkeeran et al. 2013).

Leaf Blight and Dry Fruit Rot: *Alternaria alternata*

Tamil Nadu and Karnataka states witnessed a severe outbreak of leaf blight, for the first time during 2008–2009. The causal agent was identified as *Alternaria alternata* (Manjunath Hubbali et al. 2010). The same pathogen causes dry fruit rot which is characterised by the presence of black necrotic sunken spot of 2–3 mm diameter on the green unripe fruits. During favourable environment the spot turns to dark black lesion and coalesced. The centre of the lesions is black in colour with alternate concentric zonations. As the fruit expands, it leads to splitting and drying of the fruits coupled with the saprophytic infection of other moulds (Nakkeeran et al. 2013).

Soft Rot of Fruits: *Pantoea agglomerans*

Typical symptoms of brown water-soaked lesions appeared on the surface of matured but unripe fruits. Later, within 24–48 h after infection, the lesion spread to the entire fruit, and the infected fruits emit a bad odour. The affected tissue becomes softened and rots subsequently. Later, the infection extends up to the peduncle and the fruits fell down 24–48 h after infection. The soft rot of noni fruits was reported for the first time by Nakkeeran et al. (2013).

Root-Knot Nematode (RKN): *Meloidogyne incognita*

RKN is one of the serious diseases on noni causing considerable yield loss. The infestation could be observed both in the nursery and main field. Typical above ground symptoms include yellowing of leaves in between veins, stunted growth and sickly appearance of the trees. The foliar symptoms may resemble iron and nitrogen deficiency. The below ground symptoms are root galling, distorted roots; the primary, secondary and tertiary roots are affected. Young lateral roots may become stubby with swollen tips; severely affected roots may show decaying due to secondary infection. Kavitha et al. (2012) established the host-parasite relationship, and the yield loss was estimated (42–46%) (Kavitha et al. 2011a, b). Kavitha et al. (2011b) studied the complete life cycle of *M. incognita* affecting noni.

Glory Lily: *Gloriosa superba*

Pests of Glory Lily

Glory lily is the state flower of Tamil Nadu and cultivated commercially in an area of 7000 acres. Major pests infesting glory lily are the lepidopteran pests, viz. lily caterpillar (*Polytela gloriosae* Fabricius), semilooper (*Plusia signata* Fabricius), tobacco cutworm (*Spodoptera litura* Fabricius) and a sucking pest, thrips (*Thrips tabaci* Lind) (Suganthy et al. 2012).

Thrips: *Thrips tabaci* (Thripidae, Thysanoptera)

When infestation begins, adults position themselves deep between leaf sheaths where they can find protection. Later, they spread preferentially on the lower surfaces of young leaves and flowers. Flower petals will become discoloured and deformed. They are responsible for transmission of a virus disease – necrosis. The plants infected by necrosis virus show symptoms of bronzing or purple discolouration of leaves. Leaves curl down and they become distorted. Affected leaves show numerous dark spots which subsequently wilt and die. Stems often show dark streaks near the growing point and the whole plant may die.

Lily Caterpillar: *Polytela gloriosae* (Noctuidae, Lepidoptera)

Early instar larvae feed on the chlorophyll of the leaves, while later instar larvae feed voraciously leaving only the hard stem of the plant. They also feed on the pods and seeds resulting in complete devastation.

Semilooper: *Plusia signata* (Noctuidae, Lepidoptera)

The caterpillars feed the leaves and growing tip voraciously and cause heavy defoliation. Defoliation up to 28% was recorded in the farmers' holdings.

Tobacco Caterpillar: *Spodoptera litura* (Noctuidae, Lepidoptera)

The caterpillars feed the leaves and cause defoliation. Defoliation up to 17% was recorded in the farmers' holdings in Moolanoor, Dharapuram and Ottanchathiram areas of Tamil Nadu.

Diseases of Glory Lily

Root Rot: *Macrophomina phaseolina*

Plants affected by root rot caused by *M. phaseolina* show characteristic symptoms like yellowing of leaves, development of dark lesions on the stems showing black sclerotial bodies and rotting of roots. The pathogen could survive in the soil for several years. The yield loss ranged from 50% to 60% as reported by Meena and Rajamani (2016).

Leaf Blight: *Alternaria alternata*

Leaf blight to begin with occurs as small brownish spots on leaves which later develop into concentric rings; several such spots coalesce leading to blighting of the entire leaf (Maiti et al. 2007).

Makoi (*Solanum nigrum*)

Pests of Makoi

Mealybugs, aphids, hadda beetles, red cotton bugs, stink bugs, tobacco caterpillar and semilooper are some of the pests observed on this crop (Suganthy et al. 2011).

Mealybugs: *Paracoccus marginatus* and *Phenacoccus solenopsis* (Pseudococcidae, Hemiptera)

In case of severe infestations with mealybugs, clusters of mealybugs could be seen on the lower surface of the leaves giving an appearance of thick mat with waxy secretions. Such cottony white patches could be observed on all parts of the plant. The ants are attracted due to honeydew secretion, thus leading to sooty mould growth. Due to desapping by nymphs and adults, the leaves become yellow and finally wither. The fruits of affected plants may drop prematurely. Heavy infestation may lead to death of the plant.

Aphids: *Aphis craccivora* (Aphididae, Hemiptera)

Both nymphs and adults occur in colonies on lower surface of leaves and terminal shoots. They suck the sap resulting in curling and crinkling of leaves. Honeydew excretion and sooty mould are the typical symptoms of damage. Ants are the uninvited guests to feed on honeydew excretion.

Thrips: *Thrips tabaci* (Thripidae, Thysanoptera)

The nymphs and adults lacerate the leaves and suck the sap causing upward curling of leaves.

White Flies: *Bemisia tabaci* (Aleyrodidae, Hemiptera)

The nymphs and adults suck the sap causing chlorotic spots on the leaves which later coalesce to form irregular yellow patches on the leaves which may dry and fall. If the plant is shaken, a cloud of tiny mothlike insects flutter out but rapidly resettle.

Red Spider Mite: *Tetranychus urticae* (Tetranychidae, Acari)

Yellowish white speckles, blotches, yellow bleaching of leaves and webbing on the undersurface of leaves are some of the common symptoms.

Yellow Mite: *Polyphagotarsonemus latus* (Tarsonemidae, Acari)

The yellow mite is polyphagous in nature and feed from the lower surface of young leaves. The infested leaves curl downwards at margin and tips. Severe infestation

leads to elongation of petiole and stunted growth of the plant. Presence of large number of mites is common on young leaves.

Tobacco Caterpillar: *Spodoptera litura* (Noctuidae, Lepidoptera)
Small caterpillars congregate on the undersurface of leaves and cause severe defoliation. The caterpillars cause maximum damage during night time. During daytime, they hide under the leaf litters and in cracks and crevices.

Hadda Beetle: *Henosepilachna vigintioctopunctata* (Coccinellidae, Coleoptera)
The grubs and adult beetles scrap the chlorophyll of the leaves in between veins resulting in skeletonised patches which could be seen on the upper surface of leaves giving lace-like appearance. The leaves turn brown, dry up and fall off. Usually the adults feed on the upper surface of leaves, while the grubs feed on the lower surface.

Semilooper: *Plusia signata* (Noctuidae, Lepidoptera)
Adult moth is stout brown with wavy markings and two small golden-coloured spots on forewings. Abdomen is covered with tuft of hairs in female. Larva is slender green with white wavy lines and stripes on the lateral sides. They feed on leaves causing defoliation.

Leaf Miner: *Liriomyza trifolii* (Agromyzidae, Diptera)
The female punctures the leaves for its oviposition thus making the foliage look stippled. Serpentine mines are typical symptoms of leaf miners as the larvae mine into the leaves. Mines are irregular and increase in width ranging from 0.25 to 1.5 mm by the time the larva matures. They are often easily visible within the mines where they remove the mesophyll between the surfaces of the leaf leading to drying of leaves.

Fruit Borer: *Leucinodes orbonalis* (Pyraustidae, Lepidoptera)
The larvae bore into the tender shoots causing drooping of terminal shoots. They also bore into the flower buds and berries and cause shedding of flower buds and unripened berries. The damaged fruits are seen with excreta.

Diseases of Makoi

Powdery Mildew: *Leveillula taurica*
The occurrence of powdery mildew caused by *Leveillula taurica* was reported by Sudha and Lakshmana (2007) which is the first report on *S. nigrum* in Tamil Nadu. The symptom appears on leaves as powdery patches which enlarge and cover the whole leaf resulting in drying up of leaves. *S. nigrum* might serve as a source of infection for cultivated *Capsicum annuum*.

Medicinal Coleus (*Coleus forskohlii*)

Pests of *Coleus*

Coleus forskohlii root has been traditionally used for medicinal purposes and contains the active constituent, forskolin. The growing demand for forskolin in international trade has made Indian farmers take up commercial cultivation of medicinal coleus.

The root-knot nematode (RKN), *Meloidogyne incognita*, is considered to be one of the major constraints to coleus production (Suganthy and Vijayakumar 2013). Fifteen species of insects and three crustaceans are reported on *Coleus* spp. from different parts of the world (Manju Lata Kapur et al. 2002). Muralibaskaran et al. (2007) recorded few insect pests on coleus during survey with different damage potentials ranging from 9.4% to 12.8% by thrips, *Scirtothrips dorsalis*, and 9.3% to 15.3% by a defoliator, *Orphanostigma abruptalis*. Mealybug is yet another serious pest of coleus.

Mealybug: *Coccidohystrix insolita* and *Paracoccus marginatus* (Pseudococcidae, Hemiptera)

The nymphs and adult mealybugs could desap the tender leaves and shoots. In severe infestations, one could see the clustering of mealybugs on lower surface of leaves as a thick mat with waxy secretions. Affected plants present a sickly appearance. Sooty mould growth is very common because of honeydew secretions from the mealybugs.

Diseases of Coleus

Coleus is vulnerable to many diseases like leaf spots, leaf blight, root rot, wilt and root-knot nematodes; however, the major devastating diseases are root rot, wilt and nematodes which could cause considerable economic loss to the growers (>50%). Leaf spots caused by *Cercospora cassicola* and *Botryodiplodia theobromae* have been reported by Fernandes and Barreto (2003) and Ramprasad (2005), respectively.

Wilt: *Fusarium chlamydosporum* and *Fusarium solani*

The wilt-affected plants show yellowing and wilting of leaves, ultimately leading to complete death of plants. The affected plants show discolouration and decay of tap and lateral roots (Singh et al. 2009a, b). Bhattacharya and Battacharya (2008) reported the occurrence of *Fusarium solani* causing wilt.

Bacterial Wilt: *Ralstonia solanacearum*

The vascular wilt on coleus was reported by Chandrashekara and Prasannakumar (2010). The affected roots become brown changing to black showing decay and oozing. Finally the affected plants completely wilt and collapse.

Root Rot: *Macrophomina phaseolina*

Kamalakannan et al. (2006) reported the occurrence of root rot caused by *M. phaseolina*. The typical symptoms are yellowing and drooping of leaves. The

lower portion of the affected stem shows blackening and the bark peels off. The roots are completely rotten, and black sclerotia could be observed on the affected part of the stem/root.

Root-Knot Nematode (RKN): *Meloidogyne incognita* (Heteroderidae, Tylenchida)

Leaves become pale yellow. Stunted growth and wilting of plants in patches are common symptoms of RKN. Large number of knots could be observed on the roots when the plants are pulled and examined. The larvae feed on the vascular system of the root resulting in swollen knots or galls on tubers. In severe case of infestations, tubers are not properly developed resulting in loss of tuber yield up to 86% (Senthamarai et al. (2006).

Senna (*Cassia angustifolia*)

Pests of Senna

Leaves and pods of senna contain glycosides called sennosides for which the plant is being exploited. Aphids, two pierid butterflies, pod borer and root-knot nematode are the major pests of senna.

Aphids: *Aphis craccivora* (Aphididae, Hemiptera)

Both nymphs and adults occur in colonies on the undersurface of leaves and terminal shoots. Due to desapping, the leaves become curled and present a crinkling appearance. Honeydew secretion is not uncommon which often attracts sooty mould growth. Ants are invited by honeydew excretion.

Pierid Butterfly: *Catopsilia pyranthe* and *Eurema hecabe* (Pieridae, Lepidoptera)

The caterpillars feed voraciously on leaves and growing shoots causing heavy defoliation.

Pod Borer: *Etiella zinckenella* (Pyralidae, Lepidoptera)

Bore holes could be seen on the pods as the larva bores in to the green pods feeding the seeds.

Diseases of Senna

Alternaria Blight: *Alternaria alternata*

Alternaria species are reported to cause foliar and seedling blights in several species of *Cassia* in India and the USA. *Alternaria alternata* and *A. tenuissima* cause foliar blight of *C. fistula* and *C. tora* in India and Pakistan (Lenne 1990). Seedling blight is the result of coalescing of several lesions on young leaves. Raj and Tetaawal (2010) studied the biochemical changes due to infection of *A. alternata* on *C. angustifolia* Vahl.

Cercospora Leaf Spot: *Cercospora* sp.

Several species of *Cercospora* are reported to cause leaf spots and blights on *Cassia* species (Lenne 1990).

Leaf and Pod Spot and Dieback: *Phomopsis* spp.

Leaf and pod spots are reported on several species of *Cassia* in several countries (Lenne 1990).

Wilt and Dieback: *Phomopsis cassiae*

Phomopsis cassiae is reported to cause wilt and dieback of *C. alata* in Tanzania (Ebbels and Allen 1979) and *C. fistula* in the USA (Baker and Dale 1948).

Wilt: *Fusarium oxysporum*

The leaves of affected plants become yellow and droop. The stem near the soil and roots becomes black. The discolouration of vascular system is a common symptom of wilt. Soil application of biocontrol agent – *Trichoderma viride* – was most effective in checking the disease (Magar and Barhate 2013).

Root-Knot Nematode (RKN): *Meloidogyne incognita* (Heteroderidae, Tylenchida)

Root-knot-infected plants showed yellowing of leaves, with reduction in leaf size. Small knots on the infected roots are common. Severe infection resulted in stunting and wilting of the plants in patches.

Ashwagandha (*Withania somnifera*)

Pests of Ashwagandha

Ramanna (2009) documented 11 species of pests on ashwagandha which included 5 species of defoliators (Coleoptera and Lepidoptera), 5 species of sucking pests (Hemiptera, Acarina) and 1 species of flower and fruit feeder (Lepidoptera). Among the different species of pests observed, the defoliators, viz. *Henosepilachna vigintioctopunctata* Fab., *Deilephila nerii* Linn., *Myllocerus viridanus* Fab., *Myllocerus discolor* Fab. and leaf miners, were found occasionally at initial stages of the crop growth causing reduction in foliage. The sucking pests, viz. *Aphis gossypii* Glover, mealybug, *Ferrisia virgata* Fab., green plant bug and *Nezara viridula*, were observed during different growth periods. The lepidopteran caterpillar, *Helicoverpa* sp., was found feeding on leaves and also boring into flowers and fruits. Mite, *Tetranychus urticae* Koch, caused yellowing and drying of leaves which ultimately reduced the yield.

Diseases of Ashwagandha

Leaf Spot Disease: *Alternaria alternata* (Fr.) Keissler

Leaf spot is one of the most common and serious diseases of *W. somnifera.* Infection ranged from 20% to 30%. Severe infection led to reduction of withaferin A and withanolides (Pai et al. 2008). Shivanna et al. (2014b) established the seed-borne nature of *Alternaria alternata* which caused severe leaf spot disease. They have also established that *Fusarium oxysporum* caused wilt in *W. somnifera*. Foliage infection resulted in reduction of steroids and alkaloids warranting effective control measures.

Wilt: *Fusarium oxysporum and Fusarium solani*

Gupta et al. (2004) reported the prevalence of root rot and wilt caused by *F. solani* in Lucknow (UP). The infected plants showed drooping and withering in the initial stage and at later stage showed severe wilting and death of the plants. The roots turn brown and the growth of fungus could be seen at the base of infected plants. Young seedlings at nursery stage also showed similar symptoms leading to death of plants (>50%). The causal agent was found as *F. solani*. The occurrence of wilt caused by *F. oxysporum* causing wilt in *W. somnifera* was reported by Shivanna et al. (2014b) which may lead to complete loss of the crop in case of severe infection.

Costum (*Costus speciosus*) (J.Konig) Sm.

Pests of Costum

Kumar (2007) recorded 11 species of phytophagous insects on *Costus speciosus*. They belonged to the order Orthoptera that includes short-horned grasshoppers *Anacridium flavescens* (F.) and *Atractomorpha crenulata*, and these were found making broad holes on the leaves. Hemiptera order includes *Oxyrachis tarandus* Fab., *Chrysocoris stolli* and a plant bug, *Nezara viridula*. The nymphs and adults of these pests desap tender shoots. Similarly, the Lepidoptera, *Spilosoma obliqua* Walk, and the Coleopteran *Myllocerus discolor* Bohemian were also recorded mainly feeding on the leaves. Muralibaskaran et al. (2009) recorded two species of sucking insect pests, viz. thrips (*Scirtothrips dorsalis*) and mealybug (*Orthezia insignis*), on leaves.

Amruthaballi (*Tinospora cordifolia*)

Pests of Amruthaballi

Hanumanthaswamy et al. (1993) reported that the larvae of *Othreis materna* completely fed on the leaves of *T. cordifolia* during September and recorded 20% infestation. The adults of *Myllocerus viridanus* Fab. caused notching of leaf margin. Mulberry grasshopper, *Neorthacris acuticeps acuticeps*, was found to feed on the leaves throughout the year.

Diseases of Amruthaballi

Phoma Leaf Spot: *Phoma putaminum*

The disease severity of the leaves infected with *P. putaminum* ranged from 0% to 100% in Bhadra Wildlife Sanctuary, Karnataka. It was also reported that the alkaloid content decreased considerably due to infection (Shivanna et al. 2014a).

Flat Stem Disease: Phytoplasma

A new flat stem disease of *T. cordifolia*, a medicinally important plant used to treat many human diseases, caused by phytoplasma, which are wall-less, minute, pleomorphic prokaryotes that inhabit phloem elements in plants (Lee et al. 2000), prevalent in Western Ghats of Karnataka was reported by Somashekhara Achar et al. (2015). All the affected branches expressed flattening of stems during winter season.

Integrated Eco-friendly Non-chemical Methods of Pest and Disease Management

Management of Pests of Medicinal Plants

Sucking Pests

It is very common that most of the medicinal plants are affected by insect and noninsect pests and diseases caused by fungi, bacteria and virus. Leaf, seed, seed cakes, crude oils, etc. are reported to help in managing these stresses. Commercial products derived from neem leaf or kernels are common in use for the management of pests, nematodes and diseases (Gahukar 2012). Botanical products containing neem and/or pungam as major constituents could be applied as sprays. These are available as liquid (EC) or powder (WP) formulations. Soap formulations of botanicals are also acceptable and can be used in organic farming. Examples of such products include Neemgold, Ponneem, Sallotrap and Sun Agro Neemal (Sithanantham et al. 2010). Insect pests like thrips and white flies could be physically prevented by growing two rows of maize as barrier crops around the field as a border crop to avoid the damage due to thrips and white flies. Roguing and destroying the infected shoots could also help in reducing the incidence.

Insect pests could be monitored by installing yellow sticky traps at 50/ha. The pests could also be trapped by the sticky traps. Eco-friendly, non-chemical methods like application of neem oil (3%) or neem seed kernel extract (5%) could greatly reduce the incidence of sucking pests.

Microbial biopesticides based on fungal entomopathogens like *Metarhizium anisopliae*, *Beauveria bassiana* and *Verticillium lecanii* can also be used for controlling some of these pests. These can be used alone or in combination for controlling thrips, hoppers, bugs, scales and mealybugs. Simple water spraying alone could also help as it washes down some pests like aphids.

Defoliating Pests

For caterpillar pests, bacterial biopesticides (like *Bacillus thuringiensis*) are used. For the management of *Spodoptera*, viral biopesticides (NPV) are recommended. Botanical biopesticides like neem can be used for short-term relief through repellent action. Mixtures of different botanical products can also be used against most defoliating pests. Pheromone traps are useful for controlling the adult (mobile) stage as moths in *Spodoptera litura*. Light traps can also be used for attracting moths and some defoliating beetles. Sticky yellow sheet traps are useful to control flea beetles, thrips and hoppers. Application of new-generation products, like insect growth regulators (IGRs), could also be used for managing some of these pests.

Rathikannu (2005) reported NSKE 5% and NeemAzal 1% at 900 ml/ha to be effective against the insect pests of *G. superba* with highest reduction of population of *S. litura* by 53.34% in NSKE 5% applied fields after 5 days of application. Suganthy and Sakthivel (2012) reported that flavonoids (Max Ranger-D2®) were recommended as the best alternative to chemical pesticides in gloriosa ecosystem and are recommended as one of the components in organic pest management.

Meshram et al. (2015) established that application of *Bacillus thuringiensis* at 1% followed by neem-based pesticide at 1% could be very effective against *P. gloriosae* on *G. superba*.

Suganthy and Sakthivel (2013) reported the effectiveness of azadirachtin at 1% or 2% aqueous leaf extract of *Andrographis paniculata* in containing the insect pest population and also conserved more number of natural enemies like predatory coccinellids in *S. nigrum*. Growing cowpea as a border crop to conserve the coccinellid predators and marigold for the management of root-knot nematodes is recommended.

IPM module comprising of designer seed treatment [polymer (3 g), carbendazim (2 g), Gaucho (5 g), and *Azospirillum* (120 g) per kg of seed], cowpea as border crop, erecting yellow sticky traps at 12/ha, and need-based spraying of azadirachtin 10,000 ppm at 1 ml/litre recorded the lowest incidence of sucking pests, viz. aphids, thrips and whiteflies, at 25 days after sowing (DAS) and afforded protection up to 35 DAS, followed by farmer's practice (chemical control) (Suganthy et al. 2015).

Field release of coccinellid predators like *Cryptolaemus montrouzieri* at 600 beetles/ha and field release of *Acerophagus papaya*e at 100 per hamlet could contain the incidence of *Paracoccus marginatus*. In the early stages of infestations, spray application of neem oil at 3% or fish oil rosin soap at 25 g/l could be very effective in checking the pests.

Bio-intensive modules

1. [vermicompost 2 t/ha + karanj cake 250 Kg/ha + bio-fertilisers 2 Kg/ha + NPK (20:60:50 Kg/ha); *C. carnea* 50,000 eggs/ha (three releases on 15, 30 and 105 DAP); *T. chilonis* 6.25 cc/ha (two releases on 45 and 60 DAP); *B. thuringiensis* 750 g/ha (three sprays on 50, 80 and 140 DAP); and fish oil rosin soap 25 g/l (five sprays on 35, 55, 75, 95 and 115 DAP)] was effective in

reducing the incidence of thrips, scale insects and defoliator of coleus (Thangavel 2010).

2. Use of bio-intensive module consisting of vermicompost 2 t/ha + karanj cake 250 Kg/ha + bio-fertilisers 2 Kg/ha + NPK (40:40:40 Kg/ha); *C. carnea* 50,000 eggs/ha (three releases on 15 and 30 DAS, 15 DAH); *T. chilonis* 6.25 cc/ha (two releases on 45 and 60 DAS); *B. thuringiensis* 750 g/ha (three sprays on 50 and 80 DAS, 50 DAH); neem oil 3% (three sprays on 20, 40 and 70 DAS); and NSKE 5% (two sprays on 30 and 70 DAH) was found effective in reducing the incidence of thrips, aphids, defoliators and pod borer in main and ratoon crop of senna and recorded the highest yield of 2028.0 Kg dry leaves/ha and 834.8 Kg pods/ha with a cost-benefit ratio of 1:1.55 (Senthilkumaran 2008).

Biopesticides

Sithanantham et al. (2011) have summarised the R & D scenario on organic pest management in noni in India. In the case of noni, R & D efforts with WNRF support have been made to opportunistically evaluate the relative efficacy of biopesticides (both botanical and microbial) which are summarised by Sithanantham et al. (2010). They compared identified two microbial biopesticides – *Beauveria bassiana* and *Verticillium lecanii* – besides two botanical products, both of which were mix of neem and pungam (Ponneem/Sallotrap) as promising for field control of the lace-wing bugs. Similar studies have also clarified the efficacy of neem plus pungam mix at 5% spray for control of defoliating flea beetles, while sprays of *Bacillus thuringiensis* at recommended concentrations can help control caterpillar pests, besides applying any locally available botanical repellents including sweet flag to control occasional attack by grasshoppers.

Studies conducted by Shanthakumar et al. (2013) on the management of mealybugs on noni (*Maconellicoccus hirsutus* and *Planococcus citri*) revealed the efficacy of neem sprays, besides the usefulness of microbial pesticide based on *Metarhizium anisopliae*, either alone or in combination with Ponneem – a mixture of neem+pungam oils. Noni leaf extracts with ethyl acetate were also found promising; more recently, the phytochemistry of the essential oil from noni leaves has been studied for its insecticidal property against the mealybug – *Maconellicoccus hirsutus* has been confirmed from laboratory bioassays (Shanthakumar et al. 2014).

Recent studies conducted by Venkatachalam et al. (2014) showed the potential of neem in reducing the internal fruit damage caused by tephritid fruit flies The potential for sweet flag sprays, either alone or in combination with neem, Ponneem, in minimising the internal fruit damage, with clear beneficial effects on increasing the yield of damage-free fruits, and promising cost-benefit ratios were brought out.

Botanical Pesticides

The use of botanical biopesticide has also received attention in managing the insect pest on *Gloriosa superba* (Suganthy and Sakthivel 2012; Suganthy and Rajamani 2015; Rathikannu et al. 2011), while similar emphasis has been given to pest of *Solanum nigrum* (Suganthy et al. 2015; Suganthy and Sakthivel 2013; Suganthy,

2014). Significant progress made with such organic pest control methods includes (Rathikannu 2005) NSKE 5% and NeemAzal 1% at 900 ml/ha that were effective against the insect pests of *G. superba*, with significantly higher reduction in *Spodoptera litura* population (by 53%) in NSKE 5% at 5 days after application. Suganthy and Sakthivel (2012) recommended flavonoids (Max Ranger-D2®) as the best alternative to chemical pesticides in gloriosa ecosystem as one of the components in organic pest management.

Suganthy and Rajamani (2015) found that application of spinosad 45 SC (200 ml/ha) twice at 15 days interval during flowering stage is most effective for the management of thrips and gloriosa necrosis. Meshram et al. (2015) reported the effectiveness of application of *Bacillus thuringiensis* at 1% followed by neem-based pesticide at 1% against *P. gloriosae* on *G. superba*. Upadhyay and Mishra (1999) have reported on the efficacy of neem in controlling the aphids on isabgol (*Plantago ovata*).

Further information on pests of other medicinal plants are provided in manuals on pest spectrum and eco-safe management of pests on cultivated medicinal plants (Suganthy et al. 2011, 2012; Suganthy and Vijayakumar 2013).

Root-Knot Nematodes

Meloidogyne incognita causing root-knot nematode (RKN) is one of the serious problems in medicinal plants causing considerable yield loss. Application of neem cake in nurseries and to the main field greatly reduced the incidence of RKN in ashwagandha and Indian senna (Sharma and Pandey 2009). Ramakrishnan and Senthilkumar (2009) found that application of humic acid and biocontrol agent, *Pseudomonas fluorescens*, to the soil was more effective than neem cake application.

Soil application of *Trichoderma viride* at 2.5 kg/ha combined with 250 kg of neem cake/ha could effectively manage the RKN. Medicinal plants like coleus which is propagated through stem cuttings and dipping in commercial preparation of 0.1% *Pseudomonas fluorescens* at the time of planting are recommended besides soil application of bioagents. Planting marigold (*Tagetes erecta*) as intercrop and incorporating the same into soil were found effective.

Bio-management of Diseases on Medicinal Plants

Ever since biological control was defined by Cook and Baker (1983), the potential of this tool has been very well recognised. Rhizosphere-competent fungi or bacteria in addition to their antagonistic activity are capable of inducing growth responses by either controlling minor pathogens or producing growth-stimulating factors (Cook and Weller 1986). Biocontrol agents fit well with organic farming, a proposition which is quite relevant to medicinal plants.

A cursory perusal of literature reveals that *Pseudomonas fluorescens*, *P. putida* and *Bacillus polymyxa* and *B. amyloliquefaciens* strain MET0908 showed powerful antagonistic effect against *Colletotrichum and Alternaria* (Kim and Chung 2004).

Fungal antagonist *T. viride* significantly reduced the growth of *A. solani* and *A. alternata* under in vitro condition (Veerasamy 1997).

Vivekananthan et al. (2006) tested different biocontrol agents against *C. gloeosporioides* causing anthracnose disease in mango. They found that the rhizobacterial strains FP7 in combination with chitin significantly reduced the growth of *C. gloeosporioides* in vitro as well as in field condition.

Leaf Blight and Anthracnose of Noni

Eco-friendly, non-chemical method like soil application of vermicompost, neem cake and *Pseudomonas fluorescens* (Pf1) and +EPC8+ *Trichoderma viride* (TV1) mixture in the ratio 8:1:1 besides foliar application of liquid formulation of *P. fluorescens* (Pf1) + EPC8+ *T. viride* (TV1) mixture at 0.2% effectively checked the diseases and increased the fruit yield by 38%.

Fruit Rot

Nakkeerran et al. (2013) have developed an IPM strategy for the management of noni diseases. The results of field experiments conducted showed that soil application of vermicompost, neem cake and Pf1(*P. fluorescens*) + EPC8 (*Bacillus subtilis*) + TV1(*T. viride*) mixture in the ratio 8:1:1 and foliar application of liquid formulation of Pf1 + EPC8 + TV1 mixture at 0.2% were highly effective in containing the incidence of leaf blight both under glass house and field conditions. In addition, the same treatment recorded higher yield compared to other treatments. Similarly, for the management of anthracnose, soil application of vermicompost, neem cake and TDK1 (*B. subtilis*) + SVPR4 (*B. subtilis*) + TV1(*T. viride*) mixture in the ratio 8:1:1 and foliar application of liquid formulation of TDK1 + SVPR4 + TV1 mixture at 0.2% were found to be very effective in reducing containing anthracnose besides increasing yield. Vermicompost- and neem cake-based mixture of Pf1 + BS4 + TV1 + Azophos coupled with foliar application of Pf1 + BS4 at 0.2% effectively checked the incidence of fruit rot.

Induced Systemic Resistance (ISR)

Hammerschmidt and Kuc 1995 defined ISR as an enhancement of the plant's defensive capacity against a broad spectrum of pathogens and pests that is acquired after appropriate stimulation. The resulting elevated resistance due to an inducing agent upon infection by a pathogen is called induced systemic resistance (ISR) or systemic acquired resistance (SAR). Chester (1933) coined the term "acquired physiological immunity" when induced protection in plants against various biotic or abiotic stresses is achieved.

Later different authors used different terms to describe the phenomenon of induced resistance such as translocated resistance (Hubert and Helton 1967) and plant immunisation (Tuzun and Kuc 1991). Plant has natural endogenous defence mechanisms that can be induced in response to insect and pathogen attack (Bostock et al. 2001). Defence reactions occur due to accumulation of PR proteins (chitinase, β-1,3-glucanase), chalcone synthase, phenylalanine ammonia lyase, peroxidase, phenolics, callose, lignin and phytoalexins. The ISR stimuli were shown to be

salicylic acid (De Meyer and Hofte 1997), avirulent pathogens (Kuc and Richmond 1977) and non-pathogens such as rhizobacteria (Wei et al. 1996). However, reports of induced resistance in the field by classical inducing agents are scanty and infrequent. Systemic protection of tomato against late blight caused by *Phytophthora infestans* de Bary was demonstrated with *B. pumilus* strain SE34 incorporated into the potting medium (Yan et al. 2003). This implies that the same PGPR strain can induce resistance against multiple pathogens in the same crop including medicinal plants.

Bioformulation of PGPR and Fungal Antagonists

The success of biological control of diseases largely relies on suitable bioformulations which could sustain the activity for bioagents for a longer period of time. Attempts are being made to mass multiply the PGPR and incorporate in a suitable medium liquid/powder formulations to be effective in the field. The formulation should be cheap and easy to handle and apply to niche area for successful management of diseases.

Boral and Dekas (2007) made comparisons of three organic substrates, viz. vermicompost, farmyard manure and mustard oil cake, for mass multiplication of *P. fluorescens* strain PfD-1. They demonstrated that vermicompost-based biopesticide being more effective than others in checking bacterial wilt incidence of tomato. Assays performed to study the population dynamics of the bacterial antagonists and the shelf life revealed that a steady population of the bacterial antagonist was maintained up to duration of 250 days at ambient temperature.

Experiments conducted by Jayaraman et al. (2007) to understand the population dynamics of introduced *P. fluorescens* revealed that organic amendments like poultry manure and FYM could sustain the population and could reduce the incidence of damping-off.

Soil application of composted coir pith (CCP) enhanced *Trichoderma* population in rhizosphere of black gram (Rukmani and Mariappan 1993) and brinjal (Sheela 1992). Vidhyasekaran et al. (1997) reported that the increase in *Trichoderma* population was obviously due to soil application of organic amendments like rice bran and farmyard manure (FYM). Palanna et al. (2007) showed that application of FYM + goat manure extensively supported the growth of *T. viride* followed by vermicompost + poultry manure, FYM + poultry manure, FYM and goat manure which accounted for reduction of disease and increase in production. It is also important that the biocontrol agents shall be incorporated with suitable organic amendments and incubated for a reasonable period for population build-up before field application. Rajendran (2006) demonstrated that incubation of *B. subtilis* and *P. fluorescens* with FYM for 20 days helped in population build-up which when applied to soil will effectively contain the pathogens.

Delivery of PGPR

Seed treatment, root dip (Maurhofer et al. 1994), sett treatment in sugarcane (Viswanathan and Samiyappan 1999), sucker treatment in banana (Saravanan et al. 2003), soil application (Vidhyasekaran et al. 1997; Nakkeeran et al. 2005) and foliar

application (Mew and Rosales 1986; Chatterjee et al. 1996) are some of the methods developed for delivering the biocontrol agents to achieve maximum benefits.

Vidhyasekaran and Muthamilan 1995 and Vidhyasekaran et al. 1997 demonstrated that seed treatment followed by soil application of talc-based powder formulation has effectively checked chickpea wilt and pigeon pea wilt under field conditions besides increasing the yield.

Combination of different methods of application might prove useful and more effective in disease management strategies than depending on a singly method of application as evidenced by the findings of Vidhyasekaran et al. (1997), Meena et al. (2000), Nandakumar et al. (2001) and Saravanakumar (2006).

Root Rot (*Macrophomina phaseolina*)

Root rot-causing pathogens being soilborne, the management strategy shall follow more than one method of application/delivery of biocontrol agents. Dipping of tubers of *G. superba* in *P. fluorescens* at 2 g per litre of water for 20 min followed by drenching the soil at the same concentration on the 30th day of planting was found effective in reducing the root rot (Meena and Rajamani 2016). Judicious combinations of methods of delivery and timely application of quality biocontrol agents shall go a long way in managing soilborne pathogens.

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Biodiversity Conservation in Medicinal Plants and Protection of Plant Varieties and Farmers' Rights Act

Rayappa R. Hanchinal, Jyoti Jaiswal, and Dinesh Kumar

Introduction

Our ancestors always considered plant genetic resources (PGR) to be the heritage of humankind and were of the opinion that this treasure would provide the foundation for attaining food, nutritional, and health security. Though evolution on Earth started over 3.5 billion years ago, it is with human interference, coupled with natural processes, that biodiversity has expanded. Human civilization is closely associated with the refinement of biodiversity. Looking to their day-to-day needs, humans started selecting plants from the available natural biodiversity. In ancient times, when men used to go hunting, it was women who developed the art of gathering and selecting plant species according to the needs of the family/society. Along with the advance of civilization, a natural evolutionary adjustment process took place in nature, of course aligned with human interference in different ecologies and changing environmental and biotic conditions. The resultant plant biodiversity was an irreplaceable resource and was the lifeline of humans, providing a sustainable ecosystem to meet the food, clothing, shelter, nutritional, and health requirements of the population. Among developing countries, India is considered as a cradle of agricultural biodiversity, known for its rich heritage of plant, animal, and fish genetic resources, as well as microorganisms, which constitute biodiversity. With 17% of the world's population, only 4.0% of the world's area, and 40% of its water bodies, India is considered to be one of the world's 17 mega biodiversity countries, with 12 of the world's mega-diversity centers, accounting for 7–8% of the world's recorded species. India is also considered as a major center for the domestication of crop plants. Among the 34 biodiversity hot spots identified across the world—which are largely superimposed over the phyto-geographical regions—the Indian Gene Centre has three: the Western Himalayas, North-Eastern region, and Western Ghats. The Indian Gene Centre is divided into eight regions, including biodiversity-rich zones. The Andaman

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and Nicobar Islands are rich treasure houses of agro-biodiversity and are connected with Indo-Burman, Indo-Malyasian, and Indo-Indonesian biodiversity. The Indian Protection of Plant Varieties and Farmers' Rights (PPV&FRA) Authority has identified 22 agro-biodiversity hotspots across the country. Looking to the rich biodiversity of the country, Vavilov (1951) identified India as one of the eight primary centers of the origin of cultivated plants; the country hosts about 49,000 species of flowering and nonflowering plants (18.8%), out of 260,000 described across the globe. India is rich in endemic plant species, which represent 33% of its flora. Within the spectrum of crop species and wild relatives, thousands of varieties, cultivars, landraces, and ecotypes occur in India. The country is known to have more than 18,000 species of higher plants, including 160 major and minor crop species and 325 wild relatives. Around 1500 wild edible plant species are widely exploited by native tribes. In addition, nearly 9500 plant species with ethno-botanical uses have been reported in the country, of which around 7500 are used for ethno-medical purposes and 3900 are edible species. Medicinal plants account for nearly 3000 species (India's 4th national report to the Convention on Biological Diversity [CBD] 2009).

Historic Perspective of Intellectual Property Right (IPR) Issues in Medicinal Plants

The history of the protection of intellectual property (IP) in plant varieties found in different parts of the globe goes back to the eighteenth century, when various attempts were made by different countries in Europe to extend intellectual property protection (IPP) for agricultural innovations. However, the first attempt to recognize the IPR of plant breeders was the enactment of the Plant Patent Act in the United States in 1930. Similar legislation was subsequently enacted in Germany, Hungary, Italy, The Netherlands, Austria, and other countries in the 1930s, and this led to private monopolization of plants and associated businesses (Hanchinal 2014). In 1961, representatives of five European countries, meeting as the International Convention for the Protection of New Varieties of Plants, initiated *sui generis* IPR protection for plant varieties and formed the International Union for the Protection of New Varieties of Plants (UPOV), whose recommendations came into force in 1968.

The Convention aimed to ensure the protection of plant breeder's rights (PBR) by the granting of an exclusive right in the protected new plant variety on the basis of a set of uniform and clearly defined principles: distinctiveness, uniformity, stability (DUS), and novelty. The UPOV Convention recommendations were revised in 1972, 1978, 1991, and 1998. At present, there are 74 members of UPOV, with other countries acting as observers or in the process of becoming members (Hanchinal 2014).

This development in developed countries was followed by a concern for farmers' rights, in particular by the developing world, expressed under the auspices of the Food and Agriculture Organization (FAO). As a result, the Convention on Biological Diversity (CBD) came in force on December 29, 1993. This is perhaps

the most comprehensive intergovernmental agreement concerning conservation, sustainable utilization of PGR, and sharing the benefits arising out of such use in equitable ways. Article 8 (j) of the CBD recognizes the contributions of local and indigenous communities to the conservation and sustainable utilization of biological resources through traditional knowledge, practices, and innovation and provides for equitable sharing of benefits with such people arising from the utilization of their knowledge, practices, and innovations. Further, in harmony with the CBD, to promote the conservation of PGR and to protect farmers' rights to access and to have fair and equitable sharing of benefits arising out of their sustainable use to achieve food and nutritional security, an International Treaty on Plant Genetic Resources for Food and Agriculture (ITPGRFA), a legally binding instrument, was adopted in 2001. This instrument also highlights the necessity of promoting farmers' rights at national and international levels, and the instrument established a multilateral system (MLS) for facilitated access to a specified list of PGRFA, including 35 crop genera and 29 forage species for food security and interdependence, balanced by benefit-sharing in the areas of information exchange, technology transfer, capacity-building, and commercial development. The Nagoya Protocol, which came into existence on October 29 2010, is a new international agreement that aims at sharing the benefits arising from the utilization of genetic resources in a fair and equitable way, thereby contributing to the conservation and sustainable use of biodiversity. This protocol advances the CBD's third objective and creates greater legal certainty and transparency for both the providers and users of genetic resources. Communities will benefit from the use of their knowledge, innovations, and practices.

Another important international agreement, the Trade Related Aspects of Intellectual Property Rights (TRIPS), under the auspices of the World Trade Organization (WTO), was ratified by the CBD countries in 1994. As the TRIPS agreement requires WTO members to introduce an effective system for the protection of plant varieties, this commitment by WTO member countries implies that the countries which hitherto had not extended IPR to their agricultural sector would have to do so.

