Chapter 1
Medicinal Plants Domestication, Cultivation, Improvement, and Alternative Technologies for the Production of High Value Therapeutics: An Overview

Kishan Gopal Ramawat and Jaya Arora

Abstract Medicinal plants are source of several valuable drugs known as natural products or secondary metabolites. Only a handful of medicinal plants are cultivated while most of them are still collected from wild. Due to the high demand for these products, over-exploitation resulted in endangering the species, loss of biodiversity, adulteration of plant materials and products, and the effect on ecosystem. Plants and plant products are used in many traditional medicines for several centuries. To meet the demand of raw plant material for direct use or industrial use, agrotechnologies have been developed for several medicinal plants, alternative biotechnologies (micropropagation, production in cell cultures grown in shake flasks and bioreactor, transfer of gene/s in plant and microbes, modification of biosynthetic pathways, etc.) and microbial production system have been attempted. Understanding seed and floral biology, development of agrotechnologies and introduction into new habitat may improve the availability of raw medicinal plant material associated with the improved downstream process can affect high recovery. Similarly, the use of sophisticated detection methods, high throughput screening methods, genomics and proteomics can through light on genes involved, types of biomolecules, and new sources of known drugs. Biotechnological methods (elicitation, immobilization, cloning of selected strains, hairy root cultures, and gene manipulation) including gene editing can help in improvement in the production system. With ever-increasing population and reliability of herbal medicine, demand for medicinal plants continues to increase; hence, domestication of plants along with new technologies is a demand of time to meet the challenge of supply of uniform raw material. This brief overview presents state of research on medicinal plants and their products.

Keywords Medicinal plants · Plant biotechnology · Secondary metabolites · Agrotechnology · Domestication

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1.1 Introduction

Plants are called medicinal because of the present of certain secondary metabolites in them which impart profound physiological effect on the mammalian system. These compounds impart curative, additive, or synergistic effects on human health. Though these may be present in the whole plant body, higher concentrations may be present in one or many parts such as stem, root, bark, seeds, or leaves. Though secondary metabolites (not involved in metabolism) are considered as waste products of the plant, many evidences are produced to demonstrate them as plant growth modulators or reused by the producer plant as primary metabolites (involved in primary metabolism). Secondary metabolites are also involved in plant defence (Erb and Kliebenstein 2020). In developing countries, most of the medicinal plants are collected from the wild. In Europe, out of 1300 species used, about 80% medicinal plants are collected from wild (Ramawat 2019a; Ramawat and Goyal 2008; Balunas and Kinghorn 2005). Because cheap labour is involved, most of these materials are collected by illiterate labour resulting in problems of identification, deliberate adulteration, inappropriate part collection, and damaging to the plant and ecosystem. The consequence of uncontrolled collection and commercialization is habitat loss, the encroachment of land by alien species, and unavailability of medicinal plants. This puts pressure on the wild population of medicinal plants and the rate of disappearance has accelerated particularly in developing countries like India, China, Nepal, Kenya, Tanzania, and Uganda (Chen et al. 2016). Therefore, the development of cultivation practices, understanding floral and seed biology, improvement of secondary metabolites content in plants, and developing alternative technologies are ways to save medicinal plants from becoming endangered. It is important to introduce and domesticate wild plants for their sustainable utilization and ensure a continuous supply of uniform material for human welfare (Hua et al. 2018; Tanga et al. 2018; Ramawat 2019b).

In developing countries, herbal medicine is the backbone of the traditional systems of medicine like Ayurveda in India (4500–1600 BC), Chinese traditional medicines (3000 BC), Jamu (Indonesia 800 AD), Kampo (Japan 500 AD), Thai medicine (1200 AD) or Unani medicine (Astutik et al. 2019; He 2015; Ramawat and Goyal 2008; Sheehan and Hussain 2002). Most of these countries, where traditional medicine is used by a large number of people, have invested substantially in herbal research. Traditional systems of medicine, whether Indian Ayurveda or Chinese, are based on plant extract whereas allopathic system works on a pure active molecule. Thus, plants of interest to Ayurveda are different than those required for allopathic system (Ramawat and Goyal 2008). Traditional systems of medicine are safe, time tested, and cheap for the people. Traditional systems contain a wealth of information about use of medicinal plants and scientific study of this information has led to new knowledge about medicinal plants and several drug discoveries (Suntar 2019). With increase in living standards in the recent past, consumption of herbal drugs for wellbeing and longevity has increased tremendously. These countries are also major producers of herbal drugs (Vashist et al. 2016) which are further handled by traders, not trained or

educated in knowledge about medicinal plants (Barata et al. 2011). Because of high bulk is involved, now value addition is done by preparing and selling extract or active ingredients which involve good manufacturing practices (GMP), and many of them either not follow them or ignore. It is estimated that about 0.5 million tons of raw material comprising of about 60,000 medicinal, nutraceutical, and aromatic plants have been traded (WHO 2015). The world market for pharma and related compounds in 2022 will be about $1.12 trillion (Subramani 2018).

In this review, we summarize the importance of medicinal plants, efforts for their cultivation towards availability, alternative technologies for their production, conservation, and improvement. Biotechnological methods (elicitation, immobilization, cloning of selected strains, hairy root cultures, and gene manipulation) including gene editing will not only save the medicinal plants but also improve the quality of human life.

1.2 Historical Use of Medicinal Plants

Some of the landmarks in the description and use of medicinal plants are Ayurvedic, Egyptian descriptions, and Chinese medicine system where the time of collection, preparation of herbal medicine and dosage to be given are described in detail. Particular emphasis is given in Ayurveda about the collection and combination of herbs with minerals which is about 5000 years old. Cultivation and/or use of medicinal plants like *Cannabis sativa*, *Papaver somniferum*, and *Conium maculatum* is as old as human civilization. Modern herbal knowledge includes first pharmacopoeia by Greek physician Galen (129–200 AD), isolation of morphine from *P. somniferum*, quinine from *Cinchona* species, and pilocarpine from *Pilocarpus jaborandi* (Ramawat 2019a; Ramawat and Goyal 2008). Details of historical account and old literature describing medicinal plants are given elsewhere (Khan 2018; Petrovska 2012). Several human diseases in the history of human civilization are well documented, and plants are used as curative agents. Not only humans but animals and insects can recognize the presence of secondary metabolites in plants and their biosynthesis in plants or their neutralization by insects/animals, both have co-evolved with the evolution of these species (Ramawat and Goyal 2019). It is evident that old descriptions are about plant morphology and its usage, whereas (see Chap. 25) plant-based drugs were developed with the development of modern scientific tools and a strong base in chemistry. In the last 2–3 decades, metabolomics, molecular markers, high throughput screening, and genetic manipulations added new dimensions in the medicinal plant research.

