Chapter 9
Cultivation of Herbal Drugs, Biotechnology, and In Vitro Production of Secondary Metabolites, High-Value Medicinal Plants, Herbal Wealth, and Herbal Trade

Abstract Medicinal plants are used directly as therapeutic agents in various traditional practices, and medicinal plants like *Dioscorea deltoidea*, *Papaver somniferum*, *Atropa belladonna*, *Rauvolfia serpentina*, *Hyoscyamus niger*, *Digitalis lanata*, *Datura metel*, *Digitalis purpurea*, *Pilocarpusa bonandi*, *Cinchona ledgeriana* are contributing directly several prescribed medicinals. The ever-increasing trend in the use of medicinal herbs and herbal products in therapeutic purpose, research, and trade has created tremendous pressure on supply from their wild source. Under the circumstances of increasing demand, indiscriminate and over extraction from the wild, habitat destruction, etc., many of the naturally growing medicinal herbs are on the verge of extinction and thus unsustainability in the supply of medicinal plants from natural source. Systematic cultivation of medicinal herbs would be a viable alternative to overcome this unsustainability problem of medicinal plants from the wild source and cultivation offers opportunity to optimize yield and achieve a uniform, high-quality product. Several drugs like cardamom, cannabis, cinnamon, ginger, cinchona, opium, linseed, and fennel are now obtained almost exclusively from cultivation source. Benefits of cultivation of medicinal plants are widely viewed as a means for meeting current and future demands for large volume production of plant-based drugs; cultivation can reduce growing pressures on wild medicinal plants and ensure pure and smooth supply; means of earning livelihood, etc. The WHO has published wide-spectrum guidelines for good agricultural and collection practices for sustainable production of raw material of quality and standardized herbal ingredients to ensure quality of herbal medicines. Medicinal plants may be cultivated by the following: (a) agricultural practice at field level and (b) in vitro production of secondary metabolites. Commercial cultivation at field level (open field, homestead garden, forest floor) is an agronomic practice and offers the opportunity to overcome the problems that are inherent in herbal extracts like misidentification, genetic and phenotypic variability, extract variability and instability, toxic components, and contaminants. The agronomic method of crop cultivation includes systematically the steps like site and season selection, selection of crop, true seed or vegetative propagule, land preparation and basal manuring and fertilization, spacing, seed sowing or seedling transplantation,

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split application of fertilizer, irrigation, intercultural operation, and weed control and harvesting. Large-scale plant tissue or organ culture for the production of secondary metabolites is an attractive alternative approach to traditional methods of cultivation of drug plants. The advantages of this method are many; it is independent of soil, paste, climatic interference, geographical location, and it can ultimately provide a continuous and reliable source of natural products. Considering the cost involvement, plant tissue culture for secondary metabolites is now limited to only high-value compounds such as diosgenin-derived steroid hormone precursors, digitalis glycosides, berberine isoquinoline alkaloid, taxol, paclitaxel, and several other toxoids—complex diterpene alkaloids. Herbal plants have global market worth about US$62 billion per annum, and so they may be a good export item and wealth of a country and should be cultivated in commercial scale like any other conventional cash crops. As cultivation of medicinal plants at commercial scale is comparatively a new concept in many countries to meet the demand of internal and foreign markets and earning livelihood of the rural people, medicinal plants are important natural wealth and herbal wealth, play significant role in providing primary health-care services to rural people, and serve as raw material in traditional and modern pharmaceutical as well as in cosmetic, agricultural, and food industries. Substantial amount of foreign exchange can be earned by exporting medicinal plants to other countries. In this way, indigenous medicinal plants may play a significant role in the economy of a country.

Keywords Medicinal plant cultivation · Genetic erosion · Habitat destruction · GACPs

9.1 Field-Level Cultivation of Medicinal Plants

Cultivation of medicinal plant is gaining popularity because of the worldwide use of herbal medicine and high demand of herbs in the internal and world market. Musk mallow (*Abelmoschus moschatus*), senna (*Cassia senna*), ashwagandha (*Withania somnifera*), long pepper (*Piper longum*), black pepper (*Piper nigrurm*), Indian goose berry (*Emblica officinalis*), king of bitters (*Andrographis paniculata*), Indian borage (*Coleus amboinicus*), Rauvolfia (*Rauvolfia serpentina*), yam (*Dioscorea deltoidea*), opium poppy (*Papaver somniferum*), deadly nightshade (*Atropa belladonna*), heart-leaved moonseed (*Tinospora cordifolia*), Gymnema (*Gymnema sylvestre*), wood apple (*Aegle marmelos*), black oil plant (*Celastrus paniculatus*), Asparagus (*Asparagus racemosus*), Aloe (*Aloe vera*), drum stick (*Moringa oleifera*), basil (*Ocimum basilicum*), Indian pennywort (*Bacopa monnieri*), Calendula (*Caledula officinalis*), longevity spinach (*Gynura procumbens*), daruharidra (*Berberis aristata*), guggal (*Commiphora wightii*), jatamansi (*Nardostachys jatamansi*), jatropha (*Jatropha carcus*), saffron (*Crocus sativus*), lavender (*Lavedula*

angustifolia), lemongrass (*Cymbopogon citrtus*), parsely (*Petroselinum crispum*), Stevia (*Stevia rebaundiana*), Vanilla (*Vanilla planifolia*), licorice (*Glycerrhiza glabra*), neem (*Azadirachta indica*), foxglove (*Digitalis purpurea*), Eucalyptus (*Eucalyptus globules*), tobacco (*Nicotiana tabacum*), periwinkle (*Catharanthus roseus*), rose (*Rosa damascene*), coriander (*Coriandrum sativum*), *colocynth* (*Citrullus colocynthes*), fennel (*Foeniculum vulgare*), *senna* (*C. senna*), Calabar bean (*Physostigma venenosum*), nux vomica (*Strychnos nux-vomica*), castor bean (*Ricinus communis*), kurchi (*Holarrhena antidysenterica*), cinnamon (*Cinnamomum zeylanicum*), henbane (*Hyoscyamus niger*), bitter-wood (*Quassia amara*), sandalwood (*Santalum album*), ipecac (*Carapichea ipecacuanha*), turmeric (*Curcuma longa*), zinger (*Zingiber officinale*), valerian (*Valeriana officinalis*), mayapple (*Podophyllum peltatum*), *Artemisia annua*, *Taxus wallichiana*, *Strophanthus* spp., *Chondrodendron tomentosum*, *Cinchona* spp., etc., are some of the common herbs used in manufacture of herbal drugs and active constituents of modern drugs.

Many medicinal plants of therapeutic importance are low input economically important medicinal crops. They are presently cultivated commercially in different countries along with traditional agricultural and horticultural crops as sole crops, intercrops, sequential crops, mixed crops, etc., as well as in the agroforestry systems. Agroforestry systems have the advantage over agricultural systems because of the presence of a wide range of ecological niche or microclimates ranging from nearly full sun, partial shade, and partial sun to full shade (heliophyte–sciophyte). A specific crop can be located in near-ideal conditions as close to its natural habitat as possible. Many countries have developed a number of high-yielding varieties, worked out agrotechnologies and processing technologies for cultivation of HYV medicinal and aromatic plants.