Protection of Plant Varieties and the Farmers' Rights Act, 2001

Notwithstanding the fact that the UPOV system is the first system for plant variety protection (PVP), India evolved its own *sui generis* legislation for the protection of plant varieties, enshrining the rights of farmers and plant breeders and encouraging the development of new varieties of plants; this was the Protection of Plant Varieties and Farmers' Rights (PPV&FR) Act, 2001. The PPV&FR rules were notified in September 2003 and the Act came into force in November 2005. This legislation provides a more comprehensive framework for PVP, and contains several deviations from the UPOV model. This is the only IP law in India that gives dual IP proprietorship for a variety and its denomination. Another special feature of this legislation is that the protection accrues to a person from the date of filing the application, giving priority and provisional protection.

The PPV&FR Act, 2001 is unique in regard to the national situation, yet it matches the larger global commitment. It attempts to optimize and balance claims for protection by both plant breeders and farmers and is the first of its kind in the world. India is the first country to provide substantial rights to farmers, and the registration of their plant varieties is one of such rights. The PPV&FR Act recognizes the multiple roles played by farmers in cultivating, conserving, developing, and selecting varieties. With regard to developing or selecting varieties, the Act refers to the value added by farmers to wild species or traditional varieties/landraces through selection and identification for their economic traits. Accordingly, farmers' rights encompass the roles of farmers as users, conservers, and breeders. Farmers are entitled to save, use, sow, re-sow, exchange, share or sell their farm produce, including seeds of a protected variety, in the same manner as they were entitled to before the operation of the PPV&FR Act, provided that they shall not be entitled to sell branded seeds of a protected variety.

Objectives of the Act

- To establish an effective system of plant varieties, and rights of farmers and plant breeders and to encourage increased breeding activity and new types of breeders, such as private breeders, researchers, and farmer breeders.
- To accelerate agricultural development in the country, protect plant breeders' rights, stimulate investment, in both the public and private sectors, for the research and development of new plant varieties to facilitate the growth of the seed industry, which will ensure the availability of high-quality seeds and planting material to farmers.
- To recognize and protect the rights of farmers in respect of their contributions made at any time for conserving, improving, and making available plant genetic resources for the development of new plant varieties.

Various Rights Under the Act

(i) *Breeders' Rights*

The Act provides an exclusive right to the breeder or their successor, or their agent or licensee, to produce, sell, market, distribute, import, or export the variety registered under the Act. A breeder may authorize any person to produce, sell, market, or otherwise deal with the variety registered under this Act.

(ii) *Researchers' Rights*

A researcher can use any of the varieties registered under this Act for conducting experiments or research. However, authorization from the breeder of a registered variety is required where the repeated use of such a variety as the parental line is done for the commercial production of another newly developed variety.

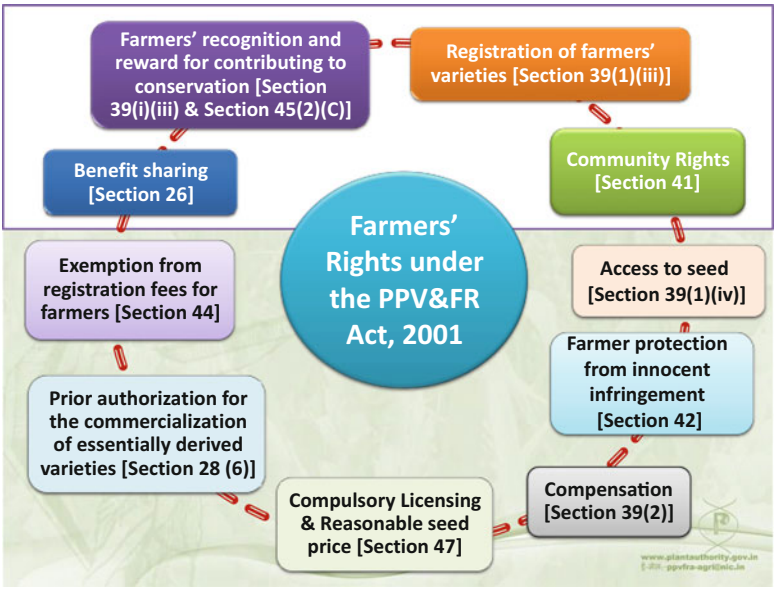

Fig. 1 Provisions related to farmers' rights

(iii) *Farmers' Rights*

The Act treats the farmer as a plant breeder insofar as the farmers' variety is concerned and the farmers can register them under the Act. The other provisions related to farmers' rights are presented in Fig. 1. Farmers are entitled to recognition and reward from the Gene Fund, provided that the material so selected and preserved (landraces and wild relatives) has been used as gene donors in varieties registrable under the Act.

(iv) *Community Rights*

If any village or local community has made a significant contribution to the evolution of any variety and if such a variety is registered by any other person then the said village or local community can claim compensation.

Other Provisions

Compulsory License A compulsory license is granted by the Authority to a competent person when a registered variety falls short of public demand 3 years after its registration.

Benefit-Sharing When the genetic material of a plant variety is used in the development of a registered variety by any person or group of persons who are citizens of India or any firm or governmental or non-governmental organization formed or established in India, such a community can claim benefit-sharing from the registered breeder.

Registration of a Plant Variety

A variety is eligible for registration under the Act if it essentially fulfils the criteria of distinctiveness, uniformity, and stability (DUS), which means that the candidate variety must be distinguishable by at least one essential characteristic from a variety that is a matter of common knowledge in any country at the time of filing the application, and the variety must be sufficiently uniform in the expression of its essential characteristics, which should remain unchanged even after repeated propagation. The variety should also have a single and distinct denomination.

The Authority has established 107 DUS test centers for different crop species; these centers are responsible for conducting DUS tests of varieties for which registration has been applied and for maintaining, multiplying, and characterizing reference/example varieties as per DUS descriptors. For a new variety, DUS tests are carried out for 2 years at two locations and for an extant variety, DUS testing is done for 1 year only. When the DUS test result is found to be satisfactory, a certificate of registration is issued to the applicant and its details are published in the *Plant Variety Journal of India.*

Notification of Medicinal Plant Species for Registration of Plant Varieties

The central government of India has notified 140 crop species for the purpose of registration, which include 17 medicinal plant species (Table 1). For these crop species the PPV&FR Authority has developed guidelines for the conduct of species-specific (DUS) tests and specific guidelines for individual crop species. The purpose of these specific guidelines is to provide detailed practical guidance for the harmonized examination of DUS, and in particular, to identify appropriate characteristics for the examination of DUS and production of harmonized variety descriptions.

Table 1 Medicinal crop species notified for registration

Group	No.	Crop species			
Medicinal plants	17	Garlic, ginger, turmeric, isabgol (*blond psyllium*)	Fenugreek, coriander, small cardamom, black pepper, menthol mint	Damask rose, periwinkle, eucalyptus	Brahmi (Indian pennywort), noni (*Morinda citrifolia*), neem, nutmeg, safflower

Categories of Varieties Eligible for Registration Under the Act

New Varieties

A new variety shall be registered for breeder's right if it conforms to the criteria of novelty and DUS. The variety should also have a denomination in accordance with the provisions of the PPV&FR Act, 2001. Novelty criteria apply, if, at the date of filing of the application for registration for protection, the propagated or harvested material of such variety has not been sold or otherwise disposed of by or with the consent of its breeder or their successor for the purposes of exploitation of such variety in India, earlier than 1 year; or outside India, in the case of trees or vines, earlier than 6 years, or in any other case, earlier than 4 years.

Extant Variety

An extant variety is defined as a variety available in India that is notified under Section 5 of the Seeds Act, 1966 (Subsection 54 of 1966); or a farmers' variety; or a variety about which there is common knowledge; or any other variety that is in the public domain;

Farmers' Variety

Farmers' variety is defined as a variety that has been traditionally cultivated and evolved by the farmers in their fields; or is a wild relative or landrace or a variety about which farmers possess common knowledge.

As per the Act, "farmers" means any persons who cultivate crops by cultivating the land themselves; or those who cultivate crops by directly supervising the cultivation of land through any other person; or those who conserve and preserve, severally or jointly, with any other person any wild species or traditional varieties or add value to such wild species or traditional varieties through selection and identification of their useful properties.

Variety of Common Knowledge

A variety of common knowledge (VCK) refers to a variety that has not been released and notified under the Seeds Act, 1966, and has not been sold or otherwise disposed of in India for more than a year from the date of filing the application. If a variety that is under cultivation in a State/region/country, even as a "truthfully labeled" variety, is an entry in an official list/register of varieties in any country granting PVP, including the filing of an application for PBR, or is included in a recognized publicly accessible collection, including accession in a national/international gene bank and

adequate description of the variety in a publication that may be considered part of the public technical knowledge, it may be found to be eligible under the VCK criteria .

Essentially Derived Variety

A variety (the initial variety), shall be said to be an essentially derived variety (EDV) from such initial variety when it is predominantly derived from such initial variety, or derived from a variety that itself is predominantly derived from such initial variety, while retaining the expression of the essential characteristics that results from the genotype or combination of genotypes of such initial variety; is clearly distinguishable from such initial variety; and conforms (except for the differences which result from the act of derivation) to such initial variety in the expression of the essential characteristics that result from the genotype or combination of genotype of such initial variety.

National Gene Fund for Promoting PGR Activities

Based on the richness of agro-biodiversity ,i.e., number of crop species, crop varieties, wild relatives of various crop species cultivated, social relevance and ancientness of the agriculture, wild relatives of crop species occurring in the region, number of species domesticated, and the uniqueness of the agro-ecosystems, the Authority has identified 22 agro-biodiversity hotspot regions in India (Fig. 2).

Farmers who have been engaged in the conservation and preservation of PGR of landraces and wild relatives of economic plants and their improvement through selection and preservation in these 22 identified agro-biodiversity hotspots receive recognition and rewards from the National Gene Fund. This provision, when taken in conjunction with the provisions relating to farmers' privilege, is similar to the concept of Farmers' Rights contained in the ITPGRFA.

The National Gene Fund receives contributions from the central government, national and international organizations, and other sources [section-45 (1-d) of Protection of Plant Varieties and Farmers' Rights Act 2001]. The Gene Fund also receives funds from benefit-sharing [section-45 (a)] from the breeder of the variety or an essentially derived variety or propagating material registered under the Act, the compensations deposited [section-41 (4)], and the annual fee payable to the Authority by way of royalties [section-35 (d)]. The expenditures of the Fund are earmarked to support the conservation and sustainable use of PGR, including in-situ and ex-situ collections. Thus, in this way it can be considered to be a national equivalent to the global benefit-sharing fund operating within the ITPGRFA.

PGR conservation, protection, and promotion for sustainable use has been practiced by farmers and their families from ancient times. This has allowed them to cultivate a large number of different local varieties of different crop species of economic importance. Thus India is regarded as one of the 17 mega bio diversity countries in the world. To support PGR activities, the Authority has selected

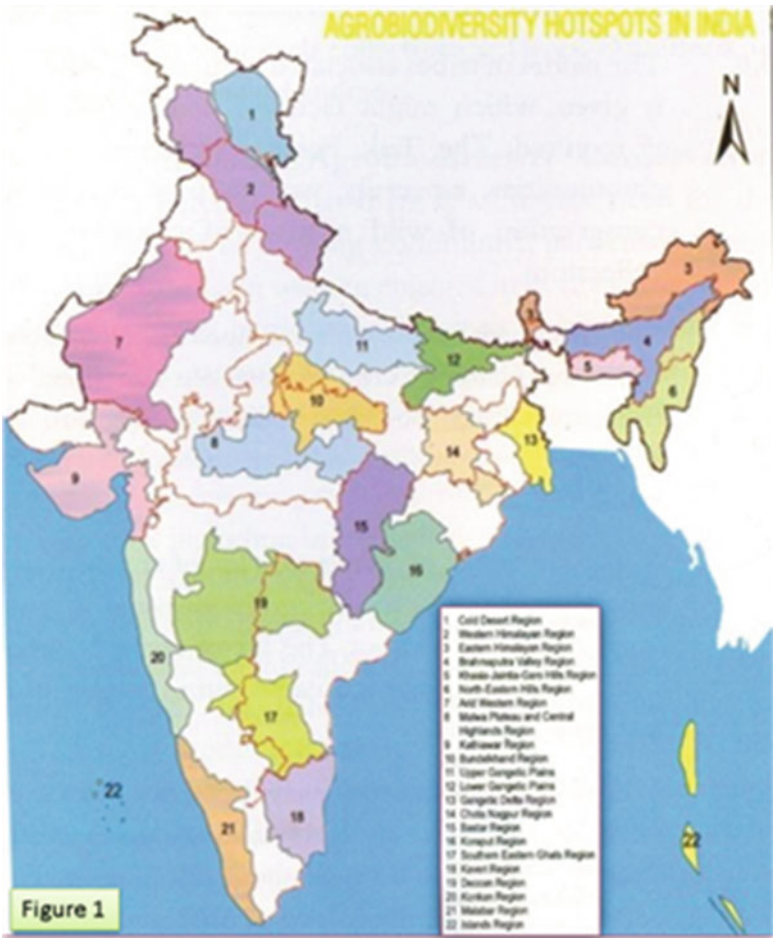

Fig. 2 Agro-biodiversity hotspot regions of India

Genome Saviour Award Communities to support their efforts in saving local varieties and landraces. As climate change has a significant impact on agricultural production, growing local varieties that have a high degree of genetic diversity is highly important, because these varieties have the ability to better withstand and adapt to environmental stresses. Setting up community seed banks/community nursery banks may help farmers to acquire varieties that are adapted to local conditions; these varieties may not be accessible through formal seed systems, may be costly, or may suffer from erratic supplies. To make available quality seeds/planting material of popular local varieties through informal seed/nursery chains, the Authority promotes a "community seed bank/community nursery bank concept" at different agro climatic biodiversity hotspots where improved varieties

have not made an impact on production and productivity. The Authority has identified regions in agro biodiversity hotspots and farmers' varieties are being mainstreamed there.

Progress in Filing of Applications for Medicinal Plants and the Issue of PVP Certificates

Applications that have fulfilled all requirements and have been finally accepted by the Registrar for registration are issued with PVP certificates. The details of applications received by public and private organizations and also by individual farmers for medicinal plant species are presented in Table 2. It is quite encouraging to note that farmers have taken so much interest in the registration of their varieties (n=388), followed by public organizations (n=45), although there has been no response from private organizations. Applications received, categorized according to the variety, are presented in Table 3. It is quite interesting to note that public institutions have invested in research and development in a few important crop species: black pepper, eucalyptus, fenugreek, garlic, ginger, medicinal plants, safflower, small cardamom, and turmeric. As a result, applications were received for the new variety and extant notified variety categories. One private company, Noni Biotech, has invested in research and development to develop improved varieties of noni, which are will be mainly used for a health drink. As a result, two varieties in the Extant-VCK category were received by the Authority to grant IPR. The certificate of registration issued will be valid for 9 years in the case of trees and vines and for 6 years in the case of other annual crops. It may be reviewed and renewed for the

Table 2 Applications received for medicinal plants, shown according to applicant

Serial number	Crop name	Farmer	Private	Public	Grand total
1	**Black pepper**	9	0	4	**13**
2	**Brahmi**	1	0	0	**1**
3	**Coriander**	67	0	2	**69**
4	**Eucalyptus**	0	0	1	**1**
5	**Fenugreek**	25	0	0	**25**
6	**Garlic**	54	0	11	**65**
7	**Ginger**	66	0	3	**69**
8	**Isabgol**	2	0	0	**2**
9	**Menthol mint**	4	0	1	**5**
10	**Neem**	1	0	0	**1**
11	**Noni**			2	**2**
12	**Nutmeg**	15	0	0	**15**
13	**Safflower**	5	0	9	**14**
14	**Small cardamom**	12	0	3	**15**
15	**Turmeric**	127	0	9	**136**
	Grand total	**388**	**0**	**45**	**433**

Table 3 Applications received for medicinal plants, shown according to category

S. no.	Crop name	Farmer	New	Extant (notified)	Extant (VCK)	Grand total
1	**Black pepper**	9			4	**13**
2	**Brahmi**	1				**1**
3	**Coriander**	67				**67**
4	**Eucalyptus**	0	1			**1**
5	**Fenugreek**	25				**25**
6	**Garlic**	54	2	9		**65**
7	**Ginger**	66			3	**69**
8	**Isabgol**	2				**2**
9	**Menthol mint**	4			1	**5**
10	**Neem**	1				**1**
11	**Noni**				2	**2**
12	**Nutmeg**	15				**15**
13	**Safflower**	5	1	7	1	**14**
14	**Small cardamom**	12		1	3	**16**
15	**Turmeric**	127	2	4	4	**137**
	Grand total	**388**	**6**	**21**	**18**	**433**

VCK Variety of common knowledge

Table 4 Certificates issued for medicinal plants, shown according to applicant

S. No.	Crop name	Farmer	New	Extant (notified)	Grand total
1	**Black pepper**	3			**3**
2	**Garlic**			5	**5**
3	**Safflower**			6	**6**
4	**Small cardamom**	5		1	**6**
5	**Turmeric**			2	**2**
	Grand total	**8**	**0**	**14**	**22**

remaining period on payment of renewal fees, subject to the condition that the total period of validity in the case of trees and vines shall not exceed 18 years from the date of registration of the variety, 15 years from the date of notification of the variety under the Seeds Act, 1966, and in other cases 15 years from the date of registration of the variety.

Of 433 applications received, only 22 variety applications for five crop species have been granted IPR certificates so far (Table 4). This is mainly because, in regard to farmers' varieties, one season of testing is required, and for new varieties two seasons of testing are required. Progress in the case of extant notified varieties is quite encouraging, as the DUS testing process in the field is not involved.

Impact of Awareness Programs on Registration of Farmers' Varieties and Response to Plant Genome Saviour Awards

The filing of applications for the registration of farmers' varieties (Fig. 3) in medicinal plant species came into force after the notification of two medicinal plant species, i.e., ginger and turmeric, in 2009, and other crop species notified in subsequent years. The data (Fig. 3) indicated that in the first 5 years, not a single application was filed by farmers. However, during the year 2012, the Authority received one application. In regard to the lukewarm response of the farmers regarding the registration of their varieties in different medicinal crop species, the Authority, in collaboration with National Agricultural Research System and Agriculture Science Center (Krishi Vigyan Kendras;KVKs) and non-governmental organizations (NGOs), carried out massive awareness programs that were accepted from the year 2013. As a result, 23 applications were received in 2013, 82 applications were received in 2014, 130 applications were received in 2015, 119 applications were received in 2016, and 33 applications had been received by the time of writing in 2017. It is a matter of pride to note that, owing to the awareness program, the farmers also sent applications for their medicinal crops.

Although awareness programs on the PPV&FR Act and the provisions of Farmers' Rights in the Act have been reflected in the submitting of applications for PVP entitlement in farmers' varieties (Table 2) and in eligibility for the Genome Saviour Award (Table 5), there is still not much awareness in NARS systems, as reflected by the non-registration of extant notified varieties in all medicinal plant species. The concerned Universities/Institutes are yet to submit applications for PVP entitlement. Since the varieties are developed through public funding and these crop species are known for their medicinal value, there is an urgent need to obtain PVP entitlement for IPR to avoid the patenting of molecules for medicinal/pharmaceutical use.

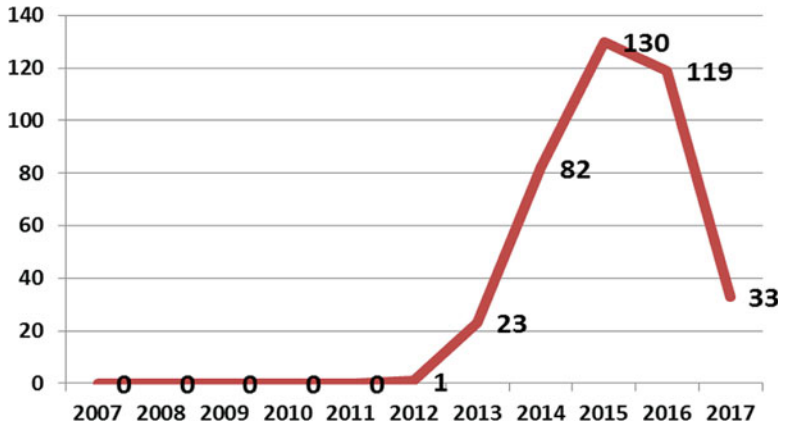

Fig. 3 Yearly trend of filing applications for intellectual property right (IPR) for farmers' varieties in medicinal plants

Table 5 Details of applications received for Plant Genome Saviour Awards

S. No.	Name of the award	2009–2010			2010–2011			2011–2012		
		Total no. of applications received	No. of States participating	Total no. of award given	Total no. of applications received	No. of States participating	Total no. of awards given	Total no. of applications received	No. of States participating	Total no. of awards given
1.	Plant Genome Saviour Community Award	20	11	2(1)	18	11	4(−)	27	11	4(−)
2.	Plant Genome Saviour Farmer 'Award	–	–	–	–	–	–	30	13	10(4)
3.	Plant Genome Saviour Farmers' Recognition	–	–	–	19	11	7(−)			15(2)

S. No.	Name of the award	2012–2013			2013–2014			2014–2015			Total no. of awards given
		Total no. of applications received	No. of States participating	Total no. of awards given	Total no. of applications received	No. of States participating	Total no. of awards given	Total no. of applications received	No. of States participating	Total no. of awards given	
1.	Plant Genome Saviour Community Award	28	15	5(1)	26	14	5(1)	14	10	5(2)	25(5)
2.	Plant Genome Saviour Farmer 'Award	80	20	10(4)	78	16	3(1)	47	11	10(2)	33(15)
3.	Plant Genome Saviour Farmers' Recognition			4(3)			11(1)			20(−)	57(6)

Numbers in parentheses indicate the numbers of awards granted for the conservation of medicinal plants

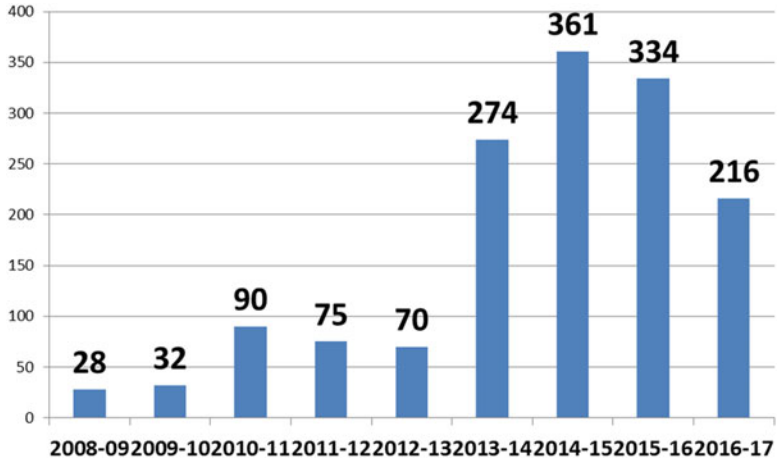

Fig. 4 Details of Protection of Plant Varieties and Farmers' Rights (PPV&FR) Authority awareness programs organized by the Authority

As suggested by Pratibha Brahmi et al. (2004) and Hanchinal et al. (2014), there is a need to create awareness among scientists, policy makers, and breeders, as well as farmers and village communities, as the PVP law in India is relatively new. Although the Authority has jurisdiction over the entire country of India, it cannot travel the length and breadth of the country to organize awareness programs, but can only facilitate these. The programs facilitated by the Authority (Fig. 4) have had an impact on both the filing of the applications for farmers' varieties and the receipt of applications for Plant Genome Saviour Awards (Table 5). The Act came into force from the year 2005, but it has taken some time to formulate the guidelines and prepare the format for the Award applications.

In the first year (2009–2010) there was a good response in the receipt of applications for Plant Genome Saviour Awards. Twenty applications were received from 11 States and three communities were conferred with Genome Saviour Community Awards, of which one award was conferred for conserving medicinal plants. The good response in receipt of applications was due to the effective wide publicity gained through 32 awareness programs carried out in agro-biodiversity-rich states. However, the trend was not continued during the year 2010–2011 in spite of a greater number of awareness programs (90) being conducted, suggesting that rather than organizing a greater number of awareness programs, it would important to determine how effective these training programs were in reaching the community. During the year 2011–2012, the trend was different. Seventy-five awareness programs, covering 14 states, were organized, which helped to get 27 applications for Community Awards and 30 applications for Farmers' Rewards and Recognition. No Plant Genome Saviour Community Awards were given for the conservation of medicinal plants, but four farmers were felicitated with Rewards and two farmers received Recognition Certificates. During the periods 2012–2013 and 2014–2015,

four communities and 11 farmers were conferred with Plant Genome Saviour Awards.

Success in gaining the receipt of a greater number of applications was obtained by reaching previously un-reached regions of agro-biodiversity hot-spots through awareness programs and print and electronic media, involving all the stakeholders. The 637 KVKs in India, covering eight agro-climatic zones located in all the districts of the country, co-ordinate with the farmers at a grass-roots level in regard to frontline technology transfer. Awareness programs were conducted in 161 districts in all eight agro-climatic zones, particularly in agro-biodiversity hot-spots. Zonal level capacity-building programs, covering information about the PPV&FR Act in general and Farmers' Rights in particular, were organized by the Authority for the benefit of KVK scientists, who are the master trainers at the grass-roots level. Further, capacity-building activities for the administrators, scientists, and students at 27 State Agricultural Universities and at 38 ICAR Research Institutes (NARS), covering important biodiversity regions, were also organized by the Authority. Many NGOs are working in different biodiversity regions and they play a key role in the dissemination of information. Relevant NGOs were identified and seven programs were conducted through these NGOs. The Authority also facilitated the organization of seminars/conferences on PVP laws. Exhibitions about PPV&FR were arranged at Farmers Fair or Exhibition, Agricultural Fairs, etc. During such exhibitions, video clippings, Street Play, posters, and unique material about farmers' plant varieties were displayed. Articles about the PPV&FR Act were published in magazines and daily newspapers in many vernacular languages. Bulletins were distributed and radio talks and TV programs, as well as a National Dialogue on Farmers' Rights, were organized.

All these programs helped in reaching farmers/farming communities, particularly the tribal communities who are the custodians of medicinal plant species. Further, resource centers on Farmers' rights and entitlements were established, both at the Authority head office and at regional centers. Innovative ideas were shown for supporting SAUs/scientists in biodiversity-rich regions, by way of establishing projects and providing financial support to facilitate the collection of the farmers' plant varieties for filing applications for IPR. Confidence-building with farmers/farmer communities, gained by mainstreaming their traditional medicinal plant species and establishing community clonal banks and market linkages involving NGOs, SAUs, KVKs, and all other stakeholders, may further boost the morale of farmers in filing applications for IPR for their plant varieties. However, there is a misconception with some farmers/groups that if the seeds of the planting material of their medicinal crop varieties are deposited with the Authority they will lose their ownership. Such misconceptions are to be cleared from the minds of farmers through proper education and capacity-building. The Indian government has notified that although 17 medicinal crop species await filing for IPR, applications have been filed for only 2 species. Hence, sincere efforts are needed to help farmers to obtain IPR for their farm innovations.

It is suggested that to increase the effectiveness of the Indian PVP law, continued efforts should be made to provide periodic training to trainers from Indian Council of Agricultural Research/State Agricultural University/Non Government Organizations/Krishi Vigyan Kendras and other stakeholders. Further, there is a need to continue the awareness programs among scientists, policy-makers, farmers, and village communities in the agro-biodiversity regions of India.

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Part IV

New Age Uses of Herbs

Exploring the Medicinal Value of Green Wealth with Special Reference to Neuronutrient Medhya Rasayana Plant Drugs

Ram H. Singh

Introduction

Plants have been the core source of food and medicine since antiquity. More recently the green pharmacy has resurged once again as the major field of drug development and therapeutics gradually shaping up as a powerful movement for medicinal green revolution. The reason for this resurgence is the unfolding usefulness of many medicinal plants in the treatment of a range of intractable diseases proving safe and cost-effective medicare and functional food as opposed to the significant adverse effects and cost barriers of conventional medicine today. Obviously there has been a big boom in the market of natural products world over. The number of pharma companies producing herbal drugs has increased manyfold during the last few decades, the number going well over 7500 in India alone. So also the rate of production of such products pouring in the market is increasing every day. Similarly the clients seeking health care through green pharmacy products and traditional medicine is increasing all over the world. Even in the developed countries, people prefer this approach for their routine health-care needs over unsafe chemical medicines. However this upsurge of interest has now simultaneously started posing a range of newer questions before this sector which is still not well organized to address the coming up issues. The main questions are validation of the traditional claim of efficacy and safety of plant-based medicines. Today's mind is looking forward to an evidence-based practice of traditional medicine beyond belief and claim alone. This trend warrants rapid translational research in traditional medicine from book to bedside bringing traditional medicine in the mainstream.

India is one of the richest countries in the world in terms of its biodiversity and green wealth. Because of high safety and cost-effectiveness besides global acceptability, the green pharmacy is emerging as a major scientific and industrial hub.

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Analytical chemistry seems to be the central player in all spheres of R&D activity in this sector. There is a need to develop appropriate technology and novel approach to study the plants and to design quality standards for medicines of plant origin. It would be seen that researches conducted in recent past have shown encouraging outcomes.

"Green wealth" in this context refers to the green belt of the biodiversity in general and medicinal plants (Paranjape 2001; Singh and Chunekar 1972) in particular. India is considered as one of the richest countries of the world in terms of its green wealth, which has not yet been fully utilized and conserved. The "green" has not yet been converted into wealth also because of lack of awareness and national initiatives. During the last few decades, lot of work has been carried out on medicinal plants by different investigators which need to be updated. There is a need to develop appropriate research methodology for the study and value addition to the nature's gift. Green pharmacy is now emerging as the most promising field of R&D activity where chemistry plays an important role. The term "green pharmacy" refers to preparation and use of holistic medications from green resource, i.e., medicinal plants. The green pharmacy is fast growing as an acceptable tool in biotherapy of different lifestyle-related noncommunicable diseases because of its high safety level and holistic pro-nature approach. Chemical analysis of green pharmacy is essentially needed for standardization of its preparations and for monitoring their safety, not necessarily for development of new phytochemical drugs.

The Scope and Approach

There is a need of strategic planning for obtaining intermediaries as required steps for processing the vegetable raw material into finished product yielding desirable pharmaceutical presentation. Quality control is essential right from conservation, cultivation management, and harvesting of plant species passing through the production of intermediate product up to the final formulation. Adhering to the predesigned criteria will help to characterize the raw plants, their derivatives, and the formulation, designing to establish the parameters of quality control. It is necessary to identify standardized markers or to develop methods for quantification of purified principle before, during, and after the production process. Fingerprinting technique is now being commonly used for this purpose. It is essential to establish protocols that ensure quality control of raw material, vegetable products, and ingredients used in their production, as well as validation of qualitative and quantitative techniques appropriate to the chemical markers chosen; only then one can get finished product of ensured quality further to be validated with adequate clinical evaluation before it becomes a safe and effective medicine for professional use.

The abovementioned vertical approach is considered ideal, but because most of such medicinal plants are already in use as medicine in the hands of traditional practitioners, it is advisable to soften the rigor of scientific validation. There is growing consensus to suggest that the traditionally in-use medicinal plants and formulations should be first validated for their efficacy in practice settings or in a

pilot clinical trial and later if needed further laboratory studies may be contemplated. This is called "reverse pharmacology" approach.

Neuronutrient and Immunomodulator Plant Drugs

Out of the range of medicinal activities of different medicinal plants, the most attractive area is the sector of *Rasayana* drug plants specially the *Medhya Rasayanas* which are believed to be neuronutrients and rejuvenators of neural tissues. Ever since Kuboyama and associates (2005) from Japan published their in vitro studies showing that Withanolide-A isolated from the *Medhya Rasayana* plant *Ashwagandha* (*Withania somnifera*) had significant regenerative impact on neurons, this field has become more attractive for researchers. It was demonstrated that neurons cultured with Withanolide-A had elongated dendrites and their synopses got reconstructed as compared with controls. It was also claimed that experimentally induced amyloid plaques of the brain substance got resolved when cultured with the Withanolide-A suggesting that *Ashwagandha* could prove to be a logical herbal remedy for many degenerative neural diseases including Parkinson's disease and Alzheimer's disease. This model of study is likely to be replicated in many other *Medhya Rasayana* drug plants, some of which have already been studied for their neural effect showing memory-enhancing, anxiolytic, and anti-stress activities. Such plants where new scientific evidence has accumulated are Brahmi (*Bacopa monnieri*), Mandukaparni (*Centella asiatica*), Shankhpushpi (*Convolvulus pluricaulis*), Vacha (*Acorus calamus*), Jatamansi (*Nardostachys jatamansi*), and Jyotishmati (*Celastrus paniculatus*).

The Ayurveda scholars and practitioners believe that the so claimed therapeutic activity of *Rasayana* plants is through nutrition dynamics, not necessarily through phamaco-dynamics, because as per Ayurvedic concepts the *Rasayana* remedies are essentially micronutrients. They nourish the vital cells and tissues of the body and in turn produce positive health impact, viz., efficient memory, good sleep, relief from stress, etc. Many of the *Rasayana* drug plants also possess immunomodulating effect. At this juncture in order to clarify the core effect of *Rasayana* remedies, it is imperative to discuss the ancient classical concepts of *Rasayana* therapy specially *Medhya Rasayanas* (Singh 2001, 2002, 2008, 2010; Singh RH and Associates 2005). Many medicinal plants have been studied for their possible utility in prevention and treatment of cancer which is believed to be a potential area of enquiry on the role of *Rasayana* therapy in such diseases (Smit et al. 1995, 1997).

What Is Rasayana

Ayurveda, the ancient Indian traditional medicine, is essentially the science of life, longevity, and holistic health care. Since antiquity this unique science has been in an unbroken professional practice in India through its eight specialties. One of these

eight specialty branches specially deals with the uplift of molecular nutrition, immunomodulation, and healthy longevity. This special discipline is designated as *Rasayana Tantra*. This review article will describe in detail the definition and scope of *Rasayana*, the mechanism of action of various *Rasayana* remedies, and their classification and therapeutic applications and future potential for revival and development to enrich the mainstream of promotive and preventive health care today (Caraka 700 BC; Mehra and Singh 2001; Mishra 1600 AD; Pant 1013; Rastogi et al. 2012; Satyawati 1976; Sharangadhara 1300 AD; Singh 1998, 2012, 2017, 2015b; Sinha and Singh 1979; Susruta Samhita et al. 2002; Uabundit et al. 2010; Vagbhatt 300 AD; Vagbhatta 300 AD).

The technical Sanskrit word *Rasayana* refers to the process of procurement of nourishment for formation of quality Dhatus, i.e., cells and tissues of the body including the genes leading to improved physiological state, enhanced bio-strength including immunity, improved mental and cognitive functions, and healthy longevity. Thus *Rasayana therapy* has multifaceted impact on biological system with positive nutrition, immune enhancing, longevity, and sustenance of mental and sensorial competence. Because of such important attributes, *Rasayana* therapy offers a logical pan-health-promoting impact and preventive role against different diseases. The *Rasayana* remedies produce their core effect through following three probable modes of action.

1. At the level of *Rasa or* plasma by directly acting as a nutrient in itself enriching the nutrient value of *Posaka Rasa* in the plasma. Few common examples are the nutrient *Rasayanas* like Shatavari (*Asparagus racemosus*), *Sharkara*, *milk*, *Ghrita*, etc.
2. At the level of *Agni* by enhancing the biofire system of the body acting as digestive and metabolic boosters and in turn promoting the nutritional status as is often observed in case of *Rasayanas* like pippali/long pepper (*Piper longum*), marich/black pepper (*Piper nigrum*), and shunthi/ginger (*Zingiber officinale*).
3. At the level of *Srotas*, i.e., microcirculation by inducing *Srotoprasadana* effect improving the competence of inner transport system, microcirculation, and tissue perfusion as is seen in case of Vacha (*Acorus calamu*) and Guggulu (*Commiphora wightii*) Rasayana.

Classification of Rasayana Measures

In principle *Rasayana* is not merely a remedy or a recipe; rather it is a rejuvenative regimen and is an approach to positive health care. *Rasayana* therapy comprises of the elements of positive lifestyle and conduct, healthy dietetics, and rejuvenative herbs and minerals. *Rasayana* therapy is practiced as a routine open-air form or as an intensive indoor regimen in consideration of the need and the affordability for a particular patient. Textually the *Rasayana* therapy is categorized as below.

A. As per the therapy procedures:
   1. *Vatatapika Rasayana* or a casual outdoor practice
   2. *Kutipraveshika Rasayana* or intensive indoor regimen
B. As per scope of application and indications:
   1. *Kamya Rasayana* – For promotion of health of the healthy, viz.:
      (a) *Sri Kamya* – To promote glow and beauty
      (b) *Prana Kamya* – To promote longevity
      (c) *Medha Kamya* – To promote mental competence
   2. *Naimittika Rasayana* – Disease-specific *Rasayanas*
C. *Adjunct Rasayana* – Non-recipe rejuvenative regimen to be practiced singly or as an adjunct for all forms of *Rasayana* therapy, remedies, and recipes, viz.:
   1. *Àcara Rasayana* – Healthy rejuvenative positive lifestyle and conduct
   2. *Àjasrika Rasayana* – Daily dietary *Rasayana* approach consuming *Sattvika*, nourishing diet

Age-Specific Rasayana

Ayurvedic classics consider aging as the *Swabhawa* or the very nature of a living creature. The life of a human being in this world has been conceived to last for a time-bound span of 100–120 years designated as *Ayu* and *Param Ayu*, respectively. During the estimated span of life, the body undergoes progressive involution and degeneration leading finally to death. Ayurveda describes the process of aging and sequential senile changes in different ways in different contexts. The three *Dosas*, namely, *Vata*, *Pitta*, and *Kapha*, are known to dominate specifically during old age, youth, and childhood, respectively. The *Vata Dosha*, because of its drying and decaying effect, plays the central role in the aging process.

Vagbhatta and Sharangadhara Samhita describe a unique scheme of biological aging in a 12-decade frame suggesting the specific sequential loss of certain biological qualities in the body specific to respective decades of the estimated life span. This classical information opens the newer vistas of investigation exploring the possibility of designing specific *Rasayana therapy* to restore the likely biological involutions of the particular decade. It is believed that if *Rasayana* therapy is executed in relation to age, there is a possibility of retarding the aging process. The following table describes the pattern of the sequence of biological aging as conceived in Ayurveda and proposes certain age-specific *Rasayanas* for the purpose.

Pattern of biological aging and age-related bio-losses and some suggested *Rasayana drugs*

S. no.	Decades of life span	Natural bio-losses	Suggested *Rasayana*
1.	0–10 years	*Balya* – corpulence	*Gambhari*, *Ksira*, *Ghrita*
2.	11–20 years	*Vriddhi* – growth	*Bala*, *Àmalaki*
3.	21–30 years	*Chhabi* – luster	*Àmalaki*, *Haridra*
4.	31–40 years	*Medha* – intellect	*Brahmi*, *Sankhapuspi*
5.	41–50 years	*Twaka* – skin quality	*Bhringraja*, *Haridra*

(continued)

6.	51–60 years	*Dristi* – vision	*Triphala*, *Jyotismati*
7.	61–70 years	*Shukra* – virility	*Ashwagandha*, *Kapikacchu*
8.	71–80 years	*Vikrama* – physical strength	*Àmalaki*, *Bala*
9.	81–90 years	*Buddhi* – thinking	*Brahmi*, *Sankhapuspi*
10.	91–100 years	*Karmendriya* – locomotion	*Bala*, *Sahacara*

Tissue and Organ Specific Rasayana

Rasayana in general is a holistic restorative and rejuvenative modality, but one can visualize some *Rasayana* remedies and recipes for specific promotion and protection of certain tissues and organs. Such *Rasayanas* can be prescribed in need-based manner for promotive or even for curative purposes for organ protection. Some examples are proposed below.