1.3 Domestication of Medicinal Plants

Plants are sources for food, cloth and shelter, and humans have learned to select and cultivate plants for their needs. Domestication of plants and animals is an evolutionary process spreading over centuries involving farmers, crop adaptation, and its environment. Domestication involves changes in traits and is a slow co-evolutionary process leading to new species or cultivars. Domestication of plants and animals is described as greatest advance in development of human civilization. Various selection pressures lead to evolution of more desirable and suitable cultivars (Diamond 2002; Purugganan 2019). The process of natural selection and wide seed dispersal contributes significantly in the adaptation and evolution of crop plants. Out of 452,000 plant species, currently <500 is in cultivation. Therefore, domestication has given rise to a lesser germplasm pool and contributes towards loss of diversity.

The various centres of crop origin and plant domestication are MesoAmerica, the Southern Andes, the Near East, Africa (probably the Sahel and the Ethiopian highlands), Southeast Asia, and China, from where they spread to other regions. The probable centres of various present-day crop plants are presented in Fig. 1.1 from which it is evident that important crops were domesticated all over the world and related plant species were selected the man for their use.

It takes several years or decades to develop agrotechnologies for the cultivation of plants and understand seed and floral biology. Cultivation practices for various species evolved with human being's civilization and about 38% of the total land is used for cultivation purposes. Gepts (2014) defined the process of domestication

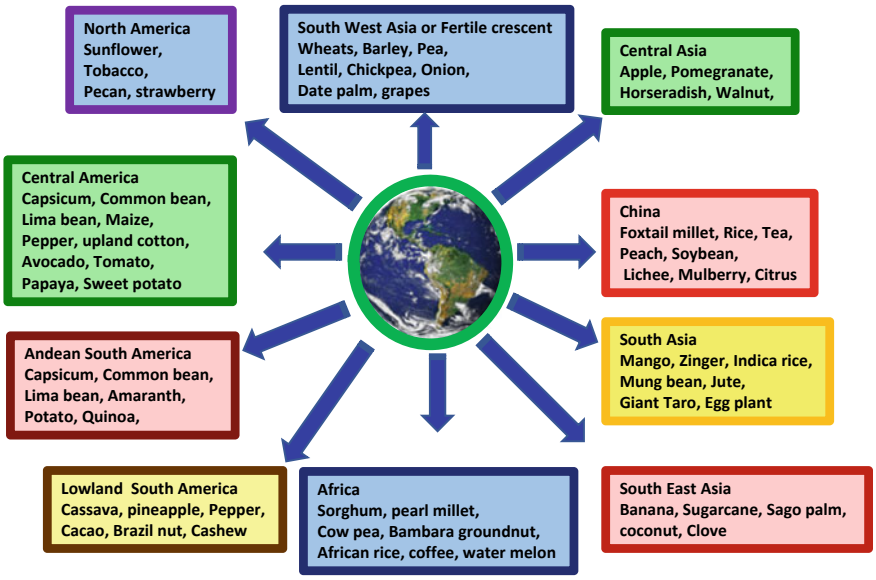


Fig. 1.1 Centres of agricultural crop origin and examples of plant species

as an "evolutionary process driven by natural and human (whether conscious or unconscious) selection applied to wild plants or animals and leading to adaptation to cultivation and consumption or utilization. Domestication can be complete, whereby organisms become entirely dependent on humans for their continued existence or can be partial or incipient, whereby they still reproduce independently of human intervention".

The domestication of plants followed by agriculture leads to the settlement of humans around such places. Therefore, the process of gathering, selecting, and cultivating started with the start of civilization at the end of recent ice-age during 10–12,000 years ago (Fuller et al. 2014). How, when, and where plants have been domesticated in time to time is a point of discussion in various disciplines. Inputs by archaeological and new molecular tools have helped in developing understanding about the relationship between wild and cultivated plants and their domestication (Larson et al. 2014). Man has always an attraction for better fruits and grains, colour, or size of fruits, and all these factors contributed to the slow process of selection and evolution leading to better-cultivated plants. Therefore, early breeding was based on simply visible traits. These characters provided improved fruit and seeds and enable use of technology for harvest, storage, and cultivation. Still very large variation in fruit size, taste, and colour exists in plants like tomato and brinjal demonstrating wide genetic variation within the species and model to trace wild relatives. Next-generation sequencing techniques are being employed to identify these conserved characters (alleles) in some crop plants for their exploitation (Li and Olsen 2016). Similarly, the uniform size of plants, branching, and non-seed shattering habits paved the way for mechanization in wheat and rice crops. During this, domestication, development, and selection of specific plant parts like cabbage (leaf), kale (leaf), cauliflower (unripe flower), and broccoli (unripe flower and stem) took place from a lean and thin mustard plant (*Brassica oleracea*) during the evolution process (Ramawat 2019a; Gepts 2014; Larson et al. 2014). Some examples of domestication of crop plants during last 12,000 years are: wheat, rice, pearl millet, sweet potato, and cotton (Fig. 1.2). Medicinal plants come after realization of primary need and man learned to use the plants for diseases and health. Cytogenetic and molecular markers are useful tools to compare and determine the wild relatives and genetic structure of the plants. At the beginning of domestication, wild germplasm is rich in diversity but development of homogeneous crops for cultivation may result in loss of valuable biodiversity (Chinthiya and Bhavyasree 2019).

India being a major producer and exporter of medicinal herb, a separate medicinal plant board has been established under the Ministry of Health and Family Welfare (a central government initiative). The worldwide demand for medicinal herbs and related products is estimated to be ~US $60 billion (Shepherd 2007). This board funds research and takes measure for the availability of medicinal herbs and provides 30, 50, and 70% subsidy for the development of agrotechnology (Table 1.1) and cultivation practices of medicinal plants (https://www.nmpb.nic.in/content/prioritised-list-medicinal-plants-cultivation). Similarly, other countries have prioritized plants for their local demand and domestication efforts for medicinal plants (Katumba et al. 2004). Selected examples of medicinal plants promoted by the Indian National