Cultivation of medicinal plants requires intensive care and management. The conditions and duration of cultivation required vary depending on the quality of medicinal plant materials required. If no scientific published or documented cultivation data are available, traditional methods of cultivation should be followed, or a method should be developed through research. Factors that affect medicinal plant cultivation are altitude, temperature, rainfall, humidity, irrigation, soil types and fertility levels, fertilizers, pest and pest control, plant hormones, polyploidy, green house effects, hybridization, etc. Conservation Agriculture (CA) techniques (resource-efficient/resource-effective agriculture) including 'no-tillage' systems should be followed where appropriate, especially in the build-up of organic matter and conservation of soil humidity. CA aims to conserve, improve and make more efficient use of natural resources through integrated management of available soil, water and biological resources combined with external inputs. The principles of good plant husbandry including that of WHO on good agricultural and collection practices (GACPs) may be followed, and tillage should be adapted to plant growth and other requirements.

9.1.1 WHO's Guidelines on Good Agricultural and Collection Practices (GACPs)

The WHO has published guidelines for GACPs for medicinal plants, and member countries are required to develop country-specific guidelines for sustainable production of raw material of quality and standardized herbal ingredients to ensure quality of herbal medicines and ecologically sound cultivation practices. The GACPs cover a wide spectrum of cultivation and collection activities.

Some Basic Guidelines Under GACPs for Cultivation of Crop
Selection of proper site for cultivation of a particular medicinal plant, selection of correct time for cultivation, selection of proper variety, adoption of organic farming, etc., are considered important for cultivation.

GACPs for Collection (Harvest)
For collection, only desired mature part(s) to be selected without harming the mother plant and whole population (at least 30–40% to be left for regeneration), twigs/branches are not to be cut for the collection of plant parts, proper equipment is necessary for cutting, shearing, peeling, etc. Collected material should be cleaned (removal of dust and other undesirable matter), sun dried, or processed immediately after collection up to complete drying before packing and storage, but aromatic herbs and delicate fruits in shade separately (two or more herbs not in close vicinity). Herbs should be packed in suitable packaging material to avoid losses due to external factors and to be stored in proper storage conditions to minimize loss on storage.

Collection of Underground Part(s), Bark, and Whole Plant
To facilitate regeneration, collection should be made after seed shed, collection of the underground part should be done when the mother plant fully matures, digging should be minimum and to facilitate regeneration, some parts to be left underground, fleshy parts should be dried before packing and storing, large parts should be cut into smaller pieces, bark should be collected not from immature plant, instead, from the branches of main trunk, strip the bark longitudinally and not from all over the circumference of trunk/branches, to be cut into small pieces to facilitate complete drying, only mature branches for stem is recommended for harvest, and herbs are to be dried properly before packing or storing.

Collection of Leaves, Flowers, Fruits, Seeds, Floral Parts, etc
For collection of leaves, flowers, fruits, seeds, floral parts, etc., only mature parts from healthy plants are to be selected for harvest and not collect all material of the plant at a time, and it is advised not to cut branches for collecting leaves, fruits, flowers, and so on; some floral parts on the plants are to be left to facilitate natural regeneration, fleshy flowers may be dried in the sun, but shade is preferable. Parts like stigma, anthers, buds, etc., should be collected at appropriate time and seeds, when the fruits are completely mature.

Collection of Gums, Oils, Resins, Galls, etc

For collection of gums, oils, resins, galls, etc., incisions should be made only vertically on some portions of the tree and not horizontally; the incisions to be treated after collection of the desired material; the gum or resin not to be collected from a tree continuously but precisely in right season; gum or resin not to be left exposed in the field; but to be packed in appropriate containers or drums with polyethylene lining; the galls are to be collected only from prescribed species (e.g., Karkatshringi from *Pistacia integerrima*), and no live insect should be present inside the galls.

9.1.2 Necessity, Benefits, and Limitations of Commercial Cultivation of Medicinal Plants

Medicinal plants are the oldest known health-care products, and their importance is still growing all over the world. Medicinal plants have been playing a significant role in Ayurveda and Unani systems of medicine in Indian subcontinent, Chinese traditional medicine, Kampo of Japan, and other traditional systems of medicine developed by the indigenous people of many countries of Europe, America, Australia, and Africa since long past. The World Health Organization (WHO) supports Member States in their efforts to formulate national policies on traditional medicine taking into accounts its potential, safety, efficacy, etc. A genuine interest in various traditional practices now exists among practitioners of modern medicine. A survey among Member States of the European Union in 1991 identified about 1400 herbal drugs used in the European Economic Community. Medicinal plants are important for pharmacological research and drug development directly as therapeutic agents as well as basic materials and models for the synthesis of drugs and pharmacologically active compounds, respectively. Some of the important medicinals from plant sources are enlisted in Table 9.1.

Consumption of herbal medicines is widespread and increasing. Naturally growing medicinal plants are collected for use in traditional medicine and in pharmaceutical industries. Indiscriminate and over extraction from the wild by uneducated and unskilled local people is a major cause of loss of genetic diversity (genetic erosion) and habitat destruction and thus unsustainability, and it is also downgrading the herbal products due to adulteration, inferiority, substitution, admixture, etc. Local communities, traditional medicinal herbalists, and herbal medicine vendors popularly collect roots, bark, and whole shrubs, and thus subject the naturally grown plant species in the verge of extinction. The uncontrolled collection and sale of large quantities of plant material from the forest leads to the destruction of many forest plants. These problems associated with wild medicinal plants have necessitated systematic cultivation of many medicinal plants, and domestic cultivation is a viable alternative to overcome the problems that are inherent in the production of herbal medicines including species misidentification,