Some suggested tissue- and organ-specific Rasayana drugs

S. no.	*Rasayana* quality	Purpose	Suggested remedies
1.	*Medhya Rasayana*	Neuroprotection and cognition	*Brahmi*, *Shankhpushpi*
2.	*Hridya Rasayana*	Cardioprotective	*Arjuna*, *Puskarmula*
3.	*Mutra Janana*	Nephroprotective	*Punarnava*, *Guksuru*
4.	*Twacya Rasayana*	Skin health	*Haridra*, *Somaraji*
5.	*Caksusya Rasayana*	Eye health	*Triphala*, *Jyotismati*
6.	*Kanthya Rasayana*	Throat and speech	*Vacha*, *Yastimadhu*
7.	*Vrisaya Rasayana*	For virility	*Ashwagandha*, *Kapikacchu*
8.	*Stanya Rasayana*	To promote lactation	*Satavari*
9.	*Srotoprasadana*	Promoting microcirculation	*Guggulu*, *Vaca*
10.	*Nasya Rasayana*	To help nose and sinuses	*Katphala*, *Apamarga*

Acara and Ajasrika Rasayana

Acara Rasayana is a unique concept in Ayurveda which implies that a moral, ethical, and benevolent conduct and lifestyle bring about *Rasayana*-like rejuvenative effect on the mind-body system of the organism. An individual who adapts such a healthy lifestyle and conduct gains all benefits of *Rasayana* therapy without physically consuming any material *Rasayana* remedy. All forms of *Sadvritta*, *Acara*, and practice of Yoga and spirituality produce such a quantum *Rasayana* effect. This is practiced singly or in a combination with material substance *Rasayana* therapy of different kinds. Similarly *Ajasrika Rasayana* refers to daily rejuvenative dietetics with adequate quantity of duly nourishing *Sattvika* diet. *Ajasrika Rasayana* may be used singly or as an adjunct to material substance *Rasayana* medications

Divya or Divine Rasayanas

The Ayurvedic classics as well as the Vedic texts propound a unique concept of *Divya Rasayana* which is claimed to possess divine power to bring about divine transformation in an individual. The Ayurvedic classics such as Caraka, Susruta, and Vagbhatta describe several *Divya Rasayana Mahousadhis*, i.e., great medicines with paranormal attributes. These divine medicinal plants grow in *Soumya* Himalayan range and are rarely found and are believed to possess a special class of spiritual/quantum pharmacology, and their actions are due to their *Divya Prabhava*. Caraka Samhita Ckikitsa Sthana Chapter 1, Pada-4 (Ayurveda Samutthaniya Rasayana), Susruta Samhita Chikitsa Sthana Chapter 30 (Nivritta Santapiya Rasayana), and Astanga Hridaya Chapter 39 mention a number of *Divya Ausadhis* such as *Brahma*, *Subarchala*, *Soma*, *Padma*, *Varahi*, *Golomi*, *Ajagari*, etc. The identity of these drugs is presently unknown.

Rasayana and Plant Drug Research

Plant drug research has been pursued during several decades with two distinct motives: firstly to standardize and validate the efficacy and safety of in-use crude plant drugs or a formulation in order to reestablish a particular medication for contemporary use and secondly the plant drug research is pursued as an attempt to develop a new modern drug from plant resource by isolating and characterizing the probable active chemical constituent of a chosen plant species. The first approach is largely a short-term program and is most useful and cost-effective because such an exercise provides immediate benefit to a large number of traditional medicine practitioners world over particularly in India. Hence it is the priority program at governmental level and is in public interest.

The experiences of the last five decades on medicinal plant research with the motive of new drug development have not been rewarding because new active chemical principles isolated from plants have not proved safe for human use. There are more failure stories than success stories in this approach. Experiences suggest that out of few thousand such active principles, hardly one or two may show the hope for a new drug which may subsequently flop before evaluated in human use settings. It is something like searching a needle from the heap of hay. Another greater problem is that so isolated active principle no more remains a natural drug and is associated with all safety problems as any other modern chemical drug. The success/failure story of isolation of reserpine from *Sarpagandha* (*Rauwolfia serpentina*) is the most glaring example. The alkaloid reserpine isolated, characterized, and adequately tested for its powerful antihypertensive effect proved to be a successful modern medication for treatment of hypertension during the early 1950s, but it was soon discovered that this new chemical drug was unsafe for human use as it produced severe depression in patients, and hence within few years, the drug had to be withdrawn from the market, while *Sarpagandha* as crude whole drug is still in popular use.

The *Sarpagandha* story proved to be a turning point in plant-based drug research. Now most of the researchers feel that a natural product or formulation should be in the natural whole material form. Of course such natural whole substance will have to be duly standardized for quality and quantity of its marker compound. Thus under this scheme, phytochemistry of the plant material is done for the purpose of standardization of the product, not for the purpose of developing a new drug. This is truer in case of nutraceuticals and neuronutrient drugs. However it does not mean to completely rule out active principle approach of plant medicine research. It is only to point out the problems involved. Both approaches may be adopted by different investigators as per their convenience, expertise, and interest with due appraisal.

Standardization of Plant Products and Quality Control

Standardization of a plant material or a product involves following two issues:

1. To identify the standard process of production authentically known for the same.
2. The following three aspects need to be duly standardized:
    (a) Standardization of the raw materials
    (b) Standardization of the methods of production and the tools used
    (c) Standardization of the finished product, batch to batch

The important issues involved are (1) source of procurement of raw materials including harvesting and storage besides identification, chemistry, characterization, and biological testing; (2) clinical trials, vertical controlled trials or through reverse pharmacology approach; and (3) safety studies – general safety, microbiological safety, heavy metal contamination, and presence of special toxic constituents if any present in the plant or the formulation.

Regulatory Issues and Accreditation

Lot of vagueness still prevails on regulation, licensing, and prescription of ASU drugs and phytomedicine in India and world over. Central Council of Indian Medicine (CCIM) act regulates the education and registration of practitioners in this sector with lot of gaps. Quality control and licensing of ASU medicines and natural products is regulated by old Drug and Cosmetic Act which has very scratchy provisions. It initially ruled that the formulations described in a set of 54-year-old Ayurvedic books be taken as official Ayurvedic medicines with the facility of autoregulation and registration. Few years ago the Government of India enacted a law for Good Manufacturing Practices (GMP) which is now implemented. Further, provisions are now being made to regulate the licensing of newer herbal products, extracts, and phytochemicals with quality descriptions and the authority of prescribers. There is a need of clear national policy and regulatory mechanism to

control the entire sector which is growing fast and warrants clear and robust regulatory provisions (ADMA 2014; AYUSH 2009).

Research and Development Program

The last five decades have attempted a range of interrelated research and development activities on medicinal plants supported by R&D organizations and individual activists and scientists besides universities and research institutions. Organized efforts have been made by Botanical Survey of India, by forest conservation organizations, and more recently by the National Medicinal Plants Board (NMPB) to conserve, to develop, and to utilize the plant resources for medicinal uses. The creation of the Ministry of AYUSH under the Central Government, Central Council for Research in Ayurvedic Sciences, and the establishment of National- and State-level Medicinal Plants Boards are some of the organized activities still awaited to yield good results. More in the past, the CSIR laboratories especially CDRI, RRL, CIMAP, and NBRI have done commendable job in developing strategies and action plans. The major national research projects like Composite Drug Research Scheme (CDRS), Golden Triangle Project, and the Science Initiative in Ayurveda and the task force on Ayurvedic Biology and Traditional Knowledge Digital Library (TKDL) deserve mention, all this gradually shaping into a "Medicinal Green Revolution." Research, development, and utilization of medicinal plants for human health care have to pass through the following spectrum of R&D activities:

- National and regional survey of medicinal plants
- Climate and cultivation needs of different plant species and their local usage
- Conservation of medicinal plants on priority
- Cultivation strategies and management
- Harvesting, transportation, preparation of extracts and intermediaries, and storage
- Identification and pharmacognostic studies
- Chemistry and identification of marker compounds and characterization
- Biological testing and safety studies
- Pharmaceutical studies and formulation development
- Clinical evaluation
- IPR issues and actions and product accreditation
- Marketing and post marketing surveillance

Glimpses of Research on Plant-Based Drugs

The medicinal plant research has already yielded a range of useful information which has helped developing strategies for developing new chemicals and drugs besides providing leads for standardization of traditional drugs and formulations for their safety and efficacy. Hundreds of new chemicals have been isolated from these plants many of which have rich medicinal potential. Such an information has enriched the

branch of pure chemistry on one side and has provided leads for new drug development for patient care. The following is a select list of some new chemicals isolated from medicinal plants showing potential value in new drug development besides the therapeutic efficacy.

Some leading phytochemicals isolated from medicinal plants and their use

Active chemical constituents	Medicinal plants	Therapeutic use
Reserpine	*Rauvolfia serpentina*	Hypertension
Piperine	*Piper longum*	Bio-enhancer
Digoxin	*Digitalis*	Cardiotonic
Levo Dopa	*Mucuna pruriens*	Parkinson's disease
Curcumin	*Curcuma longa*	Anti-inflammatory
Vincristicine	*Vinca rosea*	Anticancer
Kutakin	*Picrorhiza kurroa*	Hepatoprotection
Ephedrine	*Ephedra* spp.	Antiasthmatic
Strychnine	*Nuxvomica* spp.	Neurostimulant
Atropine	*Atropa belladonna*	Antispasmodic
Punarnavine	*Boerhaavia diffusa*	Diuretic
Bacosides	*Bacopa monnieri*	Nootropic
Withanolides	*Withania somnifera*	Adaptogen
Glycyrrhizin	*Glycyrrhiza glabra*	Anti-inflammatory

Besides the development of new phytochemicals of potential medicinal value, hundreds of plant species and traditional formulations have been proved to have very useful therapeutic effect and are ready to be used as medicine in patient care with reenforced conviction among the prescribers and consumers. This article has no scope to go to the exhaustive details of such researches. However the following is the glimpse of some researches emerging out of the work of the author of this article and his associates presented here as an example to illustrate the issue under discussion with particular reference to neuronutrient impact of *Medhya Rasayana* medicinal plant drugs.

Tables 1, 2 and 3 display anti-stress effect of *Ashwagandha* (*Withania somnifera*) with comparatively lower Ed50 as compared to the Chinese herb ginseng indicating that *Ashwagandha* and *Tulasi* (*Ocimum sanctum*) are more effective than ginseng in experimental settings. A prolonged use of *Ashwagandha* in elderly population has shown significant antiaging effect as is evident from the reduced scores of biological aging scales, improved immediate memory, and better mental health in human subjects (Dwivedi and Singh 1997, 1992; Singh et al. 1987, 2008; Singh and Malaviya 1978).

Table 1 Anti-stress activity of *Ashwagandha* and *Tulasi* in terms of Ed 50 in stressed rats showing notably lower Ed50 in Ashwagandha and Tulasi groups as compared to ginseng (Singh et al. 1987)

Drugs tested in albino rats	Botanical names	Anti-stress unit/mg/g Ed 50
Ashwagandha	*Withania somnifera*	14.9 ± 1.50
Tulasi	*Ocimum sanctum*	13.7 ± 1.30
P. ginseng	*Panax ginseng*	25.2 ± 2.30

Table 2 Antiaging impact of *Ashwagandha* in elderly persons after 3 months of treatment (Dwivedi and Singh 1997)

Variables	Before treatment Mean ± S.D.	After treatment Mean ± S.D.	t	P
Biological age scores	19.33 ± 4.57	14.95 ± 4.53	3.77	<0.01
Immediate memory score	4.55 ± 0.86	4.85 ± 1.00	6.16	<0.01
BPRS scores for mental health	32.40 ± 5.22	22.93 ± 2.86	6.12	<0.01

Table 3 Humoral basis of the anti-stress activity of *Ashwagandha* (J. Ethnopharmacol 64(1999), 91–93)

Observation Mean ± SE	Control	Ashwagandha	Swimming stress	Swimming stress plus Ashwagandha
Plasma corticosterone μg/dl	98.95 ± 0.5	98.95 ± 0.27	107.2 ± 0.38 $p < 0.05$	99.77 ± 0.14
Phagocytic index	68.50 ± 0.56	69.60 ± 0.70	78.0 ± 0.58 $p < 0.05$	68.83 ± 1.20
Total swim time			5.30 ± 0.24	8.90 ± 0.50 $p < 0.05$

Table 4 Effect of *Brahmi* (*Bacopa monnieri*) on certain mental health indices in human subjects

Observations ($n = 22$)	Mean scores ± SD Before treatment	After treatment	*P*
Immediate memory span	5.94 ± 1.18	6.37 ± 1.17	<0.01
Mental fatigue index			
1. Work output/5 Mt.	711.15 ± 270.15	855.85 ± 233.55	<0.01
2. Error score/5 Mt.	7.85 ± 12.65	1.15 ± 2.49	<0.05

Certain authors have also reported a significant reduction of plasma corticosterone and phagocytic activity with significantly enhanced swim time in stressed rats (Archana and Namashivayam 1999). Besides, a large number of other studies on Ashwagandha have been reported in various scientific journals. The readers are advised to refer to the original sources (Bhattacharya et al. 1997; Kuboyama et al. 2005; Prakakash et al. 2013). *Brahmi* (*Bacopa monnieri*) has shown increased memory span with reduced mental fatigue rate in human volunteers treated with *Brahmi* (Table 4). There are many other such studies reported on Brahmi (*Bacopa monnieri*). The readers are advised to refer to the primary sources (Amitava et al. 2002a; Bhattacharya et al. 1999, 2000; Bhattacharya and Ghosal 1998; Calabrese et al. 2008; Caraka (700 BC); Channa et al. 2003; Charles et al. 2012; Stough and Scholey 2013; Stough et al. 2013; Dar and Channa 1999; Das et al. 2002b; Dhawan and Singh 1996; Dhyanasekaran et al. 2007; Dulcy et al. 2012; Emmanuval Rajan et al. 2011; Holcomb et al. 2006; Hota et al. 2009; Jayakumar et al. 2014; Jyoti and Sharma 2006; Kuboyama et al. 2005; Limpeancholo et al. 2008; Maher et al. 2002; Matthew et al. 2012; Morgan and Stevens 2010; Sheikh et al. 2007; Pase et al. 2012; Pathak and Singh 1986; Preethi et al. 2014; Rasso et al. 2003; Rastogi et al. 2012; Roodenrys et al. 2002; Russo and Borrelli 2005;

Table 5 Pattern of mental performance in cases of educable mental retardation after treatment with *Mandukaparni* (*Centella asiatica*) – Agrawal and Singh (1998)

Observations Mean ± SD	Before treatment	After treatment	*P*
Performance I.Q.	56.395 ± 04.65	60.385 ± 05.931	$P < 0.05$
Immediate memory span	3.00 ± 0.87	3.66 ± 0.88	$P < 0.01$
Social quotient	59.03 ± 14.56	66.20 ± 14.04	$P < 0.01$

Table 6 Impact of Rasayana therapy on blood sugar PP, *Ojas*, and *Agni* status in diabetes

	Blood sugar PP		Negative Ojas score		Agni status score	
	Mean ± SD		Mean ± SD		Mean ± SD	
Groups	BT	AT	BT	AT	BT	AT
Group A (n = 33)	266.40	209.40**	10.07	6.07**	4.94	0.55**
Only Ay. drug	± 71.77	± 52.12	± 8.45	± 6.09	± 3.27	± 0.62
Group B (n = 23)	242.90	178.07**	8.21	5.42**	4.61	1.09**
Add Ay. drug	± 41.38	± 24.97	± 6.21	± 5.37	± 4.36	± 1.44
Group C (n = 28) control	237.60	225.40*	9.50	9.15*	5.11	3.39**
	± 29.00	± 31.32	± 7.90	± 7.34	± 3.47	± 2.33

* = $p < 0.05$, ** = $p < 0.001$

Sairam et al. 2002; Singh and Dhawan 1982, 1997a, b; Singh and Singh 1978, 1980; Singh et al. 1979; Singh 2015a; Stough et al. 2001, 2008; Uabundit et al. 2010). Similarly as displayed in Table 5, *Mandukaparni* (*Centella asiatica*) shows an evidence of improved performance quotient, memory span, and social quotient in educable mentally retarded children after 3–6 months of open trial (Agrawal and Singh 1998). All these studies indicate significant lead for further studies. Table 6 shows the effect of a special therapeutic *Rasayana* drug on blood sugar PP level with improved *Ojabala* and *Agnibala* in cases of diabetes mellitus type II after 3 months of treatment. The same series of patients is simultaneously examined for their immune status rated by an *Ojas* status scale developed by the author, and they exhibited significant decrease in the negative *Ojas* score suggesting that treatment given not only corrected the hyperglycemia but rather also improved the immune status of these patients because the trial drug was basically a *Rasayana* remedy used in cases of mild to moderate diabetes. These patients also showed improved *Agni* status after treatment which is suggestive of improved digestive and metabolic functions.

Conclusion

Ayurveda is the oldest system of life science and health care in the world, its antiquity going back to the ancient Vedas. *Rasayana Tantra* is one of the eight specialty branches of Ayurveda which deals with micronutrition, positive health, immune enhancing, rejuvenation, and longevity. The *Medhya Rasayanas* form the most important category of *Rasayana* remedies. These special *Rasayanas* are

basically neuronutrients and work through the nutrition dynamics. Further it cannot be overemphasized that India is one of the richest countries in the world in terms of its biodiversity and green wealth. Because of high safety and cost-effectiveness besides popular acceptability, the green pharmacy is emerging as a major scientific and industrial hub. There is a need to develop appropriate new scientific research methodology and novel approach to study the medicinal plants and to design quality standards for all range of medications of plant origin. The investigations conducted in recent years have shown encouraging results warranting further research and development in order to bring herbal medicine in the mainstream.

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Medical Radiation Countermeasures for Neuroprotection: Herbal Solutions, Evidences, and Challenges Therein

Madhu Bala and Vanita Gupta

Use of ionizing radiation in diagnostics and therapy is increasing. Nausea, vomiting, and various other forms of neurotoxicity are often observed in the patients undergoing tumor radiotherapy. These ill effects are due to severe radiation injuries and consequent toxicity to the normal cells within the non-target tumor volume. World over efforts are in progress to develop the drugs, which are suitable for countering the ill effects of radiation to the normal tissues. Such drugs (medical interventions) have been termed as radiation countermeasures. More specifically, medical radiation countermeasures are the interventional agents, which are essentially required to provide medical support and management of radiation injuries caused by total body or partial body exposures. For the purpose of understanding, the radiation countermeasures are divided into three broad categories, i.e., radioprotectors, mitigators, and therapeutics. Radioprotectors are meant for administration prior to radiation exposure and are intended to counter the radiation injuries, mitigators are meant for use shortly after the exposure (but before the manifestation of illness) and are intended to accelerate the repair or recovery process, and the therapeutics are meant for treatment of radiation injuries (after the explicit manifestation of symptoms) and are intended to promote tissue recovery and regeneration process. In the true sense, the radioprotectors remain in the body even after the exposure and, therefore, continue to act beyond prophylactic action.

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Technology Gap

Despite decades of research, no safe and efficacious radioprotective drug has been approved for human use till date. Efforts are in progress to develop protective agents against the lethal as well as sublethal/therapeutic doses of radiation. Behavioral alterations induced by therapeutic doses ($\leq$ 2 Gy radiation dose) are important concerns. Incomplete understanding of mechanisms of radiation-induced neurotoxicity, nausea, vomiting, etc. are important reasons for the limited progress in this field. Availability of appropriate research models is yet another serious concern. For ethical reasons, the humans cannot be exposed to radiation for research purposes. Experimental rats are accepted models for studying important behavioral alteration by using conditioned taste aversion (CTA) assay. In rats, total body irradiation (TBI) at dose less than or equal to 2 Gray or ($\leq$2 Gy) often causes CTA. It is considered that process of CTA in rats has parallel mechanisms to nausea and vomiting in humans (Cairnie 1983). It is broadly understood that during CTA, the serotonin (5-HT) is released by enterochromaffin cells, which are located in the epithelial layer of the gastrointestinal (GI) tract. The 5-HT then activates the 5-hydroxytryptamine-3 (5-HT_3) receptors present on vagal afferent nerves. These receptors in turn send impulses to chemoreceptor trigger zone (CTZ) of area postrema (AP) of the brain causing vomiting reflex (Scarantino et al. 1994; Smith 1971). However, the serotonin 5-HT_3 receptor antagonists such as ondansetron, granisetron, and tropisetron have been only partially effective in the prevention as well as treatment of emesis. This suggests that more complex interplay between the brain and GI tract regulates this process. The radiation-induced CTA in rats is often recommended for evaluating the performance of pharmaceuticals or drugs intended for use as radiation countermeasures (Cairnie and Leach 1982). WR-2721, the only radiation countermeasure approved for clinical use with radiotherapy, was unable to counter the radiation-induced CTA in rats (Cairnie 1983). It was later found to be neurotoxic in humans (Czerwinski et al. 1972). Thousands of single molecule drugs have been tested world over for the past seven decades but have failed to achieve the desired results. The most important reason of failure is their toxicity at doses which are very close to radioprotective doses. Thereafter, the studies with combination of molecules rendered better results. More recently, the plant extracts, which are a natural combination of many secondary metabolites of therapeutic properties, have been found to be more efficacious with almost negligible toxicity.

New Leads from *Hippophae rhamnoides* (Sea Buckthorn)

Studies in our laboratory have demonstrated significant radioprotective properties of a specific patented preparation (coded as SBL-1) from leaves of sea buckthorn [*Hippophae rhamnoides* L. (Elaeagnaceae)]. Sea buckthorn is a high-altitude plant known for nutraceutical properties. Leaves are reported to be rich in α-tocopherol, plastochromanol-8, β-carotene, chlorophyll, quercetin 3-galactose, kaempferol, rutin, and gallic acid (Guan et al. 2005; Kumar et al. 2011; Górnaś et al. 2014,

2016). SBL-1 demonstrated significant radiation protection in mice against cobalt-60 γ-irradiation (^{60}Co-γ-irradiation). A single dose of SBL-1 [30 mg/kg body weight (b.w.)] administered 30 min before total body ^{60}Co-γ-irradiation (TBI) at lethal dose 10 Gy rendered survival of more than 90% mice population, whereas all of the non-SBL-1-treated irradiated (10 Gy) mice died within 14 days (Bala et al. 2009). This communication briefly summarizes the recent progress made in understanding the role of SBL-1 on radiation-induced neuroprotection in rats using CTA assay. Experimental studies showed that radiation CTA was coupled with injuries to the brain, changes in levels of neurotransmitters, and antioxidant defenses. The scope of developing SBL-1 as radiation countermeasure for human use and challenges involved are also discussed.

Neuroprotection by Sea Buckthorn Against Radiation Injuries: Preclinical Studies

TBI causes a state of oxidative stress and damages multiple tissues. The brain has relatively much higher amount of polyunsaturated fatty acids and also consumes higher amount of oxygen. Because of these important distinct features, the brain suffers much higher oxidative damage in comparison to other organs. The oxidation of cellular protein and nucleic acid leads to damage of neuronal cells, glial cells, and membrane phospholipids.

Radiation CTA is a complex behavioral trait and involves multiple functions such as learning, memory formation, retrieval of stored information, consolidation of taste, visceral information, etc. (Welzl et al. 2001; Scott 2011). In this assay, the experimental rats are first conditioned to drink water only once in a day at a specified time; thereafter the solution of "preferred taste" (e.g., saccharin) is introduced, which is followed by "the malaise" (radiation in this case); and then the preference of animals toward "preferred taste" (saccharin) after "the malaise" (irradiation) is observed. Figure 1 describes the experimental design and the methods used to

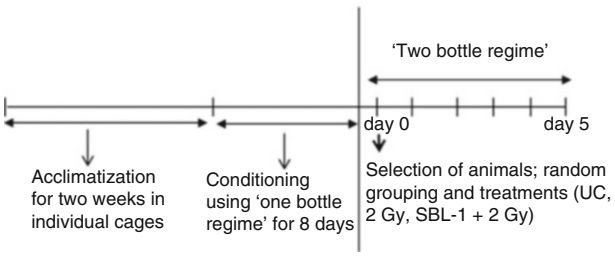

Fig. 1 After acclimatization in individual cages for 2 weeks, the rats were conditioned to take water only once a day (only for 30 min) at a specified time on each day. On day 0 the conditioned rats were selected on the basis of preference for intake of saccharin, where animals were given both water and saccharin simultaneously for 30 min only. Rats showing ≥50% intake of saccharin of the total fluid intake were selected. Selected rats were randomized and divided in three groups such as untreated control (UC), gamma-irradiated (2 Gy), and treated with SBL-1 before irradiation (2 Gy)

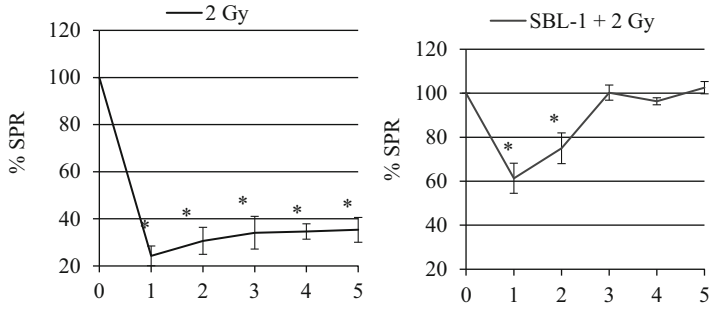

Fig. 2 Shows SPR change (%) in different groups. The SPR was 100% for untreated control. * statistically significant at $p < 0.05$, in comparison to untreated control

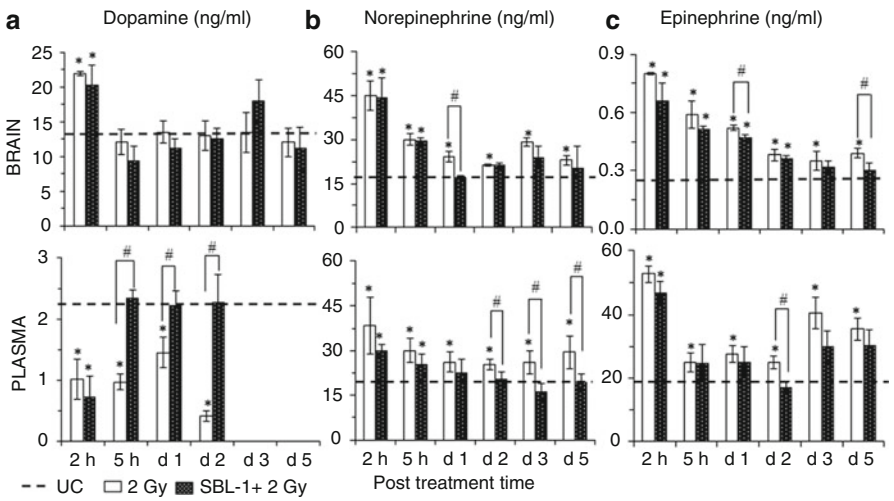

Fig. 3 Modifying effect of SBL-1 on changes in the levels of (**a**) dopamine, (**b**) norepinephrine, and (**c**) epinephrine in the brain and plasma of rats. Data was presented as mean ± SD of six rats in each group. *significantly different at $p < 0.05$ in comparison to untreated control, #significantly different in comparison to irradiated animals at $p < 0.05$

quantify the radiation CTA in experimental rats. CTA was quantified in terms of saccharin preference ratio (SPR) {SPR (%) = [saccharin intake/(water intake + saccharin intake)] × 100}; decrease in SPR indicated acquisition of CTA. The irradiated animals showed significant CTA within 24 h (Fig. 2).

The neurotransmitters are important determinants of a plethora of behavioral responses. In irradiated animals, the dopamine (DA) levels in the brain were significantly ($p < 0.05$) increased (63.4%) at 2 h only, while DA levels in plasma were decreased significantly ($p < 0.05$) at all observation time points (Fig. 3a). The SBL-1 treatment before irradiation normalized the DA levels in plasma from 5 h onward (Fig. 3a). The levels of norepinephrine (NE) in the brain and plasma were

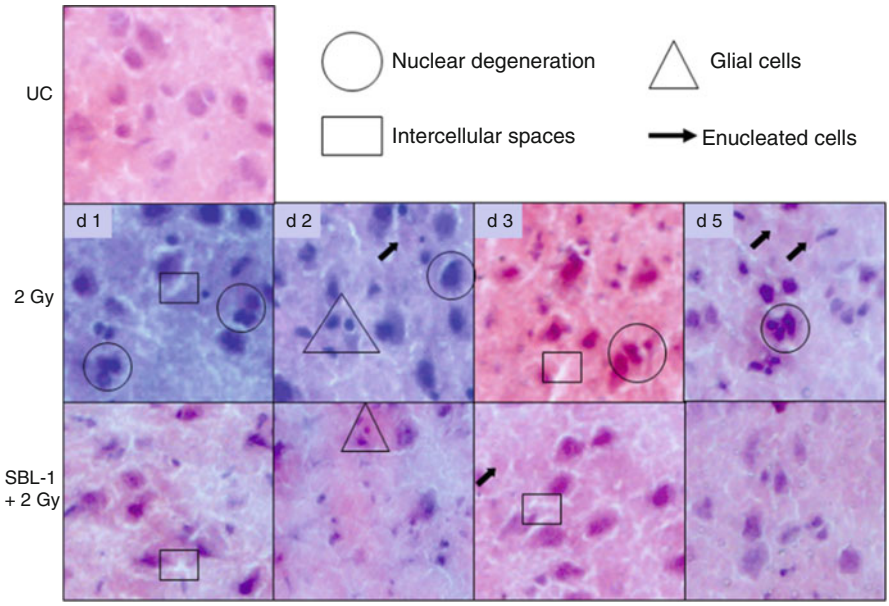

Fig. 4 Radiation-induced histological changes in the cerebral cortex and modifying effects of *Hippophae* leaf extract (SBL-1). Microscopic observations of hematoxylin and eosin stained tissue sections are presented at 200× magnification

increased significantly ($p < 0.05$) at all observation time points after ^{60}Co-γ-irradiation (Fig. 3b). The increase was maximum at 2 h in the brain (157%) as well as in plasma (103%). The SBL-1 treatment before irradiation normalized the levels of NE in the brain and plasma from day 1 onward (Fig. 3b). Irradiation caused significant ($p < 0.05$) increase in the levels of epinephrine (E) in the brain as well as in plasma (Fig. 3c). Treatment with SBL-1 before irradiation normalized the levels of E in the brain from day 3 onward and in plasma from 5 h onward (Fig. 3c).

The normal neuronal architecture in the cortex, hippocampus, and amygdala region of the brain of untreated animals is presented in Figs. 4, 5, and 6. The neuronal cells were compactly arranged, there were no nuclear degenerative changes, and clear demarcation of nuclear material from the cytoplasm was visible. The irradiated animals showed nuclear degeneration in cortex on all the observed days (Fig. 4). The intense and darkly stained nuclear material was observed in multiple cells of the cortex which indicated degenerative changes in the nuclei. In the same group, formation of intercellular spaces on day 1 and day 3, enucleated cells on day 2 and day 5, and increased glial cells on day 2 were observed in the cortex. The hippocampus region showed loss of neurons and dispersion of neurons (Fig. 5). The amygdala region showed nuclear degeneration and formation of intercellular spaces (Fig. 6). In animals treated with SBL-1 before irradiation, the tissue histology was normalized by day 5, suggesting the recovery of lesions in the cortex, hippocampus, and amygdala region.

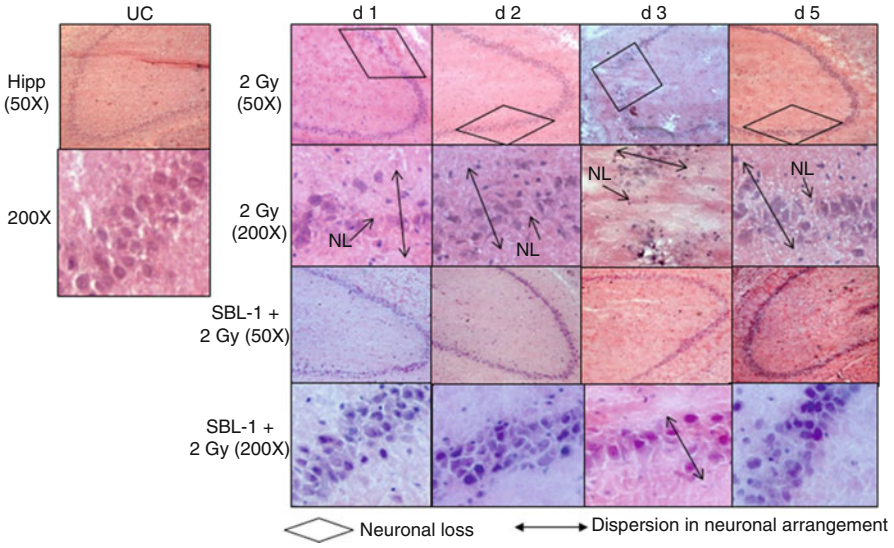

Fig. 5 Histological changes in the hippocampus (Hipp) of rat brain after irradiation and modifying effects of SBL-1. Microscopic observations of hematoxylin and eosin stained tissue sections are presented at 50× and 200× magnifications

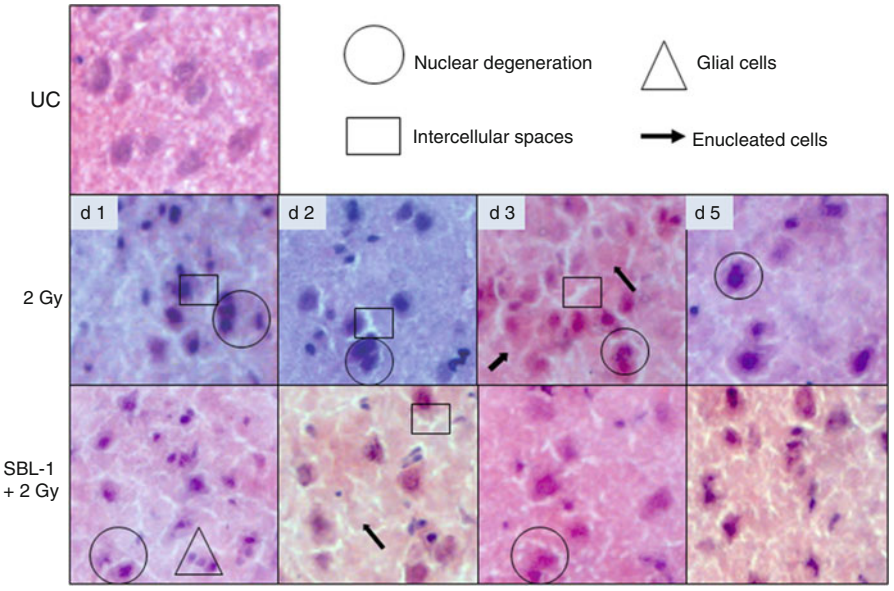

Fig. 6 Radiation-induced histological changes in the amygdala of rats and modifying effects of *Hippophae* leaf extract (SBL-1). Microscopic observations of hematoxylin and eosin stained tissue sections are presented at 200× magnification

The inherent antioxidant enzymes such as SOD and CAT as well as nonenzymatic antioxidant such as GSH play important roles in countering oxidative stress (Sen and Chakraborty 2011). SOD dismutates the toxic superoxide radical into hydrogen peroxide, which is subsequently removed by CAT and/or glutathione peroxidase in the presence of GSH. GSH is one of the major nonprotein thiols and plays an important role in cellular antioxidant defense. GSH scavenges free radicals, removes hydrogen and lipid peroxides, and prevents oxidation of biomolecules. In this study, the decrease in levels of SOD, CAT, and GSH at most observation time points in the brain and blood in irradiated animals indicated that the antioxidant defenses were weakened after irradiation (Table 1).

Potential of Sea Buckthorn as Radiation Countermeasure

The neuronal injuries observed in the cerebral cortex and amygdala (nuclear degeneration, increase in intercellular spaces and glial cells, and formation of enucleated cells) as well as in the hippocampus (neuronal loss and dispersion of neuronal cells), within 24 h (day 1) in irradiated animals (Figs. 4, 5, and 6), could be primarily due to oxidative damage caused by irradiation. In SBL-1 pretreated irradiated animals, normalization of DA, NE, and E in the brain and plasma (Fig. 3) at most observation time points indicated that this was one of the important mechanisms by which SBL-1 countered radiation CTA (Fig. 2). Another mechanism by which SBL-1 rendered protection against CTA was by countering the radiation-induced increase in levels of 5-HT in jejunum and in plasma. This was reported in another study (Gupta et al. 2011). SBL-1 treatment prior to irradiation countered radiation-induced changes in SOD, CAT, and GSH (Table 1) indicating that the normalization of antioxidant defenses was yet another mechanism by which SBL-1 countered radiation CTA (Fig. 1). Earlier studies showed that SBL-1 treatment prior to irradiation countered the huge flux of radiation-induced ROS, free radicals (Tiwari et al. 2009; Saini et al. 2010), and was able to counter radiation-induced inflammation (Tiwari and Bala 2011) and derangement of tissue architecture of the kidney (Saini et al. 2014) and gastrointestinal tract (Bala et al. 2015). Countering of radiation-induced oxidative stress by SBL-1 could be responsible for countering the neuronal injuries observed in the cerebral cortex, hippocampus, and amygdala (Figs. 4, 5, and 6). The SBL-1 contained quercetin dihydrate (4.66 mg), rutin trihydrate (8.72 mg), gallic acid ethyl ester (12.09 mg), thiols (0.827 M total thiols), tannins (0.32 $\pm$ 0.006 g), proanthocyanidins (2.5% of total constituents of *Hippophae* leaves) (Bala and Saini 2013), and ellagic acid (Bala et al. 2015). These constituents are likely to be responsible for rendering strong antioxidant potential to SBL-1. Quercetin is an important flavonoid and crosses the blood-brain barrier (Youdim et al. 2004). Proanthocyanidins are the oligomers of flavonoids, viz., catechin, epicatechin, and their gallic acid esters (epigallocatechin). Catechin and epicatechin also cross the blood-brain barrier (Faria et al. 2011). They have shown neuroprotection by decreasing oxidative stress and promoting spatial cognitive learning ability (Haque et al. 2006). Dietary polyphenols have shown neuroprotection in patients of Alzheimer's

Table 1 Radiation-induced changes in levels of superoxide dismutase (SOD), catalase (CAT), and reduced glutathione (GSH) in the brain and blood of rats and medication caused by treatment with *Hippophae* leaf extract (SBL-1)

Time	Groups	SOD		CAT		GSH	
		Brain	Blood	Brain	Blood	Brain	Blood
	Group I	1.42 ± 0.08	10.5 ± 1.54	6.62 ± 2.04	153.6 ± 6.67	0.99 ± 0.14	3.30 ± 0.18
2 h	Group II	0.66 ± 0.04*	5.2 ± 0.53*	2.77 ± 0.49*	113.3 ± 12.2*	0.49 ± 0.03*	1.67 ± 0.05*
	Group III	0.83 ± 0.31	5.9 ± 0.96*	5.01 ± 0.34#	126.9 ± 13.2	0.55 ± 2.10	1.86 ± 0.22*
5 h	Group II	0.57 ± 0.01*	9.7 ± 2.52	2.68 ± 0.55*	106.2 ± 4.33*	0.59 ± 0.07*	2.00 ± 0.18*
	Group III	1.06 ± 0.32	10.6 ± 2.61	3.70 ± 0.45	140.6 ± 42.8	1.15 ± 0.01#	2.79 ± 0.1#*
Day 1	Group II	0.71 ± 0.03*	10.1 ± 1.49	4.91 ± 1.37	116.4 ± 5.03*	0.81 ± 0.09	2.47 ± 0.49*
	Group III	1.36 ± 0.26#	13.8 ± 1.57#	7.74 ± 1.51	156.2 ± 5.61#	1.20 ± 0.14#	3.22 ± 0.13#
Day 2	Group II	0.83 ± 0.09*	9.9 ± 3.07	6.09 ± 2.02	152.4 ± 5.48	0.93 ± 0.48	2.09 ± 0.35*
	Group III	1.44 ± 0.40	10.6 ± 1.79	9.57 ± 0.89	177.2 ± 12.3#	1.33 ± 0.23	2.31 ± 0.53
Day 3	Group II	0.47 ± 0.14*	7.6 ± 1.26	6.86 ± 1.94	147.5 ± 15.3	1.10 ± 0.46	2.19 ± 0.43*
	Group III	1.76 ± 0.21#	10.3 ± 0.89#	12.41 ± 2.31#	192.8 ± 10.1*	2.22 ± 0.3#*	2.32 ± 0.52
Day 5	Group II	1.08 ± 0.39	4.4 ± 1.91*	6.57 ± 1.72	145.8 ± 18.9	1.02 ± 0.06	2.52 ± 0.13*
	Group III	1.50 ± 0.11	10.5 ± 1.65	6.61 ± 1.03	179.1 ± 11.5	1.39 ± 0.14#	3.12 ± 0.32

Group I, untreated control (UC); Group II, 2 Gy; and Group III, SBL-1 + 2 Gy. Each group had six rats. Data is mean ± SD. *statistically significant at $p < 0.05$ in comparison to UC; #statistically significant at $p < 0.05$ in comparison to 2 Gy. GSH is expressed as nmol/mg protein; SOD as U/mg protein; CAT as μmol H_2O_2 consumed/min/mg protein

and Parkinson's by attenuating oxidative stress and neuronal damage. Some of the flavonoids like epigallocatechin 3-gallate and epicatechin provide neuroprotection not only by their antioxidant properties but also via activation of the various cellular signaling pathways. Some important ones are phosphorylation of kinases such as extracellular protein kinases, phosphatidylinositol-3 kinase/protein kinase B, and protein kinase C (Schroeter et al. 2007).