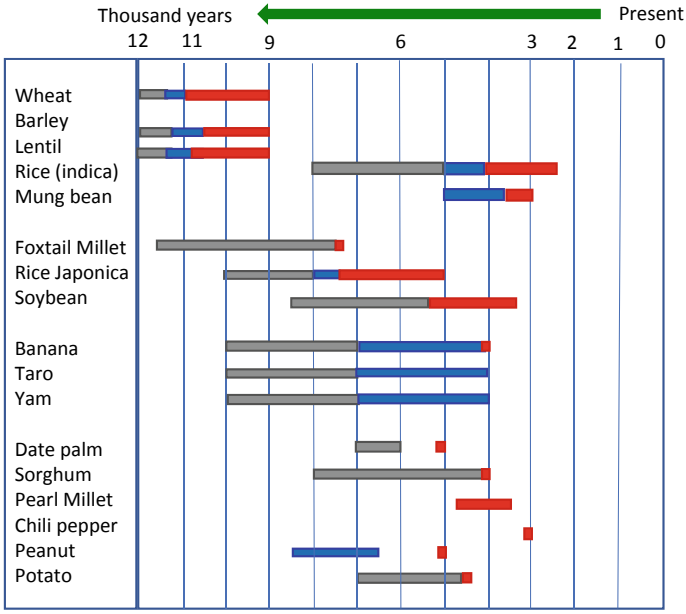


Fig. 1.2 Chronological chart listing the time frames over which a few selected examples of plants were domesticated. Grey bars ( ) represent documented exploitation before domestication, blue bars ( ) represent pre-domestication cultivation of plants, red bars ( ) represent the period over which morphological changes associated with domestication. Simplified and developed from Larson et al. (2014)

Medicinal plants board for domestication in India and worked out by the authors are presented in Table 1.2. For these plants, in vitro techniques were used to study seed biology, micropropagation, and production of secondary metabolites towards conservation. Similarly, the medicinal plants promoted in Pakistan are *Asparagus recemosus, Carum carvi, Rauvolfia serpentina, Atropa accumonicata, Valeriana jatamansi,* and *Linum usitatissimum*, (Sher et al. 2010), in Poland are roseroot (*Rhodiola rosea*, Weglarz et al. 2008), in Mexico are cultivars of *Agastache mexicana* (Carrillo-Galván et al. 2020), and in Israel are basil, caraway, chamomile, and dill (Dudai 2012). These plants were put for domestication based on size of vegetative or reproductive parts, their useful metabolites contents, and growth and yield parameters (Carrillo-Galván et al. 2020). Therefore, it is of utmost importance to develop agrotechnologies towards domestication of medicinal plants collected from wild. However, uniform cultivation of selected germplasm may result in permanent loss of biodiversity for some species (Ramawat 2019a). Improvement of germplasm of the opium poppy through hybridization is also a continuous process to develop varieties suitable for factory-based extraction to avoid illegal drug trafficking (Fig. 1.3). Note the differences in wild and cultivated plants.

Before supporting the cultivation practices, there is need to prioritize the medicinal plants based on several parameters of demand and supply as well as their biology,

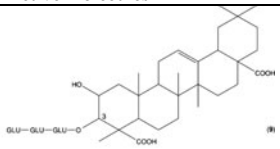
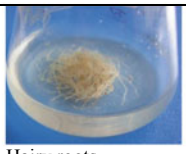
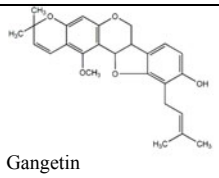
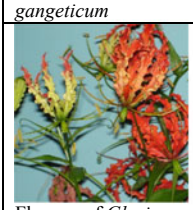
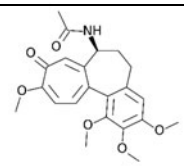
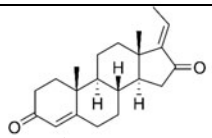
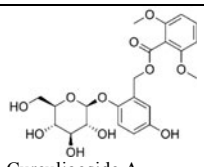
Table 1.1 Selected examples of medicinal plants promoted by the Indian medicinal plants board for development of agrotechnology towards domestication and cultivation

% Subsidy for cultivation cost	Selected examples of plant species promoted for cultivation and development of agrotechnologies towards domestication
30%	*Abrus precatorius* Linn., *Acorus calamus* Linn., *Andrographis paniculata* (Linn.) Burn, *Artemisia annua* Linn, *Asparagus racemosus* Willd., *Celastrus paniculatus* Willd., *Chlorophytum borivillianum* Sant., *Embelia ribes* Burm. f., *Emblica officinalis* Gaertn, *Gymnema sylvestre* R. Br., *Hemidesmus indicus* R.Br., *Plantago ovata* Forssk., *Psoralea corylifolia* Linn, *Rubia cordifolia* Linn., *Terminalia bellirica* Gaertn., *Terminalia chebula* Retz, *Vitex negundo* Linn., *Withania somnifera* (Linn.) Dunal
50%	*Acacia catechu* Willd., *Atropa belledona* Linn., *Desmodium gangeticum* (Linn.) DC., *Gloriosa superba* Linn, *Glycyrrhiza glabra* Linn, *Mesua ferrea* Linn., *Pueraria tuberosa* DC, *Pterocarpus marsupium* Roxb., *Rheum emodi* Wall., *Smilax* china Linn., *Valeriana wallichi* DC
70%	*Aconitum heterophyllum* Wall. ex Royle, *Berberis aristata* DC., *Commiphora wightii* (Arn.) Bhandari, *Nardostachys jatamansi* DC., *Oroxylum indicum* Vent, *Picrorhiza kurroa* Benth. ex Royle, *Podophyllum hexandrum* (Royle) T.S. Ying, *Pterocarpus santalinus* Linn. f., *Saussurea costus* C.B. Clarke, *Swertia chirata* Buch-Ham

phytochemistry, and traditional usage. Work related to the collection, storage, marker compounds associated with quality control and microbial load, etc. need to be investigated. Vast data generated in the last two decades about ancestry, relationship, and possible origin of crop plants using next-generation sequencing and molecular markers will through light after in-depth analysis about domestication and selection of characters (Chinthiya and Bhavyasree 2019; Larson et al. 2014). The details of domestication of plants are beyond the scope of this article and can be found elsewhere (Gepts 2014; Larson et al. 2014).

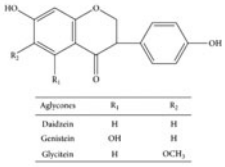
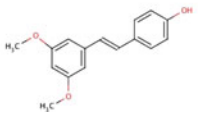
It is certain that to meet the demand of medicinal plants for ever-increasing population, more plants should be collected from wild resulting in endangering the species and loss of habitat. This can be avoided by the cultivation of medicinal plants but a uniform crop may be detrimental for the biodiversity of that species (Ramawat 2019a) and we may lose it forever if in situ conservation is not practised. Therefore, conservation and cultivation should be practised hand-in-hand. Most of the medicinal plants have been completely vanished from localities nearby to human habitat as witnessed by the eradication of *Taxus baccata* from Jageswar locality (Almorah district), *Chlorophytum borivilianum*, *Curculigo orchioides,* and *Gloriosa superba* from all nearby forests to Udaipur, and *Commiphora wightii* from the wild near Jodhpur. Therefore, there is a need to conserve them in situ as well as develop methods of agrotechnology and cultivation (Ramawat 2019a; Goyal et al. 2014). In India about 20% out of 400, in China 5–20% out of 5000 species, in Europe ~130