Table 9.1 Some most common medicinals from plant sources

Medicinal plant source	Medicinals	Activity
Aglaia foveolata	Silvestrol	Cytotoxic
Artemisia annua	Artemisinin	Antimalarial drug
Atropa belladonna	Atropine	Anticholinergic
Camptotheca acuminata	Camptothecin	Antitumour
Capsicum frutescens	Capsaicin	Counterirritant
Cassia angustifolia	Sennosides A and B	Laxative
Cassia quinquangulata	Resveratrol	COX-1 enzyme inhibitor
Castanospermum australe	Castanospermine	Glycoside inhibitor
Catharanthus roseus	Ajmalicine	Antihypertensive
Catharanthus roseus	Vinblastine, vincristine	Anticancer
Cephaclis ipecaccuanha	Emetine	Powerful emetic
Cinchona spp.	Quinine, Quinidine	Antimalarial
Colchium autumnale	Colchicine	Antitumour
Coleus forskolii	Forskolin	Bronchial asthma
Coptis japonica	Berberine	Antibacterial, anti-inflammatory
Datura metel, *D. stramonium*	Scopolamine	Anticholinergic, antihypertensive
Digitalis lanata, *D. purpurea*	Digoxin, Digitoxin	Cardiotonic glycosides
Dioscorea deltoidea	Steroids	Antifertility agents
Erythroxylum pervillei	Pervilleine A	Anticancer
Galanthus woronowii	Galantamine	Anti-Alzheimer's drug
Hyoscyamus niger	Hyoscyamine	Anticholinergic
Lithospermum erythrorhizon	Shikonin	Antibacterial, Antiseptic
Orchrosia elliptica	Ellipticine	Antitumor
Panax ginseng	Ginsenosides	Health tonic
Papaver somniferum	Opium alkaloids, Codein	Analgesic, antitussive, sedative
Pilocarpus abonandi	Pilocarpine	Cholinergic
Podophyllum petalum	Podophyllotoxin	Antitumor
Rauvolfia serpentina	Reserpine	Antihypertensive
Sanguinaria canadensis	Sanguinarine	Antiplaque
Taxus brevifolia	Taxol	Anticancer drug
Trichosanthes sp.	Trichosanthin	Cytotoxicity against HIV-infected cells

genetic and phenotypic variability, variability and instability of extracts, toxic components, and contaminants. Cultivation offers the opportunity to optimize yield and achieve a uniform, high-quality product. Several plant-derived drugs like cardamom, cannabis, cinnamon, ginger, cinchona, opium, linseed, and fennel are now obtained almost exclusively from cultivated plants. Medicinal plants play a central

role as therapeutic agents and also as trade commodities of the international markets. Cultivation of medicinal plant is gaining ground because of the sky rocketing prices of allopathic medicines which also have side effects. Cultivation of medicinal plants is widely viewed as a means for meeting current and future demands for large volume production of plant-based drugs and herbal remedies, and also as a means for relieving harvest pressure on wild populations (Palevitch 1991; WHO/IUCN/WWF 1993; FAO 1995; Lambert et al. 1997; de Silva 1997).

Benefits of Commercial Cultivation of Medicinal Plants

Cultivation Can Reduce Growing Pressures on Wild Medicinal Plants

The World Health Organization (WHO) has estimated that more than 80% of the world's population in developing countries depends primarily on herbal medicine for basic health-care needs, and the use of herbal medicines in developed countries is also growing; about 25% of the UK population takes herbal medicines regularly (Vines 2004). Approximately two-thirds of the 50,000 different medicinal plant species in use are collected from the wild (Edwards 2004) and, in Europe, only 10% of medicinal species used commercially are cultivated (Vines 2004). There is growing concern about diminishing populations, loss of genetic diversity, local extinctions, and habitat degradation. Well-known species threatened by wild harvesting include *Arcostaphylos uva-ursa*, *Piper methysticum*, and *Glycyrrhiza glabra* (Vines 2004). Between 4000 and 10,000 medicinal species might now be endangered (Edwards 2004).

Ensures Pure and Smooth Supply

Importance of cultivation of medicinal plants may be justified on the basis of the following points. (i) It ensures a correct natural source of the drug. (ii) The process of collection and harvesting of the drugs can be effectively monitored under cultivation, i.e., they can be collected at the right time and in the proper manner. (iii) Drying and storage of the drugs from cultivated sources can be more effectively regulated and controlled ensuring the production of good-quality drugs. (iv) Purity of the finished product is assured under cultivation as weeds and other contaminants can be removed by careful weeding during the growth of the crop. (v) Quality and production of the drug can be improved under cultivation by the selection of high-yielding and disease resistant seeds and varieties, by the use of natural and synthetic fertilizers, which increase the total yield of the plants and their active constituents, e.g., nitrogenous fertilizers increase alkaloid content of Solanaceous plants and by the production of hybrids with high-yielding and disease resistant properties. (vi) Cultivation ensures constant and regular supply of genuine drugs. (vii) Prices of crude drugs and monopolies of their production can be controlled and reduced by cultivation. (viii) Illegal trade of dangerous drug like cannabis and opium can be restricted by controlled cultivation of such drugs. As evident from these points, it is preferable that crude drugs are obtained from cultivated sources.

Means of Earning Livelihood

Cultivation of medicinal plants is economically very attractive. Advantages of commercial of medicinal plants over wild harvest for production of plant-based medicines include (i) wild collection often offers material adulterated with unwanted, sometimes harmful other plant species while cultivation provides reliable botanical identification and production of uniform materials; (ii) wild harvest volumes are dependent on many factors that cannot be controlled and the irregularity of supply is a common feature while cultivation guarantees continuity and steady supply of raw material; (iii) wholesalers and pharmaceutical companies can agree on volumes and prices over time with the grower; (iv) the selection and development of genotypes with commercially desirable traits from the wild or managed populations may offer opportunities for the economic development of the medicinal plant species as a crop; (v) cultivation allows controlled post-harvest handling and therefore (vi) quality controls can be assured through cultivation; (vii) cultivation can provide opportunities for value addition through processing; (viii) product standards can be adjusted to regulations and consumer preferences; (ix) cultivated material can be easily certified organic or biodynamic, although certifiers are also presently developing wild crafting standards; (x) commercial cultivation of medicinal plants helps to conserve endangered species in their natural habitat; (xi) provides good income to the farmers; and (xii) provides a better environment through utilizing waste and unproductive lands (Palevitch 1991; Pierce et al. 2002).

Limitations of Commercial Cultivation

The principal disadvantages of cultivation of medicinal include (i) failure of crops due to (i) adverse weather conditions such as flood, drought, frost, or heavy rain during growth and harvesting seasons; (ii) fungal and viral diseases which spread rapidly among closely growing plants of the same species, e.g., attack of Belladonna by *Phytophthora* species; (iii) large-scale damage of the crops by the attack of insects (like the flea beetle) and rodents in the field; (iv) high production cost; and (v) lack of required environmental conditions for cultivation of a particular medicinal plant. For example, Indian hemp requires a typical tropical climate for the production of the narcotic resin. However, all these drawbacks, which are also common to other cultivated crops, should not discourage the cultivation of drug plants as the benefits derived from cultivation greatly outweigh these disadvantages. Beside these limitations, there are some other constraints to commercial cultivation of medicinal plants.

As compared to other economic crops, medicinal plants have received much less attention in genetic and cultural improvements. Only a few countries are now cultivating improved cultivars, while the rest still depend on wild material collected for cultivation. Their cultivation techniques are quite primitive, resulting in poor yield and quality of the materials. Several constraints include (i) biotic—unimproved cultivars, long life cycle, susceptibility to pests and diseases; (ii) abiotic—low soil fertility, flood and drought, improper light intensity and duration, extremes of temperatures, and physical injuries or damage; (iii) technological—lack of good agronomic practices, lack of technology and technology transfer, and lack of

facilities; and (iv) socioeconomic—competition with other economic crops and modern drugs; lack of market channels, a domestic pharmaceutical industry, and organized cultivation and no price support. In spite of these constraints, medicinal plants continue to play a significant role in the welfare of rural people in Asia and other parts of the world. Due to higher demand of raw material for industrial processing coupled with the loss of natural habitats of most medicinal plants, large-scale cultivation of promising species has recently been attempted in several countries.