Sea Buckthorn as an Interventions for Human Use

The development of medical countermeasures for human use has been a challenge for the past many decades, and the demand is yet unmet. The enormity of challenge in developing radioprotective drug stems from the fact that ionizing radiation causes multiple damages simultaneously and deranges many bodily functions concurrently. The single molecule drugs have failed primarily because of their toxicity to human body. As understood, the toxicity arises because single molecule drugs act via action on selected biochemical pathways. The remaining deranged biochemical pathways in irradiated subjects become the potential source(s) of progressive cellular injuries, which ultimately result in tissue toxicity. The SBL-1 from leaves of sea buckthorn acts by countering multiple pathways, such as by countering radiation-induced redox disturbances, DNA damage, inflammation, and immunosuppression. It promotes tissue recovery and normalization of levels of neurotransmitters in the irradiated animals. Multiple actions are possible because sea buckthorn leaves are rich in multiple compounds, which have shown bioactivity. These may be flavonoids, tannins, triterpenes, and vitamin C. The nutraceutical value of sea buckthorn leaves is well recognized. The tea prepared from *Hippophae* leaves is popular for nutraceutical properties in China, Tibet, and the Indian Himalayan region. In traditional medicinal systems, sea buckthorn is recommended for treatment of hepatic injuries, asthma, gastric ulcers, coronary heart diseases, and skin diseases. The nontoxic nature of sea buckthorn makes it an ideal choice for developing medical countermeasure for human use.

The Challenges of Developing Herbal Medical Interventions as Radiation Countermeasures

The issue of batch-to-batch variation and maintenance of quality control has plagued the development of herbal drugs. Because of multiple bioactive constituents present in botanical preparations, it is difficult to standardize the pharmaceutical preparations. The mass scale cultivation and tissue culture practices are being promoted to address these concerns. The development of approved radiation countermeasures has additional complications, because they cannot be tested for efficacy in human beings as it is unethical. The Food and Drug Administration (FDA) of the USA has issued the Animal Efficacy Rule in year 2002 to expedite the development of medical countermeasures against radiation. According to the Animal

Efficacy Rule, the potential agent must undergo rigorous testing utilizing the animal models which have similar pathophysiological mechanisms for radiation injury and treatment response in humans. While this rule may help in extrapolation of the medical effects of single molecule drugs, it is likely to be unsuitable for Ayurvedic/Unani or herbal medicines because they have multiple bioactive components. It is therefore imperative that AYUSH, the regulatory body for traditional drugs in India, may device appropriate guidelines for such cases. Another option is that it may permit use of herbal radiation countermeasure after they are found to be safe in humans. It is now being proposed at several platforms that a standardized herbal drug, prepared from cultivated plantations, and also that appears in Ayurvedic Formulary may be permitted its use after the indication of reasonably sufficient efficacy in animals. A nutraceutical preparation, which has already passed the human body for many years without notable side effects, may be another good option. Considering the total absence of safe and approved medical radiation countermeasures globally, the leaves of sea buckthorn are highly promising for developing medical countermeasure for human use.

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DRDO Herbal Technologies: Military and Civil Applications

Rajesh Arora

Introduction

India's military is a human-intensive workforce with nearly 1.3 million defence personnel and over 2.0 million personnel in reserve. In addition, to cater to the nation's security needs – both external and internal – India has over 1.0 million paramilitary personnel. The military personnel are deployed in diverse harsh environmental conditions to guard national sovereignty under diverse geoclimatic conditions and diverse terrains exposing them to unique conditions that are not perhaps encountered anywhere elsewhere on the globe. Homeland security is an important component of national security. The paramilitary and military personnel inevitably have to participate in low-intensity conflict (LIC) theatres also. Besides, the Indian military has always risen to the occasion and helped in disaster management, whenever there has been a natural calamity. The Indian military personnel have also been an integral part of several successful UN peacekeeping missions.

Keeping the physical and mental health of soldiers is paramount from an operational perspective. In addition, they need to be updated and trained to be able to utilize state-of-the-art technologies. They need to be ever vigilant, be prepared for handling any situation and protecting national interests in a rapidly changing world. In order to keep the military fighting fit for operations, there is a need to equip the soldiers with state-of-the-art technologies, often disruptive technologies, products, processes and requisite paraphernalia with a human engineering perspective to support them and handle today's VUCA (volatile, uncertain, complex and ambiguous) environment. DRDO well realizes that this can be only accomplished through continuous, concerted R&D with a focus on new technologies and product development, synergizing the private industry sector along with academia. Today's military operations have to be conducted seamlessly, irrespective of environmental stressors

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like sub-zero temperatures, torrential rains, snow, extremes of humidity, lashing winds, extreme heat, etc. and/or in a nuclear, biological and chemical (NBC) environment. In the Indian military context, the environmental hazards posed due to the geographical locale often prove more lethal to the health of a soldier than the military operation itself. More than the threat from enemy bullets is the threat of fluid loss from the body under desert conditions or frostbite and high-altitude pulmonary oedema on mountaintops in winter. High pressure of the water column can limit the performance of submariners. Even those who escape sickness may face problems of performance at altitudes in the Eastern and Western Himalayas, where the atmosphere is rarefied due to low oxygen availability. In fact in Siachen sector, more lives have been lost due to the environmental conditions rather than military operations in this ruthless sector.

During the combat active period, the Indian military personnel are exposed to almost all of the environmental and occupational hazards in a single lifetime. The military personnel, depending on their field of activity, are exposed to various stresses in micro-environments like engine rooms of ships, submarines, aircraft cockpits, crew compartments of tanks and other combat vehicles which are usually cramped, hot, polluted by noise, smoke and toxic gases and radiation, and hence these also pose real threats to efficient performance and health of the warfighter.

The operational needs of the Indian Defence Forces necessitate operations under unforgiving and inhospitable environments, which can severely impact both physical and cognitive performance of the personnel. The Life Sciences laboratories of DRDO are engaged in R&D with the aim to develop processes, products and technologies and effective strategies to protect and enhance the operational efficiency of the Indian Armed Forces. The mandate of the Life Sciences laboratories in Defence Research and Development Organisation (DRDO) is to optimize performance of the of the human-intensive combat force, create soldier-system fit in the various weapon development programmes and help them in meeting the unique operational requirements in conventional and nonconventional warfare situations. The R&D activities of the laboratories are focussed on recruitment, selection and development of appropriate human capital, development of intricate life support systems and technologies to protect against extreme and toxic/lethal environments, promotion of health and well-being of warfighter and development of strategic support systems. The fields of research encompass protective equipments and clothing, physiological-psychological aspects, protection against nuclear, biological and chemical (NBC) warfare and high-altitude agro-animal technologies to meet fresh food requirement of troops at high altitudes, exploiting bioenergy as emergency fuel and meeting instant food requirements for high-altitude battlefield operations.

Over the last five decades, the endeavours of the Life Sciences cluster of laboratories have resulted in:

- Creating specialized human capital through selection & training
- Enhancing efficiency through customized nutrition
- Optimizing human efficiency through traditional systems
- Optimizing performance through human engineering approach
- Protecting against health hazards like CBRN and vectors
- Reducing combat stress by counselling, training and resilience building
- Reinforcing adaptation through acclimatization processes
- Saving lives through life support systems

The Life Sciences group of laboratories of DRDO have made significant contributions towards improving the health and performance of soldiers deployed at high altitude, cold, desert, under water, both in air and space and low-intensity conflict areas. The various labs have also made immense progress in agriculture and food technology for adverse environments such as extreme altitude, space, etc. Further, these labs are also engaged in the development of novel technologies to detect, decontaminate and protect against chemical, biological, radiological and nuclear (CBRN) threats. Research in the various laboratories and sponsored projects cover an entire gamut of areas ranging from biomedical, food and high-altitude agro-animal and bioenergy research. Development of herbal technologies is an important area of research where attention is being focused in DRDO, in keeping with prevalence of rich traditions of diverse systems of medicine in India that have been tried, tested and proved over a period of thousands of years. Perhaps no other system of medicine has stood the test of time for such a long period.

Resurgence of Interest in Herbal Technologies in India

Affordable, accessible and acceptable healthcare for all sections of society is the need of the hour. Meeting the health needs of population of over seven billion people, majority of which reside in developing/underdeveloped countries where the population just cannot afford costly healthcare, has necessitated the revival of traditional systems of medicine and the development of cost-effective and pragmatic strategies for the masses. Besides, the disease preventive as well as curative aspects of Indian herbal medicine, its safety and efficacy proved over thousands of years, have made it a preferred choice amongst the masses. The Indian system of medicine, Ayurveda, has evolved as a result of years of medical practice with medicinal plants in humans. India is endowed with extremely rich biodiversity and is home to innumerable medicinal and aromatic plant species which have been used extensively to prevent and/or cure diseases. Similarly, other alternative systems of health like Siddha, Unani and Homoeopathy are now officially recognized, and a ministry known as AYUSH – Ayurveda, Yoga and Naturopathy, Unani, Siddha and Homoeopathy – has been set up. In view of the huge population of India, it is imperative to develop cost-effective and sustainable health solutions for the public at large. Only one particular system of medicine cannot meet the health challenges posed by the burgeoning population. It is the author's firm belief that we have to consider the rich, Indian traditional systems of medicine for both their preventive and therapeutic aspects and utilize them for meeting the health challenges of our country. Besides, treatments for all diseases are not available in the allopathic system of medicine. The undesirable side effects of modern medicines, the mounting costs of conventional hospital-based healthcare and the emergence of lifestyle-related diseases are some of the reasons for the population to consider alternative simpler and cheaper forms of treatment. Effective and holistic treatment for several diseases like cancer, AIDS, emerging and reemerging bacterial, fungal and viral diseases, Alzheimer's, Parkinson's and several emerging and reemerging diseases are still not available in modern systems of medicine leading to more and more patients taking recourse to alternative therapies for improving the quality of life. Table 1 enumerates the reason why recourse to herbal medicine is the natural choice for sustainable, affordable and acceptable healthcare for the population.

DRDO has been working on herbals for the last several decades to solve the problems of the soldiers, who guard the borders. There are several problems for which holistic and pragmatic solutions are just not available. It is in these areas that the DRDO herbal technologies lay emphasis on.

Harnessing Herbal Technologies to Meet Military Challenges: DRDO Endeavours

DRDO has been making concerted efforts towards research and development of herbal products with the aim of providing solutions to the unique problems faced by the Armed Forces. Some areas of research where DRDO is focussing its attention

Table 1 Why herbal medicine is a natural choice

S. No.	Synthetic medicine	Herbal medicine
1.		
2.	Synthetically derived; often nature-imitated or nature-identical	Plants have grown in nature and evolved over millions of years during the course of natural selection
3.	Mostly therapeutic	Both prophylactic and therapeutic; prophylactic action more profound. Herbal medicine focuses on prevention rather than treating a disease or ailment once it arises
4.	Usually acts on a particular biological pathway	Holistic in action; overall wellness
5.	Expensive	Cheaper
6.	Accessible only to section of society, which can afford	Affordable, acceptable and accessible to all sections of society
7.	Few years of animal and human testing	Years (sometimes thousands) of usage in traditional systems of medicine
8.	Requires prescription from medical practitioner	Easier to obtain than prescription medications, mostly available OTC
9.	Limited choice for chronic and acute medical ailments	Possess beneficial, healing properties for the treatment of chronic and acute conditions and various ailments for which treatment is not available in Western systems of medicine
10.	Very few medicines for terminally ill patients	Preferred choice of terminally ill patients
11.	Toxicity/side effects: a major issue	Lesser toxicity/side effects; presence of multiple components countering the toxic action of one compound
12.	Few antiviral drugs	Source of antiviral drugs

Fig. 1 DRDO herbal technologies encompass various areas that touch the lives of the man behind the weapon in multifarious ways in their day-to-day working in difficult terrains and under various operational conditions

vis-à-vis herbal technology development are highlighted in Fig. 1. Documentation of traditional knowledge on herbs of some of the inaccessible areas of the country, mainly high altitude, desert and remote regions of the country, has been carried out. Research to generate scientific data on herbs and their products are under way to explore their potential as antioxidants, immunomodulators, CBRN countermeasures and antistress agents, which would help in improving and protecting the health of troops who are exposed to various occupational and other hazards.

Management of High-Altitude Maladies Encountered by Soldiers

Composite Indian Herbal Preparation

Exposure of living organisms to adverse climatic conditions is known to cause stress, which is a non-specific response to the excess demands imposed on the body. A plethora of stressors like physical, chemical, immunological, biological and emotional factors are involved in the manifestation of stress. Soldiers staying and working in adverse climatic conditions such as cold, heat and high altitude also suffer from stress-induced disorders and at times exhibit decreased or suboptimal levels of physical/cognitive performance due to stress. Adaptogens are biologically active substances that are known to improve physical and mental performance under adverse environmental conditions and induce resistance against stressors,

Fig. 2 Composite Indian herbal preparations: unique adaptogens

e.g. extreme cold and heat, pain and infections. A plethora of herbals possess adaptogenic properties and are used in different traditional systems of medicine for improving overall stress resistance. Taking leads from India's traditional system of medicine – the Ayurveda, a formulation – Composite Indian Herbal Preparation I (CIHP I) has been developed by DIPAS to increase the physical performance of our soldiers who have to operate in high-altitude environments under extremes (Fig. 2). The formulation CIHP I comprises of ingredients (derived from seven different plants used in Ayurveda) and the following aqueous herbal extracts: *Asparagus racemosus* wild roots, 29.23%; *Withania somnifera* Dunal roots, 16.92%; *Pueraria tuberosa* DC tubers, 16.92%; *Mucuna pruriens* DC seeds, 8.46%; *Dioscorea bulbifera* Linn. rhizomes, 8.46%; *Argyreia speciosa* sweet whole plant, 8.46%; *Piper longum* Linn. fruit, 4.32%; and Asphalt extract 7.32%.

CIHP I was tested for its adaptogenic activity using C-H-R animal model. CIHP I helped in improving resistance to C-H-R-induced hypothermia and enhanced post-stress recovery to regain Trec 37 °C. In Border Security Force volunteers, CIHP I intake was found to restrict combat stress-induced deterioration in both physical and mental performance (Srivastava et al. 1996). Another Composite Indian Herbal Preparation II (CIHP II), a combination of numerous plant ingredients and minerals (Table 2), was tested for its adaptogenic action using C-H-R animal model. The acclimatization to hypoxia of extreme altitude was facilitated by the administration of CIHP II during exposure (Grover et al. 1995). Both CIHP I and II augmented oxygen delivery to biological system by increasing red cell 2,3-diphosphoglyceric acid levels during hypobaric hypoxic exposure and improved cellular membrane permeability and maintenance of blood glucose and muscle glycogen levels. CIHPs also regulated the mobilization of lipids from adipose tissue and their preferential utilization for thermogenesis as compared to carbohydrates (Grover et al. 1995; Kumar et al. 1999, 2000). The above studies conducted at DIPAS have indicated that the Ayurvedic herbal preparations/rasayanas with adaptogenic activity increase the high altitude and cold tolerance and relieve the incapacitating effect of stress on operational performance.

Table 2 Composition of CIHP II (460 mg tablet)

Ingredient	Content (mg)	Ingredient	Content (mg)
Withania somnifera	30.0	Abhrak bhasma	10.0
Makardhwaj	10.0	*Adhotoda vasica*	10.0
Asparagus racemosus	20.0	*Argyreia speciosa*	10.0
Mandur bhasma	5.0	*Carum copticum*	05.0
Caesalpinia digyna	10.0	*Celastrus paniculatus*	05.0
Crocus sativus	5.0	*Curcuma longa*	05.0
Asparagus adscendens	10.0	*Eclipta alba*	10.0
Tamarix gallica	3.2	*Elettaria cardamomum*	05.0
Achillea millefolium	3.2	*Eugenia caryophyllata*	05.0
Amber	2.0	*Exts. Berberis aristata*	10.0
Centella asiatica	20.0	Jasad bhasma	05.0
Glycyrrhiza glabra	20.0	Loh bhasma	05.0
Terminalia arjuna	6.4	Mace	10.0
Cichorium intybus	13.8	*Mucuna pruriens*	10.0
Solanum nigrum	6.4	*Myristica fragrans*	10.0
Cassia occidentalis	3.2	*Piper longum*	10.0
Chyavanprash concentrate	100.0	Shilajeet (purified)	20.0
Exts. Capparis spinosa	13.8	*Terminalia chebula*	15.0

Processed in: *Allium cepa*, *Allium sativum*, *Asparagus racemosus*, *Berberis aristata*, *Boerhavia diffusa*, *Eclipta alba*, *Phyllanthus emblica*, *Phyllanthus niruri*, *Raphanus sativus*, *Terminalia chebula*, *Tinospora cordifolia*, *Tribulus terrestris*

Herbal Antistress Adaptogen: DIP-91

Stress is a part and parcel of modern-day life. Prolonged and persistent stress can lead to psychophysiological and metabolic disorders like hypertension, diabetes, gastric ulcer, cardiovascular ailments, etc. A non-specific, antistress procedure/process and herbal or chemical intervention can help in the restoration of normal homoeostasis of the body. The soldier faces innumerable stresses while on military assignments. A potent herbal antistress adaptogen (DIP-91) has been developed for soldiers using a single herbal component, which is specifically effective in alleviating high-altitude stress (Fig. 3).

DIP-91 has numerous benefits: (i) it acts as an anxiolytic, promotes endurance and is a potent rejuvenator/revitalizer; (ii) doesn't alter metabolism during the resting phase; (iii) exerts adaptogenic effect only under conditions of threat to the system; (iv) possesses exceptional antioxidant potential and thereby alleviates oxidative stress; (v) possesses non-cumulative potent adaptogenic activity; (vi) is better than available products in the market (polyherbal/multicomponent preparations; and (vii) is cost-effective, non-toxic, safe and free of heavy metal-associated toxicity.

DIP-91 is very effective as a health food supplement/nutraceutical. It is useful for overall health and well-being under stressful situations as it improves stamina and

Fig. 3 The herbal antistress adaptogen – DIP-91– is useful in high-altitude regions

Fig. 4 *Hippophae salicifolia*: potential application in high-altitude stress management

immunity and helps in adapting to adverse environmental conditions. DIP-91 has been reported to be a potent antistress agent for management of day-to-day stresses faced by the soldier during operations.

Hippophae spp. for High-Altitude Stress Management

The high-altitude medicinal plant species, viz. *Hippophae salicifolia* and *Hippophae rhamnoides turkestanica*, are widely distributed in the Himalayan region and possess adaptogenic properties, which alleviate high-altitude stress. *Hippophae salicifolia* has been shown to have potential application in high-altitude stress management (Fig. 4). Rathor et al. (2015) evaluated the mechanism of action of both the species against multiple stresses [cold-hypoxia-restraint (C-H-R)]. They reported the adaptogenic activity of *Hippophae salicifolia* in facilitating tolerance to CHR in rats. Pretreatment with *Hippophae salicifolia* significantly attenuated reactive oxygen species production, protein oxidation and lipid peroxidation and also helped maintain antioxidant status. Pretreatment of *Hippophae salicifolia* resulted in decreased protein oxidation, and protein homoeostasis was sustained by regulation of heat shock proteins (HSP70 and HSP60). Interestingly, heme oxygenase-1, vascular endothelial growth factor and nitric oxide level were also

increased in *Hippophae salicifolia* pretreated rats proving its adaptogenic activity against cold-hypoxia-restraint. Conclusively, aqueous extract of *Hippophae salicifolia* could be used as an adaptogen for high-altitude-associated multiple stress.

Sharma et al. (2015) have attributed the potent in vitro antioxidant potential of the extracts of the leaves of *Hippophae salicifolia* and *Hippophae rhamnoides mongolica* (as compared to *Hippophae rhamnoides turkestanica*) to the in vivo adaptogenic performance in animals during cold and hypoxia exposure under restraint stress. For adaptogenic studies, rats with oral drug supplementation were exposed to cold-hypoxia-restraint (C-H-R) stresses-induced hypothermia to determine endurance. Aqueous extracts of *Hippophae salicifolia* showed maximum (99%) resistance compared to the other *Hippophae* spp. The levels of biochemical parameters such as reactive oxygen species, malondialdehyde, lactate dehydrogenase, superoxide dismutase, glutathione and catalase in blood samples also revealed that the aqueous leaf extract of *Hippophae salicifolia* exhibited superior antioxidant and adaptogenic potential and established its usefulness for high-altitude stress management.

Sea Buckthorn Soft Gel Capsule for Better Acclimatization

With a view to developing prophylactics for soldiers who operate in high-altitude regions, supercritical fluid extracted sea buckthorn seed oil-based soft gel capsules has been formulated by the Defence Institute of High Altitude Research (DIHAR), Leh (J&K). The formulation is extremely rich in unsaturated fatty acids (omega 3,6,9), vitamin 'E' and carotene and contains minerals that confer it with significant antioxidant activity. The high antioxidant activities possessed by the product make it promising for use as a prophylactic for the management of high-altitude maladies (Fig. 5). The formulation improves heart function and blood circulation and increases myelination of neurons, thereby improving cognitive faculties.

Fig. 5 Sea buckthorn seed oil-based soft gel capsule helps in better acclimatization at high altitudes

Herbal Adaptogenic Appetizer for Appetite Modulation

Research has shown that at high altitudes, there is significant weight loss, which is attributable to decreased energy consumption mainly due to lack of appetite. A nutraceutical herbal adaptogenic appetizer has been formulated using spray-dried powder of sea buckthorn and apricot pulp along with the extract of *Rhodiola,* the indigenous medicinal plants of high-altitude Himalayas with exceptionally rich nutritional and pharmacological potential (Fig. 6). The main ingredients include *Hippophae rhamnoides*, *Prunus armeniaca and Rhodiola imbricata.* It is rich in flavonoids, polyphenols, sterols, vitamin C, vitamin E, riboflavin, niacin, isorhamnetin, rosavin and carotene. The formulation improves digestion and appetite and augments serum antioxidants.

Protection Against UV Radiation

The military units deployed at high-altitude regions face challenges posed by the harsh environment. The excessive ultraviolet radiation at high-altitude regions, together with hypoxia, cold, wind, dryness and solar radiation, adversely impacts the performance of troops. Providing health service support to the Armed Forces is the key mandate of DRDO. A herbal formulation has been developed that acts as a prophylactic for low humidity and UV-mediated skin damage at high altitude (Fig. 7).

The UV protective cream has anti-photoageing and anti-blemish properties. The formulation contains *Hippophae rhamnoides* (sea buckthorn) seed oil as the major ingredient. The formulation also contains uvinul t 150-ethyl hexyl triazone, uvinul mc 80-ethylhexyl methoxy cinnamate, tinisorb m-methylene bis-benzotriazolyl tetra methylbutyl phenol, uvinul a-diethyl amino hydroxyl benzoyl tetra methylbutylphenol, tinosorb-bis-ethylhexyloxyphenol methoxyphenyl triazine, petroleum jelly, isopropyl myristicate (IPM) and other compounds. The formulation

Fig. 6 Herbal adaptogenic appetizer

Fig. 7 UV protective anti-blemishes cream developed by DRDO for use in high-altitude region by the troops

does not contain zinc and has UV protective efficacy equivalent to SPF > 45 along with anti-blemishes and skin-healing properties. The topical formulation can be effectively used as a prophylactic for low humidity and UV-mediated skin damage caused at high altitude.

Another high-altitude herbal UV screen "Umbriel" has been specifically developed to protect the subjects from damage caused by the UV flux prevalent at high altitudes. Umbriel (a registered trademark of DRDO) is a unique combination of herbal agents developed by INMAS, DRDO. It is non-toxic, prophylactic, dermal ointment which has been scientifically tested to cut off more than 90% UV rays, prevent DNA damage, regulate melanin content in the skin, prevent immunosuppression, and counter oxidative stress and photosensitivity. This product has passed highly sensitive tests such as infrared thermography. This innovation is useful for people living in high-altitude regions, tourists visiting high-altitude areas and sportsmen participating in snow/winter games at high altitudes and people who migrate from plains to high altitudes and are unacclimatized to the extreme UV radiations prevalent at high altitudes.

Ensuring Survival in Land Locked Regions During Military Exigencies

The concept of survival garden has been developed by DRDO to meet the health and survival needs of the Armed Forces in case of exigencies arising locally during operations. The ethnic medicinal plant resource especially in the North West, Central and North East Himalayan region are being harnessed for this purpose.

The purpose of survival garden is that the soldiers should be able to utilize the locally available indigenous medicinal and aromatic plants can be utilized for treating common ailments and survival during operational conditions in diverse terrain (Fig. 8). India is extremely rich in phytobiodiversity in view of the prevailing

Fig. 8 The survival garden for soldiers can be immensely helpful during emergency situations

divergent ecosystems and considerable altitudinal variations in the country. The Himalayan ranges possess huge phytobiodiversity in terms of various plant species, including angiosperms, gymnosperms, pteridophytes, bryophytes, lichens, bacteria and fungi. Of this diversity, nearly 25% species are endemic to the Himalayas. The Himalayas are considered to be a treasure house of medicinal, aromatic, edible wild and other important plants, which have been used by the local populations for meeting their daily necessities since ages, and this long dependence on plant wealth enriched their knowledge about the multifarious uses of phytodiversity. The high-altitude regions of Ladakh and Lahaul-Spiti in the North West and Tawang in the North East are strategically important because of the international borders with China and Pakistan. The Armed Forces, deployed in these regions, have to face extreme environmental conditions, as the region is prone to landslides, heavy rainfall, cloud bursts, avalanches and natural calamities. At times, these landlocked areas become inaccessible by road and/or air. In view of the likelihood of availability of food and medicines in such areas becoming compromised under certain operational conditions, a need was felt to develop databases on wild plants that can help sustenance and survival of troops in such regions in case of an emergency. The survival garden can play an important role in helping the troops operate unhindered under any circumstances.

Improving Combat Performance and Operational Efficiency

DIP-G-FIT: A Unique Formulation for Improved Soldier Performance

An oriental fungus that has been used for promoting health and longevity in the Himalayan tracts of India, China, Japan and several other Asian countries has been used to develop a medicinal formulation - DIP-G-Fit for enhancing performance in soldiers (Fig. 9). The fungus grows at high elevations in the Himalayas and has been recognized as a very powerful medicinal mushroom for several hundred years. It is widely used due to its numerous pharmacological properties including antistress, antifatigue, immunomodulatory, health-promoting and longevity-enhancing properties, etc. and is quite popularly accepted as a dietary supplement in Western countries. In preclinical studies conducted at DIPAS, supplementation of DIP-G-Fit enhanced exhaustion time in experimental animals significantly both under

Fig. 9 Performance enhancing DIP-G-Fit formulation

normoxic and hypoxic conditions. DIP-G-Fit can be extremely useful for enhancing performance of soldiers posted at high altitude and improving endurance in sports personnel.

Cordyceps Capsules for Improved Physical Function

Cordyceps sinensis is a high-value medicinal fungus growing on caterpillar in Himalayan region (11,000–14,000 ft), locally known as Kira Ghas or Yarsha Gamboo. The extract of the fungus is effective against hypertension, diabetes, pneumonia, leukaemia, cirrhosis, impotence, tuberculosis, insomnia, joint pain, cough, hypoxia, arthritis, erythropoiesis and chronic bronchitis. Laboratory cultures for the fungus have been established and can serve as a stable source for obtaining its extract, removing the dependence on harvesting from the natural environment.

Cordyceps improves the respiratory function and can be used for treating chronic bronchitis and asthma; improves the functioning of the heart and is helpful in heart rhythm disturbances, cardiac arrhythmias and chronic heart failure; alleviated LDL cholesterol and increases HDL cholesterol; possesses antitumour properties; improves immune system function by modulating the activity of different cytokines like NK cells, ILs and IFNs and protects against free radical-induced damage; improves stamina by increasing ATP synthesis, reduces fatigue; and improves physical function. A formulation developed from *Cordyceps* has been developed, which improves physical function of soldiers (Fig. 10).

Herbal Performance Enhancer

A herbal adaptogenic performance enhancer performer has been formulated using different plant parts, viz. root, leaves, fruits, etc., of high-altitude medicinal plants and exotic plants based on ethnobotanical information (Fig. 11). The herbal formulation enhances physical and mental performance and can be used as a herbal prophylactic for high altitude and as a dietary supplement. The main constituents are *Codonopsis pilosula*, *Astragalus membranaceus*, *P. cocos*, *Rhodiola imbricata*, *Aloe vera*, *Zingiber officinale*, *Ginkgo biloba*, *Bacopa monnieri*, *Withania somnifera*

Fig. 10 *Cordyceps* for improved physical function of soldiers

Fig. 11 Perfomax: a herbal adaptogenic performance enhancer

and Tribulus terrestris. The formulation is rich in vitamins A, B series, C, D, E and GSH, besides astragalosides, choline, beta-sitosterols, pachymaic acid, withanine, protodioscin, triterpenes, ginkgolides, anthraquinones, aloin, shogaol, gingerol and bacosides. The herbal prophylactic is useful for high-altitude problems and as a dietary supplement.

Antistress Multivitamin Sea Buckthorn-Based Herbal Beverage

A multivitamin herbal beverage has been developed by the Defence Institute of High Altitude Research (DIHAR), Leh, from the fruits of the plant *Hippophae rhamnoides* that grows wild in the mountains (Fig. 12). The lab has developed the technology for processing of fruits of sea buckthorn (*Hippophae rhamnoides*) with high retention of bioactive compounds. This tasty drink is rich in vitamin A, B1, B2, C, E and K, carotenoids, flavonoids and phytosterols. The drink is being supplied to the Army for the troops located in the Siachen area. The beverage is very popular amongst the troops not only because of its rich vitamin content but also because it does not freeze even at sub-zero temperatures and, furthermore, possesses antistress properties.

Fig. 12 Multivitamin sea buckthorn-based herbal beverage developed by DRDO has proved useful for the soldiers operating in the Siachen sector

DRDO Herbal Tea for Alertness and Efficiency

Oxidative stress has been implicated in the development of several degenerative diseases. Utilizing ethnobotanical leads from the locals residing in the high-altitude region, Defence Institute of High Altitude Research (DIHAR), Leh, has formulated an antioxidant-enriched herbal health tea based on indigenous high-altitude medicinal plants of the Ladakh Himalayas (Fig. 13).

The product is a combination of high-altitude herbal plants having a high antioxidant content. Sea buckthorn (*Hippophae rhamnoides* var. turkestanica), salam panja (*Dactylorhiza hatagirea*), local tea (*Bidens pilosa*), local caraway (*Carum carvi*), black caraway (*Bunium persicum*), oregano (*Origanum vulgare*), local mint (*Mentha longifolia*), yarrow (*Achillea* sp.), rose root (*Rhodiola* sp.), etc. are the key ingredients of this herbal tea formulation. The herbs used in making this herbal tea are widely used since ages in the Tibetan system of medicine (Amchi system) for the treatment of various ailments such as the body ache, cold, cough, fever, gastritis, headache, stress, high-altitude mountain sickness, high blood pressure, indigestion, memory loss and weakness, etc.

Studies in animal models have shown that the herbal tea reduces the onset of oxidative stress. The tea is rich in natural antioxidants like polyphenolic compounds, flavonoids, etc. The herbal tea is invigorating, stimulating and stress relieving, apart from being a thirst quencher.

Pain Management

Pain management often becomes an issue, especially in the cold and low barometric pressure conditions in high-altitude environment for the soldiers. A composite herbal formulation – Joint Care Gel meant for targeted topical application on joints to get relief from joint pain – has been developed by the Defence Institute of High Altitude Research,

Fig. 13 DRDO herbal tea for alertness and efficiency

Fig. 14 A herbal formulation for pain management especially in the cold and low barometric pressure conditions (high-altitude environment) for the soldiers

Leh, from essential oil extracted from leaf of winter green, eucalyptus and kernel of apricot mixed at standardized optimal ratio with *Aloe vera* gel base (Fig. 14).

The formulation is rich in methyl salicylate, oleic acid and cineole. The Joint Care Gel can be topically applied in area of pain, by gently rubbing it and covering the area with dry warm cloth for 15 min. It should not be applied on cuts, burns and wounds. The botanical ingredients include *Eucalyptus globulus labillardiere*, *Gaultheria fragrantissima* and *Prunus armeniaca*. The gel is reddish in colour and has a characteristic odour due to the presence of aromatic camphoraceous compounds and has sweet woody smell.

Management of Toothache

Toothache can be a problem anytime, especially when soldiers are on the move and have no access to modern medical care in the remote jungles, desert or mountainous regions. The nearest dental clinic may be several kilometres away. An anti-toothache formulation has been developed incorporating the ingredients of locally available Himalayan medicinal plants, which can come in handy during operations to alleviate

Fig. 15 Amtooth and its use for toothache management by soldiers

toothache in emergencies (Fig. 15). The formulation contains ethanol isolates of five plants and essential oil of two plants.

The product relieves pain within 2 min of application and reduces swelling of gums, and there is no burning sensation on gums. It is also effective in hot and cold sensation of gums (sensitive teeth).

Herbals for Low-Intensity Conflict (LIC) Operations and Crowd Management

Low-intensity conflict entails armed conflict between law enforcement agencies and non-regular armed militias, which could include terrorist groups, guerrilla fighters, rioters, etc. (Chadha 2005). The involvement of the local populace either overtly or covertly is a common feature of most low-intensity conflicts. The low-intensity conflicts usually involve contiguous regions/states and ultimately aim to destabilize and weaken the nation. India has witnessed several low-intensity conflicts of varying nature, degree and contexts, and these pose immense problems to law enforcement agencies in general and the military in particular for handling such issues with a sense of responsibility. Use of force under difficult inevitable circumstances often results in death or maiming of local populations leading to immense furore, e.g. the use of pellet guns to disperse mobs in North India resulted in serious eye injuries to several people. Subsequently, the Supreme Court of India ruled that being a welfare state, it is the duty of the government to ensure safety of its people as well as security forces. With a view to managing LIC operations less fatally, oleoresin-based products like Capsispray™/Capsigrenade™ – an eco-friendly, less-than-lethal weapon, based on oleoresin extract of chilli (Bhut Jolokia, *Capsicum assamicum*) developed by DRDO (Fig. 16) – can come very handy.

Capsi-based products are suitable for the law enforcement agencies, police and paramilitary forces for use in the LIC-affected areas, for riot control and for protection of vital installations from sabotage, crime prevention, flushing out hiding people as well as civilian applications like personal or area protection and self-defence. Capsi-based products can be used as a handheld spray or as a hand grenade or thrown through projectile/launchers as ball or shell or through water cannon as

Fig. 16 *Capsicum assamicum* plant (left) and fruits (right) – the source of oleoresin-based products like Capsispray™/Capsigrenade™ produced by DRDO

Fig. 17 OR-based grenade for crowd management

fine spray. They have no explosive fuse for burning chilli powder and thus do not need any approval from explosive agency.

One of the aims of the civil/military authorities during operations is to catch hold of the miscreants alive and get complete information about their ring leaders. Particularly when they take shelter in hideouts or in house/building, it is very difficult to take them out without collateral casualties. During counterinsurgency operations, security personnel have to disarm/incapacitate agitated individuals, disperse the crowd or capture militants held up in a bunker or building. The available methods in vogue are normally lethal in nature, e.g. small arms and grenades, which result in the loss of life/bodily injuries. There is a need for a non-lethal aerosol chemical-based weapon system, which is instantaneously effective and totally safe in controlling agitated and misguided individuals and can be used to incapacitate them leading to their capture.

In the Defence Research and Development Establishment (DRDE), Gwalior has developed a unique non-lethal munition, named as oleoresin-based grenades (Fig. 17). The product is totally safe, simple to operate, user-friendly and extremely useful for the above purpose. The body of the grenades is made of plastic, which melts when the grenade bursts thus making its throwing back difficult. The grenade

Fig. 18 Capsispray™ is a novel and eco-friendly less-than-lethal chilli spray derived from *Capsicum assamicum* (Bhut Jolokia) oleoresin. Capsispray™ is highly suitable for Indian troops for use in low-intensity conflict areas as well as for riot control, personal protection self-defence and crime prevention, etc.

can be thrown by hand up to 30 m to the hideouts/agitated mob. The grenades when applied emit tear gas for 60–80 s after a delay of 1–2 s, making the person come out due to lachrymation, irritation and suffocation. The product has been inducted into the services and is being used for the riot control agents by services, CAPFs, state police and other paramilitary forces. The product is useful for peacekeeping purposes and combating terrorism. It can be used to incapacitate terrorists and flushing them out from hideouts (tunnels, fields, dense jungles, broken grounds, etc.) without casualties. The grenade can be effectively used for controlling agitated and misguided individuals and can be used to incapacitate them within no time.

Capsispray™ is a unique, eco-friendly less-than-lethal chilli spray developed from Bhut Jolokia (*Capsicum assamicum*) (Fig. 18). It contains Bhut Jolokia oleoresin dissolved in a suitable carrier solvent and pressurized under propellant. Capsispray™ is available in container can (aluminium make) of length 11 cm and diameter 3.5 cm. The water capacity of the container can is 80 ml, net weight is 35 gm, and volume content is 55 ml net. The effective range is up to 20 ft. The spray end of the nozzle should face the desired area of contamination. The nozzle is pressed downwards to discharge spray. The ideal usage pattern is short bursts of 2–3 s to contaminate the area. It is advisable not to store the container above 50 °C temperatures and in areas prone to heating because excessive heating may cause this unit to burst. It causes coughing and skin and eye irritation to exposed person. It incapacitates the person for 20–45 min. Simple ‘lock-unlock’ mechanism is provided for using the product by any responsible person. The salient features of the product include the following: is an effective human/animal deterrent device, has diverse applications (use against individual or mob) and is useful in stationary and mobile systems; safety of usage has been established as the effects are reversible. The product is useful from personal security point of view, for self-defence, crime prevention, mob control, etc.

Ensuring Survival in Extreme Environments

Managing Frostbite Using Herbals

In snowbound areas of the high mountains, one of the major problems encountered is frostbite, which if not treated timely can lead to irreparable tissue damage leading to amputation of the affected parts. DRDO has attempted to develop appropriate prophylactics and curative measures to solve this problem often encountered by soldiers operating at high altitude. Based on extensive research, an *Aloe vera*-based cream has been developed by DRDO for the management of frostbite – the cream is non-greasy and does not cause any sweating or flaking and does not freeze even at low temperature (0 °C). Besides its application for management of cold injuries, this cream can be used for treating burns, wounds, ulcers, cracked and chapped skin and cuts and as an antiseptic dressing.

During trials in the Siachen glacier region, a combination of pentoxifylline, vitamin 'C' and low dose of aspirin along with ALOCAL (*Aloe vera* cream) application in soldiers showing early signs of cold injury was found effective in reducing morbidity (Fig. 19). Topical application of *Aloe vera* cream (50%) prophylactically along with tab aspirin 150 mg once daily, tab pentoxifylline 400 mg and tab vit C 500 mg three times daily and rewarming in decoction of tea leaves as therapy has been proved to be an effective remedy in preventing and ameliorating cold injuries in high altitude. The limbs of several soldiers were saved from amputation as a result of the use of this formulation in the cold, ruthless Siachen glacier sector.

Managing Difficult to Heal Wounds

Wound healing is classically divided into haemostasis, inflammation, proliferation, and remodelling. Abnormalities arising due to wound per se and during the wound repair process cause a great deal of physical and mental ordeal to patients. A unique herbal formulation, viz. Herbo Healer for management of normal as well as chronic, non-healing wounds, has been developed from a single constituent of herbal origin by the Defence Institute of Physiology and Allied Sciences (DIPAS) – a constituent laboratory of DRDO (Fig. 20). It promotes rapid and aesthetic healing, is rich in natural antioxidants and bioactive polyphenols/flavonoids and aids in healing of wounds by acting at cellular and molecular level. It has been proven to augment healing via wound contraction, re-epithelialization, cellular proliferation, collagen accumulation and neovascularization. Herbo Healer has antibacterial properties and has been shown to be way better than silver sulfadiazine and other iodine-based ointments. The safety and toxicological studies have proven it to be safe for dermal applications. ToT has been transferred to reputed firms.

Fig. 19 ALOCAL (an *Aloe vera*-based cream developed by DRDO) application in soldiers exhibiting early signs of cold injury has been found to be very effective in reducing morbidity in glacier region

Fig. 20 Herbo Healer is an effective wound healer and promotes scar-free healing

Prevention Against Snakebites

Snakebites are common in jungles and pose environmental hazard that is associated with significant morbidity and mortality, if the snake is poisonous and the individual is not timely treated with anti-snake venom (Singh et al. 2008). The soldiers have to operate in jungles, where wildlife is in plenty. For the military, it is definitely an occupational hazard. The best method to reduce human-snake encounters is to be cautious and avoid them; however, this is not always possible in snake-infested areas, and snake repellents can be quite useful in such scenarios. Murdock et al. (1990) and Krysa-clark et al. (2004) have highlighted the importance of prevention and emergency field management of venomous snakebites during military exercises.