Table 1.2 Plant species for domestication by developing seed biology, agrotechnology and cultivation/micropropagation methods at author's laboratory

Plant species	Plant part	Active molecules	Use
 Flowers of *Chlorophytum borivilianum*	 Tuberous roots	 Several saponins	Adaptogenic activity
 *Desmodium gangeticum*	 Hairy roots	 Gangetin	Gastroprotective
 Flowers of *Gloriosa superba*	 tubers	 Colchicine alkaloid	Cell division inhibitor (anti-microtubulin polymerization agent)
 *Commiphora wightii*	 Plantlet in vitro	 Guggulsterone	Antihyper-cholesterolemia
 Germinating tubers of *Curculigo*	 Tubers from leaf explants	 Curculigoside A	active on β-amyloid aggregation

(continued)

Table 1.2 (continued)

	 Seeds		Green energy
 *Pueraria tuberosa*	 Tuber		cardiovascular disease, osteoporosis, hormone-dependent cancer and loss of cognitive function
 *Pterocarpus marsupium*	 Wood	 Pterostilbene (also marsupsin, pterosupin)	Antihyperglycemic activity

Aglycones	R_1	R_2
Daidzein	H	H
Genistein	OH	H
Glycitein	H	OCH_3

After Goyal et al. (2014, 2015)

Fig. 1.3 Breeding the opium poppy (*P. somniferum*) towards domestication and improvement. Wild (inset) has small flower and capsule with almost no production of alkaloids while cultivated variety has large leaves, flowers and capsules with high production of the opium latex. Photo by KGR

species, in Hungary 40 species, and in Germany 3–6% plants are obtained from cultivation (Schippmann et al. 2002). In general, people prefer medicinal plants collected from wild (as slow-growing wild plants accumulate more secondary metabolites as compared to cultivated, e.g. in ginseng) whereas pharmaceutical companies prefer uniform certified biomass for processing. There are certain advantages of cultivation and domestication over wild collections, such as uniform, certified material without adulteration, taxonomically identified, producers, and pharmaceutical companies (buyers) can enter in an agreement for long-term supply of material with price, post-harvest processing, quality control at each point, product standard can be adjusted for price and consumer preference, etc. However, all medicinal plants cannot be cultivated due to inherent biological problems like seed set, germination, and edaphic factors, and wild plant population will serve the requirement. Therefore, management of sustainable utilization is the need of the hour and proper protection of wild species/relatives and exploitation of available genetic resources (Chinthiya and Bhavyasree 2019; Yuan et al. 2010) (see Chaps. 2, 3, 5, 7, 23 in this book).

1.4 High Value Metabolites from Plants

There are several hundred species of recognized medicinal plants and remaining all are not explored completely. Still, new bioactive molecules are discovered and their biosynthetic pathways are determined. Drug discovery from the medicinal plant is a very long, tedious, time-consuming, and highly expensive research (Suntar 2019; Li et al. 2019). There are many challenges in isolation and determination of biological properties like correct identification of the plant, collection of large quantities of biomass from the wild, presence of low amount of bioactive molecule in plant, and this may be frustrating if desired success in finding novel drug is not achieved at the end (Atanasov et al. 2015). This is the main reason for the decline of research on drug discovery from plants. The details of the pros and cons of drug discovery from plants are discussed in detail elsewhere (Suntar 2019; Li et al. 2019; Atanasov et al. 2015). A few examples of selected long-standing drugs of plant origin are presented in Table 1.3.

Medicinal plants such as *Catharanthus roseus* (>200 alkaloids), *Podophyllum* species (several podophyllotoxins), *P. somniferum* (several benzoisoquinoline alkaloids), and *Cannabis sativa* (cannabinoids, terpenes, phenolics), are rich sources of many bioactive molecules. Most of the high-value therapeutics are produced in very minute quantities, for example, taxol in *Taxus* (500 mg out of 12 kg bark), vincristine and vinblastine in *C. roseus* (1 g vinblastine out of 500 kg dried leaves), and camptothecine in *Camptotheca acuminata* (0.042% in shoots) (Oberlies et al. 2009). Only a few compounds are produced in plenty like morphine in latex (minimum 55 kg raw opium/ha should be returned to government in plants cultivated in Rajasthan, India) of *P. somniferum* or oleo-gum (400 g/young plant to 1600 g old plant equals to 3200 kg/ha) yielding plants producing secondary metabolites in large quantities (Anonymous 2007, 2020; Krishna et al. 2014). Yet, there are no alternative synthetic

Table 1.3 Selected examples of long-standing drugs obtained from plants and their biological effect (after Ramawat 2019b; Aslam and Ahmad 2016)

Natural compound	Plant species	Biological effect/use
Atropine	*Atropa belladonna*	Mydriatic, anhidrotic, antispasmodic
Cocaine	*Erythroxylon coca*	Narcotic, local anaesthetic
Curcumin	*Curcuma longa*	Anti-inflammatory, anticancer
Digoxin	*Digitalis purpurea*	Cardiac glycoside
Glycyrrhetic acid	*Glycyrrhiza glabra*	Anti-inflammatory, peptic ulcer treatment
Menthol	*Mentha arvensis*	Local anaesthetic
Morphine	*Papaver somniferum*	Narcotic, analgesic
Quinine	*Cinchona officinalis*	Antimalaria
Reserpine	*Rauwolfia serpentina*	Antihypertensive, tranquilizer
Vincristine, vinblastine	*Catharanthus roseus*	Leukaemia, cancers

drugs for several plant-based drugs. These bioactive molecules are large and complex, contains specific arranged aromatic rings and chiral centres, make them a difficult candidate to obtain by chemical synthesis but may serve as a base for novel synthetic drugs (Ajayi et al. 2019). These plants were known primarily for their medicinal values, and it took several years to identify several of these compounds. Taxol was isolated in 1966 with structure established in 1971 but took several years to establish the drug before it was approved by the Food and Drug Administration, USA (FDA) in 1994 (Weaver 2014). Similar was the case for FDA approval for camptothecine. Plants are sources of several types of drugs and are continuously find their way as FDA approved drug in vast number of diseases (Newman and Cragg 2020).

Still it is not clear to us, why such complex compounds are produced in such a low quantity in plants but are effective against dreaded diseases like cancer. Yet, many minor compounds, biosynthetic intermediates, and biosynthetic pathways are not known clearly. Metabolomics and transcriptomics may through light on these aspects as evident in *Podophyllum hexandrum*, where transcriptome analysis showed six enzymes involved in podophyllotoxin biosynthesis (Lau and Sattely 2015). A new opportunity is the production of bioplastic from Cannabis stem fibre (Andre et al. 2016), which may provide the use of by-products and support cultivation of medicinal plants.