9.1.3 Factors Affecting of Cultivation of Medicinal Plants

Cultivation of some herbs has proved difficult because of low germination rates or specific ecological requirements (Vines 2004). There could simply be a lack of knowledge about the specific requirements for pollination, seed germination, and growth. Low germination rates frequently result from fungal infection or mechanical damage to seeds and can be improved by seed treatments and by ensuring optimum storage conditions. Stratification, the artificial emulation of environmental conditions required for seed germination such as soaking or chilling, can sometimes provide the key to success. In *Panax quinquefolium* (American ginseng), the use of a controlled environment substantially shortens the stratification period required, increases germination rate and seed viability, and enables seed germination at any time of the year (Li et al. 2000). Similarly, it might be necessary to optimize the conditions for pollinators or to conduct artificial pollination. The use of controlled environments including hydroponics could be one way in which difficult-to-grow medicinal plants can be cultivated on a commercial scale.

Other problems associated with cultivation of medicinal plants are in no way different from those associated with other agricultural crops. The factors that create great problems for cultivation of medicinal plants may be roughly divided into two groups: (a) climatic factors and (b) ecological factors.

Climatic Factors

The climatic factors that directly affect the growth of a plant include (i) altitude, (ii) temperature, (iii) sunlight, and (iv) rainfall.

(i) Altitude refers to the specific elevation of a land surface in comparison with the sea level. This factor influences the growth of plants very seriously, and thus, plants of different altitudes vary greatly from each other in type, nature, and constituents. A plant of the higher altitude cannot therefore be profitably cultivated in a land of lower altitude. (ii) Temperature influences plant growth considerably. Plants growing in a tropical climate do not normally survive in a temperate region. (iii) Sunlight or length of day or photoperiod plays a significant role in plants' growth and production of chemical constituents. Short-photoperiodic plants do not grow well under longer photoperiodic condition. (iv) Rainfall determines the type of vegetation of a region. Every plant requires enough rainfall for its growth and

survival, but the requirement varies from plant to plant. While rainfall is an important factor for plant growth, heavy rainfall or drought is highly detrimental to their growth, particularly under cultivation.

Ecological Factors

Ecological factors like soil condition, soil pH, and associated plant growth (weed) are of great importance in the cultivation of medicinal plants. Soils differ from each other both in physical and chemical properties and may be a clay or loamy soil. The loamy soils may be either sandy barns or loamy sands. These different soils have different water retention capacity which determines the type of plants grown or cultivated in them. Selection of the correct type of soil, which is not always practicable, is very important in the cultivation of various plants including the medicinal ones. Weeds often pose a serious problem in the cultivation of medicinal plants. They affect the crop adversely in a number of ways: (i) use up the essential food elements and manures used for cultivation and thus compete with the drug plant; (ii) prevent sunlight reaching the drug plant; (iii) choke the drug plant by occupying essential land space; (iv) introduce difficulty in collection and contaminate the collected drug, and (v) attract and harbor insects, fungus, and other microorganisms.

Cultivation of medicinal plants especially high-value medicinal plants is creating new dimension in the field of agriculture. For active drug principles of therapeutic importance, medicinal plants are now cultivated following: (a) field-level cultivation through agricultural practices and (b) biotechnology and in vitro production of secondary metabolites.

9.1.4 Field-Level Commercial Cultivation of Medicinal Plants (e.g., Rauvolfia Serpentine*,* Cinna *spp.,* Atrpa Belladonna, and Catharanthus Roseus*)*

Commercial cultivation at field level (open field, homestead garden, forest floor) is a viable alternative and offers the opportunity to overcome the problems that are inherent in herbal extracts like misidentification, genetic and phenotypic variability, extract variability and instability, toxic components, and contaminants. Field crop cultivation in a scientific way at field level is an agronomic practice. Agronomy is the science and technology of crop producing in the field for food, fuel, fiber, and others. Agronomy encompasses work in the areas of plant genetics, plant physiology, meteorology, and soil science. The agronomic method of crop cultivation includes systematically the following steps: selection of seeds or propagules of the desired crop, land preparation and basal manuring and fertilization, spacing, seed sowing or seedling transplantation, split application of fertilizer, irrigation, intercultural operation, and weed control and harvesting.

Cultivation of medicinal plants involves all the common processes and methods utilized for cultivation of other agricultural crops. They can be cultured by true seeds as well as by vegetative propagules.

Some of the Basic Steps of GACPs for Medicinal Plants

(i) Selection of medicinal plant for cultivation,
(ii) Botanical identity,
(iii) Seeds and other propagation material,
(iv) Method of cultivation,
(v) Harvest, and
(vi) Personnel.

(i) Selection of medicinal plant for cultivation

Species or botanical variety selected for cultivation should be same as that specified in national pharmacopoeia or recommended by other authoritative national documents of the end—user's country. In case of newly introduced medicinal plant, the species or botanical variety selected for cultivation should be identified and documented as the source material used or described in traditional medicine or the original country.

(ii) Botanical identity

Scientific name (genus, species, subspecies/variety, cultivar, family) should be verified and recorded. Cultivar name, ecotype, chemo-type, or phenotype may also be provided, as appropriate name of the material supplier should be recorded. In case of land races collected, propagated, disseminated, and grown in a specific region, records are kept of the locally named lines, including the origin of the source seeds plants or propagation material.

(iii) Seeds and other propagation material

All information relating to identity, quality, and performance (as well as breeding history where possible) of the propagation material (seed or vegetative propagule) is obtained from the supplier and recorded. Planting material should be free from contamination and disease to promote healthy plant growth. It is necessary to be careful to exclude extraneous species, variety, or strain. Any genetically modified germplasm should comply with regional and/or national regulations and be appropriately labeled and documented, as required.

(iv) Method of cultivation

If no scientific published or documented agrotechnology data are available, traditional method of cultivation to be followed or agrotechnology is developed through research work. Principles of good plant husbandry, including appropriate rotation of plants selected according to environment suitability, should be followed. Conservation agriculture technique (CA) is followed where appropriate, particularly in the build-up of organic matter and conservation of soil humidity. CA aims to

conserve, improves, and makes more efficient use of natural resources through integrated management of available soil, water, and biological resources combined with external inputs. It contributes to environmental conservation as well as to enhanced and sustained agricultural production. Cultivation process involves site selection, ecological and social impact, climate, soil, irrigation and drainage, plant maintenance, and protection.