Defence Research Laboratory (DRL), Tezpur, has developed a snake repellent formulation, which is effective against deadly poisonous snakes including spectacled cobra (*Naja naja*), banded krait (*Bungarus fasciatus*), monocled cobra (*Naja kaouthia*), saw-scaled viper (*Echis carinatus*) and Russell's viper (*Daboia russelii)* (Fig. 21). The formulation is eco-friendly; it does not harm the snake, it only repels the snake so that it goes away from the area where the formulation has been used. The formulation is available in liquid and granular form; it can be used in gardens and home also. The formulation is of herbal origin and has a shelf life of 1 year under any environment conditions. Ethnic herbs, mainly from eastern India, have been used to develop the formulation. The formulation is non-toxic, non-hazardous and non-mutagenic and contains no phenolic compounds. The formulation has been found to be effective in warding off snakes from Army units, installations and residential areas in the North East region.

Herbals for Alleviating Seasickness

Seasickness is a form of motion sickness; it is hardly fatal, but symptoms such as nausea, stomach cramps and vomiting caused can be quite debilitating, affecting performance of the seafarers. In critical military operations, seasickness can impede mission accomplishment. The Indian Navy had reported that the Somalian pirates use a plant called khat (*Catha edulis*) for alleviating symptoms of seasickness and improving performance. *Catha edulis* is native to the Horn of Africa and the Arabian

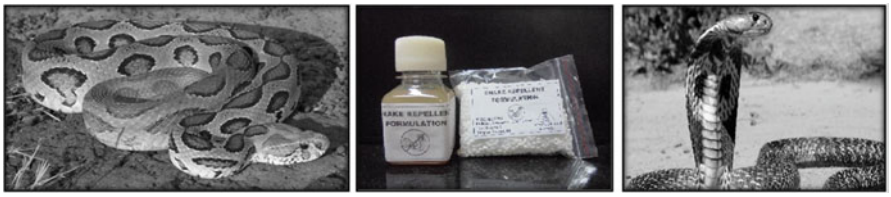

Fig. 21 The snake repellent formulation developed by the Defence Research Laboratory (DRL), Tezpur, is effective in warding off snakes from Army units, installations and residential areas of the North East

Peninsula, where khat chewing is a social custom dating back thousands of years. *Catha* (khat) contains a monoamine alkaloid – cathinone (an amphetamine-like stimulant), which causes excitement, appetite loss and euphoria. The World Health Organization (WHO) classified it as a drug of abuse in 1980 as it can induce psychological dependence. Based on the information provided, khat was evaluated for its ability to reduce conditioned taste aversion in rats (a model system for motion-induced nausea and vomiting) and compared to some Indian medicinal plants. IBG-15 was found to be more effective and safe in alleviating CTA as compared to *Catha edulis* (Fig. 22).

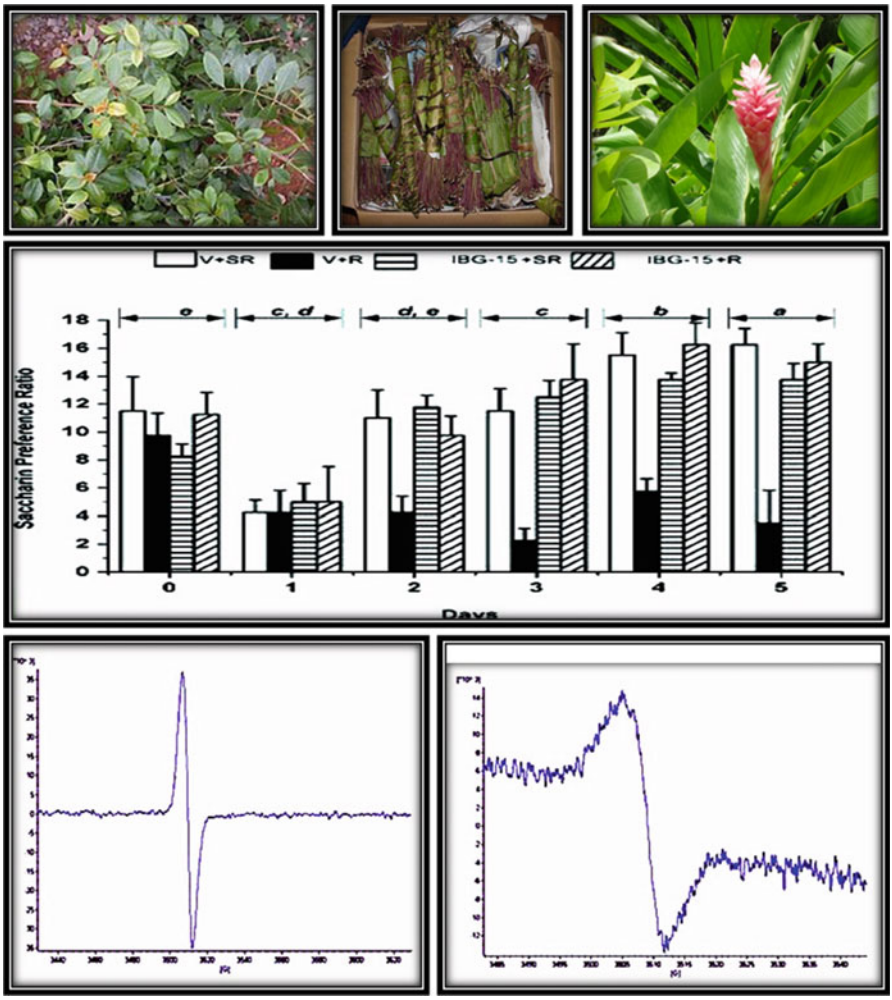

Fig. 22 Evaluation of some medicinal plants for alleviation of seasickness utilizing conditioned taste aversion (CTA) as a model system

Mitigating CBRN Hazards

One of the major threats faced by the Armed Forces is attack by CBRN agents. Very few medical countermeasures are available in the armamentarium of the services to deal with such hazards, which is life threatening in nature. DRDO labs are engaged in R&D on developing safe and effective CBRN countermeasures from herbals, and several leads have been obtained. Some such leads obtained vis-à-vis CBRN countermeasures are discussed in the ensuing sections.

Radiological/Nuclear Countermeasures

Attempts have been made by various researchers to classify radioprotectors into different categories. However, there is no universal classification that is unanimously acceptable. A radioprotector can be defined as a substance [element, chemical or a compound of synthetic or molecular nature or biological compound(s)] that reduces the deleterious effects of radiation, when administered to living organisms, usually prior to irradiation (prophylactic administration), while radiorecovery agents are those that help in recuperation and augment recovery once the living organism has been exposed to radiation. Most agents available today fall in the former category. Since the damage resulting due to ionizing radiation in most cases cannot be totally reversed, the currently available radiorecovery agents exhibit limited efficacy. There is immense interest now to develop both categories of agents so that radiation damage can be treated holistically.

Vasin 1999 of the Institute of Aviation and Space Medicine, Moscow, classified prophylactic antiradiation drugs into the following categories: (i) drugs having short-term and long-term action drugs, (ii) drugs that stimulate radioresistance, (iii) drugs that suppress symptoms of primary radiation reaction, (iv) drugs that detoxify early and (v) drugs that act by absorption or elimination of radionuclides from an organism. Nair et al. (2001) have classified radioprotective agents into three categories: (i) radioprotectors, (ii) adaptogens and (iii) absorbents. Radioprotectors include compounds like antioxidants and others that possess sulfhydryl groups. Adaptogens in general are non-toxic stimulators of radioresistance. Usually these are natural biological protectors that offer protection against low levels of ionizing radiation primarily by regulating immunity and modulating the endogenous antioxidants, thereby improving non-specific resistance in biological systems. The last category comprises of absorbents that protect against internal radiation injuries that result due to ingestion of radionuclides. Such agents prevent the incorporation and absorption of radionuclides like ^{90}Sr, ^{137}Cs, ^{239}Pu, ^{131}I, etc. A large number of plant-derived compounds are known to possess highly effective absorbent capability.

The High-Altitude Himalayan Plant Species as Radiation Countermeasure Agents (Fig. 23)

Fig. 23 The high-altitude medicinal plants from the Himalayan region were evaluated and have shown immense potential as radiation countermeasure agents

Podophyllum hexandrum Royle

Podophyllum hexandrum is a medicinal plant species of the Himalayas listed as an endangered species. It is the natural source of podophyllotoxin and other aryltetralin lignans and several other bioactive constituents. In modern systems of medicine, podophyllotoxin finds use as a precursor for the semi-synthetic anticancer drugs etoposide, teniposide and etopophos and other antiarthritic drugs. These drugs and other derivatives, based on podophyllotoxin, find use for the treatment of leukaemia, Kaposi's sarcoma, lung and testicular cancers, dermatological disorders like warts, rheumatoid arthritis, psoriasis, malaria, etc. In ancient Indian traditional system of medicine, *Ayurveda* plant has been mentioned as 'Aindri' – a divine drug in ancient literature (Singh and Shah 1994). *Podophyllum hexandrum* has been used as a cure for allergic and inflammatory conditions of the skin; biliary fever; burning sensation; cold; constipation; cancer of the brain, bladder and lung; erysipelas; Hodgkin's disease; insect bites; mental disorders; monocytoid leukaemia; non-Hodgkin's lymphoma; rheumatism; septic wounds plague; and venereal warts since ages (Chatterjee and Pakrashi 1996).

The radioprotective properties of *Podophyllum hexandrum* have been evaluated in detail at INMAS. The plant *Podophyllum* belongs to the family Berberidaceae and is commonly known as: Bankakri, Papra, Himalayan mayapple, Banwangam and Venival Patvel. *Podophyllum hexandrum* is found at altitudes above >2500 m, in the Himalayan ranges of India, including Sikkim, Jammu and Kashmir, Himachal Pradesh and Uttaranchal. Two other species, viz. *Podophyllum montanum* and *Podophyllum peltatum*, are found in Western China and North America, respectively. The plant contains picropodophyllotoxin, podophyllotoxin, epipodophyllotoxin, podophyllotoxin-β-D-

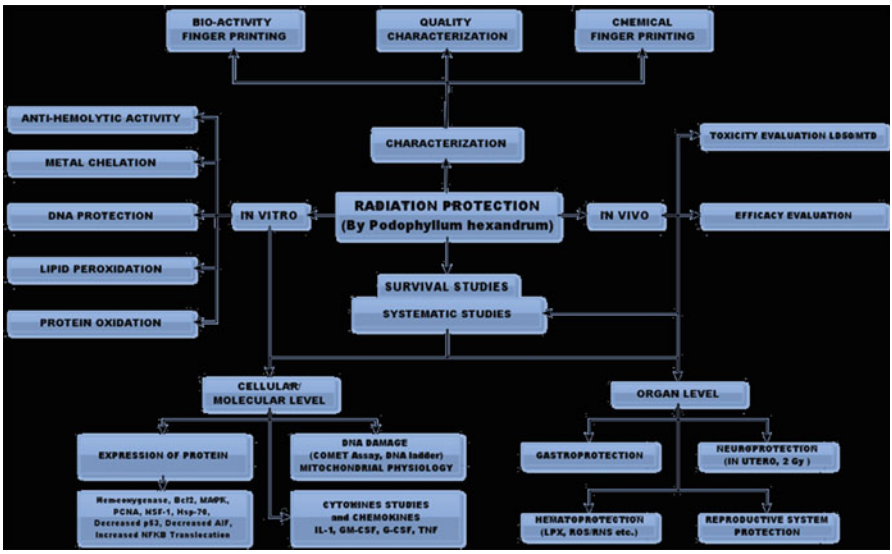

Fig. 24 The multifaceted mode of action of *P. hexandrum* has been elucidated via experimental studies

galactopyranoside, 4′-demethyl podophyllotoxin, quercetin-3-O-β-D-galactopyranoside, kaempferol-3-O-glucoside, deoxypodophyllotoxin and podophyllin.

Radiation is one of the most lethal sources that is capable of inducing severe state of oxidative stress in living systems causing mortality in mammals. In a 30-day survival study using strain 'A' Swiss Albino mice, our group showed that the crude extract of *Podophyllum hexandrum* possesses the ability to save more than 80% animals with administration of a single dose 2 h prior to 10 Gy exposure (Goel et al. 1998, 2000a, b; Arora et al. 2005d). Subsequent studies established that using *Podophyllum hexandrum* extract, a dose reduction factor (DRF; LD_{50}: treated group/untreated group) of 1.33 was achievable. In order to unravel the mode of action, the radiation-induced multi-organ dysfunction needs to be investigated at different levels of hierarchy of organization, and, therefore, our group investigated the organ-level protection rendered by *P. hexandrum* in greater detail (Fig. 24). *Podophyllum* has been shown to protect various organs and systems, including the haemopoietic system, gastrointestinal system, reproductive system and central nervous system (Arora et al. 2005a,b,c, 2006, 2007a,b, 2008a,b,c, 2009, 2010a,b,c,d,e, f,g, 2011a,b; Gupta et al. 2008), (Arora et al. 2010a,b,c,d,e,f,g; Singh et al. 2009a, b; Sagar et al. 2006; Chawla et al. 2005a, b, 2006; Gupta et al. 2003, 2004; Yashavarddhan et al. 2016).

Studies by our group have shown that one of the mechanisms by which *Podophyllum hexandrum* renders radioprotection is modulation of radiation-induced oxidative stress. Podophyllotoxin and other aryltetralin lignans in combination with rutin have been shown to play a crucial role in radioprotection. Whole-body radioprotection by *P. hexandrum* is now well established (Fig. 25).

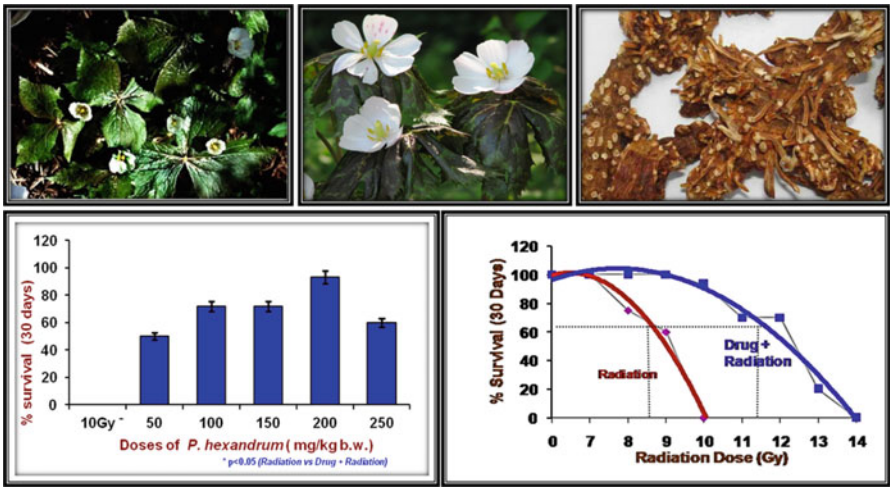

Fig. 25 Plants and rhizome of *Podophyllum hexandrum* (Himalayan mayapple) yield compounds that act as effective radiation countermeasures

Hippophae rhamnoides L.

Hippophae rhamnoides L. belongs to the family Elaeagnaceae and is commonly known as sea buckthorn or chuma, Ames, dhurchuk or milech and tarru. The plant is a spiny shrub or small tree, with a height between 2–4 m, and berries are fleshy and an important source of antioxidants. The plant is hard, drought and usually cold-tolerant plant, and shoots are modified into spines. The leavesare lanceolate – linear, obtuse and with peltate and stellate scales on lower surface. Male flowers are clustered at the base of shoot; petals free suborbicular, while female flowers are pedicellate inclusions (2–4), petals: 3–4 mm long, united utricles subglobose, succulent, red and orange coloured. Seeds are solitary, unequally bilobed, light, black and very hard. The plant is widely distributed throughout the temperate zones of Asia and Europe. Distribution ranges from the Himalayan region, India, Nepal, Bhutan, Pakistan, Afghanistan to China, Mongolia, Russia, Kazakhstan, Hungary, Romania, Switzerland, Germany, France and Britain to Scandinavian countries, including Finland, Sweden and Norway. In India, it is found in North West Himalayas.

The main chemical constituents include carotene and soluble sugars. The berries are rich in vitamin C content, and calcium, chromium, cobalt, iron, magnesium, manganese, molybdenum, potassium, sodium, strontium and zinc have been reported to be present. Malic and quinic acid are the major (>90%) organic acids present in fruit juice of species of different origin. The volatiles responsible for aroma include 3-methyl butanol, butyl pentanoate, 2-methyl propyl 3-methyl butanoate, ethyl 2-methyl butanoate and ethyl hexanoate. Quercetin, isorhamnetin, rhamnetin, steroids, terpenoids, alkaloids, fatty oil, etc. are also reported to be present. *Hippophae rhamnoides* has been widely used for the treatment of

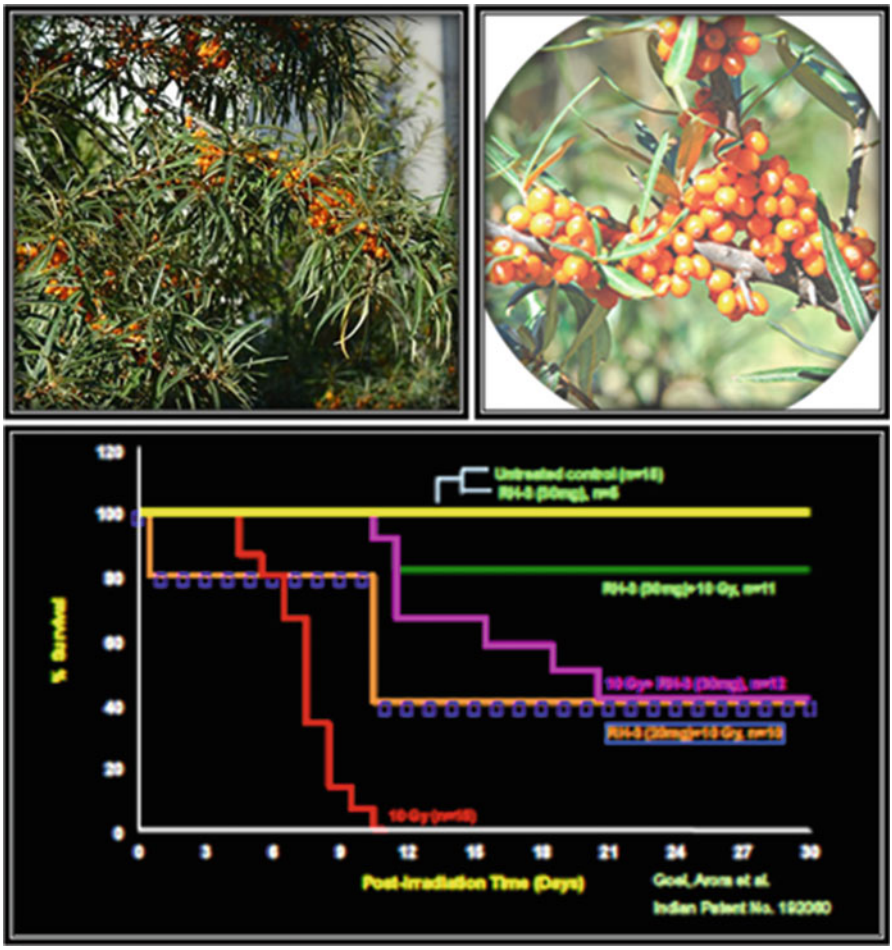

Fig. 26 *Hippophae rhamnoides* (sea buckthorn) – a promising radioprotector

circulatory disorders, vaginal mucositis, oral mucositis, wound healing, cutaneous eruptions, lung complaints, stomach malfunctioning, duodenal ulcers, cervical erosion, scalds, skin ulcers, hepatic injury, neoplasia and gastric cancers. It is also useful for treating skin ulcers, gastric disorders/cancers, mucositis, wounds and other high-altitude disorders. *Hippophae* has been shown to effectively protect mammals against lethal ionizing radiation and acts via multifarious ways, including free radical scavenging, immune modulation, boosting antioxidant defence, etc. *Hippophae* protects the haemopoietic system, gastrointestinal system and central nervous system against the deleterious effects of radiation and also mitigates gamma-radiation-induced genotoxicity (Swaroop et al. 2005, 2007; Chawla et al. 2007; Fig. 26). At molecular level, it has been shown that a large number of genes and proteins are modulated, thereby rendering radioprotection.

Rhodiola imbricata Edgew.

Rhodiola imbricata Edgew. (roseroot or arctic root or golden root or shrolo) belongs to the family Crassulaceae and is an important medicinal plant and food crop of the Indian trans-Himalayan cold desert. It is an erect, perennial herb with rose-scented massive rootstock and Fleshy, succulent stem. It has pale yellow flower which grows in clusters and is found at >4000 m in the high-altitude Himalayan ranges from Leh, Ladakh, India. It is also found in Pakistan and Nepal.

The biologically active phyto-constituents present in different *Rhodiola* species vary greatly according to the species. *Rhodiola* contains several bioactive compounds, e.g. phenolic glycosides, phenylpropanoids (rosavin, rosin, rosarin), flavonoids (rhodiolin, rhodionin, tricin, acetylrodalgin, catechins), proanthocyanidins (rhodioloside), tannins, phenylethanol derivatives (tyrosol), phenolic acids (chlorogenic, hydroxycinnamic, caffeic and gallic acid), monoterpenes (rosaridin, rosaridol), triterpenes (beta-sitosterol, daucosterol), etc. The plant has been in use in traditional systems of medicine since antiquity, where it has been used for treating a variety of ailments and also as a prophylactic and general tonic. The plant increases bodily endurance, work efficiency and longevity and is used to treat fatigue, asthma, fever, haemorrhage, depression, anaemia, impotence, gastrointestinal ailments, infections and central nervous system disorders. It is used for restoring memory and as a general health tonic and is also useful in cold, cough and lung ailments. In recent years, the plant has been used in Russia, Mongolia, China, India, America, Kazakhstan and European countries. The therapeutic uses include application in asthenic conditions like sleep disturbances, decline in work performance, loss of appetite, irritability and hypertension. It has been a widely accepted medicinal plant possessing antidepressant, tonifying, stimulative, DNA repair enhancing, anticancer, antimutagenic, reactive oxygen scavenging, adaptogenic, antiageing, antioxidant, anti-inflammatory, cardioprotective and central nervous system protective properties.

In a 30-day survival study, *Rhodiola imbricata* (Fig. 27) was found to be maximally effective at 400 mg/kg b.wt. administered 30 min prior to 10 Gy exposure in strain 'A' Swiss albino mice model system, 1000 mg/Kg b.wt. indicating the non-toxic behaviour of the extract (Arora et al. 2005a, 2008a, c, d; Chawla et al. 2010).

The reported therapeutic activities of *Rhodiola rosea*, a close species to *Rhodiola imbricata*, and the present study matched indicating that it is an excellent candidate for investigation of biological activity particularly related to radiation protection. Comparative analyses of the biological activities associated with the plant have also been performed. Simultaneously, screening of the novel compounds as well as the different reported categories of compounds from this plant species, presumably responsible for radioprotection, is also continuing. Studies have revealed the ability of extract to effectively tackle radiation-induced oxidative stress.

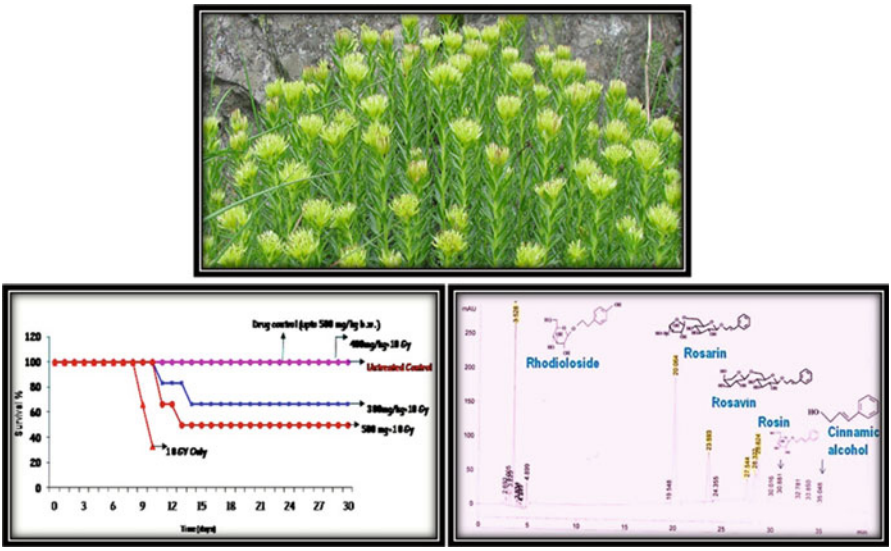

Fig. 27 Radioprotection by *Rhodiola imbricata* and identification of its key bioconstituents

Ocimum sanctum Linn. (Fig. 28)

The radioprotective properties of tulsi (*Ocimum sanctum*) have been demonstrated at INMAS and taken to phase II clinical trials at the Advanced Centre for Treatment Research and Education at the Tata Memorial Centre in Mumbai. Undoubtedly, the preliminary results have been promising; however, the limitation of using total body irradiation as a clinical model for testing of radiation countermeasures has to be kept in mind, and its extrapolation to normal individuals who might be exposed to radiation needs to be considered in an unbiased manner. The radioprotectors developed at INMAS can ameliorate the adverse effects of radiotherapy in cancer patients and have immense potential.

Behavioural Radioprotectors

From a military perspective, behavioural radioprotectors are of immense importance since they can help improve the performance of soldiers and emergency first responders who may have to operate during crucial rescue and recovery missions during radiological/nuclear incidents. They can also be used by Air Force fighter pilots and in a naval setting. At INMAS, we have investigated *Centella asiatica*, *Zingiber officinale* and *Mentha piperita* and found them to render efficient behavioural radioprotection in rodent model system if administered prior to radiation

Fig. 28 Radioprotector from *Ocimum sanctum*

exposure (Goel et al. 2006; Haksar et al. 2006, 2009). A fairly narrow window of protection currently makes them appear suitable only for planned radiation exposures. However, their efficacy in postirradiation scenario and validation in higher animal models remains to be done.

Biotechnological Production of Radioprotective Molecules

As alluded to earlier, *P. hexandrum* (Himalayan mayapple) is an invaluable source of podophyllotoxin, 4′-demethylpodophyllotoxin, podophyllotoxin glycoside and other polyphenolic compounds that find application as anticancer, antiviral, antibacterial, immunostimulating and antirheumatic drugs. The aryltetralin lignans synthesized by the taxon are in great demand worldwide due to their use in the synthesis of topoisomerase inhibitors and form an integral part of the modern chemotherapeutic regimen for the treatment of a variety of cancers. The radioprotective and anti-HIV properties are further likely to increase the demand for lignans and related molecules produced by this important plant species. In order to maintain continued production of these aryltetralin lignans, the plant has to be harvested on a large scale from its natural environment, and this has resulted in the plant's endangered status, which now finds mention in the Red Data Book. The chemical synthesis of podophyllotoxin is complicated, and in view of the difficulties in its total chemical synthesis (due to the presence of four chiral centres along with a γ-lactone and a high degree of oxygenation), problems in cultivation on large scale and failure of metabolic engineering approaches, there is a need for alternative sources of production of aryltetralin lignans. To circumvent the problem of production of radioprotective secondary metabolites, a novel endophytic fungus (*Trametes hirsuta*) was isolated from high-altitude *P. hexandrum* with the capability to synthesize podophyllotoxin and other lignans and possessing amenability for scale-up in bioreactors (Puri et al. 2006; Fig. 29). Methodology for consistent production of the aryltetralin lignans was established, and methods for characterization by HPLC, LC–MS, LC/MS–MS and 1H-NMR were developed and standardized. The lignans

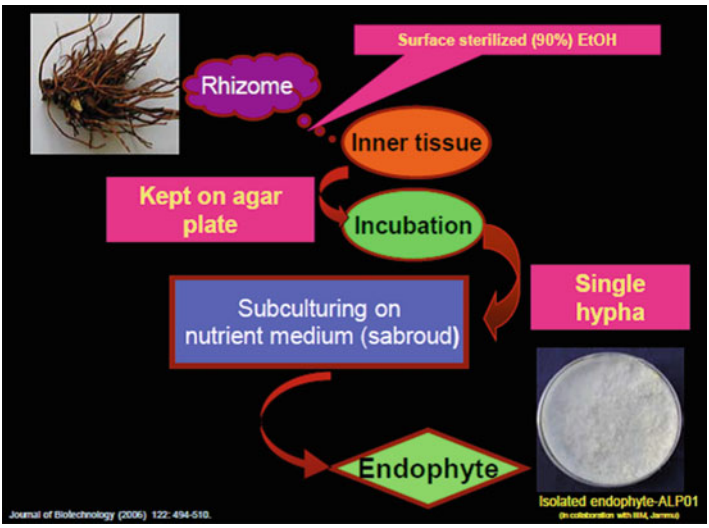

Fig. 29 Isolation of the endophytic fungus *Trametes hirsuta* from *Podophyllum hexandrum*

produced by the endophyte are biologically active and exhibited significant antioxidant, anticancer and radioprotective properties. This strategy promises to improve the production of these therapeutically important high-value bioactive radioprotective secondary metabolites at lower costs.

Biothreat Countermeasures

The rapid development of antimicrobial resistance amongst microbes poses serious public health concerns. As per the WHO, antibiotic resistance is a major threat to global health, food security and development in today's scenario. Though antibiotic resistance develops in microbes naturally, the extensive and indiscriminate use of antibiotics in humans and animals accelerates the process leading to the development of antibiotic resistance leading to plethora of microbes becoming drug-resistant posing grave concerns to humanity. Increasingly, diseases like pneumonia, tuberculosis and gonorrhoea are becoming harder to treat as the antibiotics used to treat them are becoming ineffective. Antibiotic resistance is fast becoming a major problem necessitating to longer stays in hospital and resulting in higher medical costs and increased mortality. Developing countries cannot afford the additional financial burden posed by this major problem. In addition, multidrug-resistant microbes can also pose biothreat concerns if used inappropriately by certain nefarious people to disrupt military as well as civil populations.

With antibiotic resistance becoming more prevalent and the consequent evolution of more virulent strains, there would be very few effective antimicrobial drugs available in our armamentarium to tackle antibiotic-resistant bacteria, and patients

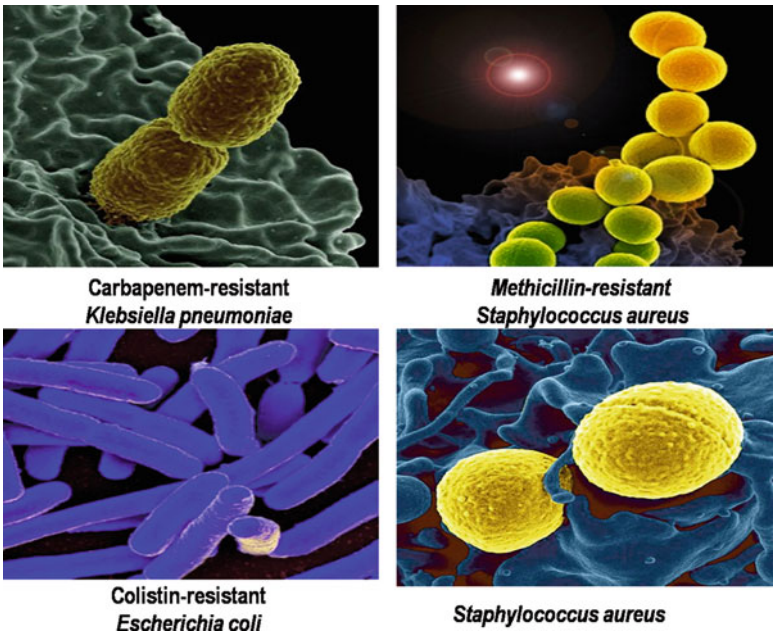

Fig. 30 The emergence of antibiotic resistance is a major challenge that needs to be tackled on priority basis (Photo Credit: National Institute of Allergy and Infectious Diseases, NIH)

might succumb to even simple infections as they would become untreatable. It is under such conditions that herbal medicine could be used as an alternative therapy for treatment.

Multiple drug resistance (MDR) refers to the resistance shown by a species of microorganism to multiple antimicrobial drugs. Several terms recognizing the varying degrees of MDR encountered have been introduced, e.g. drug resistant (XDR) and pandrug-resistant (PDR). A plethora of antibiotic-resistant bacteria have been reported, including community- and hospital-acquired methicillin-resistant *Staphylococcus aureus* (MRSA), vancomycin-intermediate *S. aureus* (VISA), vancomycin-resistant enterococci, macrolide- and penicillin-resistant *Streptococcus pneumoniae*, extend-spectrum β-lactamase (ESBL)-producing *Escherichia coli* and *Klebsiella pneumoniae*, carbapenem-resistant Enterobacteriaceae (CREs) and multidrug-resistant *Pseudomonas aeruginosa* and *Acinetobacter* spp. (Fig. 30).

The multidrug resistance offered by carbapenem-resistant *Escherichia coli* (CRE) (family: Enterobacteriaceae; class: Gammaproteobacteria) against third-line antibiotics can be attributed to its ability to develop biofilm(s). Such process involves adhesion and quorum-sensing induced colonization, leading to biomass development. Studies conducted at INMAS by our group have explored the anti-adhesion, anti-quorum sensing and anti-biofilm potential of 05 pre-standardized potent herbals

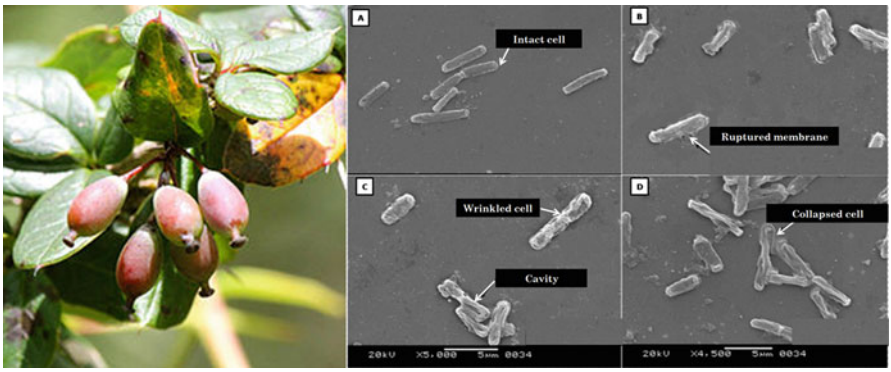

Fig. 31 Cell ultrastructure disruption as revealed by scanning electron microscopy upon treatment with *B. aristata*

(Thakur et al. 2016a,b). *Berberis aristata* (PTRC-2111-A) exhibited maximum potential vis-à-vis these activities, while *Holarrhena antidysenterica* (PTRC-8111-A) showed only anti-quorum sensing potential as compared to standards/antibiotics. These findings are in line with the molecular docking analysis of phytoligands against Lux S and Pilin receptors. Additionally, pairwise correlation analysis of the tested activities with qualitative, quantitative and bioactivity functional descriptors revealed that an increased content of alkaloid, moderate content of flavonoids and decreased content of tannins supported anti-adhesion, anti-quorum sensing and anti-biofilm potential activities. Besides, nitric oxide and superoxide scavenging activity correlated with anti-quorum sensing activity. The findings indicated clearly that *B. aristata* (family: Berberidaceae) and *C. sinensis* (family: Theaceae) are potent herbals with significant therapeutic potential for the management of CREs (Fig. 31).

Further we carried out studies using an aquo-ethanolic extract of *Camellia sinensis* (PTRC-31911-A), standardized using Fourier-transform infrared analysis, which revealed the presence of seven common functional groups (Thakur et al. 2016a,b). Combinations of PTRC-31911-A with third-line antibiotics ($n = 5$) were tested against carbapenem-resistant *Escherichia coli*. Combination modality revealed synergistic behaviour (fractional inhibitory concentration indices < 1) with tigecycline, ertapenem, meropenem, colistin and augmentin. The lead combination of PTRC-31911-A + ertapenem or meropenem showed maximum augmentative potential at a dose of 50 and 100 μg/mL, respectively, with nearly fivefold decrease in minimum inhibitory concentrations as compared with the respective antibiotics alone. The synergistic effects implied that the antibacterial combinations of PTRC-31911-A and ertapenem, meropenem, colistin, tigecycline or augmentin are more effective than monotherapy with either of these antibiotics.

Herbal Prophylactics and Therapeutic Agents: Preparing for Worst-Case Scenarios

Life-threatening opportunistic infections or biothreat agents can wreak havoc in individuals with inflammatory conditions if they are treated with available conventional therapies, and this has raised concerns in the management of national security (Tanwar et al. 2016). Highest priority opportunistic pathogens like *Salmonella species*, *Brucella abortus*, etc. pose a risk to national security as they can be easily disseminated or transmitted from person to person, which can further result in high mortality rates amongst the vulnerable populations. These pathogens in turn also play an important role in the initiation and perpetuation of such inflammatory conditions (e.g. rheumatoid arthritis and neurological disorders) in susceptible community. However, the use of biologic therapies (corticosteroids, NSAIDS, DMARDS) is also associated with increased risk of infections with intracellular microorganisms like *Salmonella* spp., *E. coli*, etc. (Thakur et al. 2016a,b and Tanwar et al. 2017a,b). Further, the combination therapy of corticosteroids and conventional drugs like anti-inflammatory drugs (naproxen, aspirin, diclofenac) etc. was shown to have antagonistic effects. Nowadays, the management of inflammatory conditions requires aggressive treatment with existing conventional drugs. Due to an increase in the unwanted effects of such therapeutic modalities, alternative therapies, particularly herbal, need to be explored as a curative remedy for the management of such vulnerable condition in terms of 'worst-case scenario' (Thakur et al. 2016a). With this aim in mind, endeavours were made to identify and develop natural remedies that could be employed in worst-case scenarios (mainly in susceptible population) to modulate the deleterious effects of natural disease outbreaks or deliberate use of biothreat agents. A herbal informatics evidence-based matrix modelling approach was envisaged, and this unique approach utilized the in silico bioprospection for identification of potent herbal leads. The model was developed using PubMed-based dynamic search engines as interactive platform. The priority index algorithms were used as a basis for evaluation of relevance factor of various physiological targets or bioactivity parameters specific to a particular microorganism(s) or inflammatory condition(s) in conferring antibiotic resistance over a period of time (Chakotiya et al. 2016; Tanwar et al. 2017a,b). Similarly, the potential herbals identified the basis of classical bioprospection were subjected to binary, weightage and fuzzy set-based analysis using assigned relevance to each activity parameter as dependent variable. The final optimized matrix provided several potential herbal leads. These identified leads were further carried forward for evaluation of their antimicrobial activity as well as anti-inflammatory potential against a novel standardized 'vulnerable animal model' (collagen type II-induced rheumatoid arthritis in combination with opportunistic infections involving multidrug resistant pathogens as worst-case scenario) (Tanwar et al. 2017a,b). The identified herbal leads (e.g. *Camellia sinensis*, *Berberis aristata*, *etc.*) could be utilized as novel holistic drugs in the future for the management of biothreats posed, particularly in vulnerable populations.

Broad-Spectrum Antiviral Drugs

There is a dearth of antiviral drugs in modern systems of medicine due to the rapid rate of mutation found in viruses. Viral pathogens pose a serious threat, especially the clinical viruses (HIV, hepatitis viruses, etc.), natural emerging viruses (avian and swine influenza strains, SARS, novel H1N1, H1N5, etc.) and viruses relevant to potential bioterrorism (Ebola, smallpox, etc.) (Rider et al. 2011). Unfortunately, there are relatively few prophylactics or therapeutics available for management of viral diseases and pandemics (Arora et al. 2010f, 2011). A plethora of medicinal plants exhibit promise for the treatment of viral infections, as several of them possess potent broad-spectrum antiviral activity (Arora et al. 2011, 2013). In the past, evaluation of antiviral activity of medicinal plants was constrained in view of the highly infectious nature of viruses and lack of suitable separation techniques for the identification of antiviral components. Development of vector-based strategies, in which non-infectious molecular clone of a virus is used for antiviral screening purposes and advancement in separation technologies, has offered promise for medicinal plants usage in modern antiviral drug discovery (Mukhtar et al. 2008). With increase in the virulence of viruses, emergence of new viral strains and non-availability of broad-spectrum synthetic drugs, there is a need to explore plants for the development of new and potent antiviral drugs.

DRDO has been engaged in the development of new candidate drugs for treating viral infections. *Hippophae rhamnoides* has been tested for its anti-dengue potential (Jain et al. 2008). Dengue virus occurs as four distinct serotypes, viz. dengue 1, 2, 3 and 4. Symptomatic dengue virus infection ranges from a self-limited febrile illness and dengue fever to a more severe disease, dengue haemorrhagic fever/dengue shock syndrome. Medical treatment of dengue poses serious issues as virtually no targeted therapeutic agents are available to treat the disease and consequently whatever treatment is given is mostly supportive in nature. Jain et al. (2008) evaluated the anti-dengue activity of *Hippophae rhamnoides* leaf extract in dengue virus type II infected blood-derived human macrophages. The dengue virus-infected cells were treated with *Hippophae rhamnoides* leaf extract and compared with the commercially available antiviral drug – ribavirin. The extract was able to maintain the cell viability of dengue-infected cells at par with ribavirin, along with the decrease and increase in TNF-α and IFN-γ, respectively. Anti-dengue activity of *Hippophae rhamnoides* extract was further determined by the traditional plaque assay. The results suggested that the *Hippophae rhamnoides* leaf extract has significant anti-dengue activity and has potential for the treatment of dengue.