1.5 Improvement and Breeding

1.5.1 Introduction, Selection, and Cloning

The introduction of a plant in a new area requires similar agroclimatic conditions as well as pollinator present in the old habitat. Uniform material in quality and assured supply are basics to run a plant-based industry. In countries like India, temperate to tropical and desertic conditions are available making them suitable for various types of plant introduction, domestication, and development of agrotechnologies. Introduction to a new country is regulated by the law of land and which differ from country to country. Generally, endangered and economically important species are closely monitored, e.g. seeds raised plants of asafoetida (Umbelliferae) were introduced recently while *Cinchona* species, *Artemisia* species, were introduced long back (The Economics times, 27 October 2020).

Because of high income from medicinal plants over cereals and other conventional crops, medicinal plant cultivation is directly related to an increase in the socio-economic level of farmers (see Chaps. 27, 28, 29 in this book). This is very well documented, e.g. growing Isabgol, the opium poppy, ajowan, fennel, Mentha, and several others cereals in India. Until now, rural tourism related to medicinal plants agriculture is not launched very well in most of the developing countries, which can further boost the rural economy.

1.5.2 Cloning by Conventional and Non-conventional Methods

Several medicinal and aromatic plants are propagated by vegetative methods, and in such plants, conventional cloning and selection are best ways to obtained improved plant material, e.g. in *Mentha* species, Vetiver, *Pandanus* species, *Zinger*, *Curcuma*, tea (*Camellia sinensis*), *Taxus brevifolia*, *Ginkgo biloba* (stem cuttings), etc. In vegetatively propagated crops, once cloned and selected, they remain stable for several generations. Clones can be selected based on active molecule production, phenotypic characters, and specific compound production as done in vetiver (*Chrysopogon zizanioides*) for khusinol, khusimol and khusilal (Singh et al. 2019; see Chaps. 15, 16 in this book).

Non-conventional cloning is done by plant tissue culture using micropropagation methods in static and agitated liquid media. Micropropagation methods for several medicinal plants have been developed using static, liquid, and bioreactor systems. Protocols for micropropagation for several medicinal plants were developed in our laboratory. Shoots on the static medium may be slow-growing but stout whereas cultures in the agitated liquid medium are fast-growing with high rate of multiplication as evident in *C. orchioides* cultures grown in liquid medium. Department of Biotechnology, Government of India, and other funding agencies encouraged and

supported research on medicinal plants for the past 3–4 decades. This results in generating large data about medicinal plants and protocols for their micropropagation. A list of selected medicinal plants for which micropropagation protocols have been developed is presented in Table 1.4. However, large-scale production of medicinal plants using these protocols is yet to be practised in most of the cases (Ramawat 2019b; Goyal et al. 2015). In medicinal plants where a regenerative protocol was available and its bioactive molecules are in demand, transgenic plants were obtained by using an Agrobacterium T-DNA-based transgenic system to obtained desirable molecules. Thus, combing genetic engineering with a propagation system to obtain bioactive molecules, modified bioactive pathways and molecules, or used as a production platform (heterologous expression leading to molecular pharming) was attempted using a highly regenerative system of Bacopa (Table 1.4; Yadav et al. 2014).

1.5.3 Improvement by Conventional and Non-Conventional Methods

Medicinal plants though important come after food, and therefore, conventional hybridization methods, which are long and time-consuming, are used to a lesser extent. Increased polyploidy in plants results in higher vigour in plant parts as compared to diploid because of presence of a greater number of chromosome sets. This method was explored in many medicinal plant species (Ajowan, Anis, *Artemisia annua*, *Eclipta alba*, *C. sativa*, Patchouli, etc.) if this also results in higher metabolic rate and production of useful metabolites (Niazian 2019).

Methods of genetic engineering, practised for the last 40 years, insert gene/s at a random site, on one/more sites, may on different chromosomes, one/multiple copies of the gene and hence not suitable for target-specific changes. This type of genetic transformation may not yield desirable qualitative/quantitative changes in the production of secondary metabolites. Recent and advanced tools of molecular biology include synthetic promoters, variable transcription factors, genome editing tools, and site-specific recombinases that can be helpful in accurate and faster plant improvement (Liu et al. 2013).

Besides conventional plant breeding, non-conventional methods of plant improvement using plant biotechnology and tools of genetic/genomic manipulation (CRISPR, clustered regularly interspaced short palindromic repeats; Cas CRISPR, CRISPR associated proteins; GWAS, genome-wide association; MAS, marker-assisted selection; TALENs, transcription activator-like effector nucleases; TILLING, targeting-induced local lesions in genomes; ZFNs, zinc-finger nucleases), help in cloning, conservation and preservation of selected material, understand structure and functioning of genome. Consequently, these methods are beneficial for higher secondary metabolites production or specific compound production and conservation of natural

Table 1.4 Recent examples of micropropagation of medicinal plants of high value as well their genetic transformation to develop altered/high secondary metabolites producing plants

Plant species Common name Family	Principal bioactive molecule	Medicinal use	Method of propagation	References
I. Micropropagation of medicinal plants				
Acorus calamus Sweet flag Acoraceae	Flavonoids, polyphenolics	Antioxidants, Antibacterial	Shoots from rhizome bud explants	Babar et al. (2020)
Andrographis paniculata Nees, 'Kalmegh, Acanthaceae	Diterpenoids, flavonoids, quinic acids, xanthones, nor-iridoids	For common cold, diarrhoea, fever, liver, and cardiovascular	Shoots from stem explant	Pandey et al. (2017)
Cannabis sativa L Marijuana, Cannabaceae	Cannabinoids (THC), terpenes	Narcotic, pain relieving,	Photoautotrophic micropropagation from nodal cuttings	Kodym and Leeb (2019)
Carum copticum L Ajowan Umbellifeae	Thymol	Antibacterial, antiulcer, improve cholesterol,	indirect somatic embryogenesis and indirect shoot regeneration	Niazian et al. (2017)
Celastrus paniculatus Willd Malkangani Celastraceae	Seed oil, lupeol, sesquiterpene polyalcohol,	Improves memory and cognitive functions	Shoots from leaf via organogenesis	Moola and Kumari (2020)
Digitalis purpurea L. Foxglove, Scrophulariaceae	Digoxin and digitoxin	Against heart failure, anticancer,	Plant from leaf segments via organogenesis	Pérez-Alonso et al. (2018)
Hypericum gaitii, goat weed Hypericaceace	Hypericin	Wound healing, bactericidal and antiinflammatory	Multiple shoots were induced from apical and axillary meristems	Swain et al. (2016)

(continued)

Table 1.4 (continued)