Site Selection

When cultivated at different sites, same medicinal plant may exhibit differences in quality due to soil, climate, and other factors. These differences may relate to physical appearance or to variations in their constituents. Risk of contamination as a result of pollution of soil, air, or water by hazardous chemicals should be avoided. The impact of past land uses on cultivation site, including the planting of previous crops and any application of plant protection products, should be evaluated.

Ecological and Social Impact

The ecological impact of cultivation should be monitored overtime, where practical because cultivation of medicinal plants may affect ecological balance and in particular, the genetic diversity of the flora and fauna in surrounding habitats; the quality and growth of medicinal plants can also be affected by other plants, living organisms, or by human activities, etc. Introduction of non-indigenous medicinal plant species into cultivation may have a detrimental impact or biological and ecological balance of the region. The social impact of cultivation on local communities should be examined to ensure that negative impacts on local livelihood are avoided. In terms of local income—earning opportunities, small-scale cultivation is preferable to large-scale cultivation provided the small-scale farmers are organized to market their products jointly. In case of the establishment of large-scale cultivation of medicinal plants, care should be taken for local communities benefit for fair wages, equal employment opportunity, and others.

Climate Conditions

Climate conditions, e.g., length of the day, rainfall (water supply), and field temperature, significantly influence the physical, chemical, and biological qualities of medicinal plants; duration of sunlight; average rainfall; average temperature; and anytime and night time temperature differences influence the physiological and biochemical activities of plants and prior knowledge should be considered.

Soil

Soil should contain appropriate amounts of nutrients, organic matter, and other elements to ensure optimal medicinal plant growth and quality; optimal soil conditions including soil type, drainage, moisture retention, fertility, and pH should prevail; correct type and quantity of fertilizers are necessary with minimum risk of leaching; human excreta as fertilizer to be avoided due to potential presence of infectious microorganisms and parasites; and animal manure should be thoroughly composted to meet safe sanitary standards of acceptable microbial limits

Irrigation and Drainage
Irrigation and drainage should be controlled and carried out in accordance with the needs of the individual medicinal plant species during its various stages of growth. Water used for irrigation should comply with local, regional, and or national quality standards. For choice of irrigation, as a general rule, the health impact of different types of irrigation (various forms of surface, subsurface or overhead irrigation), particularly on the risk of increased vector-borne disease transmission, must be taken into account.

Plant Maintenance and Protection at Field Level
Plant maintenance and protection at field level (e.g., timely topping, bud nipping, pruning, and shading) should be in favor of the growth and development characteristics of medicinal plant as well as part of the plant destined for medicinal use to improve the quality and quantity of medicinal plant material. Use of any agrochemical for growth promotion and protection should be kept to the minimum. Integrated pest management should be followed. When necessary, only approved herbicide/pesticide is applied at minimum effective level as per label instructions. All applications should be documented. Growers should comply with maximum pesticide/herbicide residues

(v) Harvest

Time of harvest depends upon the plant part to be used and for that national pharmacopoeia, official monographs, and published standards may be consulted. MPs parts are to be harvested during optimum season or time period to ensure best quality of harvested material. Concentration of bioactives varies with the stage of plant growth and development. Best time for harvest (quality peak season/time of day) should be based on maximum concentration of bioactive principles. During harvest, no foreign matter, weeds, or toxic plants are not to be mixed with the harvest and dew, rain, humidity, etc., should be avoided during harvest. Immediate drying after harvest is advisable. Cutting devices, harvesters, and other mechanical devices should be clean and adjusted to reduce damage and contamination from soil and other material. Storage under uncontaminated dry place free from insects, rodents, birds, and other pests and inaccessible to livestock and domestic animals is recommended. Contact with soil and humidity to be avoided to minimize microbial load in harvested material. Clean baskets, dry sacks, trailers, hoppers, or other well-aerated containers are suggested for use for transporting harvested material to central place. Any decomposed material should be discarded.

(vi) Personnel

Growers and producers should have adequate knowledge of the MAPs including botanical identification, cultivation characteristics, and environmental requirements. Growers and producers should receive and abide by the instructions on all issues relevant to the protection of the environment, conservation, and proper agricultural stewardship for producing quality MAPs material. All personnel (including field workers) involved in propagation, cultivation, harvest, and post-harvest stages

should maintain appropriate personal hygiene and should have received training regarding their hygiene responsibilities and in MAPs cultivation and harvesting.

Cultivation by True Seeds
Like any other crops, drug plants are raised from seeds. This method of propagation involves different steps, e.g., selection of seeds, preparation of seed beds, sowing of seeds, and transplantation of the seedlings, irrigation and weeding, protection of the crop and harvesting.

Cultivation by Vegetative Propagules
Cultivation by vegetative propagules or organs involves a number of methods including cuttings, layers, grafting and budding, fermentation.

Vegetative Organs
Cultivation of medicinal plants may be done by various vegetative organs like bulbs, corms, tubers, rhizomes. Vegetative organs are planted in large numbers to raise a crop such as (i) by division or separation of a plant into its individual constituent aerial stems or buds (each having roots and a growing point) and planting them separately, (ii) by bulbs as is done in the propagation of garlic, onion (*Alium sativum*, *A. cepa*), rhubarb (*Rheum rhabarbarum*), squill (*Drimia maritime*), and gentian (*Gentiana* spp.); (iii) by runners or offsets as produced by many plants like cocoyam (*Colocasia esculenta*, *Xanthosoma* spp.), mints (*Mentha longifolia*), etc., when the runners with the daughter plants are detached from the mother plant and planted, (iv) by suckers or stolons—in this case, the suckers are separated from the mother plant and planted separately as in liquorice (*G. glabra*), (v) by corms as in coichicum, teliga potato (*Colchicum speciosum* and other species, *Amorphophallus bulbifer*), (vi) by tubers as in winter aconite (*Eranthis* spp.), and (vii) by rhizome as in ginger (*Z. officinale*).

Cuttings
Cuttings are made by severing a stem (or root) into many parts, each having at least one node. On dipping into soil, roots and buds develop from the nodes. This method is applied in propagating large number of plants, e.g., rose, grapes, coca, and snakeroot.

Layers
In this method, a branch or shoot is induced to produce roots by partly interrupting the food supply by removing a portion of the bark at one part of the stem. This part is then embedded or covered with soil and regularly supplied with water. When roots develop at the treated part, the stem is severed from the plant with the roots and then planted. Propagating plants by means of layers always ensures exact duplication of the mother plant. This is a popular method of propagation, particularly with fruit trees.

Grafting and Budding
These are not commonly used for propagation of medicinal plants, except for some experimental purposes. Grafting is a method of growing the foliar parts of one plant, termed as action, on the main or side stem of another related plant, called the

stock. In making a graft, two stems of equal size and age of two related plants are cut obliquely using a sharp knife or a razor blade to ensure a clean cut across. The severed parts are then exchanged, fitted together, tied with a string, and covered. In a few weeks time, the scion and the stock arc naturally joined and the wound health. In budding, a piece of bark bearing a bud is removed from one plant and is introduced into a suitable cavity or a 1-shaped slit made in the bark of another plant or stock, which finally bears the developed bud. This is largely used in citrus plants for growing sweet orange branches on sour stocks.