Understanding and modulating the signal transduction pathways are imperative to the development of new therapeutic agents. It is well known that the recognition of virus infection by retinoic acid-inducible gene (RIG) I and melanoma differentiation-associated protein (MDA) 5, which are RNA helicases, and interferon-stimulated gene (ISG) 15 can activate a cascade of signal transduction pathways leading to production of type I interferons and proinflammatory cytokines that synchronize the removal of the virus from the host. However, it has been demonstrated that RNA-helicase-mediated innate immunity plays an important role in defending the host

from infection. During endeavours to identify plant-derived antivirals that could selectively enhance ISG- and RNA-helicase-mediated antiviral immune responses, *Rhodiola* was found to be effective. *Rhodiola* treatment significantly promoted ISG, RIG-I and MDA 5 gene expression and an antiviral immune response against dengue virus infection. *Rhodiola* treatment also induced interferon (IFN) β and other cytokines, including IL-1β, TNF-α, IL-6 and IL-8 in infected cells. *Rhodiola* also upregulated phosphorylated eIF-2α, PKR and NF-kB in infected cells. In addition, the number of NK cells was also increased upon treatment with *Rhodiola* in dengue virus-infected human PBMCs. Based on the studies, Diwaker et al. (2014) demonstrated that *Rhodiola* induces pharmacological modulation of RIG-I, MDA 5 and ISG signal transduction pathways supporting the induction of a favourable antiviral immune response against dengue virus and, can, therefore, be a novel therapeutic strategy for the management of dengue infection.

Chemical Warfare Countermeasures

Chemical warfare agents (CWAs) and toxic industrial chemicals (TICs) can pose problems during military conflicts and terrorism since most CWA and TIC exposures are difficult to manage as these agents have a rapid onset of action and can kill, injure or incapacitate human beings within no time. The mostly unprepared individuals get affected almost immediately; the first responders can get accidentally exposed, and the population in the vicinity can get affected rapidly. The specific mode of toxicity of these agents is varied; they are often mediated either directly or indirectly with increased oxidative stress in biological systems. Consequently, a plethora of antioxidants have been explored as potential medical countermeasures for CWA/TIC exposures. In DRDO, studies have been performed with a diverse array of CWAs, model organisms, exposure systems, target organs and antioxidants, looking at an almost equally assorted set of endpoints. Endeavours at treating CWAs/TICs with antioxidants have often met with mixed results, though exploration of antioxidants as medical countermeasures for CWA/TIC management has shown some potential. A plethora of herbal drugs have been utilized for their protective efficacy against chemical warfare agents, including extremely toxic sulphur mustard (SM). Ethanolic extract of *Hippophae rhamnoides* leaf (HL-EOH), water and ethanolic extract of *H. rhamnoides* fruit (HF-W and HF-EOH) and *H. rhamnoides* flavone from fruit (HR-flavone) were evaluated against percutaneously administered sulphur mustard (Vijayaraghavan et al. 2006). The *H. rhamnoides* extracts (1 g/kg; 3 doses; po) significantly protected SM-induced lethality. Following percutaneous administration of sulphur mustard, reduced glutathione and oxidized glutathione levels decreased, and malondialdehyde was elevated. Oral administration of HL-EOH and HR-flavone significantly protected

against loss of body weight. Following oral administration of ethanolic leaf extract and HR-flavone, recovery GSH, GSSG and MDA levels were recorded. The authors concluded that percutaneous administration of sulphur mustard induces oxidative stress, and ethanolic extract of leaf of *H. rhamnoides* and *H. rhamnoides* flavone from fruit can significantly protect against sulphur mustard-induced toxicity.

Prophylactic effect of gossypin (3,3′,4′,5,7,8-hexahydroxyflavone 8-glucoside) – an anti-inflammatory compound widely used as a herbal remedy for treating diabetes, jaundice, inflammation melanoma and glioma – has been demonstrated against percutaneously administered sulphur mustard in mice (Gautam and Vijayaraghavan 2007).

Various antioxidants like trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) and quercetin have been shown to protect the liver and lung tissues from oxidative damage caused by sulphur mustard exposure through inhalation and percutaneous routes (Kumar et al. 2001). This study showed that antioxidants could enhance survival time, protect the liver and lung from oxidative damage and reduce accumulation of purine metabolites in the blood following SM intoxication.

Conserving and Harnessing Natural Resources in Extreme Terrain

Defence Institute of High Altitude Research (DIHAR) has established a permafrost-based plant germplasm storage facility and modern laboratory at Chang La top (5360 m AMSL) in Ladakh exploiting the naturally existing sub-zero temperature in the region for most parts of the year (Fig. 32). The facility provides an insurance against both incremental and catastrophic loss of the plant diversity. The preserved diversity of the genes will serve as a safety net for current and future food security in an era of global warming and climate change. The facility is being utilized by DRDO and ICAR for storage of germplasm of economically important plant species for posterity. The facility will serve as a backup of important germplasm for posterity.

Fig. 32 Permafrost facility at Chang La with modern laboratories at a height of 17,600 ft AMSL

Improving Health and Well-Being of Armed Forces Personnel and Spin-Offs for Civil Applications

Vector Control

The Indian Armed Forces personnel are deployed at various locations and different geoclimatic locales. They are constantly exposed to a variety of bloodsucking insects such as mosquitoes, blackflies, ticks, mites, rat flea, sandflies and leeches during their operational postings. Insects are carriers (vectors) of dreaded diseases such as malaria, dengue, chikungunya, etc. Various methods have been used for the ecological condition with certain limitations. Application of insecticide residual spraying causes inconvenience to children and older persons and can cause toxicity. A better method would be to use personal protection measures to protect human from the painful bites of bloodsucking organisms such as mosquito, sandfly, blackfly, rat flea and bedbugs.

Amongst vectors, mosquitoes are responsible for causing major vector-borne diseases in India and other tropical countries. Use of repellents and insecticides is the best possible methods to keep mosquitoes at bay. Synthetic repellents used in commercially available mosquito kits are toxic to human health and detrimental to environment. A herbal mosquito repellent has been formulated using six essential oils of indigenous plant species which can be used with the help of vapourizing kit (Fig. 33). It is a formulation of essential oils of traditional herbs, is non-toxic as compared to the commercially available products, has natural fragrance and is eco-friendly. The herbal formulation wards off insects and, therefore, has immense potential for use in hot and humid areas, where vector-borne diseases are most prevalent.

The mosquito repellent formulation derived from local herbs has been value added with natural fragrance for using as mosquito-cum-room freshener (Aeromos). It has an extra advantage over conventional mosquito repellents available in the market due to additional natural fragrance. It can be used as mosquito repellent as well as a deodorant by spraying in the living rooms. It is primarily a formulation of

Fig. 33 Mosquit: an eco-friendly, herbal mosquito repellent (left) and Aeromos (right)

Fig. 34 Bio-larvicide for controlling mosquito larvae at the breeding sites

essential oils of local herbs and is effective for 3–4 h, non-toxic and eco-friendly, and the natural fragrance is acceptable to all age groups.

Control of mosquito-borne diseases at larval stage is said to be one of the best approaches to limit the control of diseases. With this aim, Defence Research Laboratory (DRL), Tezpur, has isolated a bioactive entomopathogenic bacterium, *Bacillus sphaericus* GC Sub Gr. IV, which is effective against mosquito larvae. The isolated bacterium has been formulated as a bio-larvicide for controlling mosquito larvae at the breeding sites (Fig. 34). The bio-larvicide is derived from nature and is eco-friendly, and the effective dose rate of the formulation is 0.1–0.2 kg/ha.

Herbal Adjuvant to Increase Vaccine Response

Adjuvants are substances that act to accelerate, prolong or enhance antigen-specific immune responses when used with specific vaccine antigens. A herbal adjuvant is an agent of natural origin that may stimulate the immune system and increase the response to a vaccine, without having any specific antigenic effect in itself. Adjuvants have been used for decades as important agents for generation of strong immune response to vaccine antigens. Besides generating strong immune response, they also act as immunomodulators by influencing the type and character of antibody generated. Defence Institute of Physiology and Allied Sciences (DIPAS) has developed a new herbal adjuvant, called DIP-HIP (Fig. 35), and compared its efficacy to standard adjuvants, viz. complete Freund's adjuvant and alum.

Antigen-specific immunoglobulin levels have shown to be significantly enhanced by DIP-HIP. Upon administration of DIP-HIP, cytokine profile correlates well with the Th1 and Th2 type of immune response. Immunization through different routes like intraperitoneal and intramuscular did not show any significant difference nor caused any muscular damage, granulomatous reaction or dystrophy. The shelf life of DIP-HIP is more than 3 years, whereas in the formulation with antigen, it is about 4 months at 40 ° C. No haemolytic activity is observed on the treatment of both human and animal erythrocytes with DIP-HIP. Incorporation of DIP-HIP results in

Fig. 35 A novel herbal adjuvant for increasing vaccine response

enhancing, accelerating and prolonging the antigen specific-antibody responses in animals. Further advantages of DIP-HIP include enhancement of immunogenicity, reduction of antigen amount needed for a successful immunization and reduction of frequency of booster immunization, without any muscular damage or side effects.

Herbal Hypolipidemic

A herbal hypolipidemic DIP-LIP has been obtained from a high-altitude growing plant. It is rich in essential fatty acids like linoleic acid (26%), linolenic acid (20%) and other important fatty acids such as oleic (30%) and palmitic acid (17%). The presence of these fatty acids and total carotenoids (430 ppm) and tocopherol (1175 ppm) imparts potent bioactivities. DIP-LIP has significant hypocholesterolemic activity and inhibits cholesterol deposition in cholesterol-fed animals, besides increasing HDL cholesterol levels. DIP-LIP also exhibits significant vasorelaxant activity in aortic ring model. DIP-LIP also increases resistance to hypoxia and cold stress. DIP-LIP can be widely used for atherosclerotic cases and for its hypolipidemic and vasorelaxant properties.

Anti-vitiligo Herbal Ointment and Oral Liquid

Vitiligo or leucoderma is a chronic auto-immune dermal disease with virtually no reasonably effective treatment available in modern system of medicine. Frequently even systemically administered steroids are not effective. It is a social stigma and is caused mainly due to decreased synthesis of melanin pigment by the skin resulting in white patches and discolouration of the affected areas. The current treatment

modalities have not been able to provide any satisfactory solution. Defence Institute of Bioenergy Research (DIBER) has developed a polyherbal topical cream and oral mixture of seven herbs (lukoskin) for curing vitiligo without side effects upon long-term application (Fig. 36). The combination therapy was found to inhibit reoccurrence of white patches besides preventing blister formation and has no side effects. Lukoskin enhances melanin synthesis, is a non-toxic herbal product and prevents hyper pigmentation and restores normal skin texture.

Herbal Anti-Eczema Ointment

Eczema or atopic dermatitis is an eruption of the skin characterized by pinhead-sized maculae, papules and vesicles accompanied by intolerable itching. The vesicles and papules exude yellowish material forming crusts which on scratching can bleed and secondary infection may set in. There is a seasonal relapse, and also all the clinical features need separate drugs. A polyherbal ointment (Eczit; Fig. 37) has been developed and found to address all clinical features of eczema, including psoriasis. Topical application of this preparation is effective in all kinds of eczema and devoid of any side effects. The formulation is low cost and broad spectrum and is a non-toxic

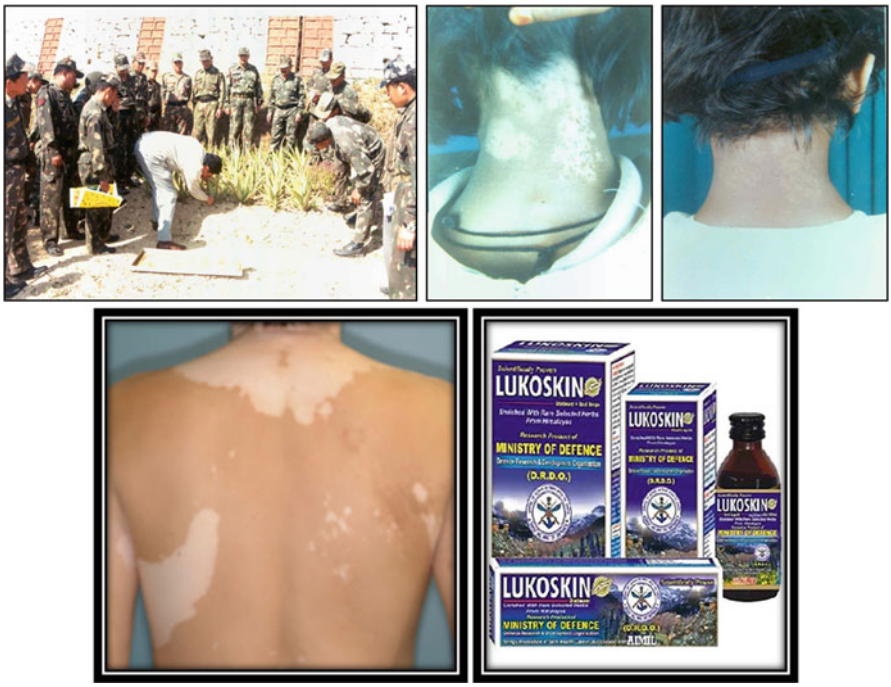

Fig. 36 Anti-vitiligo herbal formulation – lukoskin

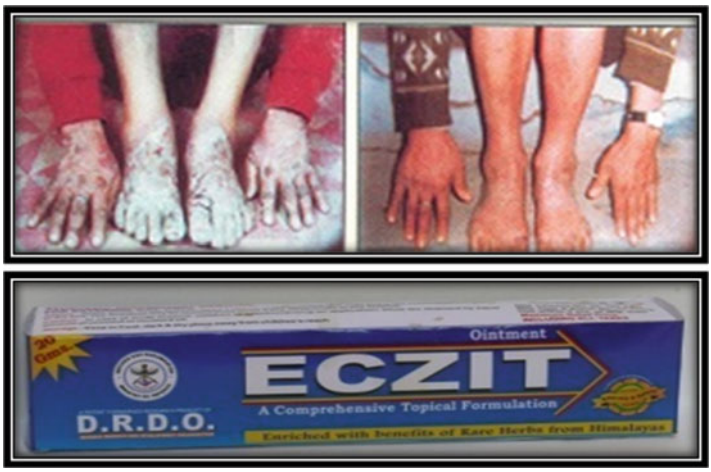

Fig. 37 Eczit (herbal anti-eczema ointment)

polyherbal ointment. The product offers better efficacy than the product in the market, is effective in all kinds of eczema, stops reoccurrence and has no side effects.

Antioxidant Beverages and Supplements

Antioxidant beverages and supplements have been developed utilizing the medicinal plants grown in the Himalayan region. Seapricot is cloud-stable blended herbal beverage based on the pulp of sea buckthorn (*Hippophae rhamnoides)* and apricot (*Prunus armeniaca)*. The blended herbal beverage is rich in vitamins, unsaturated fatty acids, carbohydrate, proteins, minerals, etc., which is the synergistic effect of both the fruits, having antioxidant, nutraceutical and health refreshing properties. The blended herbal beverage – seapricot – does not contain any hydrocolloids and artificial colour or flavour. The beverage has antioxidant properties and is rich in vit A, vit B_1, vit B_2, vit B_3, vit B_6, vit B_9 vit B_{12}', vit 'C', vit 'E' and unsaturated fatty acids (Fig. 38).

An 'antioxidant herbal nutraceutical supplement' based on sea buckthorn and other fruit pulp and extracts of high-altitude medicinal plants has been developed. The detailed nutritional profiling of the herbal antioxidant supplement has been conducted and found to be rich in various vitamins, unsaturated fatty acids, etc. Acute and subacute oral toxicity studies on the product were carried out, and no toxicity was found. The heavy metals, viz. arsenic, cadmium, mercury and lead, were found below detection level. The main ingredients are *Hippophae* sp., *Emblica* sp., *Rhodiola* sp., *Origanum* sp., *Capparis* sp., *Achillea* sp., *Rubia* sp., *Prunus* sp., etc. The supplement developed is rich in vit 'C' (124 mg/100gm), vit 'A' (121 IU/100 ml), vit 'B' complex, vit 'E', minerals, unsaturated fatty acids, minerals, etc.

Fig. 38 Seapricot beverage, herbal antioxidant supplement and antihypertensive beverage – Hridayamrith

An antihypertensive beverage – Hridayamrith – has been developed from Himalayan shrub, viz. *Crataegus crenulata*. Hridayamrith is a herbal cardiac tonic and is useful in case of hypertension, angina, arrhythmia and congestive heart failure. It is an energizer, nerve soothing and refresher health tonic. The presence of flavonoids in this beverage helps in cardiotropic and vasodilator action. The regular intake of drink lowers the serum cholesterol and triglycerides.

Challenges and Issues Ahead

For several terrain, operation and platform-specific problems faced by our Armed Forces, there are no effective solutions available. DRDO has been making concerted efforts to consolidate and streamline research and development endeavours on herbal products to provide unique solutions to the Armed Forces. In the process, at times some spin-offs emanate, which find practical application in both military and civil setting. With a view to meeting emerging challenges, the traditional knowledge on herbs of high altitudes, desert areas, Central Himalayan and North East region, etc. is being documented, and research carried out to generate scientific data that can be useful for the Armed Forces. There is a paradigm shift towards acceptance of scientifically validated herbals as effective prophylactic and therapeutic agents. The grant of the 2015 Nobel Prize in Physiology or Medicine to discoveries concerning novel therapies for some of the most devastating parasitic diseases, viz. river blindness, lymphatic filariasis (elephantiasis) and malaria, has once again highlighted the importance that herbal drugs are receiving in today's world. Drugs derived from *Streptomyces avermitilis* and *Artemisia annua* were found to be effective against parasitic diseases. However, it is a paradox that in our country, where Ayurveda has been a very successful and effective system of medicine for over 5000 years, there is still some amount of scepticism among civil health experts when it comes to acceptance of herbal drugs, and services are no exception.. This

view has to change in coming times if the full benefit of scientifically validated herbal drugs has to reach the users. The realization that herbal drugs are effective and safe and can solve the emerging problems of our Armed Forces will lead to induction of more and more products to the advantage of our soldiers who operate in harsh and extreme terrain and inhospitable operational conditions. As alluded to earlier, serious endeavours are underway in DRDO towards protecting the warfighter and promoting performance through the development of scientifically validated herbal products, processes and technologies. The need of the hour is achieving greater synergy with the services and industrial partners so that the herbal products, technologies and complementary and alternative medicine can be inducted in a rapid manner for the benefit of the soldiers.

Disclaimer

The views and opinions expressed in this article are entirely those of the author and do not necessarily reflect the official policy or position of DRDO/Government of India. The article contains only unclassified information, and the pictures have been used for representative purposes only. No endorsement of any private company or enterprise is made, as ToT in DRDO is done on a non-exclusive basis. Any herbal technology that has been left out is purely unintentional and is mainly due to space and other constraints.

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Herbals for Animal Health and Better Production

Anup Kalra, K. Ravikanth, and M. J. Saxena

Background

India has a rich and diversified flora. Ayurvedic medicines made for livestock are generally considered safe, environment-friendly and cost-effective. On the contrary, synthetic medicines are often costly and may have side effects. Moreover, the people have used them for generations. It is estimated that close to one fourth of the prescribed medicines in the world come from plants. Medicinal plants grow naturally in different states of India and are known to cure various livestock ailments pertaining to liver, reproductive, digestive, respiratory, foot and mouth, skin diseases, etc. This chapter deals with important medicinal plant species that are useful for animal health.

Introduction

Herbals are an integral part of most of the medical therapies mentioned in Ayurveda (Ayur, Life; Veda, Knowledge, in Sanskrit), the science of life. "Restoring health by harmony between various body systems and the environment" is the basic principle of Ayurveda. Recently herbals as part of the Ayurvedic system have been documented and practised on animals not only to treat them but also to improve their productivity. Indian ancient scriptures, called the Vedas and Samhitas, contain the basic and fundamental information of Ayurveda. The Rig Veda contains a number of prescriptions for human health and ailments. Nakula Samhita likewise contains the repository of knowledge for health care of animals.

Scientific and technological advances in the field of diagnostics, material analysis and instrumentation and introduction of latest biological screening models have

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revived the interest of health-care practitioners in exploring the virtue(s) of herbals. The development of resistance by pathogens and parasites against the chemicals developed in the last century coupled with the ever-growing concern for toxicity and damage to the environment heightened the interest in herbal alternatives. It is well documented that many in the world rely on the traditional systems of medicines, largely plant based, to address their primary health-care needs.

The traditional medicine (herbal medicine) is actually the integration of knowledge, skills and practices based on the theories, beliefs and experiences indigenous to different cultures, used for maintaining health and preventing diseases.

Herbal medicines based on the principle of Ayurveda are widely used in India, particularly in rural areas, where 70% of the country's population lives. Veterinary herbal medicines comprise plant-based medicines and their therapeutic, prophylactic application in animal health care. In the rural areas of India, the veterinary medicines cover knowledge, skills, methods, practices and beliefs of the smallholders about caring for their livestock.

Building Confidence and Conviction

In the last several years, the science of herbalism based on Ayurveda has become popular in India and abroad. In fact it has gained more acceptance in the developed world. This traditional knowledge-based system suffers with scepticism. Practising scholars who rely on Ayurvedic principles believe that there is no need for the validation of Ayurvedic knowledge as they feel that its usefulness can always be supported on the basis of its traditional use. On the flipside, the other stream staunchly advocates the scientific basis of its usage and its clinical validation, in a language that is understood by today's world with the scientific community. Application of newer methods of research in the treatment practices and manufacturing methods of Ayurveda is inevitable for the transition of the ancient Indian health-care system as the existing environment is always subject to changes. The journey from the mythological roots to the present-day scientific validation process shows the periodical growth of Ayurveda.

Veterinary Ayurveda Tradition in India

Shalihotras Ashwashastra (1800 BC) is considered as the first work on veterinary science. Hastyayurveda (1000 BC) by Palakapya is the most ancient text on elephants. *Pashuvaidyamattuvagadam*, a Tamil book based on an ancient Tamil palm leaf manuscript, discusses over 250 diseases in cattle and their management. Matsya Purana, Garuda Purana, Agni Purana, Brahmananda Purana and Linga Purana have veterinary information. *Arthashastra* by Kautilya describes cattle, buffalo, goat, horse, elephant and other animal rearing. It also gives the detailed account of welfare practices of livestock and regulations for the protection of wildlife. The documented ancient veterinary knowledge is available in the form of

texts and old manuscripts for health management of cattle, horses, birds and elephants. The non-codified or oral tradition also exists in India, in the form of local health traditions which are practised by the local Vaidyas. There are local healers (Pashuvaidyas) who are knowledgeable and experienced in traditional veterinary health care spread all over the country. The folk health practices are largely undocumented and are passed on from one generation to the other by word of mouth. They mostly use the locally available medicinal plants for improving animal health or for curing disease.

Veterinary Medicine in Ancient India

During Mahabharata period (1000 BC), Nakula and Sahadeva, the two Pandava brothers, were experts on horse and cattle husbandry. Graeco-Romans imported livestock from India after the invasion by Alexander the Great. These descriptions are available in *Indika*, a book authored by Megasthenes, the ambassador of Seleucus Nicator, king of Macedonia, in the court of Chandragupta Maurya. King Ashoka (300 BC) erected the first known veterinary hospital of the world.

Gaja Ayurveda Depiction of Elephant Medicine

The Gautam Samhita, Ashwa Ayurveda and Hastya Ayurveda are the only treatises on animal science till now. Palakapya, an authority on elephant medicine, belonged to the Rig Vedic period and wrote Hastya Ayurveda or Gaja Ayurveda that dealt with elephant medicine and was dedicated to Lord Ganesha. Medicines for elephants surgery were divided into four parts, viz. Maha Rogsthan or major diseases, Ksudra Rogasthan or minor diseases, Salyasthan or surgery and materia medica or diet and hygiene. Hastya Ayurveda also mentions about anatomy of elephant, treatment of different kinds of diseases, training of elephant and also classification of elephants on the basis of a number of characteristics.

Equine Medicine or Haya Ayurveda

Salihotra is known as a specialist in the treatment of horses. He composed a treatise called Haya Ayurveda or Turan-gama-sastra or Salihotra Samhita, a work on the care and treatment of the horses. Haya Ayurveda is said to have been revealed to Salihotra by Brahma himself, the fountainhead of all knowledge. Two other works, namely Asvaprasnsa and Asvalaksana Sastram, are also attributed to Salihotra.

Veterinary medicine is theoretically divisible into eight branches, corresponding to the eight divisions set out in the Ayurveda – general surgery, general therapeutics, ophthalmology and otorhinolaryngology, care of foals (corresponding to Ayurvedic paediatrics), toxicology, fortifying treatments, demonology and the use of aphrodisiacs.

Cattle Management

During the Rig Vedic period, the management of cattle was well developed and documented. The cow (Kamadhenu) was worshipped and was considered as wealth of mankind. The possible reason could be owing to milk for food security and human health. Thus, our ancestors laid great emphasis on the protection of cows. References about grazing of livestock, provision of succulent green fodder and water to drink from clean ponds, etc. can also be traced in Rig Veda.

In Krishi-Parashara (c. 400 BC), it shares the importance of cleanliness of the shed and protection of animals from diseases. It also contains the information regarding fumigation of cattle sheds with dried plant products with volatile compounds.

In Arthashastra, the ancient text clearly defined the presence of a government officer called the superintendent of cattle whose exclusive duty was to supervise livestock in the country, keep a census of livestock and see to it that they were properly reared. It also goes to explain that the abundant fodder/balanced ration and water be made available for cows and bulls. It also describes that maintenance of pastures around villages was encouraged. The surplus greens were processed to silage (an old process in the Indian subcontinent as the word suyavasa in the Rig Veda).The ancient text also explains and shares the breed preservation of the cattle in the country with its benefits.

A lot of ancient texts are available on cows and their housing and management. "Gau Ayurveda" discusses them. Shala Nirman and Goshth Suktas of Atharva Veda describe that the animal houses (Goshth) and their management were of good quality. Pashu Samvardhan Sukta of Atharva Veda indicates that Brihaspati Deva knew the animal behaviour and management well. Treatment of weak, infertile and unproductive cows for making them productive was well described.

Sustainability in Traditional Medicine

In the recent past, much emphasis has been given on natural therapies owing to the fact of limitation of modern drugs in many conditions, increasing side effects, multiple drug resistance and presence of antibiotic residues in the food chain and ever-escalating cost of the modern or chemical therapeutics. FAO reports that lack of drugs to treat diseases and infections results into losses of 30 to 35% in the breeding of livestock in many developing countries, where poor animal health remains the major constraint to breeding. Traditional medical practices have presented themselves as a dependable alternative. In recent years uses of alternative medicines have increased significantly around the world. Herbs are more compatible with the body and have less side effects and help in improving the immunity and health of the livestock and humans alike.

Let thy food be thy medicine was the mantra given by our ancestors; possibly it has linkages and documentation with our Vedas.

Indian Animal Health Industry

The Indian animal health industry has played a vital role in safeguarding the animal husbandry interests of the nation. The Indian animal health-care (AH) market is estimated to be around 42,000 million INR (2014) and is projected to be around 60,000 million by 2018. The species share in AH market is 50% for livestock, 40% for poultry, 5% for companion animals and the rest which is 5% for other remaining animals. Though there are no published data, INFAH anticipates the contribution of various categories of animal health products as 40% for feed supplements, 17% for antibacterials, 15% for biosecurity, 13% for antiparasitics, 5% for hormones and biologicals and 10% for other categories. Although there are nearly 50 major companies operating in the animal health market in India including pharma and Ayurvedic, the market is dominated by a few players in herbals like Ayurvet Limited, Natural Remedies, Indian Herbs, Himalaya Animal Health, Natural Herbs and Cattle Remedies.

The regulation of animal health products in India is under the Department of Animal Husbandry which is under the Ministry of Agriculture (MOA). Ministry of AYUSH is responsible for bringing regulations for herbal and contemporary medicines meant for animal use.

Animal husbandry in India has undergone magnificent changes over the years, thanks to the adoption of innovative technologies used for prevention and cure of farm and companion animals. There has been a paradigm shift in the business approach of animal health companies that have evolved from therapeutics to preventive to productivity enhancement and now to the overall health care of the animals.

Through new approaches and paradigm, the animal health industry has evolved and propelled the animal husbandry to new heights of glory. India's surge to the top of milk and egg production reinforces the significance of animal health industry. The animal health industry is working together and strengthening animal husbandry in the country.

Challenges of Veterinary Medicine

The high treatment cost, inaccessibility and indiscriminate use of antibiotics and hormone, which leads to user-unfriendly effects such as high antibiotic and hormone residues in the milk and other animal products, are serious limitations of modern veterinary management. Veterinary services have a crucial role in controlling highly contagious diseases and zoonotic infections, which have implications for human health as well as that of livestock. The presence of drug residues in milk, meat and eggs can cause allergies, anaphylactic shocks and toxicity in consumers. It also results in the development of drug-resistant microorganisms that are difficult to treat. Bacterial resistance to antibiotics is much more a threat to animal and human health than the low levels of residues, which may be found in animal foods. The drug-resistant pathogens like salmonella occurring in animal foods may be transferred to

and be pathogenic in man and can cause illness and death. All the antimicrobial drugs administered to cows can enter the milk to some degree. A drug administered to a milk-producing animal has a withdrawal period, during which the drug residue should fall below a predetermined level. A residue can be the drug itself or its metabolites. The testing of residue is of significance for ethical, public health, dairy, technological and environmental reasons.

Herbal formulations have reached extensive acceptability as therapeutic agents for control of several diseases. Therapeutic activity of herbal formulation depends on its constituent herbs and their phytochemical constituents. The development of authentic analytical methods which can reliably profile the phytochemical composition, including quantitative testing of analytical marker/bioactive compounds and other major constituents, is a major challenge.

Matter of Concern

The use of antibiotics and other chemical products has in fact been banned for animal health care in many countries. The world is looking for safer herbal alternatives. Thus, herbal medicine science of India has great potential to address current challenges faced by veterinary medicine as it has decentralized local resource-based applications which are safe and efficacious and create no adverse effects in the animals. However, they are facing the threat of rapid erosion.

The urgent revival of these traditional veterinary practices is a high priority in the light of the constraints of modern medicine and the benefits of these practices. So we need to have a high-level gathering to review this traditional medicine in animal health management.

Plants for Cattle Health: Ethnoveterinary Herbs in Veterinary Health Care

A lot of research work had proven that many medicinal plants are useful in the treatment of animals. Some of them are listed below as per their medicinal usage for addressing particular disease.

1. **Khadira**

Ayurveda uses the dried pieces of heartwood of *Acacia catechu*, a moderate-sized tree, found mostly in dry parts of India. It is a household remedy for weight loss.

Names:
Latin: *Acacia catechu*
Sanskrit: Khair
English: Black catechu
Parts used: Heartwood, bark, gum

Activity: Astringent, anthelminthic, antiseptic, antidysenteric and anti-inflammatory and useful in diarrhoea, anorexia, skin diseases and sore throats. Used extensively in Ayurvedic formulations.

Chemical constituents: Catechin, epicatechin, quercetin, isorhamnetin, phlobatannins and others.

Classical uses: The concentrated extract, known as Khair gum or "cutch", is cooling and digestive and beneficial for coughs and diarrhoea. It is applied externally to ulcers, boils and skin eruptions. The bark, combined with other drugs, is prescribed for snake bite.

2. **Lasunah**

The *garlic* plant's bulb is the most commonly used part of the plant. With the exception of the single-clove types, *garlic* bulbs are normally divided into numerous fleshy sections called cloves. *Garlic* cloves are used for consumption (raw or cooked) or for medicinal purposes.

Names:
Latin: *Allium sativum*
Sanskrit: Lasunah, yavanesta
Hindi: Lahasun
English: Garlic
Parts used: Cloves
Activity: Antibacterial, antiviral, carminative, expectorant, stimulant tonic, etc.
Chemical uses: The oil of *Allium sativum* is used for skin rashes, as ear drops and in dyspepsia, flatulence and colic. It is used for reducing lipids and to relieve joint pains. The juice is used for epilepsy.

3. **Nimbah**

Azadirachta indica is a moderate-sized to fairly large evergreen tree, attaining a height of 12–15 m, with a stout trunk and spreading branches, occurring throughout India up to an altitude of 900 m. Long before camphor balls, neem leaves were used as a pesticide to prevent insects from destroying our winter woollies. Neem twigs are still used as toothbrushes throughout India.

Names:
Latin: *Azadirachta indica*
Sanskrit: Nimbah, prabhadrah
Hindi: Nim, nimb, neem
English: Margosa tree
Parts used: Stem bark, fruit, leaf

Activity: Astringent, antiseptic, anthelmintic, demulcent, expectorant, insecticidal and liver tonic and useful in skin disease, intermittent and malarial fever, tuberculosis, diabetes, inflammation, etc.

Chemical constituents: Nimbin, nimbidin, azadirachtin, margosian, rutin, catechin, epicatechin, etc.

Classical uses: Neem has been extensively used in Ayurveda, Unani and homoeopathic medicines and has become a cynosure of modern medicine. Neem oil, its bark and leaf extracts have been therapeutically used in old medicines to control leprosy, intestinal worms, respiratory disorders, constipation and skin diseases and as a general health promoter.

4. **Daruharidra**

Berberis aristata is an erect, spinous and deciduous shrub, usually 1.8–3.6 m in height, found in the Himalayan ranges at an altitude between 1000–3000 m and in the Nilgiri Hills in South India. This one can help you to be slim and keep your bowels healthy. Recently it has been used to replace metformin, the common drug for diabetes.

Names:

Latin: *Berberis aristata*

Sanskrit: Daruharidra, darvi, katamkateri

Hindi: Rasaut

English: Indian barberry

Parts used: Stems, roots

Activity: Antiprotozoal, anti-amoebic, antidiarrhoeal, antipyretic, antiseptic and stomachic and useful in diarrhoea and obesity.

Chemical constituents: Berberine, berbamine, oxyacanthine, pendulin and aromoline.

Classical uses: *Berberis aristata* is used in diarrhoea; dysentery; diseases of the eyes, ears and oral cavity; and itching, wounds and skin diseases.

5. **Purnarnava**

Boerhaavia diffusa is a trailing herb found throughout India; it is diffusely branched with a stout root stock and many long, slender and prostrate or ascending branches. The dry powder is a great help with indigestion. The plant spreads itself on the ground and produces clusters of flowers, from brilliant magenta to pale pink.

Names:

Latin: *Boerhaavia diffusa*

Sanskrit: Purnarnava, sophaghni
Hindi: Gadapurna, lalpunarnava
English: Spreading hogweed
Parts used: Whole plant
Activity: Anti-inflammatory, hepatoprotective, blood purifier, immunostimulant, diuretic, expectorant, etc.
Chemical constituents: Boeravinones, punarnavoside, punarnavine, ecdysterone and triacontanol.
Classical uses: In India, punarnava has a long history of usage by indigenous and tribal people. The roots are employed for treating liver, gallbladder, kidney, renal and urinary disorders. The plant is used as an expectorant, stomachic and a tonic for relieving abdominal pains.

6. **Guggulu**

The gum that forms on the trunk of *Commiphora mukul/wightii* relieves arthritic pain and is a good antiseptic; it is a small perennial tree or shrub up to 1.2–1.8 m high, occurring in rocky tracts of Rajasthan and Gujarat in India. The gum comes in five colours: golden, red, deep blue, blackish blue and white. Recent studies have shown its usefulness in lowering cholesterol.
Names:
Latin: *Commiphora mukul/wightii*
Sanskrit: Guggulu, mahisaksha, kausika
Hindi: Guggul, gugal
English: Gum guggul
Parts used: Gum exudates
Activity: Astringent, anti-inflammatory, carminative, antiseptic, liver tonic and rejuvenator and useful in rheumatoid arthritis, gout, sciatica, facial paralysis, cardiac disorders, coronary thrombosis, etc.
Chemical constituents: Guggulsterones E and Z, guggul sterols I–V, myrcene, cembrene, etc.
Classical uses: Gugulipid has a long history of usage in Ayurveda. It is responsible for lowering lipids and reducing fat and provides relief in inflammation, arthritis, atherosclerosis, obesity and hyperlipidaemia

7. **Haridra**

The turmeric plant grows up to about 1 m in height with large oblong-shaped leaves and incredible white/green flowers. A single turmeric plant can produce over

700 gm of its distinctive roots in one growing season and has between 2–5% curcuminoids and 5% essential oil.

Names:

Latin: *Curcuma longa*

Sanskrit: Haridra, varavarnini

Hindi: Haldi

English: Turmeric

Parts used: Rhizome

Acitivity: Anti-inflammatory, antioxidant, anticancer, carminative, diuretic, expectorant and hepatoprotective and useful in general debility, etc.

Chemical constituents: Curcumin, desmethoxycurcumin, bisdesmethoxycurcumin, turmerone, zingiberene, etc.

Classical uses: Haridra in Sanskrit means "an efficacious drug for jaundice". It is known to have been used in western and southern parts of India for thousands of years and is a major part of Ayurvedic medicine. The rhizome is used to treat coughs and colds, body pain and liver disorder and as adjuvant in cancer chemotherapy due to its anticancer property. It also applied on the skin, to protect it from infection and enhance the complexion.

8. **Bhrnga**

A common weed in damp places, it is found throughout India, up to an altitude of 1700 m. The flower looks like a daisy, with white petals and a yellow centre. You may be able to grow it in pots and use it to protect your liver and get rid of acne. It does many things, from getting rid of intestinal worms to making your hair look really good!

Names:

Latin: *Eclipta alba*

Sanskrit: Bhrnga, tekaraja

Hindi: Bhangra, babri

English: Trailing eclipta

Parts used: Whole plant

Activity: Anti-inflammatory, anthelmintic, carminative, diuretic and hepatoprotective and also used as hair tonic and applied externally.

Chemical constituents: Wedelolactone, demethylwedelolactone, wedelic acid, apigenin, luteolin and others

Classical uses: Traditionally Bhringraj oil is used for promoting hair growth. It is a powerful liver tonic and rejuvenator and helps to relive oedema and inflammation.

9. **Yastimadhu**

Glycyrrhiza glabra, a tall perennial herb up to 2 m high, is cultivated in Europe, Persia and Afghanistan and, to some extent, in parts of India. Liquorice root extract is used in many European countries and the USA, as a flavour for sweets and toffees. It soothes sore throats and is commonly used in cough mixtures.

Names:
Latin: *Glycyrrhiza glabra*
Sanskrit: Yastimadhu, madhuka
Hindi: Mulethi, jethimadhu
English: Liquorice
Parts used: Roots, stems
Activity: Anti-inflammatory, antiulcerative and expectorant and useful in coughs, bronchitis and pharyngitis, as respiratory tonic.
Chemcial constituents: Glycyrrhizin, liquiritin, isoliquiritin, glabranins and others
Classical uses: Traditionally the plant has been recommended as prophylaxis for gastric and duodenal ulcers and dyspepsia as an anti-inflammatory agent during allergic reactions. In folk medicines it is used as a tonic, laxative and galactagogue and for various respiratory ailments.

10. **Kutaja**

Holarrhena antidysenterica is a small-to-medium-sized tree and common throughout India in hilly deciduous forests. It is known also as the ivory tree because it has white wood and bark. The flowers are also white and look a bit like the champak flower.

Names:
Latin: *Holarrhena antidysenterica*
Sanskrit: Kutaja, kalinga
Hindi: Kurchi, kuda, shabdkosh
English: Ivory tree or Tellicherry
Parts used: Bark
Activity: Powerful antidysenteric, astringent and carminative and useful in diarrhoea, amoebiasis and gastric disorders.
Classical uses: The bark is considered the best medication for chronic diarrhoea. It is often prescribed to relieve ailments like piles, chronic dysentery, intestinal worms, colitis and some skin ailments. Several Indian tribes use the plant for diseases like anaemia, epilepsy and cholera. In Ayurvedic and Unani system of medicine, it is used for diarrhoea and skin ailments and as anthelmintic.

11. **Atmagupta**

It is a slender, extensive, climbing plant found all over India. Luckily, it is very common, as the seeds have many important uses, from helping to maintain health to an antidote for poisons.

Names:

Latin: *Atmagupta*, *Kapikacchu*

Hindi: Kewanch, kaunch

English: Cowhage

Parts used: Seeds

Activity: Astringent, laxative, anthelmintic, aphrodisiac and alexipharmic and tonic and useful in sterility and general debility.

Chemical constituents: L-dopa, mucuadine, mucuadinine, prurienidine, serotonin, amino acid analogues and others.

Classical uses: *Mucuna pruriens* is a very well-known, multifaceted herb, with immense health benefits. It has many uses, from aphrodisiac to treatment for neurological conditions. Its extract, L-dopa, is widely used in the treatment of Parkinson's disease and of sexual and neurological disorders.