Plant species Common name Family	Principal bioactive molecule	Medicinal use	Method of propagation	References
Rauvolfia serpentine *Rauvolfia tetraphylla* *Rauvolfia hookeri* Apocynaceae	Reserpine, serpentine, Ajmalicine,	Snakebites and mental illness, hypertension, and reduces blood pressure	Shoots from nodal explant	Hussain et al. (2018)
Securidaca longipedunculata (Fresen), violet tree Polygalaceae	Methylsalicylate, flavonoids, alkaloids elymoclavine, and dehydroelymoclavine,	As a pesticide, malaria, stomach problems, toothache, headache, sleeping sickness	Shoots formation	Lijalem and Feyissa (2020)
Vaccinium L (berries) Ericaceae	Polyphenolics, anthocyanin, tannins	Antioxidant metabolites	Axillary shoots, organogenesis	Debnath and Goyali (2020)
II. Agrobacterium-mediated genetic transformation of medicinal plants				
Artemisia annua Asteraceae	Artemisinin	Antimalarial drug	Chloroplast genome transformation	Kaushal et al. (2020)
Artemisia aucheri Boiss Asteraceae	Flavonoids, sesquiterpene lactones, lignans, acetylenes, triterpenes	Anti-tumour activity	Shoot organogenesis, Ri based hairy roots,	Sharafi et al. (2014)
Bacopa monnieri, Brahmi, Plantaginaceae	Bacopasides	Memory improvement	*Agrobacterium*-mediated genetic transformation, regeneration	Yadav et al. (2014)
Catharanthus roseus Periwinkle Apocynaceae	Ajmalicine, Several indole alkaloids	Anticancer, antihypertensive	Agrobacterium T-DNA, ovary and shoot apical meristem injection, 12% transformation rate, transgenic lines	Bahari et al. (2020)

(continued)

Table 1.4 (continued)

Plant species Common name Family	Principal bioactive molecule	Medicinal use	Method of propagation	References
Picrorhiza kurroa Royle ex. Benth Kutki Schlophularaceae	Iridoid glycosides known as picrosides (picroside-I–IV, apocynin, androsin, and kutkoside)	Hepato-protective, antioxidative, antiallergic and antiasthmatic, liver anticarcinogenic, and immuno-modulatory	Plant regeneration via direct organogenesis and *Agrobacterium tumefaciens*-mediated genetic transformation	Bhat et al. (2012)
Scutellaria ocmulgee Small Ocmulgee skullcap, Lamiaceae	Flavonoids	Anti-inflammatory, antioxidative, antiviral, anticancer	Leaf and shoot-derived transverse thin cell layer explants, transgenic cultures and plants	Vaidya et al. (2016)
Trachyspermum ammi (L.) Sprague) ajowan Umbellifeae	Thymol	Antibacterial, antiulcer, improve cholesterol	*Agrobacterium*-mediated gene transformation, for drought and salinity tolerance	Niazian et al. (2019)
Veratrum dahuricum L. (Liliaceae)	Cyclopamine, jervine, and veratramine	Anticancer drug	Transgenic plants from embryonic callus	Ma et al. (2020)
Withania somnifera Ashwagantha Solanaceae	Withanolides such as withaferin-A, withanone, withanolide D and withanolide A	Anti-cancer apoptosis, osteo-protective, cardioprotective, stress-reliever	High frequency, efficient and rapid transformation system, transgenic shoots	Mishra et al. (2016)

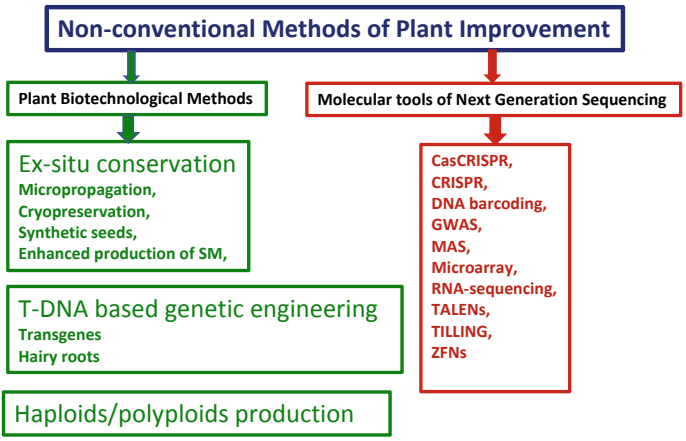


Fig. 1.4 Non-conventional medicinal plants improvement methods include applications of plant biotechnology, Agrobacterium's T-DNA-based genetic engineering and more recent molecular tools (CRISPR, clustered regularly interspaced short palindromic repeats; Cas CRISPR-associated; GWAS, genome-wide association, MAS, marker-assisted selection; TALENs, transcription activator-like effector nucleases; TILLING, targeting-induced local lesions in genomes; ZFNs zinc-finger nucleases

biodiversity. These tools can specifically modify a gene or genetic structure of medicinal plants for higher production of useful metabolites. Details of these methods are beyond the scope of this brief review and can be found elsewhere (Niazian 2019; Sinha et al. 2019; Gandhi et al. 2015) and only summarized here (Fig. 1.4).

CRISPR together with Cas CRISPR acts as a type of adaptive immunity system in prokaryotes and provides sequence-specific protection against foreign DNA or RNA (Klimek-Chodacka et al. 2018). Therefore, CRISPR/Cas9 system works by locating and identifying the foreign DNA sequence by small guide RNA (sgRNA), and then foreign DNA is cleaved by Cas9, an endonuclease (Marchev et al. 2020). Instead of random (as in T-DNA gene-based insertion) targeted regulation of gene expression can change the secondary metabolite production system (Xu et al. 2014). Tools that can create a target-based break in double-stranded DNA are of interest such as ZPNs, TALENs and CRISPR/Cas9 (three types of engineered sequence-specific nucleases). These fusion proteins have two parts; 1, Programmable and sequence-specific DNA binding domain, and 2, A non-domain specific DNA cleavage (Gaj et al. 2013). The sgRNA sequence of CRISPR/Cas can be designed; therefore, its complementary sequence can target any gene of interest in a selected genome and can carry out different functions such as gene mutations, deletion, and insertion, and also transcriptional control is possible (Xu et al. 2014). Some examples of applications of recent molecular tools such as CRISPR/Cas9 are; suppression of rosmarinic acid synthase gene in (*SmRAS*) in *Salvia miltiorrhiza* (Zhou et al. 2018), targeted mutagenesis in Dzfps gene responsible for farnesyl purophosphate synthase enzymes (consequently low squalene) in *Dioscorea zinziberansis* (Feng et al. 2018), GABA shunt pathway

in *Solanum lycopersicum* (Li et al. 2018), glycosyltransferase gene in *Nicotiana benthamiana* affecting the production of recombinant proteins lacking β-1,2-xylose and core α-1,3-fucose (Jansing et al. 2019), in hairy roots of *S. miltiorrhiza* for the production of tansinone by knocking the key gene (Li et al. 2017) and benzylisoquinoline alkaloid biosynthesis in *P. somniferum* by modified reticuline 7-O-methyl transferase and 3'hyoxy-N-methylcoclaurine4'-O-methyltransferase (Alagoz et al. 2016). Therefore, new tools are helpful in obtaining targeted desired changes in the production systems by manipulating the biosynthetic pathways and save precious time and money.