Fermentation

Mold and bacteria are propagated by a process called fermentation. In this method, strains of the microorganisms are allowed to grow by seeding a small amount of the selected strain in a suitably prepared liquid nutrient medium.

Farming for Cultivation of Medicinal Plants

World needs eco-friendly farming systems for sustainable agriculture, sustainable from environmental, production, and socioeconomic points of view. Sustainable agriculture has become the umbrella under which many alternative farming systems (e.g., organic, biological, regenerative, alternate, ecological, low input agriculture) fall. Sustainable agriculture system reduces environmental degradation, maintains agricultural productivity, promotes economic viability in both the short and long term, and maintains stable rural communities and quality of life as well as emphasizes the conservation of its own resources. Sustainable farms minimize their purchased inputs (fertilizers, energy, and equipment) and rely, as much as possible on the renewable resources of the farm itself without any adverse effect on bio-physical resources including soil, water, and biota of the environment. It embraces several forms of non-conventional agriculture that are often called organic, alternative, ecological, or low input.

Medicinal Plants the First Crops for Organic Farming

Organic farming in dry lands can be started with medicinal plants because

(i) The forest resource of medicinal plants is decreasing, but demand is increasing thus cultivation is the only solution to fill this gap;
(ii) Medicinal plants are for the curing of disease and any residue of pesticide can convert it into poison. Hence, medicinal plants should only be cultivated in organic farming; and
(iii) Use of high dose of inputs like fertilizers, irrigation may change the composition and quality of medicinal plants. Growing near to natural conditions is the best way to maintain the quality, which is possible in the organic farming.

(i) Cultivation of *Rauvolfia serpentina*

Sarpagandha or snakeroot (*R. serpentina* Benth. Ex Kurz, $2n = 22$, of Apocynaceae) is an important medicinal plant distributed in the moist deciduous forests of Southeast Asia including Bangladesh, India, Myanmar, Sri Lanka, Thailand, Malaysia, the Andaman Islands, and Indonesia. In Bangladesh, it is found

in the foothills of greater Chittagong and Sylhet districts, in Sal forest and in many parts of the country. It is an erect evergreen, perennial under-shrub, 75 cm to 1 m in height. The root system of *R. serpentina* consists of a prominent, tuberous, soft taproot, reaching a length of 40–60 cm deep into soil in a 2 year old plant. Its diameter at the thickest portion varies from 1.2 to 2.5 cm. The root, especially the root bark, possesses high alkaloid concentration (40–60% of the whole root). Root contents of alkaloids vary from 1.7 to 3% of the dry roots. The fresh roots emit a characteristic acrid aroma and are very bitter in taste. The world requirement of dried *Rauvolfia* roots is about 20,000 t/year, but only about 400–500 tons of roots are presently collected annually from wild source (Poonam and Mishra 2013; Paturkar and Khobragade 2016).

In addition to *R. serpentina,* there are two other species, e.g., *R. tetraphylla* and *R. vomitaria*. Root of *R. serpentina* has a 400-year history of use in the treatment of snakebite, insect stings, nervous disorder, and others, and the alkaloids obtained from it have been recognized by the allopathic system in the treatment of hypertension and as a sedative or tranquillizing agent.

Climate

R. serpentina can be grown under a wide range of climate conditions. It flourished in tropical or subtropical hot, humid conditions and can be grown both in the sun and in partial shade. In its natural habitat, the plant thrives under the shade of forest trees. The best areas are those which combine high rainfall (250–500 cm) with properly drained soil. In low rainfall areas, the plant can be successfully cultivated with irrigation during the drier months. The plant is sensitive to water-logging, but it can withstand water for 2–3 days without too much damage. The plant sheds its leaves during the cold months in localities with severe winters. Frost kills the top tender, green twigs only, and fresh shoots sprout up with the advent of spring from the thicker shoots which can withstand the frost.

Soil

The plant grows in a wide variety of soils, from sandy alluvial loam, clay or clayey loam to red lateritic loam of stiff dark loam, but prefers soil rich in nitrogenous organic matter with good drainage. The plant produces thicker roots in black, stiff loam soils than in heavy clayey or sandy soil. Soils containing large quantities of sand retard the growth of the plants and make them more susceptible to root and leaf diseases. The ideal soil pH for this crop is acidic (pH 4.6–6.2), and alkaline soils (pH 8 or above) are not suitable for commercial cultivation.

Land Preparation

The plant requires slightly acidic to neutral soils for good growth with medium to deep well-drained fertile soils. Clay-loam to silt-loam soils, rich in organic content are suitable for its commercial cultivation. It grows well in frost-free tropical to subtropical situations under irrigation.

Propagation

The crop may be propagated by seed, stem cuttings, root stumps, and root cuttings. Seed propagation is the best method for raising commercial plantation.

Propagation by Seed

The *Rauvolifia* is usually propagated by seeds but irregular and low percentage of germination of seeds is the main difficulty in the propagation of. This is partly attributed to the adverse influence of the stony endocarp, absence of embryos due to parthenocarpy or somato-plastic-sterility.

Collection of Seeds

Rauvolfia fruits mature between July and November, and collection of mature seeds is usually done from September to February. Only a few fruits ripen at a time and, if they are not collected immediately, they are shed and lost. Therefore, the collection of ripe fruits is easy in plantation but laborious and costly from plants growing in the wild. After collection, the fruits are freed from their pulpy covering by rubbing them against old gunny bags or on rough flooring. The cleaned seeds are thoroughly dried in the sun and stored in dry places or in airtight containers; seeds thus stored in airtight bins, retained their viability for about 6 months. The viability of the seeds drops markedly with the increase in the interval of time between collection and sowing.

Seed germination in *Rauvolfia* varies from 10 to 60% even when only heavy seeds are chosen for sowing purpose. Light and heavy seeds can easily be separated by simple water flotation. Germination of heavy seeds during May–June after soaking them in water for 24 h was 20–40, and 74% germination was recorded in case of freshly collected fully matured heavy seeds. The germination rate of the seed also differs under varying agroclimatic conditions. Direct sowing of the seeds in the field has not been successful due to this variability of seed germination, and it is therefore suggested to develop seedlings in the nursery bed. In all, 6 kg of seeds are sufficient to raise one-hectare plantation.