12. **Tulasi**

Found throughout India, it is an erect, 30–60 cm high, many-branched, annual herb. It also tastes great in salads and can be used for cooking Italian food, if fresh basil is not available. The Latin name means holy basil, because a tulasi plant is kept in the house or garden and worshipped every morning.

Names:

Latin: *Ocimum sanctum*

Sanskrit: Tulasi, surasa

Hindi: Tulsi

English: Holy basil

Parts used: Whole plant

Activity: Adaptogenic, antistress, antibacterial, antiviral, anti-inflammatory, antipyretic, antispasmodic, immunostimulant and many more.

Chemical constituents: Eugenol, ursolic acid, carvacrol, mehyl chavicol, luteolin and others.

Classical uses: Tulasi is used in Ayurvedic formulation for relief of colds and cough, for various respiratory disorders and as tonic for increasing immunity. The *Ocimum sanctum* plant has been found to be useful as an adaptogenic, immunity builder, antistress, analgesic, anticancer, antiasthmatic and expectorant.

13. **Amalaki**

Besides all the health benefits and making you younger, it makes a really nice, healthy sherbet when the juice is boiled with sugar. The tree is small or medium sized and grows in deciduous forests up to an altitude of 1300 m. High in vitamin C, it is the best known herb in Ayurveda, used by Sage Chavan.

Names:

Latin: *Phyllanthus emblica*

Sanskrit: Aamla, aanwali, amlika

English: Indian gooseberry

Parts used: Fruit, leaf, seed

Activity: The leaves are useful for dyspepsia, diarrhoea and dysentery. The fruits are astringent, antioxidant and diuretic. It protects the liver, supports the immune system and is a rejuvenator. It is good for diabetes and jaundice.

Chemical constituents: Ascorbinogens, flavonoids, gallic acid, ellagic acid, chebulagic acid and emblicanin A and B.

Classical uses: An important source of bioflavonoids, amino acids and minerals. Amalaki is considered the best medicine for rejuvenation. It is tridosanghana (rebalances the three main humours of the body – water, fire and air).

14. **Bhumyaamlaki**

In English it is called "stonebreaker" because it is believed to dissolve kidney stones. *Phyllanthus niruri* is an annual herb, 20–60 cm high, found in wastelands as a weed, in Central and Southern India, extending into Ceylon. It has male and female flowers on the same plant, saving bees a whole lot of work.

Names:

Latin: *Phyllanthus niruri*

Sanskrit: Bhumyaamlaki, tamalaki

Hindi: Jangli amli, jaramla, bhui amala

English: Stonebreaker

Parts used: Whole plant

Activity: Anti-hepatotoxic, antiviral and hepatoprotective and useful in colic, dyspepsia, dysentery, jaundice and liver disorders.

Chemical constituents: Phyllanthin, hypophyllanthin, nirphyllin, quercetin, lintetralin and others.

Classical uses: *Phyllanthus niruri* is useful in several liver-related problems such as dropsy, jaundice, intermittent fevers and urogenital and kidney disorders. The whole plant is used as a liver tonic.

15. **Katuja**

Katuja is obtained from the root of *Picrorhiza kurroa*, a perennial, more or less hairy herb common in the north-western Himalayas from Kashmir to Sikkim. It is a home remedy for vomiting and hiccups.

Names:

Latin: *Picrorhiza kurroa*

Sanskrit: Tikta, katuka, rohini

Hindi: Kutki

English: *Picrorhiza*

Parts used: Rhizomes

Activity: Anti-inflammatory, anti-hepatotoxic and hepatoprotective. Useful in liver disorders and gastric ailments and as a metabolic activity modulator.

Chemical constituents: Picroside I–III, kutkoside, pikuroside, apocynin, etc.

Classical uses: To treat disorders of the liver (American Herbal Pharmacopoeia 1999) and upper respiratory tract, to reduce fevers and to treat hepatobiliary gastric ailments.

16. **Maricham**

Maricham is the mature dried fruit of *Piper nigrum*, our homely black pepper. It is cultivated from the Konkan southwards, in Kerala, Coorg and Assam. An important part of our spice mixtures, boiled eggs and black pepper are a palatable dish in India. It is believed that for pepper the British and Portuguese entered India and ruled the country.

Names:

Latin: *Piper nigrum*

Sanskrit: Maricham

Hindi: Kali mirch

English: Black pepper

Parts used: Fruits

Activity: Anti-apoptotic, antibacterial, anti-inflammatory, antimutagenic, antitumour, antithyroid, bioavailability enhancer and hepatoprotective. It enhances the bioavailability of other herbs.

Chemical constituents: Piperine, piperetine, piperidine, piperolein and volatile oil

Classical uses: Used for intermittent fever and to promote the secretion of bile; recommended for neurological, bronchopulmonary and gastrointestinal disorders. A combination of black pepper, long pepper and ginger form "trikatu", which acts to correct the balance of three humours of the body. It is used in veterinary medicines on the same scale as for humans.

17. **Kalmegh**

Andrographis paniculata is an erect plant with height of 30–110 cm. Its stem is slender and dark green, squared in cross-section with longitudinal furrows and wings along the angles. The lance-shaped leaves have hairless blades measuring up to 8 cm long by 2.5 cm wide. The small flowers are borne in spreading racemes. The fruit is a capsule around 2 cm long and a few mm wide. It contains many yellow-brown seeds.

Names:

Latin: *Andrographis paniculata*

Sanskrit: Kirata

Hindi: Kalmegh

English: Kariyat

Parts used: Whole plant

Activity: Antibacterial, antifungal, antiviral, antipyretic, adaptogenic, anti-inflammatory, immunostimulant, liver protecting, carminative, diuretic, gastric and liver tonic, choleretic, hypoglycemic, hypocholesterolemic and blood purifying.

Chemical constituents: Andrographloide

Classical uses: *A. paniculata* has been used traditionally in the treatment of a number of liver disorders. It is used for treating leprosy, gonorrhoea, scabies, boils, skin eruptions, etc. due to its blood-purification properties. Its decoction prevents and treats liver diseases and fever.

18. **Bidarikand**

Pueraria tuberosa is a perennial climber with a very large tuberous root. It grows at an altitude below 1200 m and is distributed throughout India, except in very humid or very arid regions; thus it is not found in deserts, swamps or seasides. The root looks like a brown beetroot, but it is much larger and the inside is white.

Names:

Latin: *Pueraria tuberosa*

Sanskrit: Bidarikand

Hindi: Vidarikanda

English: Indian kudzu

Parts used: Tuber

Activity: Anti-inflammatory, lactogenic, galactagogue and tonic with positive effect on steroidogenesis.

Chemical constituents: Puerarin, stigmasterol and daidzein

Classical uses: Ayurveda uses the roots to make a sweet, cooling tonic. It works as a demulcent and a refrigerant in fevers. It is also used as an aphrodisiac, galactagogue, diuretic and adjuvant in urinary disorders and restorative tonics.

19. **Haritaki**

Terminalia chebula is a moderate- or large-sized tree and found throughout India chiefly in deciduous forests and areas of light rainfall, up to about an altitude of 1500 m. The fruits are the size of almonds and are dried to preserve them.

Name:

Latin: *Terminalia chebula*

Sanskrit: Abhaya, kayastha

Hindi: Harad

English: Myrobalan

Parts used: Fruits

Activity: Astringent, anodyne, anthelmintic, anti-inflammatory, cardiotonic, carminative, diuretic, laxative and purgative and useful in gastric disorders, hyperacidity, jaundice, skin diseases, etc.

Chemical constituents: Chebulagic acid, chebulinic acid, gallic acid, tannins, etc.

Classical uses: Haritaki is an important component of triphala (three fruits) made from *Terminalia chebula*, *Terminalia bellerica* and *Emblica officinalis*. Used in the treatment of digestive issues, asthma, bleeding piles, sore throats, vomiting and gout and used as a laxative, a detoxifying agent of the colon and a rejuvenator.

20. **Guduchi**

Although the berries look tempting, it is the stems of this creeper that are medicinal. It is found throughout tropical India, ascending to an altitude of 900 m, from Kumaon eastwards and southwards to Sri Lanka.

Names:

Latin: *Tinospora cordifolia*

Sanskrit: Amrita, guduchi

Hindi: Giloe, gurcha

English: Gulancha tinospora, tinospora

Parts used: Stem

Activity: Anti-hepatotoxic, anti-inflammatory, immunomodulator, rejuvenator and tonic and useful in general debility, rheumatoid arthritis, chronic cardiovascular disorder, skin disorders, immune-degenerative diseases, etc.

Chemical uses: It is claimed to be useful in treating leprosy, fever, asthma and anorexia. It has been indicated in diseases like skin and blood infections, jaundice, diabetes, chronic diarrhoea and dysentery.

21. **Methika**

It is an aromatic, 30- to 60-cm-tall annual herb, cultivated throughout India. It is a favourite in all Indian kitchens, especially the leaves. Methi aloo! Yummy!!

Names:

Latin: *Trigonella foenum-graecum*

Sanskrit: Methika, kalanusari

Hindi: Methi

English: Fenugreek

Parts used: Seed

Activity: Anabolic, carminative, demulcent, emollient, galactagogue and hypolipidemic (Ministry of Health, Government of India 1990) and useful in anorexia, diabetes, diarrhoea, hypogalaction and enlargement of the liver and spleen.

Chemical constituents: Trigoneoside Ia and Ib; trigoloenoside A, D, F and G; diosgenin; trigonellin; and yamogenin

Classical uses: The seeds are used as a digestive tonic and demulcent for "vata", for various digestive disorders, for reduction of obesity and control of blood sugar and cholesterol as adjuvant and also in the management of inflammation of joints and healthy bowel function.

22. **Ashwagandha**

Ashwagandha consists of the dried, mature roots of a perennial shrub, found in the Gangetic plain all the way to West Bengal and in the plains of the Deccan, in cultivated fields and open grounds; it is widely cultivated in areas of Madhya Pradesh and Rajasthan. This herb is aptly named, as it smells like a horse! It is also supposed to give you the power of a horse.

Names:

Latin: *Withania somnifera*

Sanskrit: Ashwagandha, varhakarni

Hindi: Asgandh, punir

English: Winter cherry

Parts used: Roots

Activity: Antistress, adaptogenic, antianxiety, antioxidant, anti-inflammatory, antiarthritic, aphrodisiac, diuretic, immunomodulator, rejuvenator, stimulant, tonic, etc. and strengthens immune system.

Chemicals constituents: Withaferin A, withanolides, withanine, somniferine, etc.

Classical uses: The best tonic for children when given with milk. It is useful in rheumatism, debility, leucoderma, constipation, insomnia, nervous breakdown

and goitre. The paste, formed when roots are crushed with water, is applied to reduce inflammation of the joints. It is a "Rasayan" category drug, which is mainly used as an antistress, adaptogenic and anxiolytic agent.

23. **Adrakam**

Ayurveda uses the dried rhizome of ginger, widely cultivated in India. And it is widely used in cooking almost every Indian dish. Ginger tea is very soothing when you have a bad cold. And do not forget to add dried ginger (saunth) to chutneys.

Names:

Latin: *Zingiber officinale*

Sanskrit: Adrakam, ausadha, visva

Hindi: Adrak

English: Ginger root

Parts used: Rhizome

Activity: Carminative and digestive, expectorant and antiemetic. It is useful in anorexia, bronchial asthma, coughs, inflammation, oedema, diarrhoea, flatulence, cardiac disorders, rheumatoid arthritis, etc.

Chemical uses: In the old text books written by, Sushruta, Vagbhata and Chakradutta, the use of drugs is mentioned in the form of trikatu, a famous Ayurvedic recipe for the treatment of digestive disorders. The plant has been used as a remedy for arthritis. In folk medicines, the plant is used as a carminative, expectorant and astringent.

Contribution of Private Sector R&D for Animal Health- Care Management

Herbal Product Development The private sector has taken a key initiative in this arena of combining empirical knowledge about the popular use of medicinal plants with establishing scientific basis of quality, safety and efficacy of products. By its novel approach of traditional knowledge and modern research, it has modernized the pharmacy into branded herbal ready-to-use formulations for livestock.

Quality Standards Phyto-pharmaceuticals could only be considered as rational drugs if these are standardized and their pharmaceutical quality is approved. At standard private sector firms, quantitative and qualitative multicomponent analysis by advanced analytical techniques is done which serves as a specific tool to set quality standards and specifications so as to ensure therapeutic efficacy, safety and shelf life of herbal drugs. The application of high-technology-oriented advanced

hyphenated techniques in its R&D serves as a rapid tool in unravelling the hidden medicinal benefits from complex herbal formulations.

Every product formulated undergoes stringent quality control tests. A major emphasis is given to the right selection, scientific processing and efficacy evaluation of different herbs. Toxicological and pharmacological profiles of each herb are thoroughly documented before inclusion in product formulations. Each product undergoes testing for dose standardization, palatability, preclinical and clinical efficacy evaluation and scientific validation.

Quality control of herbal formulations is done according to standard WHO guidelines. Thorough macro- and microscopic examination is done for the raw material to be used for manufacturing products. The development of authentic analytical methods which can reliably profile the phytochemical composition, including quantitative analyses of marker/bioactive compounds and other major constituents, is a major task performed by R&D scientists. Therapeutic activity of an herbal formulation depends on its phytochemical constituents.

Standardization of Herbals Standardization is an important step at R&D for the establishment of a consistent biological activity, a consistent chemical profile, or simply a quality assurance programme for production and manufacturing of herbal products. Standardization based on a single or small number of chemical markers or classes of compounds serves mainly to promote quality control and batch-to-batch consistency in terms of efficacy. Methods of standardization are taken into consideration keeping in view all aspects that contribute to the quality and pharmacological efficacy of the herbal preparations.

Herbal formulations of the private sector have reached extensive acceptability as therapeutic agents for several diseases. Therapeutic activity of herbal formulation depends on its phytochemical constituents.

In view of the above, standardization is an important step for the establishment of a consistent biological activity, a consistent chemical profile, or simply a quality assurance programme for production and manufacturing of herbal drugs.

The development of authentic analytical methods which can reliably profile the phytochemical composition, including quantitative analyses of marker/bioactive compounds and other major constituents, is a major challenge. Standardization is an important step for the establishment of a consistent biological activity, a consistent chemical profile, or simply a quality assurance programme for production and manufacturing of herbal drugs. Standardization is an important aspect for maintaining and assessing quality and safety of polyherbal formulations as these are combinations of more than one herb to attain the desired therapeutic effect. Standardization minimizes batch-to-batch variation and assures safety, efficacy, quality and acceptability of the polyherbal formulations.

Clinical Validation Clinical trials are an integral part of a new product discovery and development. Clinical trials are initiated on new formulations and existing products in order to demonstrate product benefits and safety in targeted species with a cost-effective study design. With the holistic approach to rigorously pursue

scientific validations and quality control of the product, R&D collaborates with the premier government agencies like ICAR and its institutes, educational institutes, universities and veterinary colleges, and the scientific research and clinical efficacy studies on the formulations are carried out by the competent scientists and investigators at various research centres in India and abroad. The objectives of these clinical studies are largely threefold: first, the verification, demonstration and substantiation of clinical benefits; second, the product performance across diverse geographical locations; and third, to understand the mode and possible mechanism of the product action, including the aspect of pharmacological evaluation. The clinical studies continue even after the product has been introduced. In order to fulfil these objectives, clinical trials are executed so as to assess product tolerance, determine maximum tolerable dose, estimate product efficacy for targeted indication, standardize dose rate, exclude the possibility of any serious toxicities and thereby establish product safety and efficacy profile. The data generated and the results of these investigations are shared with the veterinary fraternity for the benefit of the community. The trial reports and the results of clinical research studies are also essential for gaining approval for registration of dossiers and for marketing new formulations or new indications of existing products from regulatory agencies throughout the world.

The private sector has pioneered the launch of many scientifically validated herbal formulations and trusted brands for poultry, swine, aqua and livestock.

Role of Phytogenic and Herbal Feed Additives for Animal Health Care

Feed additives depending on the specificity of function improve the health, digestibility, productivity and immunity. It is established scientifically that inclusion of herbal feed additives in the ration of poultry and other livestock improves overall growth, performance and productivity of animals, enhances nutrient utilization and feed efficiency, possesses immunopotentiating and antioxidant effect and improves gut microflora as they also have antimicrobial activity.

Improvement in Gut Function Improvement in gut function is mainly attributed to the possible stimulatory effect of phytogenic substances on digestive secretions, such as digestive enzymes, bile and mucus. Phytogenic substances from certain herbs, viz. *Aegle marmelos*, *Plantago ovata*, *Acacia catechu* and *Coriandrum sativum*, and many more other herbs and their extracts improve gut microflora and exert pharmacological actions within the digestive tract, as evidenced by their gut function-modulating efficacy. (Satyavati and Gupta, 1987) Pathogenic bacteria are always present in the gut, but the balance between pathogenic and beneficial bacteria determines whether or not disease will occur. Maintaining a healthy balance between all microfloras found in the gut is known as eubiosis which can be influenced by bacteria endemic to the microflora. In the intestine, bacteria considered beneficial to the gut, including lactic acid-forming bacteria like *Lactobacillus* spp., prevent

proliferation of pathogens, such as *Salmonella* spp., through competitive exclusion for nutrients and for receptor sites on the gut wall. Beneficial bacteria can also produce an adverse environment for pathogenic bacteria to colonize and grow, for example, by the production of short-chain fatty acids which lower the pH and prevent growth of pH-sensitive pathogenic bacteria. The microflora has also functions in the development of the digestive and immune tissue in the host animal as it can produce nutrients that can be used by the host and also can neutralize some feed toxins and promote an environment in the gut where anti-nutritional factors and toxins are minimized. In the past, antibiotic feed additives were used for manipulation of the microflora to create eubiosis. However, with the severe restriction of antibiotic feed additive use in the EU and increasing consumer concern, alternatives to antibiotic feed additives have been investigated and found to significantly influence this balance also.

Increased Feed Intake The stimulatory effect of herbal feed additives on feed intake is due to the claimed improvement in palatability of the diet resulting from the enhanced flavour and odour. The addition of *Woodfordia fruticosa*, *Zingiber officinale*, *Allium sativum*, *Trigonella foenum-graecum*, etc. to poultry and pig ration improves feed efficiency (Satyavati and Gupta, 1987). Increased palatability of the diets associated with the addition of phytogenics also may be due to their antioxidative effects, which might contribute to preserving the desired organoleptic qualities of the diet.

Digestive Tonic and Growth Promoter The role of ruminal microflora in digestion of nutrients is vital. Hence, interactions of the normal microbial flora with the host can be manipulated to improve the efficiency of nutrient utilization. Supplementation of herbs that can modulate the rumen function may be siren to animals for efficient cellulose breakdown and digestion and maintenance of normal ruminoreticular functions, intestinal movement, optimum utilization and absorption of nutrients, thus improving feed conversion ratio, productivity and body weight gain in animals. A potent herbal formulation containing some minerals and different herbs like *Allium sativum*, *Azadirachta indica*, *Calotropis procera*, *Centratherum anthelmenticum*, *Commiphora mukul*, *Eclipta alba*, *Embelia ribes*, *Picrorhiza kurroa*, *Zingiber officinale*, *Piper longum*, etc. is used as an appetizer, restorative, stomachic, digestive tonic and growth-promoter product (Kolte et al., 2009).

Hepato-efficiency Enhancers Supplementation of certain liver tonic preparations also increases secretion and flow of bile for better digestion and for treatment of anorexia by maintaining the liver parenchyma in a healthy state and regulating liver functions like detoxification of metabolic products, toxic drugs and chemicals and treatment of hepatic dysfunction. The herbal ingredients such as *Andrographis paniculata*, *Eclipta alba*, *Picrorhiza kurroa*, *Phyllanthus niruri*, *Tephrosia purpurea*, *Tinospora cordifolia* and *Boerhavia diffusa* are used to improve feed conversion efficiency and body weight gain and reduce mortality in poultry and

swine, owing to their hepatoprotective, hepato-stimulant and growth-promoting properties (Dwivedi et al. 1986).

Another polyherbal liver tonic and growth-promoter product for cattle comprises herbs, namely, *Andrographis paniculata*, *Eclipta alba*, *Picrorhiza kurroa*, *Phyllanthus niruri*, *Tephrosia purpurea*, *Tinospora cordifolia* and *Boerhaavia diffusa*, each with documented hepatoprotective and hepato-stimulant properties. It counteracts hepatopathy and restores liver functions in bovines (Pradhan and Dey, 1996). Polyherbal liver tonic formulations also protect the hepatic parenchyma from various parasitic and liver fluke infestations such as visceral larva migrans.

Antioxidant, Immunomodulator and Antistress Effect Various stressors such as high ambient temperature and relative humidity influence the performance of animals and birds by reducing feed intake, feed efficiency, nutrient utilization and feed conversion ratio.

Ayurvedic formulations that treat stress contain herbs with adaptogenic (antistress) effects. Herbs with antistress and antioxidant activity like *amla* (*Emblica officinalis*), *ashwagandha* (*Withania somnifera)*, *tulasi* (*Ocimum sanctum*), *shilajit* and many more have proven to be potent oxygen free radical scavengers in in vitro and in vivo models. Medicinal plants or herbs owing to immunopotentiating properties can provide an alternative to conventional therapy for a variety of diseases, especially when the host's defence mechanism has to be activated under conditions of impaired immune response.

Herbs, namely, *Mangifera indica*, *Echinacea purpurea*, *Phyllanthus emblica*, *Uncaria tomentosa*, *Withania somnifera*, *Ocimum sanctum*, *Tinospora cordifolia*, *Asparagus racemosus* and many more, are scientifically validated to possess antistress, immunomodulator and adaptogenic properties. Mangiferin, active constituent of herb *Mangifera indica*, is a strong inducer of in vivo and in vitro activation of peritoneal macrophages. Induction of interferon release from macrophages by *Mangifera* has a potent lymphoproliferative effect on macrophage activation, thus establishing the therapeutic potential of *Mangifera* as an immunomodulator. Another herb *Echinacea purpurea* stimulates macrophages, but does not increase resistance to a wide variety of stressors (e.g. physiological stress). However, *Uncaria tomentosa* stimulates interleukin-1 and interleukin-6 in macrophages, stimulates endothelial cells to produce a lymphocyte-proliferating regulating factor and enhances recovery of leukopenia induced by doxorubicin. Similarly, *Withania somnifera* is well established to augment endogenous antioxidants, maintenance of myocardial antioxidant status, significant restoration of most haematobiochemical and oxidative stress marker parameters by its free radical scavenging activity in addition to potentiating agglutinin antibody titres and complement fixing antibodies (Ziaddun et al. 1996). The antioxidant potential of methanolic extracts of *Ocimum sanctum* indicated their prime role in free radical elimination, thus combating immunosuppression. It can be interpreted that the characteristic dysregulation of stress hormones and neurotransmitters and the immunosuppression thereby can be successfully ameliorated by the botanical adaptogenic remedies including either single herb or combination of different herbs (polyherbal formulation). (Manoharan

et al. 2004) Another such polyherbal immunomodulator, adaptogenic and antistressor formulation, namely, "Stresroak", was also scientifically validated in amelioration of various stressors, e g. production, vaccination, overcrowding, water deprivation, lead toxicity and cadmium toxicity, and to potentiate an immune response during disease conditions, e.g. infectious bursal disease and Newcastle disease in poultry (Pradhan, 1995 and Sujatha et al. 2010).

Antimicrobial Effect The medicinal or antimicrobial properties of plant-derived substances have been well known for centuries. This property is mainly attributed to the essential oils of these plants, namely, *Trachyspermum ammi*, *Cinnamomum camphora*, *Mentha piperita* and many more. Oregano and thyme are among those which have received a great deal of interest. These plants contain monoterpenes, carvacrol and thymol, respectively, and have demonstrated high in vitro efficacy against several pathogens found in the intestinal tract, suggesting that phytogenic feed additives may be suitable replacements for in-feed antibiotics to improve pig health and growth performance, particularly during the first few weeks postweaning.

Herbal Formulation for Cure of Diseases An herbal formulation which contains onion extract, garlic extract, karanj oil, camphor powder, lemon extract, turmeric powder and sesame oil is very effective against mange, ringworm, pyoderma, eczema, FMD lesions, foot rot lesions, surgical wounds, burns, cuts, abrasions, etc. It was developed by Dr. Sharma and Dr. Dwivedi during the period of 1985 to 1992 at the Indian Veterinary Research Institute (IVRI), Izatnagar. This herbal formulation "Olinall" has been commercialized in the year 1997 and available in the market for treatment of skin ailments. It is the first technology of IVRI which has been commercialized to the private sector. IVRI had developed many other herbal formulations, which are very effective against hepatitis, diarrhoea, dysentery, renal disorders, gastric ulcer, diabetes, etc.

Toxin Binder Aflatoxins reduce growth and feed efficiency, suppress the immune system, reduce antibody titre and cause mortality and morbidity leading to severe economic losses to poultry farmers. Synergistic action of herbal toxin binders along with liver tonic formulations is scientifically well proven to improve growth and reduce mortality in broiler chickens by better protein and energy utilization by limiting the adverse effects of aflatoxins. In addition to it, other polyherbal toxin binders, namely, Vilocym and Vilocym Z, are popular in the poultry industry, among farmers and veterinarians for control of mycotoxicosis.

Natural Methionine Source Methionine is required in free as well as conjugated form to perform various metabolic functions. It assists in the breakdown of fats and thereby prevents the build-up of fat in liver and arteries, as well as assisting the digestive system and removing heavy metals from the body since it can be converted to cysteine, a precursor to glutathione, which is of prime importance in detoxifying the liver. Its deficiency may manifest in symptoms like fatty liver, retarded growth, reduced feed efficiency, weakness, oedema, skin lesions, poor feather condition,

decreased egg production, small egg size, immunosuppression, low carcass yield and disturbances in various metabolic pathways (Rama rao et al. 2003). Improper conversion of methionine can lead to atherosclerosis. Though most plants contain very little amounts of methionine, some have significant amounts. High levels of methionine are found in fish, meats and some plant seeds. Almost all feed ingredients of plant origin used for compounding poultry ration are deficient in methionine used, and hence DL-methionine is commonly added as supplement in poultry feeds. In the past, the requirement of amino acids of chickens was used to be met by more than one source of protein of plant and animal origin (Kanchi et al. 2009).

However, due to increase in prices of quality fish meal and availability of comparatively cheaper soybean meal, use of all soya feed started with an addition of methionine as soybean meal is deficient in methionine which again is a costly supplement. In poultry ration, just like protein, the vitamins and minerals have an equally important role in the development of musculature. In the list of vitamins, choline (growth promoter) plays a vital role by acting as lipotropic agent, thereby preventing abnormal fatty infiltration in the liver, thus ensuring proper metabolism of the body and effective utilization of nutrients. Moreover, it helps in formation of an excitatory neurotransmitter acetylcholine, which is responsible for proper functioning of the nervous system and maintains harmony. There is no consistency in the choline content in the natural feed stuffs, and also their bioavailability is not predictable. Therefore, in broilers as well layers, choline in combination with chloride (CC) is added as an important feed ingredient.

However, herbal or natural products, which have proven results in various areas of poultry production, can be combined as a mixture, and premix can be prepared, which may be used in feed formulations. Supplementation of polyherbal formulations containing ingredient herbs that mimic methionine-, choline- and biotin-like activity prevents fatty liver syndrome and improves overall carcass quality, yield and egg production in broilers. These herbal amino acid supplements have an added advantage over harmful effects of synthetics, use of which is totally prohibited in organic poultry production (Moritz et al. 2005).

The key activities to be undertaken are as follows:

- Evaluate the present status of the codified veterinary herbals, and prepare an annotated list of literature available in India.
- Promote cultivation of medicinal plants; currently less than 20% of medicinal plants are contributed through cultivation only. The government and industry should come forward with PPP model for promotion of medicinal plant cultivation.
- Document and rapidly assess the herbal medicine practices and related fromulations, to shortlist a number of ecosystem-specific packages of herbal medicine remedies that are safe, efficacious and cost-effective for their promotion across representative locations in the country.

- Promote the use of ecosystem-specific package of herbal medicine remedies to meet the primary health-care needs of livestock and reduction in the cost of health care among the milch animals of dairy farmers.
- Prepare appropriate medicinal formulations for selected products as per the Ayurvedic references, and standardize (herbs, products and processes) herbal veterinary products for the shortlisted conditions for wider use.
- Conduct clinical trials according to the research protocol and recommendations by the Technical Advisory and Ethical Committee for production and marketing of the standardized herbal veterinary products by a number of community-owned enterprises located across the country.
- Reduction in the antibiotic and hormone residues in the milk and other animal products by using the safe, effective and standardized products based on time-tested local traditions.

Conclusion and Prospects

Traditional systems of medicine have stood the test of time for over 4000 years and are best suited as complements to modern veterinary medicine for enhancing livestock production. Since ancient times, herbs and their products have been constantly used for curing illness. However, herbal additives have aroused much scientific interest over the past few years to explore their role as performance enhancers in livestock production. The cited instances in this text are just glimpses from the vast and virgin world of Ayurveda through the clear eye of modern science. It is up to our scientists and the industry how best to use this precious gift from Mother Nature for health and welfare of livestock and in turn mankind.

Abundant traditional knowledge is available, but due to limited flow of information from generation to generation and improper documentation of knowledge on the scientific platform, a lot of questions have remained unanswered. Leading companies like Ayurvet Limited, Himalaya Animal Health and Natural Remedies are working towards providing the scientific solutions to the available traditional knowledge for improving animal health. It is time that the focus shifts from providing herbal solutions to cost-effective, scientifically validated solutions, which are safe and efficacious.

Most of the medicinal plants/herbs are currently being sourced from the forest. This has led to destruction of medicinal flora available in wild population, which has also declined over a period of time. Thus, there is also an urgent need to encourage cultivation of prioritized medicinal plants and research in this sector. Companies like Ayurvet have taken initiative towards cultivation of medicinal plants for sustainable and quality raw material, which means better results.

The quality specifications of veterinary herbal medicines need to be looked at closely. Though steps have been taken in this direction, however, a lot needs to be done. Possibility of harmonization/collaboration efforts may be explored to improve animal health care at the national and international levels. Similarly the labelling requirements need to be stricter, so that the right information, which has scientific basis, can be considered for labelling requirements.

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Medicinal Plants as Novel Promising Therapeutics for Neuroprotection and Neuroregeneration

Gurcharan Kaur, Hardeep Kataria, and Rachana Mishra

Natural Medicines: Old Avenues and New Approaches

Ayurveda is the most ancient and the traditional medicinal system with historical roots in the Indian subcontinent. Modernized practices derived from traditional Ayurvedic medicinal system are a type of complementary or alternative medicine (Jafari et al. 2014). High costs and increased side effects of new drugs, lack of curative treatment for several chronic diseases, and microbial resistance are some of the reasons that have contributed in directing the public interest towards complementary and alternative medicine. Ayurvedic therapies have been integrated in complementary and alternative medicine as preventive measures to treat wide range of chronic diseases due to their least adverse effects compared to conventional medicines (Humber 2002; Patwardhan et al. 2004; Jafari et al. 2014). Moreover, Ayurveda formulations may also be used in combination with other drugs without any adverse drug-drug interactions (Humber 2002; Patwardhan et al. 2004). The concept of "one drug-one target-one disease" in modern medicines has provided remarkable success in providing highly selective and potent magic bullets, especially for certain highly contagious diseases. In several other diseases, especially lifestyle disorders, this approach has shown major disadvantages where a disease manifestation and progression involves multifactorial and complex signaling pathways. Herbal products are being actively used as means of alternative medicine because of their multicomponent approach to target multiple sites for their mode of action

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(Bent and Ko 2004; Tachjian et al. 2010; Pallas et al. 2013). Moreover, their formulation in single delivery system and their least side effects make them promising candidates to treat several central nervous system ailments (Borisy et al. 2003; Keith et al. 2005). Furthermore, these herbal formulations can be used in combination with other drugs without any adverse drug-drug interactions (Hopkins 2008).

Neurodegenerative diseases such as Alzheimer's disease, Parkinson's disease, Huntington disease, stroke, spinal cord injuries, etc. present a major health problem associated with aging worldwide. Efforts are being made currently to discover the underlying mechanism of neurodegenerative diseases and prospective therapy that can prevent/slow down the onset of these diseases. Since pathogenesis of the neurodegenerative diseases involves multiple factors, therefore the major challenge for neuroscientists is to first identify such multiple underlying causes in order to identify the targets and prevent age-associated neurodegenerative diseases. For majority of these neurodegenerative diseases, currently only palliative therapies are available, and none of them is capable to slow down or halt the underlying pathology. Polyphenolic compounds such as flavonoids and many other antioxidative molecules which are present in vegetables and fruits have been shown to have antiaging properties and reduce the risk of neurodegenerative diseases. Despite the abundance of natural products in dietary items, scientific studies of their potential benefits in human health have only recently begun. Several recent preclinical and clinical studies have demonstrated the potential beneficial effects of natural products for the treatment of neurodegenerative diseases, and a large number of animal models and cell culture studies have shown a great promise in developing these compounds as suitable therapeutic targets (Solanki et al. 2016).

Ashwagandha: *Queen of Ayurveda*

Withania somnifera (WS), commonly known as Ashwagandha, is one of the important herbs being used in Ayurveda since time immemorial. It has been classified as a "rasayana" herb owing to its adaptogenic and rejuvenating properties. Ashwagandha is widely distributed across Southeast Asia and from the Mediterranean region to South Africa. In India, it is widely grown in Punjab, Rajasthan, Haryana, Mandsaur district of Madhya Pradesh, Uttar Pradesh, and Gujarat (Bhatia et al. 1987; reviewed by Kulkarni and Dhir 2008). The traditional use of WS is focused on its rejuvenating and life-prolonging properties for various muscular, cardiac, and neuronal conditions, wherein WS vitalizes and invigorates several body organs to promote general health and longevity (Singh et al. 2011). Different parts of the plant such as leaves, roots, stems, bark, and even whole plant extract are being used as therapeutic interventions for the treatment of a wide array of nervous, cardiac, gastric, and psychiatric conditions (Dar et al. 2015, 2016). Preclinical research and clinical studies have supported the therapeutic use of this plant for cognitive- and memory-related ailments (Minhas et al. 2011; Pawar et al. 2011; Ahmed et al. 2013; Pingali et al. 2014).

This chapter focuses on the effects of *W. somnifera* in various CNS disorders. Various preclinical studies investigating the use of *W. somnifera* in modulation of neuroplasticity, anxiety, neuroinflammation, and neuroprotection have been discussed in detail. A plethora of studies confirm the use of *W. somnifera* and its active phytochemicals (withaferin A, withanone, withanoside IV, withanolide A, sitoindosides VII–X) alone or in combination as potential therapeutic agents. *W. somnifera* can be incorporated as an important dietary supplement for the management of anxiety and associated cognitive and functional impairments.

Neuroprotective Role of Ashwagandha in Neurodegenerative Disorders Neurodegeneration is characterized by pathologic conditions, ranging from Alzheimer's disease to glaucoma, which have devastating social and economic effects. These diseases involve a complex process implicating a series of molecular and cellular events, such as oxidative stress, mitochondrial dysfunction, protein misfolding, excitotoxicity, and inflammation. Natural medicines due to their broad spectrum of pharmacological and biological activities may be possible candidates for the management of such multifactorial morbidities (Bagli et al. 2016), but their therapeutic potential against neurodegenerative diseases has been complicated due to their poor bioavailability and subsequent insufficient delivery to the brain through the blood-brain barrier (Sharma et al. 2012). Ashwagandha has general stimulating and regenerative qualities and is used among others for the treatment of nervous exhaustion, memory-related conditions, insomnia, etc. Clinical trials and animal research support the use of Ashwagandha for the treatment of neurological disorders, and studies have proved that Ashwagandha preparations have potential therapeutic role in almost every CNS-related disorders. It modulates GABAergic, cholinergic, and oxidative systems, and the phytochemicals present in it have been shown to be responsible for overcoming the excitotoxicity and oxidative damage (Parihar and Hemnani 2003; Russo et al. 2001) in various in vitro and animal models.

The potential of Ashwagandha for regeneration has been explored in some of the in vitro as well as in vivo studies. It is well known that the extension of dendrites and axons in neurons may compensate for and repair damaged neuronal circuits in the dementia brain. Ashwagandha root extract significantly increased the percentage of cells with neurites in human neuroblastoma SK-N-SH cells in dose- and time-dependent manner which was associated with the increase in expression of dendritic markers MAP2 and PSD-95 (Tohda et al. 2000). The methanol extract of Ashwagandha has been characterized to contain withanolides such as withanolide A, withanoside IV, and withanoside VI, which induce neurite outgrowth in human neuroblastoma SH-SY5Y. Withanolide A, withanoside IV, withanoside VI, and coagulin Q have been shown to possess significant neurite outgrowth activity on a human neuroblastoma SH-SY5Y cell line (Zhao et al. 2002). In another study using methanolic extract of Ashwagandha, withanolide A, withanoside IV, and withanoside VI showed neuritic regeneration and synaptic reconstruction in Aβ (25–35)-induced damaged cortical neurons (Tohda et al. 2005). Ashwagandha extract was tested for its potent neuroprotective properties in H_2O_2 and Aβ(1–42)-

induced cytotoxicity for novel approaches to treat dementia, especially dementia of the Alzheimer's type (AD). Aqueous root extract significantly protected the differentiated PC-12 cells against both H_2O_2 and Aβ(1–42)-induced cytotoxicity, in a dose-dependent manner, demonstrating the neuroprotective properties of Ashwagandha (Kumar et al. 2010).

Parkinson's Disease (PD) and Ashwagandha Parkinson's disease is a neurodegenerative disorder which belongs to a group of conditions called motor system diseases due to loss of dopamine (DA) neurons in substantia nigra. The exact mechanism of the cell death of DA neurons is still unknown. In a clinical study of 18 clinically diagnosed parkinsonian patients, Ashwagandha treatment (a concoction in cow's milk of powdered *Withania somnifera*, *Mucuna pruriens*, and *Hyoscyamus reticulatus* seeds and *Sida cordifolia* roots) showed significant improvement in activities of daily living and on motor examination following Ayurveda medication (Nagashayana et al. 2000). One of the acceptable models used for screening drugs for Parkinsonism is haloperidol- or reserpine-induced catalepsy in mice. Administration of BR-16A (Mentat®), a polyherbal formulation of Ashwagandha (50 and 100 mg/kg, p.o.), significantly reversed the haloperidol- or reserpine-induced catalepsy suggesting that Ashwagandha has protective effect against neuroleptic-induced catalepsy (Kumar and Kulkarni 2006).

6-Hydroxydopamine (6-OHDA) elicits its toxic manifestations through oxidant stress and is one of the most widely used rat models for Parkinson's disease. Ahmad et al. (2005) in their study pretreated animals with 100, 200, and 300 mg/kg b.w. of the Ashwagandha extract orally for 3 weeks before 6-OHDA infusion into the right striatum. Ashwagandha extract was able to revert all the physiological and biochemical parameters of oxidative stress in dose-dependent manner as tested by neurobehavioral activity and oxidative enzymes, catecholamine content, dopaminergic D2 receptor binding, and tyrosine hydroxylase expression as compared to 6-OHDA-treated animals (Ahmad et al. 2005). In another study on Parkinson's model, parkinsonism was induced by 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP). Ashwagandha root extract (100 mg/kg body weight) oral treatment resulted in a significant improvement in the mice's behavior and antioxidant status, along with a significant reduction in the level of lipid peroxidation (Sankar et al. 2007). Ashwagandha leaf extract (100 mg/kg b.w.) has been shown to normalize the levels of GSH, GPx, and TBARs in the MPTP-induced PD animals. There was improvement in the motor function of the Ashwagandha-treated animals as evident by rotarod and hang test proving it to be strong contender in treating catecholamines, oxidative damage, and physiological abnormalities seen in the PD (Rajasankar et al. 2009a, b).

Alzheimer's Disease The amnesia induced by scopolamine is also associated with main symptom of Alzheimer's disease. In a recent study by Konar et al. (2011), Ashwagandha was shown to ameliorate scopolamine-induced downregulation of expression of BDNF and GFAP in a dose- and time-dependent manner in animal

model. Ashwagandha leaf extract and its bioactive component, withanone, were able to revert the scopolamine-induced cytotoxicity in the brain cell IMR32 neuronal and C6 glial cells which was associated with downregulation of neuronal proteins (NF-H, MAP2, PSD-95, GAP-43, and GFAP). It was also able to augment the scopolamine-induced upregulation of DNA damage-γH2AX and oxidative stress-ROS markers in these cell lines (Konar et al. 2011).