1.5.4 Conservation Using Alternate Technologies

The major challenge in the field of medicinal plant research is to protect, conserve, and propagate rare/endangered medicinal plants and at the same time also meet the demand for plant material. Extensive work has been carried out in different parts of the world to conserve and sustainable use of medicinal plants as well as develop alternative conservation technologies. These include setting up of medicinal plants board or task forces for medicinal plants under the Ministry of Science and Technology (India) and several other countries. Biotechnological studies are supported for conservation in situ and ex situ, development of micropropagation methods, production in cell cultures, and genetic manipulation to alter biosynthetic pathways leading to enhanced/alter production of principal molecule or novel molecules. Plant biotechnological methods are used for the last half-century, whereas molecular tools are widely used only recently. Application of plant biotechnology for the production of secondary metabolites has its production limitation, and only a few products reached at industrial level production such as taxol while micropropagation are used more widely on industrial level for the production of a wide range of species of medicinal and ornamentals plants (Haldar and Jha 2020; Ramawat 2019a) (see Chap. 17 in this book).

1.5.5 Production Bioactive Molecules in Cell Cultures

Plant cell culture technology is used to produce bioactive secondary metabolites of high value and interest. Details of production strategies are beyond the scope of this article and discussed elsewhere (Ramawat 2019a; Espinosa-Leal et al. 2018; Wang et al. 2017; Ochoa-Villareal et al. 2016). However, in brief: callus and cell cultures are optimized for growth by empirical approach manipulating medium constituents one by one, specifically nitrogen, sugar, and plant growth regulators, known as optimization (Goyal and Ramawat 2008; Ramawat 2019b). The cultures optimized for growth are then subjected to various effectors like precursors, biotic, and abiotic elicitors along with modified sugar concentration (usually high) and auxin (usually low) to

enhance the production of secondary metabolites (Goyal and Ramawat 2007; Mathur and Ramawat 2008). In this approach, fed-batch culture is another alternative to add production medium at the stationary phase cultures (Suthar and Ramawat 2010). Use of abiotic and biotic elicitors like plant gum (Dass and Ramawat 2009), fungal elicitors (Roat and Ramawat 2009), angiosperm parasite extract (Goyal et al.2011), and combining more than one effector may be a useful strategy to enhance the production of secondary metabolites in cultures (Arora et al. 2010; Suthar and Ramawat 2010). The selected cultures with optimized conditions are grown in a bioreactor to develop scale-up technology towards industrial production (Espinosa-Leal et al. 2018; Wang et al. 2017; Sharma et al. 2009). Some selected examples of production of secondary metabolites are presented in Table fv showing effectors and bioactive molecules in normal cell cultures and hairy roots, genetically modified Ri plasmid cultures. Hairy roots are organized plant tissues induced by the ability of Ri plasmid of *Agrobacterium rhizogenes* to induce roots, which are fast-growing, negatively geotropic, and grow without auxin in the medium (Sarkar et al. 2018). Because of organized tissues and grown in auxin free medium, hairy roots are supposed to produce higher amounts of secondary metabolites. Random insertion of Ri plasmid genes may affect the genes of host plant, thereby affecting the production/new product formation. The details of these methods are not possible here and can be found elsewhere (Haldar and Jha 2020).

Thus, the optimization of nutrients and culture conditions is a prerequisite for the use of scale-up technology in a bioreactor. Refinement of the conditions is further required for industrial-scale production of a bioactive molecule such as taxol, ajmalicine, etc. This technology has a direct impact on the conservation of the species and making available bioactive molecules by alternative technology. From laboratory to industrial-scale production is a long and tedious process and may not be successful for all the molecules investigated because of inherent biological problems associated with the plant species. Some of the successful examples of bioactive molecules production at large scale are given in Table 1.5. In last four decades, plant biotechnology for medicinal plants has transformed from reporting presence of a secondary metabolite in culture to grown in shake flask and bioreactor to targeted manipulation of desired gene for chosen compound. Moreover, all these techniques help in conserving the plants.

1.5.6 Novel Biomolecules from Medicinal Plants

A large number of plants are yet to be investigated phytochemically leaving aside the pharmacological properties (Ramawat 2019a). We know the compounds present in higher amounts in the plants but several compounds present in minor quantities are yet to be known. Large quantities of pure compounds are required for identification and determining biological properties. New tools of chemistry like Flash®, Ultra High-Pressure Liquid Chromatography (UHPLC), Medium Pressure Liquid Chromatography (MPLC), Electrokinetic chromatography, droplet counter-current,

Table 1.5 Few selected examples to demonstrate effectiveness of added effector on bioactive molecules production in plant cell cultures and transformed cultures (hairy roots) using shake flasks and bioreactor

Class	Effector/culture	Secondary metabolite	Plant species	References
PGR	Morphactin +2, iP, cell culture	Isoflavonoids	*Pueraria tuberosa*	Goyal and Ramawat (2008)
	Benzylaminopurine/callus culture	Anthocyanin	*Angelica archangelica* L	Siatka (2019)
Elicitor	*Cuscuta* extract, cell culture	Puerarin	*P. tuberosa*	Goyal et al. (2011)
	Plant gums, cell cultures	Guggulsterone	*Commiphora wightii*	Dass and Ramawat (2009)
	Gamma irradiation, callus culture	Camptothecin	*Nothapodytes foetida*	Fulzele et al. (2015)
	Yeast extract, silver nitrate	Camptothecin	*Ophiorrhiza mungos* Linn	Deepthi and Satheeshkumar (2016)
Precursor	Sugars, precursors, and morphactin	Guggulsterone	*C. wightii*	Mathur and Ramawat (2008)
Inhibitor	ALAR (N,N-dimethylamino-succinamic acid), chlormequat chloride (CCC)	Guggulsterone	*C. wightii*	Suthar and Ramawat (2010)
	Methyl jasmonate, spermidine, salicylic acid, paclobutrazol	Sweetener, phenolics	*Stevia rebaudiana*	Lucho et al. (2019)
	CCC, paclobutrazol, Daminozide	Sweetener, phenolics	*S. rebaudiana*	Karimi et al. (2019)
Fed-batch culture	Fed-batch process	Guggulsterone	*C. wightii*	Suthar and Ramawat (2010)
Bioreactor	Cell culture	Isoflavonoids	*P. tuberosa*	Sharma et al. (2009)
	Shoot cultures, 10-L nutrient sprinkle	Rosmarinic acid	*Dracocephalum forrestii* W. W. Smith	Weremczuk-Jezyna et al. (2019)
	Hairy roots, 1-L airlift	Rosmarinic acid	*Coleus blumei* L	Bauer et al. (2015)
	Cell suspension, 3-L balloon type airlift	Resveratrol	*Vitis amurensis Rupr*	Sun et al. (2016)
	Cell suspension, 5-L stirred tank	Resveratrol	*Vitis labrusca* L	Chastang et al. (2018)
	Methyl jasmonate, stirred tank	Rosmarinic acid	*Ocimum basilicum*	Pandey et al. (2019)