Preparation of seed bed in nursery

The nursery should ideally be located in partially shaded areas with irrigation facilities. The land is cleared of weeds and plowed to a depth of 30 cm. Raised beds, each of 10 × 10 m dimension, are made containing one-third quantity of well-matured farmyard manures (FYM) and leaf mold, and two-thirds of medium-fine silt-loam soil. Seeds should be soaked in water overnight before sowing and light seeds which float can be discarded. The seeds can be treated with Thiram, a non-systemic fungicide, at the rate of 3 g/kg of seeds. About 5.5 kg of seeds sown in a 500 m^2 area will yield seedlings sufficient to plant one hectare. The seeds are sown 2–3 cm apart in rows in shallow furrows in the middle of May. The furrows are then covered with a fine mixture of soil and FYM, and the bed was kept just moist by light irrigation. The germination is gradual, starts after 15–20 days and continues up to 40–45 days, and the growth of the seedlings is slow. Seedlings are ready by mid-July for transplanting.

Propagation by vegetative propagules

Propagation by vegetative propagules like root or shoot cuttings has been advocated for easy propagation and quick multiplication of the genetically superior clones. Alamgir and Ahmed (2005) described the easy way of propagule development from

R. serpentina by stem (42.85%), root (62.82%) and stem-root junction (78.57%) cuttings. Pretreatment of cuttings by rooting hormones like NAA (10 ppm), IBA (50 ppm) or 2, 4-D (5 ppm) may increase the number of % of successful propagules.

Propagules Development in the Nursery Bed

Large taproots with a few filiform lateral secondary rootlets or stem are used. Nearly 2.5–5.0 cm long (or more up to 15 cm long and 2.5 cm diameter from 1.5- to 2-year-old plant) root or stem cuttings are planted during spring season in nursery beds containing well matured FYM, sand, and saw-dust. The beds are kept moist through watering. The cuttings begin to sprout within 3 weeks. These can be planted in field during rainy season after 8–10 cm rains are received; the seedlings are transplanted at 45-cm row-to-row and 30-cm plant-to-plant distance. In this manner, an estimated 100 kg of root or stem cuttings is found sufficient for planting one-hectare area. Direct plantation of cuttings may be done but seedlings developed from root or stem cuttings in nursery is more preferable for uniform population.

Transplantation

Seedlings of 40–50 days, which have 4–6 leaves, are ready for transplanting by mid-July. The seedlings are carefully dug out, and the top root should be cut. They are then dipped in a 0.1% fungicide solution to avoid soil-borne fungus causing damping-off disease. Well-rotten FYM at 25–30 t/ha is added during land preparation. The field is then divided into small plots for irrigation. About 15-cm-deep furrows are dug at a distance of 45 cm. The seedlings are transplanted in rows into the furrows in holes large enough to receive the seedlings along with the accompanying clump of earth at 30 cm distance (45 × 35 cm = space between rows and plants). The seedlings are buried up to the first pair of leaves, and soil around them is lightly pressed. Irrigation after transplanting is essential for better stand. *Rauvolfia* is long-duration (18 months) and slow-growing crop particularly in the initial stage; thus, different intercrops have been tried.

Manures and Fertilizers

The medicinal plants have to be grown without chemical fertilizers. The use of organic manure (decomposed leaf and compost, FYM, green manure, vermi-compost, etc.) has been recommended to increase the quantity of nutrients in the soil and improve the drainage. Initially before sowing, 10–15 tonnes of FYM/ha are used. However, the plant responded better to chemical fertilizers than to organic manures. Nitrogenous fertilizers induce more vegetative growth, followed by organic manure. Nitrogen fertilizer in combination with FYM and phosphates results in better root growth than nitrogen alone. Application of phosphates induces more growth of thick as well as thin roots. It is suggested to apply 25–30 t of FYM at the time of land preparation and 10 kg N, 60 kg P_2O_5, and 30 kg K_2O/ ha as a basal dose. Later two equal doses of N, each of 10 kg/ha in moist soil is given at 50 and 170 days after planting.

Irrigation

Rauvolfia can be raised as rainfed crop under subtropical conditions if the growing area receives 150 cm or above rainfall distributed uniformly throughout the growing season. It needs regular irrigation that is necessary under tropical condition with high temperature and low rainfall. About 15–16 irrigations at 20-day interval in summer and at 30-day interval in winter are suggested. The crop can be cultivated under rainfed conditions also, but the yield is considerably poorer.

Intercropping

It is possible to grow intercrops in *Rauvolfia* plantation, particularly where good irrigation facilities are available. It is reported that although the yield of roots was higher under monoculture, but the highest net return is possible when *Rauvolfia* was intercropped with soybeans and onions or soybeans and garlic.

Weeding

In order to maintain the satisfactory development of roots, about two weeding are necessary during the monsoon and one hoeing at the end of the growing season (December). This may be done in large plantations using a tractor-drawn cultivator which is cheaper than manual labor. Hoeing by means of a tractor-drawn wheel-hoe is the most economical.

Disease

Leaf spot caused by *Cercospora rauwolfia* manifests as dark-brown colored spots on the upper surface of the leaf and yellowish-brown on lower surface. Dithane Z-78 or M-45 (0.2%) is to be sprayed in early June with a monthly interval until November; *Alternaria tenuis may* cause minute, brownish or dark-colored circular spots with a yellowish margin on ventral side of leaves, and spray of watery solution of Blitox (3/l) is suggested as remedy; mosaic disease may be avoided by proper selection of the germplasms (Shetty et al. 2014; Paturkar and Khobragade 2016).

For the control of root knots or galls of various sizes associated with stunted growth, etiolation and decrease in leaf size are due to the presence of mites, various soil fungi and nematodes (*Heterodera* sp.), especially in brown clay soils (but not dark clay soils), application of 25 kg of 3G Carbofuran or 20 kg of 10G Phorate granules/hectare is recommended; and for the control of pyralid caterpillar (*Glyphodes vertumnalis*) or some other caterpillars (*Daphnis nerii*, *Deilophola nerii*), 0.2% Rogor spray is suggested; mixing of phorate granules with the soil at the time of nursery preparation is recommended to check attack of Cockchafer and Haygrubs.

The other diseases reported include target leaf spot caused by *Coryneospora cassicola* and *Pellicularia filamentosa*, leaf-blotch caused by *Cercospora serpentina*, anthracnose caused by *Collitotrichum gloeosporoides*, die back caused by *Collitotrichum dematium*, powdery mildew caused by *Leviellula taurica* and fusarium wilt (*Fusarium oxysporum*, *F. rauvolfii*). The root-knot nematode (*Meloidogyne* spp.) is also reported on this crop.

This medicinal plant, however, can be grown without chemical pesticides using biopesticides prepared (either single or mixture) from neem (kernel, seeds, and leaves), Chitrakmool (*Plumbaga zeylanica*), Dhatura, cattle urine, etc. However, following measures have been reported to control different diseases.