Another landmark study showed that withanolide A, isolated from the Ashwagandha root extract, could regenerate neurites and reconstruct synapses in severely damaged neurons both in vitro and in vivo systems (Kuboyama et al. 2005). Withanoside IV also induced neurite outgrowth in cultured rat cortical neurons. Oral administration of withanoside IV (10 μM/kg) significantly improved memory deficits in Aβ(25–35)-injected mice and prevented loss of axons, dendrites, and synapses. Sominone, an aglycone of withanoside IV, was identified as the main metabolite after oral administration of withanoside IV. Sominone induced significant axonal and dendritic regeneration and synaptic reconstruction in cultured rat cortical neurons damaged by Aβ(25–35). Ashwagandha constituent withanoside IV has been reported to ameliorate neuronal dysfunction in Alzheimer's disease model (Kuboyama et al. 2002, 2006). Sominone was further found to reinforce the morphological plasticity of neurons by activation of the RET pathway and thus enhance memory. It has been proposed to be a GDNF-independent stimulator of the RET pathway and/or a novel modulator of RET signaling (Tohda and Joyashiki 2009). In another study related to AD, withanamides from Ashwagandha fruit were tested for their ability to protect the PC-12 rat neuronal cells, from beta-amyloid-induced cell damage. Withanamides negated the amyloid-induced cell death, and it was shown that withanamides uniquely bind to the active motif of Aβ(25–35) and thus prevent the fibril formation (Jayaprakasam et al. 2010). Similarly, aqueous Ashwagandha extract was able to inhibit fibril formation by the amyloid-β peptide in vitro as evident by transmission electron microscopy and ThT fluorescence assay in a concentration-dependent manner as compared with control samples, thus proving to be an important candidate in AD therapeutics (Kumar et al. 2011).

Tardive Dyskinesia (TD) Tardive dyskinesia is a serious motor side effect of chronic neuroleptic therapy. The protective effects were observed in haloperidol-induced vacuous chewing in animal model, when Ashwagandha (100 and 200 mg, p. o.) was administered concomitantly with haloperidol for 28 days (Bhattacharya et al. 2002). Vacuous chewing movements in rats are widely accepted as an animal model of tardive dyskinesia. Chronic treatment with Ashwagandha root extract for a period of 4 weeks to reserpine-treated animals (TD-induced animals) significantly and dose-dependently (50 and 100 mg/kg) reduced the reserpine-induced vacuous chewing movements and tongue protrusions and reversed reserpine-induced retention deficits. It also significantly reversed the reserpine-induced decrease in brain SOD and catalase levels in rats. Thus, Ashwagandha root extract could be a potential drug for the treatment of drug-induced dyskinesia (Naidu et al. 2006). 3-Nitropropionic acid (3-NP)-induced HD animal model has also been used to

investigate the effects of Ashwagandha root extract. Ashwagandha extracts (100 and 200 mg/kg) for a period of 2 weeks dose-dependently improved 3-NP-induced behavioral, biochemical, and enzymatic changes via its antioxidant activity (Kumar and Kumar 2009).

Antidepressant Activity from Ashwagandha Total alkaloid extract from the roots of Ashwagandha has been studied for its effects on the CNS (Malhotra et al. 1965). Ashwagandha exerted a mild depressant tranquilizer effect on the CNS in monkeys, cats, dogs, albino rats, and mice. Effects of sitoindosides VII–X and withaferin isolated from aqueous methanol extract of roots of Ashwagandha enhanced acetylcholinesterase (AChE) activity in the lateral septum and globus pallidus and decreased AChE activity in the vertical diagonal band in male Wistar rats. There was increase in cortical muscarinic acetylcholine receptor capacity which might partly explain the cognition-enhancing and memory-improving effects of Ashwagandha extracts in animal models and human studies (Schliebs et al. 1997). The anxiolytic and antidepressant actions of the bioactive glycowithanolides from Ashwagandha roots have been studied by Bhattacharya et al. (2000). There was a dose-related reversal of the stress effects as evident by augmentation of SOD and LPO activities and enhanced activities of CAT and GPX, lending support to the clinical use of Ashwagandha as an antistress adaptogen (Bhattacharya et al. 2001). The neuroprotective effects of Ashwagandha were studied on stressed adult female Swiss albino rats. Treatment with root extract of Ashwagandha (Stresscom capsules, Dabur India Ltd) significantly reduced the number of degenerating cells in the brain (Jain et al. 2001). In another study, the Ashwagandha extract was examined to retard or reverse excitotoxic neuronal injury induced by kainic acid (KA) in female Swiss albino mice. Ethanolic extract of Ashwagandha mitigated the effects of excitotoxicity and oxidative damage in the hippocampus possibly due to its antioxidative properties (Parihar and Hemnani 2003). Ashwagandha extract has been shown reducing oxidative damage in the cortex and hippocampus induced by streptozotocin (STZ) in diabetic mice model, possibly via antioxidative mechanisms (Parihar et al. 2004).

In a study, to explore the underlying molecular mechanism of neuroprotective action of the root extract of Ashwagandha, restrain-induced stress model was used (Bhatnagar et al. 2009). Activity of NADPH diaphorase (NADPH-d) and factors (acetylcholine, serotonin, and corticosterone), which regulate NADPH-d activity, were studied. Treatment with Ashwagandha extract significantly reversed the stress-induced NADPH-d activation. It was proposed that inhibition of NADPH-d by Ashwagandha was not a direct effect of extract on NADPH-d; instead, it was inhibited via suppressing corticosterone release and activating choline acetyltransferase, which in turn increase serotonin level in the hippocampus to inhibit NADPH-d. Thus, the main mechanism underlying the neuroprotective effects of Ashwagandha could be attributed to its role in the downregulation of nNOS and neurochemical alterations of specific neurotransmitter systems. Ashwagandha extract could also suppress glucocorticoid release in chronic stress which could be

exploited for treatment of neurodegenerative disease like Alzheimer's as well as in oxidative stress (Bhattacharya et al. 2001; Bhatnagar et al. 2009). Therefore, Ashwagandha constituents withanone, sominone, withanolide A, withanoside IV, and withanoside VI are important candidates for the therapeutic treatment of neurodegenerative diseases such as PD, AD, and HD (Tohda 2008; Konar et al. 2011).

Antianxiety and Anti-inflammatory Activity of Ashwagandha Recently, we have reported the anxiolytic effect of water extract from leaves of *W. somnifera* (ASH-WEX) in acute sleep-deprived female Wistar rats (Manchanda et al. 2017; Kaur et al. 2017). Sleep deprivation often leads to anxiety disorders and depression. Pretreatment with water extract of Ashwagandha leaf (ASH-WEX) on anxiety-like behavior in acute sleep-deprived rats was seen to ameliorate the anxiety-like behavior in these acute sleep-deprived animals. Oral feeding for 2 weeks with ASH-WEX was observed to enhance cell survival and reduce apoptosis in the brain. Similarly in another in vivo study from our lab, administration of leaf powder of *W. somnifera* was instrumental in ameliorating anxiety in high-fat diet-induced obesity model (Manchanda and Kaur 2017). Rats were divided into four groups: normal low-fat diet (LFD) on regular chow, high-fat diet (HFD) group on feed containing 30% fat by weight, low-fat diet plus extract (LFDE) group on regular chow supplemented with dry leaf powder of Ashwagandha 1 mg/g body weight (ASH), and high-fat diet plus extract (HFDE) group on diet containing high-fat diet supplemented with ASH for 12 weeks. Rats on HFD showed high level of anxiety in the elevated plus maze test, which was linked to the occurrence of reactive gliosis and inflammation as indicated by marked increase in the expression of specific markers. Hence, all the abovementioned studies scientifically validate the anxiolytic properties of *W. somnifera*, which are summarized in the graphical abstract (Fig. 1). The effects of *W. somnifera* have been found to be equivalent to those of the chemical drugs already being used in clinical setting. So, this plant may serve as an effective dietary supplement for management of stress and associated functional impairments.

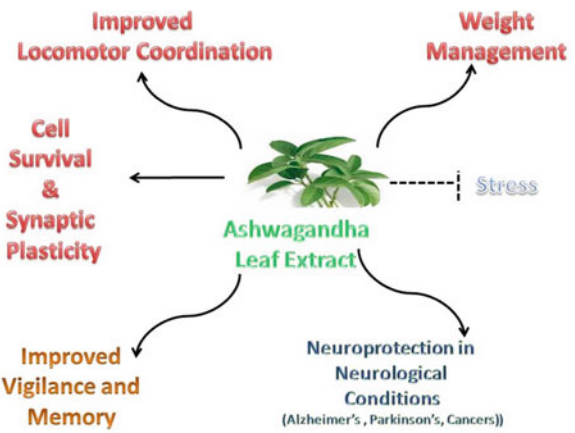

Fig. 1 Graphical abstract depicting multisite mode of action of *Withania somnifera* in neuroprotection. ASH-WEX mediates its neuroprotective role by modulating learning and memory functions, synaptic plasticity, cell survival pathways, motor coordination, body weight, and stress management

Tinospora cordifolia in Ayurvedic Practice

The herbaceous climber of family Menispermaceae, *T. cordifolia*, is commonly known as "amrita," "giloy," or "guduchi." Owing to its high medicinal values, this plant has been described as "heavenly elixir" in Ayurveda. *T. cordifolia* is frequently used in Ayurvedic formulations for its general body and mental health-promoting properties. It is used as an adaptogen to improve general body and mental health along with other herbal medicines in various herbal decoctions (Nair et al. 1992; Guruprasad et al. 2010, Saha and Ghosh 2012). For a long time, the extract of this plant has been used as a remedy for jaundice and hepatoprotective against various toxic agents such asCCl_4 and lead (Bishayi et al. 2002; Sharma and Pandey 2010). This plant possesses antidiabetic (Agrawal et al. 2012), cardioprotective (Rao et al. 2005), and antipsychotic (Jain and Shete 2010) properties and has been in use as a remedy for jaundice. Extract of this plant is a rich source of biochemicals with potential therapeutic value in treating dysregulated carbohydrate metabolism which causes diabetes (Reddy et al. 2009; Patel and Mishra 2011; Nadig et al. 2012). Various bioactive components belonging to different classes of compounds such as alkaloids, diterpenoids, lactones, glycosides, steroids, sesquiterpenoid, phenolics, aliphatic compounds, and polysaccharides have already been isolated from *T. cordifolia*.

T. cordifolia is a rich source of antioxidants which induces drugs/carcinogen metabolism and protects from chemotoxicity (Singh et al. 2006; Chaudhary et al. 2008; Dhanasekaran et al. 2009; Hamsa and Kuttan 2012). Importantly, this plant protects normal cells from negative effects of radiations (Subramanian et al. 2003; Sharma et al. 2011) but induces radiosensitivity to cancerous cells (Rao and Rao 2010). Both chemoprotective and radioprotective potentials of *T. cordifolia* have been attributed to its strong antioxidant and free radical-scavenging activity (Goel et al. 2002, 2004).

T. cordifolia in Neuroprotection

T. cordifolia is categorized under Medhya Rasayanas in Ayurveda, meaning herbs that help in improving memory and cognition. Extract of *T. cordifolia* is an important ingredient of Ayurvedic decoction used for enhancing memory (Kulatunga et al. 2012). It is very useful in enhancing focus and memory in children (Sarokte and Rao 2013) as it facilitates inhibitory GABAergic mechanism and inhibits the activity of monoamine oxidase resulting in mood modulation in depression-like behavior model system (Dhingra and Goyal 2008; Deole et al. 2011). Apart from modulating inhibitory mechanism, extract of *T. cordifolia* modulates expression of Ca^{2+}-dependent/calmodulin proteins such as calcineurin (CaN) and CamKII-α which play critical role in the maintenance of LTP (Mishra et al. 2016). It helps in calming and relaxing the nervous system and modulates the expression of pro-inflammatory cytokines along with COX-2 and iNOS and inflammatory cytokines like TNF-α, IL-1β, and IL-6. The diseases like asthma and certain kinds of edema-heightened inflammatory reactions result in hyperresponsiveness which may lead to

anaphylactic shock. Regular dosing of *T. cordifolia* extract reduces hyperresponsiveness in these diseases (Tiwari et al. 2014).

In recent studies from our lab, 50% alcoholic extract from the stem of *T. cordifolia* (TCE) was observed to prevent reactive gliosis and microgliosis by inhibiting overexpression of astroglial cell-specific protein GFAP and microglial cell activation marker proteins like integrin alpha-M MHC class I and class II proteins (Mishra et al. 2016). Even in neurodegenerative disease like Parkinson's disease, systemic treatment with the extract of this plant is reported to enhance level of the dopamine and complex I activity. Its strong antioxidant activity ameliorated the oxidative stress and improved locomotion (Kosaraju et al. 2014). Our lab also recently reported a study on the effect of TCE in acute sleep-deprived animal model, and the data revealed that *T. cordifolia* modulate stress-induced expression of synaptic plasticity proteins like PSA-NCAM, NCAM, and GAP-43 and also proteins like SNAP25, which is a SNARE complex protein and plays a role in neural signal transmission (Mishra et al. 2016). Further, its neuroprotective activity was mediated by activation of cell survival pathways and inhibition of apoptotic pathways. The data suggests that TCE may help in managing mental stress and improving memory and cognition (potential beneficial effects are summarized in the graphical abstract in Fig. 2). In this context, *T. cordifolia* may provide safe, more

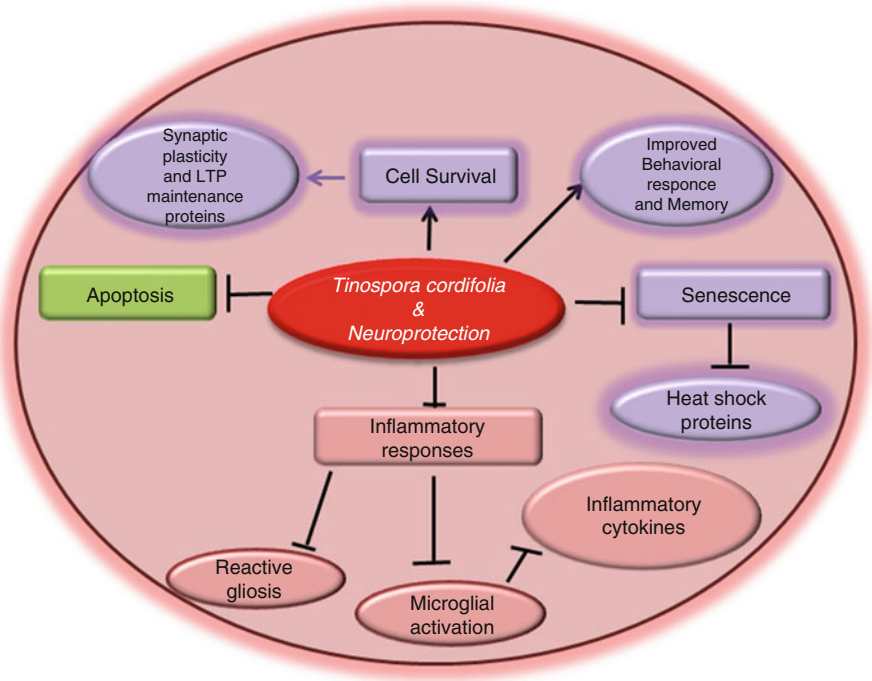

Fig. 2 Graphical abstract depicting pleiotropic mechanism of *Tinospora cordifolia* in neuroprotection. TCE mediates its protective role by activating cell survival and plasticity pathways and anti-apoptotic and anti-inflammatory pathways

beneficial, and cost-effective options to the expensive drugs that are generally accompanied by severe side effects.

T. cordifolia in Cancer and Immunomodulation

The multistep development process of tumor or any type of cancers involves sustained molecular mechanisms for the continued proliferation, evasion from growth inhibition, resisting cell death, induced angiogenesis, and finally metastasis. Cancers are result of either loss-of-function mutations in tumor suppressor genes like PTEN, p53, p16, IDH1, IDH2, etc. (Yan et al. 2009) or gain-of-function mutations in proto-oncogenes leading to recruitment of the factors which establish a microenvironment conducive for the proliferation and survival of cancer cells. Decades of research in oncology has led to the advancement of conceptual understanding of the molecular mechanisms involved in initiation and development of cancer. Generally, tumors display striking heterogeneous nature of cells. Therefore, even after surgical removal followed by chemo- and radiotherapy, their multifarious histological composition along with diffuse infiltration in surrounding tissues further complicates their treatment, which results in more aggressive relapse. The unruly development of resistance to these therapies limits their prospects. Further, severe side effects of these treatments in the patients render them to suffer with additional secondary complications. Certain drugs such as Gliadel (carmustine-loaded polymers) have been found to be highly effective in controlling the progression of tumor growth, but these effects are accompanied by frequent episodes of cerebral edema, wound infections, and breakdown in the patients (Westphal et al. 2003). Similarly, certain standard alkylating agents like temozolomide treatment lead to induced expression of DNA repair enzyme methylguanylmethyltransferase (MGMT) which further induces development of drug resistance in these cancer cells (Hegi et al. 2005).

Several studies in recent decade have shown potent anticancer property of crude as well as pure compounds obtained from *T. cordifolia* in different in vitro and in vivo model system. Initially, Chintalwar et al. (1999) isolated an arabinogalactan polysaccharide from *T. cordifolia* which was found to activate polyclonal B cells independent of macrophages. Further, aqueous ethanolic extract of *T. cordifolia* has been shown to inhibit angiogenesis by inducing production of anti-angiogenic factor IL-2 and inhibiting pro-angiogenic factors in B16F10 melanoma cells (Leyon and Kuttan 2004). Several polysaccharide compounds present in the extract of *T. cordifolia* induce the activity of tumor-associated macrophage-derived dendritic cells which results in enhanced tumor cytotoxicity and prolonged survival of tumor-bearing mice (Singh et al. 2005). In further studies with the Dalton lymphoma ascites and Ehrlich ascites, carcinoma-bearing mice have shown that treatment with extract of *T. cordifolia* to these mice reduced the volume of peritoneal ascetic fluid (Jagetia and Rao 2006). Among organic solvent extracts of *T. cordifolia*, hexane and chloroform fractions were found to be highly toxic for cancer cells (Mishra and Kaur 2013). Especially hexane fraction induces DNase-mediated apoptosis in the cancer cells which is activated by caspase-3 (Thippeswamy and Salimath 2007).

Even other organic solvent extracts like dichloromethane (DCM) extract of this plant possess potent antiproliferative property and have been found to induce cytotoxicity in very aggressive HeLa cancer cells (Jagetia and Rao 2006). Possibly, the anti-angiogenic property of *T. cordifolia* is the result of the presence of a long-chain aliphatic alcohol octacosanol which in peritoneal tumors has shown to downregulate the expression of VEGF leading to suppressed angiogenesis (Thippeswamy et al. 2008). Hence, the anticancer property of *T. cordifolia* is the result of its simultaneous targeting of several pathways at the same time. In in vivo system, *T. cordifolia* inhibits cell proliferation, induces apoptotic pathways, and increases immunogenicity of the immune cells, i.e., lymphatic and macrophage cells for cancer cells (Raghu et al. 2009; Velazquez et al. 2009; Koppada et al. 2009; Sengupta et al. 2011; Sharma et al. 2012; Aranha et al. 2012). It has also been reported to protect mice from endotoxic shock by modulating cytokines and nitric oxide release (Desai et al. 2007) and to increase the immunogenicity of the dendritic cells (Pandey et al. 2012).

T. cordifolia is also reported to decrease colony-forming capacity of cancer cells by inducing expression of genes involved in apoptosis and intracellular ROS level (Ansari et al. 2017). Our lab has recently reported the anti-brain cancer activities from chloroform and hexane extracts from TCE, and the data suggests that *T. cordifolia* is a potential candidate for differentiation-based therapy of tumors such as glioblastomas and neuroblastomas (Mishra and Kaur 2013 and 2015). As the cancer cell transformation is the result of alteration in differentiation and the self-renewal pathways, resetting this machinery results in reversal of dedifferentiated malignant phenotype of the cancerous cells to the terminally differentiated healthy cells. The terminal differentiation results in complete check on proliferation accompanied by growth arrest, senescence, and ultimately apoptosis without causing internal toxicity. The differentiation-based therapy is especially advantageous in cancers of the nervous system.

Conclusion

A large number of FDA-approved drugs such as morphine, atropine, eugenol, reserpine, etc. are derived from plants (Rajendrana and Balachandarb 2013; Godara et al. 2014; Baliga et al. 2016). Several bioactive compounds have been identified till now which are being used to treat various types of abnormalities. Among these compounds, *Ginkgo biloba* for cure of dementia and cognitive impairment; withanolides and other glycolides isolated from *Withania somnifera* as anti-inflammatory, anticancer, and memory-enhancing compounds; curcumin isolated from *Curcuma longa* for its anti-inflammatory properties; and *Lycium barbarum* for age-associated diseases are some of the compounds that have been identified to treat various ailments (Chainani-Wu 2003; Yu et al. 2005; Shah et al. 2009; Pawar et al. 2011; Gautam et al. 2013; Hashiguchi et al. 2015; Gupta and Kaur 2016; Manchanda et al. 2017). Recently, FDA has also approved various natural compound-derived drugs such as galantamine for dementia and Alzheimer's disease; paclitaxel for breast cancer; cabazitaxel for hormone refractory metastatic prostate cancer;

nabilone for chemotherapy-induced nausea; artemether, lumefantrine, and chloroquine for malaria; colchicine for gout; and many more (Kinghorn et al. 2011).

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Phytosomes: The Novel Drug Delivery System for Phytomedicine

Jubilee Purkayastha and Jayita Ghosh

Introduction

The term "phyto" stands for plant, while "some" means cell like. Phytosome is a technology for producing lipid-compatible molecular complexes of drugs and nutraceuticals, standardized plant extracts or water soluble phytoconsituents so improving their absorption and bioavailability. The novelty of the phytosome process lies in the fact that it produces a little cell, thus protecting the herbal drug from destruction by digestive enzymes and gut bacteria. Because of the presence of water-soluble herbal drug and lipophilic outer layer, phytosomes are easily absorbed and thus become easily bioavailable and provide better actions than the conventional herbal extracts (Jain et al. 2010).

The safety, efficacy, cultural acceptability, and lesser side effects of phytomedicines are well accepted now. Broadly, herbal medicines are classified into three various categories, viz., traditional Chinese medicines, derived from traditional oriental medicine; Ayurvedic medicines, which are based on Ayurveda; and Western herbal medicines, which originated from Greece and Rome to Europe and then had spread to North and South America. Unlike synthetic drugs, herbal medicines provide better patient tolerance and are sourced from renewable resources, environment friendly, locally available, and the major source of new lead molecule generation. Herbal medicines are based on indigenous systems of medicine spanning over hundreds of years and are the amalgamation of therapeutic experiences of many generations of practicing physicians. Phytomedicines also offer therapeutics for which modern medicine has no complete remedy, e.g., age-related

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disorders like memory loss, osteoporosis, immune disorders, diabetes, cancer, etc. (Shelke 2012). Biomedicine is a multidisciplinary area of medicine concerning human medicine, veterinary medicine, biology, and technology. It involves the study of disease conditions using the methods of biology, chemistry, physics, and other sciences. Applied biomedicine can also offer the prospect of an improvement in the economy by managing the cost involved in disease cure and management, globally (Berger 2011). The range of application of medicinal plants has enhanced due to increasing knowledge of metabolic processes and effects of plants on human physiology.

The compositions, biological activities, and health-promoting benefits of numerous plant products have been established by phytochemical and phytopharmacological sciences, and now it is known that most of the biologically active constituents of plants are water-soluble molecules. Being water soluble, the phytoconstituents, viz., flavonoids, tannins, terpenoids, etc., are poorly absorbed in the system due to their large molecular size and poor lipid solubility. This challenge is now overcome through "phytosomes" which have better pharmacokinetic and pharmacological parameters and can be used in the treatment of the acute and chronic diseases (Jain et al. 2010).

Herbal Biomedicine and Drug Delivery Challenges: The Intervention of Phytosomes Technology

Since ancient times, almost all the medicines were plant derived, and the plant was the only source of cure for centuries.

Again herbals have made a comeback recently, and people are taking help of herbal therapies to treat various diseases. There are mainly four reasons for the growing recognition of herbal medicines:

1. Recent concern about the safety of synthetic drugs and also of surgery.
2. Inadequateness of many modern medicines to effectively treat many of the most common health conditions.
3. Herbal medicines in many cases have shown better results than drugs or surgery without the side effects.
4. Also, many current synthetic drug therapies are known to suppress the disease symptoms, without taking care of the underlying disease causes, but many phytomedicines are known to act on the cause of many diseases and thus cures from root (Devi et al. 2010).

The method by which a herbal or a synthetic drug is delivered can have a significant effect on its efficacy. Drugs of optimum concentration are required for maximum benefit, and concentrations above or below can be toxic or may be of no benefit. Controlling the pharmacokinetics, pharmacodynamics, nonspecific toxicity, immunogenicity, biorecognition, and efficacy of drugs is required for targeted drug delivery. Multidisciplinary approach to the delivery of therapeutics to targets tissues

is required for very effective treatment of severe diseases. Drug delivery system is the method by which an ideal amount of the concerned drug is administered to the patient in such a way that it reaches exactly the "site of action" and starts working on site. The new drug delivery strategies, often called drug delivery systems (DDS), are based on interdisciplinary approaches combining polymer science, pharmaceutics, bioconjugate chemistry, molecular biology, etc. (Charman et al. 1999). Different drug delivery and drug targeting systems are being developed to prevent harmful side effects, minimize drug degradation and loss, increase bioavailability, and ensure delivery of the drug accumulated to the target zone. There are two kinds of targeted drug delivery: active targeted drug delivery, such as some antibody medications, and passive targeted drug delivery, such as the enhanced permeability and retention effect (EPR-effect). The modern drug delivery system has been developed by researchers that address the limitations of the traditional drug delivery systems. The aim is to cure a particular disease by targeting exactly the affected zone and delivering the drug to that area (Musthaba et al. 2009).The various drug carriers include soluble polymers, microparticles made of insoluble or biodegradable natural and synthetic polymers, microcapsules, cells, cell ghosts, lipoproteins, liposomes, micelles, etc. A drug's success and failure depends on the mode of delivery of a drug (Devi et al. 2010). Polymers that release the drug at a controlled rate do sustained (or continuous) release of drugs over a period of time.

If herbal medicines, phytopharmaceuticals, or phytosomes are considered together, then we can get the benefit of herbs as well as novel drug delivery systems. This necessitates the incorporation of the novel drug delivery system in Indian Ayurvedic medicines to combat serious diseases. Researchers are developing novel drug delivery systems like transdermal dosage forms, sustained and extended release formulations, mouth dissolving tablets, microparticles, microcapsules, etc. from herbs (Devi et al. 2010).

Phytopharmaceuticals are easily and more readily metabolized by the body and produce lesser side effects and enhanced absorption in the bloodstream, whereas chemical-based pharmaceuticals result in adverse side effects from minor headaches to severe ones which can be potentially lethal (Norman 2001). India is known to have a vast knowledge base of Ayurveda whose potential is only being unfolded in the recent years, but the drug delivery system used for administering the Ayurvedic medicine is still an old one. In the case of Ayurvedic medicines, there is a great possibility that the active ingredients may be destroyed or may be metabolized by the liver before reaching the blood resulting into lesser efficacy.

"Phytosomes," the lipid-based drug delivery systems, have shown their potential in controlled and targeted drug delivery. Phytosomes add better biopharmaceutical properties to the drug, thus resulting in improved bioavailability. Phytosomes also called phytolipid delivery system are known as novel compounds comprising of lipophilic complexes of plant origin with phospholipid (Semalty et al. 2009).

Phytosomes are produced by a process in which individual component of herbal extract like flavonolignans and terpenoids are bound on a molecular level to the phospholipids like phosphatidylcholine through a polar end. Phytosomes are an

advanced form of herbal extract having high lipophilicity and improved bioavailability and therapeutic properties and form a bridge between the conventional drug delivery system and novel drug delivery system.

What Is a Phytosome: Advantages

Herbal preparations of plants or parts of them were widely used since ancient times, and till today the use of phytomedicines is well accepted in most parts of the world. Many chemical and pharmacological studies have been completed for a number of plant-based herbal preparations with a purpose to know their chemical composition and confirm their safety and efficacy. However, there are a few concerns regarding use of phytoconstituents. Most of the bioactive constituents of phytomedicines are flavonoids which are poorly absorbed. The poor absorption of flavonoid is likely due to two reasons, viz., presence of multiple-ring molecules that are too large to be absorbed by simple diffusion and poor mixing of flavonoid molecules with oils and other lipids, which limits their ability to pass across the lipid-rich outer membranes of the enterocytes of the small intestine. Water-soluble flavonoid molecules can be converted into lipid-compatible molecular complexes, aptly called phytosomes.

Phytosomes are produced when the active ingredients of an herb are bound to the phospholipids on a molecular level. The phospholipid part includes a water-soluble head and two fat-soluble tails for which it acts as an effective emulsifier. The advantage of phytosomes is that they are better absorbed in the system and produce better results than conventional herbal extracts. Chemical analysis indicates that a phytosome is usually a flavonoid molecule linked with at least one phosphatidylcholine molecule. A bond is formed between these two molecules, creating a hybrid molecule. This highly lipid-miscible hybrid bond is better suited to merge into the lipid phase of the enterocyte's outer cell membrane. The phytosome process produces a little cell, thus protecting the active ingredients of the herbal extract from destruction by enzymes and gut bacteria, and their capability to transit from a hydrophilic environment to a lipophilic environment enables them to easily reach the blood and be thus more effective (Shelke 2012).

Phytosomes as a new advanced modern drug delivery technology are used for better absorption and efficacy of conventional herbal extracts. This phytosome technology results in marked improvement of bioavailability, significantly greater medical benefit, and guaranteed delivery to the tissues without compromising nutrient safety. Also, use of phytosomes results in improved pharmacokinetic and pharmacological parameters which are advantageous in the treatment of acute diseases as well as in pharmaceutical and cosmetic compositions (Patela et al. 2009).

The phytosome process has been applied to many popular herbal extracts like *Ginkgo biloba*, grape seed, hawthorn, milk thistle, green tea, ginseng, etc. As described above, the flavonoid and terpenoid components of these herbal extracts directly bind to the phosphatidylcholine present in the cell membrane. The choline head of the phosphatidylcholine molecule binds to these compounds, while the

fat-soluble phosphatidyl portion then covers the choline-bound material, resulting in a little microsphere (Shelke 2012).

Phytosome: Preparation and Properties

Preparation of Phytosomes

Phytosomes are prepared by reaction of preferably one mole of a natural or synthetic phospholipid, such as phosphatidylcholine, phosphatidylethanolamine, or phosphatidylserine, with one mole of a component, for example, flavonolignans, either alone or in the natural mixture in aprotic solvent such as dioxane or acetone to form a complex. The complex is then isolated by precipitation with a nonsolvent such as aliphatic hydrocarbons or lyophilization or by spray drying. Generally, the most preferable ratio of phospholipid to flavonoids is 1:1.

The common stages for preparation of phytosomes are shown in Fig. 1.

In preparing phytosomes, flavonoids are selected from the group consisting of quercetin, kaempferol, quercretin-3, rhamnoglucoside, quercetin-3-rhamnoside, hyperoside, vitexine, diosmine, 3-rhamnoside, (+) catechin, (-) epicatechin, apigenin-7-glucoside, luteolin, luteolinglucoside, ginkgonetine, isoginkgonetine, and bilobetine. Phospholipids are selected from the group consisting of soy lecithin, bovine or swine brain or dermis, phosphatidylcholine, phosphatidylethanolamine, and phosphatidylserine in which the acyl group may be the same or different and mostly derived from palmitic, stearic, oleic, and linoleic acid. Flavonoids are insoluble in

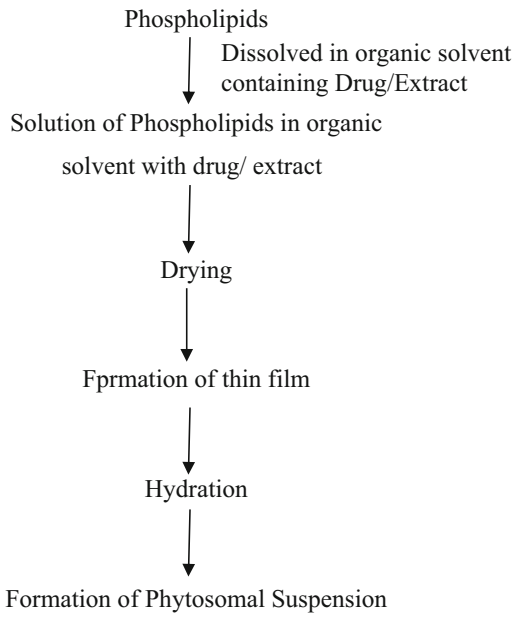

Fig. 1 Common stages for preparation of phytosomes (Patela et al. 2009)

chloroform, ethyl ether, or benzene, but after formation of phytosomes which form a stable complex, they become extremely soluble in these solvents (Shelke 2012).

Properties of Phytosomes

Chemically, a phytosome is a complex of a natural product and natural phospholipids, which is obtained by reaction of stoichiometric amounts of phospholipid and the substrate in an appropriate solvent. Spectroscopic studies revealed that the main phospholipid-substrate interaction is due to the formation of hydrogen bonds between the polar head of phospholipids (i.e., phosphate and ammonium groups) and the polar functionalities of the substrate. In presence of water, phytosomes assume a micellar shape which appears like liposomial-like structures. Biologically, phytosomes are superior forms of herbal products that are easily absorbed and utilized and thus produce better results than conventional herbal extracts, with increased bioavailability (Kumar et al. 2017).

Phytosomes are not to be confused with liposomes. Mixing a water-soluble substance with phosphatidylcholine forms a liposome where there is no chemical bond, and there may be innumerable phosphatidylcholine molecules surrounding the water-soluble compound, whereas in the case of phytosomes, depending on the constituent, the phosphatidylcholine and the individual phytoconstituent form a 1:1 or a 2:1 complex (Suryawanshi 2011).

The difference between a phytosome and a liposome is shown in Fig. 2 and Table 1.

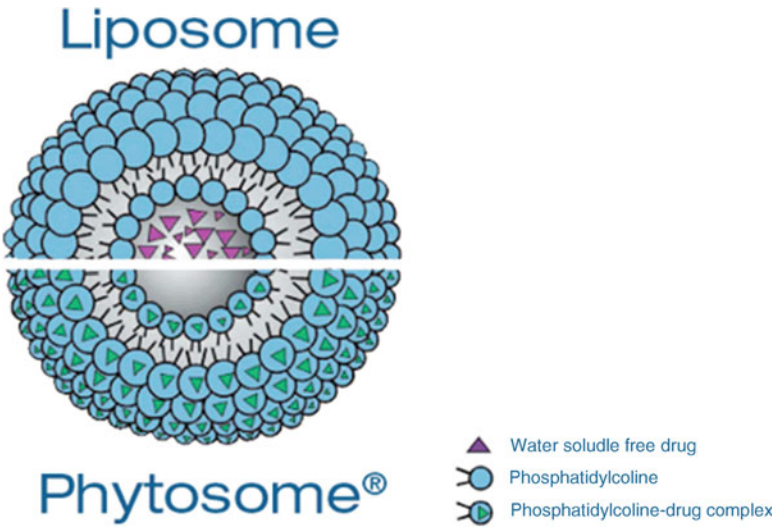

Fig. 2 Difference between phytosome and liposome (Source: https://www.phytosome.info/phyto-vs-lipo.html)

Table 1 Difference between phytosome and liposome (Gandhi et al. 2012)

Property	Phytosome	Liposome
Bonding	It is a unit of few molecules bonded together	It is an aggregate of many phospholipid molecules that encloses other phytoactive molecules without specifically bonding with them
Bioavailability and absorption	It has much better bioavailability and absorption	Its bioavailability and absorption are lesser than the phytosome
Arrangement of molecules	In phytosomes, phospholipid (phosphatidylcholine) and an individual phytoconstituent are present in 1:1 or 2:1 ratio depending on the substance	In liposomes, hundreds and thousands of phosphatidylcholine molecules surround the water-soluble molecules

Liposomes are considered as delivery vehicles only without any role as dietary supplements, whereas phytosomes have been proven as a breakthrough model with better absorption, bioavailability, assured delivery to the tissues, and superior efficacy and are already in application for many standardized flavonoid preparations.

Potential of Phytosomes as a Novel Drug Delivery System: Applications

Phytosomes are used in curing various diseases like liver disease, heart disease, etc. Many other uses of phytosomes include anti-inflammatory, lipolytic, vasokinetic, anti-edema agent, etc. In addition, it is used as a nutraceutical, immunomodulator, antioxidant, etc. Yanyu et al. (2006) demonstrated that bioavailability of silybin in rat was increased extraordinarily after oral administration of prepared silybin-phospholipid complex. Similarly, Tedesco et al. (2004) showed that in protection against the toxic effect of aflatoxin B, silymarin phytosome shows better antihepatotoxic effect than silymarin alone. Similarly, quercetin phospholipids complex exerted better therapeutic efficacy than the molecule in rat liver injury induced by carbon tetrachloride (Maiti et al. 2005).

The various advantages and applications of phytosomes are mentioned below:

1. Phytosome permeates the nonlipophillic botanical extract, making botanical extracts better bioavailable
2. Phytosome works in small quantity to give desired results and is widely used in cosmetics due to their better skin penetration.
3. Phytosome finds applications in giving liver-protectant flavonoids due to their easy bioavailability.
4. The phytosome process gives rise to little cells whereby the costly components of the herbal extract are protected from damage by digestive secretions and gut bacteria.

5. Phytosomes are used in anti-inflammatory formulations, pharmaceuticals, and cosmetic formulations. Phytosomes are also used to treat acute and chronic liver disease.
6. Phytosomes are also used as cancer chemopreventive agent, antioxidant, brain stimulant, immunomodulator, skin-improving agent, anti-wrinkle and antiaging supplement, antihypertensive agents, etc. (Dhyani and Juyal 2017). The therapeutic applications of different phytosomes are shown in Table 2.

Table 2 Therapeutic applications of different phytosomes (Patela et al. 2009)

Phytosomes	Phytoconstituent complexed with PC	Daily dosage	Indications
Leucoselect® Phytosome	Procyanidolic oligomers (PCOs) from grape seeds	50–100 mg	Systemic antioxidant, specific. Best choice for most people under the age of 50. Also specific for the eyes, lungs, diabetes, varicose veins, and protection against heart disease
Greenselect® Phytosome	Epigallocatechin 3-O-gallate from *Camelia sinensis* (green tea)	50–100 mg	Systemic antioxidant. Best choice for protection against cancer and damage to cholesterol
Ginkgoselect® Phytosome	24% gingko flavonol glycosides from *Gingko biloba*	120 mg	Best choice for most people over the age of 50. Protects the brain and vascular lining
Silybin Phytosome	Silybin from silymarin (milk thistle)	120 mg	Best choice if the liver or skin needs additional antioxidant protection
Siliphos™ Thistle Phytosome	Silybin from silymarin	150 mg	Good choice for liver or skin support
Hawthorn Phytosome	Flavonoids	100 mg	Best choice in heart disease
Panax ginseng Phytosome	37.5% ginsenosides from roots of *Panax ginseng*	150 mg	As a food product
Glycyrrhiza Phytosome	18-beta glycrrhetinic acid	–	Anti-inflammatory activity
Mirtoselect® Phytosome	Anthocyanosides from an extract of bilberry	–	These improve capillary tone, reduce abnormal blood vessel permeability and are potent antioxidants. They hold great potential for the management of retinal blood vessel problems and venous insufficiency
Salabselect® Phytosome	An extract of saw palmetto berries through supercritical CO_2 extraction	–	It delivers fatty acids, alcohols, and sterols that benefit prostate health. Also beneficial for noncancerous prostate enlargement

(continued)

Table 2 (continued)

Phytosomes	Phytoconstituent complexed with PC	Daily dosage	Indications
Polinacea™ Phytosome	Echinacosides and a unique high molecular weight	–	It enhances immune function in response to a toxic challenge
	Polysaccharide from *Echinacea angustifolia*		
Oleaselect™ Phytosome	Polyphenols from olive oil	–	As potent antioxidants, inhibit harmful oxidation of LDL cholesterol and also have anti-inflammatory activity
Lymphaselect™ Phytosome	A standardized extract of melilotus officinalis	–	Indicated for venous disorders, including chronic venous insufficiency of the lower limbs

Conclusion

Herbal medicines have been widely accepted globally since ancient times and have been recognized for their better therapeutic value and extremely lesser adverse effects as compared with modern allopathic medicines. At the same time, there are many challenges associated with herbal drugs like biological standardization; pharmacological and toxicological evaluation; investigation of sites of action/absorption, safety, toxicity, legal, and regulatory aspects of herbal drugs; etc. Moreover, Ayurvedic drugs/phytotherapeutics need a suitable delivery system for the active components to the target site to increase efficacy for which novel drug delivery systems are the most desirable one. Novel drug delivery systems like phytosomes not only reduce the repeated administration of drugs but also help to increase the therapeutic value by reducing toxicity and increasing bioavailability. Phytosomes form a connection between the conventional drug delivery system and novel drug delivery system. Phytosomes are used as a medicament and have wide scope in medical sciences, and many more areas of phytosome applications are to be revealed in the future in the view of pharmaceutical application.

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