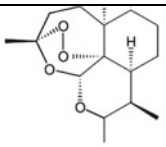
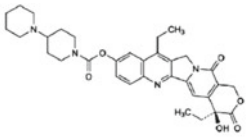
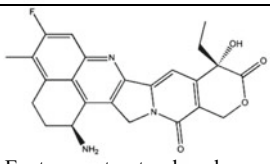
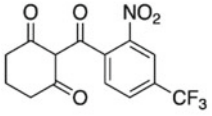
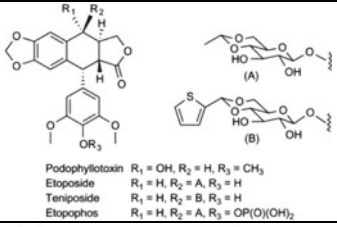
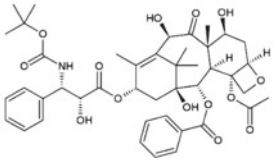
super-critical fluid, and circular chromatographies, Centrifugal partition chromatography (CPC) have revolutionized the separation and purification methods. Coupling of mass spectroscopy with other hyphenated techniques like GC-MS, LC-MS, LC-DAD-TOF-MS, circular dichroism, NMR and NMR-based correlational spectroscopies is precision tools for structure determination of isolated compounds (Khan 2018). Once the pure compounds are available in sufficient quantities, biological properties are determined. Natural compounds are obtained from plants and their derivatives or analogue can be synthesized. Initially, obtained natural products were either not effective or too toxic to use for human consumption. There are several examples that show that derivatives are more effective, less toxic, and safe than basic natural molecules (Table 1.6) (Raafat 2013). Semisynthetic derivatives of taxol (Taxotere), camptothecin (irinotecan) and podophyllotoxin (etoposide and teniposide) are not only more effective but also less toxic to human cells (Ramawat 2019b; Newman and Cragg 2020; Aslam and Ahmad 2016; see Chap. 18 in this book). These all are natural products obtained from plants and effective drugs have been developed from basic molecules present in these plants.

Halogenated secondary metabolites are of rare occurrence in terrestrial plants. It has been observed that the addition/deletion of a halide may change the pharmacological property of the secondary metabolites. Hence, a chlorinated biosynthetic machinery was genetically transferred in *C. roseus* to obtain new halogenated products paving a way to obtain such products in other plants, perhaps with more effective action (Runguphan et al. 2010). Similarly, removal of one or two hydroxyl or acetoxy groups from EGCG (epigallocatechin gallate) has resulted in more effective (antiproliferative, antiangiogenic, and antifibrotic) pro-drug with higher stability and solubility (Ahmed et al. 2019).

Cultivated medicinal plants can be used as bio-factories (known as molecular pharming) to produce molecules of pharmaceutical interest and importance even in developing countries where health infrastructure is not well developed but the population is very high (Singhabahu et al.2016; Shanmugraj et al. 2020). The health system's weaknesses are exposed throughout the world with COVID-19 pandemic. Though molecular pharming is at the initial stage, it can serve a large production system at a low cost for which more investment is required.

Secondary metabolites are generally biosynthesized through long biosynthetic pathways involving several enzymes or genes. It is difficult to manipulate this biosynthetic pathway by conventional breeding programmes but techniques of genetic engineering are involved from time to time to change the expression of genes, add new genes, or block certain genes to obtain novel molecules of interest. Biomass for downstream processing may be obtained from wild, cultivate,d or genetically modified plants or through cell cultures from bioreactors, but the processing remains more, or less the same. The process should be rapid, cost-effective, and produce a molecule of interest in pure form and undamaged (Patra and Srivastava 2017).

Table 1.6 Semisynthetic effective drugs developed from natural molecules of plant (after Ramawat 2019b; Newman and Cragg 2020; Aslam and Ahmad 2016)

Natural molecules	Plant species	New semisynthetic drug	use
Artemisinin	*Artemisia annua*	 Arteether	Antimalarial (resistant strains of Plasmodium falciparum)
Camptothecin	*Camptotheca acuminata*	 Irinotecan	Anticancer
Camptothecin	*Camptotheca acuminata*	 Exatecan, structural analogue	Anticancer
Nitisinone	*Callistemon citrinus*	 Nitisinone	Tyrosinemia
Podophyllotoxins	*Podophyllum* spp.		Anticancer
Taxol	*Taxus brevifolia*	 Docetaxel or Taxotere	Anticancer

1.6 Conclusions

Though plants are sources of many herbal drugs and demand for medicinal plants and their products will continue to increase with increased population, particularly in developing countries. This is well exemplified by the recent coronavirus pandemic. Because of lack of proper antiviral medicine or vaccine, there was a surge in immunostimulant herbs in Asian countries as the population was given a dose of herbal extract containing giloy (*Tinospora cordyfolia*), tulsi (*Ocimum sanctum*), zinger (*Zinziber officinalis*), pepper (*Piper nigra*), etc., and tons of herbs were consumed in this process, and boosting immune system is the only alternative. Therefore, dependency on herbal medicine is continued with search for bioactive molecules, analogues, and derivatives. The process of obtaining herbal drugs or even formulations is not only long, time-consuming, and tedious processes, but they are further hampered by development of formulation, poor bioavailability, solubility and stability related problems, scaling up and intellectual property problems (Anwar et al. 2019). Modern tools are helpful in the screening of plants and molecules based on their biological activity and enhancing the production in plants and cell cultures. Molecular pharming is promising technology not only to produce the desired molecule in crop plants but also anywhere in the world for human welfare. Thus, developing agrotechnologies towards domestication and cultivation along with application of modern tools to conserve and improve the medicinal plants will be helpful in conservation and meeting the demand of herbal medicine. New molecular tools have come to an edge to obtained desired targeted products.

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