Harvesting and processing

Maturity period is 3 years when the subaerial parts become dry and main root reach a depth of 0.9 m. However, roots of exploitable size are generally collected 2–3 years after planting or from 18 months onward. It is reported that roots dug out in December (winter) when the plants have shed their leaves are richer in total content of alkaloids than the roots harvested in August (summer). However, root yields at different age and season have showed that 18 months duration crop produce maximum root yield. Transplanting is done in July, and the harvesting period coincides with the shedding of leaves during early autumn season next year. At this stage, the roots contain maximum concretion of total alkaloids. At harvest, the root may be found to go up to 40 cm deep in the soil. Harvesting is done by digging up the roots, and thin roots are also collected. A light irrigation should be given in advance to facilitate easy digging of roots. The roots may be dug out carefully from the subsoil, manually or by using a board plow. After digging the roots are cleaned, washed, and cut into 12–15-cm pieces for convenience in drying and storage. The dry roots (air dried) till they become brittle possess up to 8–10% of moisture. The dried roots are stored in polythene lined gunny bags in cool dry place to protect it from mold. Care should be taken to keep the root bark intact as the bark constitutes 40–56% of the whole root and has a higher alkaloid content. Under the present system, only taproots are selected for processing. It has been observed the rootlets are also rich in alkaloids, so these should be included in the material.

Average Yield

Optimum yield of roots (including thick, thin, and fibrous) is obtained when the propagation is done by seeds. The yield of fresh roots per plant varies widely from 0.1 to 4 kg, the total yield of air dry roots in the case of plants per hectare raised from seeds and stem cuttings was estimated to be about 1175 and 1750 kg, respectively. On an average, root yield varies from 1500 to 2500 kg dry root per hectare under irrigation depending upon soil fertility, crop stand, and management. Some cultivators report that the average yield is 2700–3300 kg dry roots and 8–10 kg seed per hectare from a 2- and 3-year-old plantation under irrigated conditions on sandy, clay-loam soil. Soil NPK level, especially the nitrogen level, positively influences growth and alkaloid level in different medicinal plants including *Rauvolfia* (Alamgir et al. 1999).

(ii) Cultivation *Senna*

Senna (*Cassia* spp. of Caesalpiniaceae) is a valuable plant drug in Ayurveda and modern system of medicine for the treatment of constipation. It is a acidophilus helophyte. Senna is a small perennial shrub of less than a meter in height ascending branches. The leaves are compound pinnate, petiolate about 10 cm long, and bear 5–8 pairs of leaflets each on a small stalk. Cultivation of Senna does not require

much expenditure on irrigation, manuring, pesticides, protection, and other pre- and post-harvest care.

Senna is native to Yemen, South Arabia, and Egypt. *Cassia angustifolia* Vahl. is now naturalized and cultivated in some parts of Tamil Nadu, Bangalore, Gujarat, and Delhi in India. India is the main producer of this crop in the world and exports Senna leaves and pods worth over Indian currency 6 million annually. Leaves and pods are the usable parts in which they contain sennoside, the laxative principle extensively used as a laxative in medicine.

Soil and Climate

The crop can thrive on a variety of soils from sandy red loams to alluvial loams. The average pH ranges from 7 to 8.5. It is very sensitive to water-logging and hence requires well-drained soils. Senna is a warmth loving crop (18.30 °C), requires bright sunshine for its successful growth and can be grown as an early summer (February–March) or a winter (October–November) crop. Heavy rains and cloudy weather during growth are harmful to the crop. An average rainfall of 25–40 cm that distributed from June to October is sufficient to produce good crop.

Land preparation

The land is plowed deep, and the soil is exposed to sun for 110–115 days to dry out roots of perennial weeds followed by two cross plowing harrowing and leveling. FYM is incorporated into the soil at the time of final cross plowing. Then, the land is laid out into plots of convenient size with irrigation channels.

Seed sowing and fertilization

The crop is raised by seeds. The seeds have hard and tough seed coat. Soaking seeds for 10–12 h before sowing was reported to give 100% germination. Senna is grown as a pure crop as well as a mixed crop with gram, ginger, chilies, and cotton. Generally, two sowing seasons are recognized, i.e., February to March (irrigated crop) and November (rainfed crop). The seed rate is 27 kg per hectare under rainfed conditions and 15 kg per hectare for the irrigated crop. The seeds are broadcast or preferably sown at 30 cm lines to 30 cm apart and 1.5–2.5 cm depth in a well prepared land. Germination commences on third day and completed within a fortnight. Before sowing the seeds, the field should be perfectly leveled otherwise it hampers the uniform seed germination. It is found that the seed treatment with Thiram, Captain or Agroson G. N. at 2.5 g/kg protect the seedlings from damping-off and seedling blight diseases which are very common. The application of 20 kg of N and 40 kg of P per hectare at planting, supplemented with 40–60 kg/ha of N in 3 split doses is preferred.

Thinning and weeding

The first weeding cum hoeing is done at 25–30 days of sowing, a second at 75–80 days and a third at 110 days to keep the crop free from weeds. It flowers in about 2 months after sowing and the first flush of flowering stalks is removed to induce a higher degree of branching. Use of Treflan herbicide as pre-emergent spray at the rate of 4 kg/ha has been reported to increase the yield and anthraquinone content.

Manures, Fertilizers and Pesticides
The medicinal plants have to be grown without chemical fertilizers and use of pesticides. Organic manures like, FYM, vermi-compost, green manure, etc., may be used as per requirement of the species. To prevent diseases, biopesticides could be prepared (either single or mixture) from neem (kernel, seeds, and leaves), Chitrakmool, Dhatura, cow urine, etc.

Irrigation
Senna could be economically grown under rainfed conditions. In most years, the crop needs no irrigations except under the conditions of prolonged drought. However, when it is grown as a semi-irrigated crop, the yield increased considerably. About 5–8 light irrigations are enough to raise a good crop of Senna; however, heavy irrigations are injurious to the crop.

Plant Protection
Damping-off is common in the seedling stage. To control the disease, seed treatment with fungicides, e.g., Captain, is recommended. The pods are attacked by a borer during storage, leading to considerable damage.

Harvest and Post-harvest Operation
Senna plant produces foliage containing higher sennosides between 5 and 90 days age, depending upon the total plant growth. The picking of leaves starts in early May when leaves are fully grown, thick, and bluish, and the picking of leaves is done by hand so that most of the growing tops are removed at harvest. This also induces the plants to produce more of branching which otherwise reduce foliage growth considerably. A second picking is taken at 90–100 days and the third harvest between 130 and 150 days when picking of leaves is taken along with the plucking of pods followed by the uprooting of entire plants during August or April–May, and the harvested material includes both leaves and pods together. The pods are harvested a little before maturity to maintain their green color. The leaves and the pods are dried in a thin layer in shade for 4–7 days to reduce moisture level; the pods are lightly beaten during drying to remove the interseed material. Further drying is done in well-ventilated drying sheds that takes 10–12 days to dry completely. The dried leaves and pods should have light green to greenish yellow color. A rapid mechanical drying at 40 °C could also be attempted. The careless drying of the harvested crop spoils the color and lowers its active content. The produce is graded, baled under hydraulic pressure, and wrapped in gunny bags, for long-distance transport or for export.

Yield
A good average crop of Senna can give 15 quintals of dry leaves and 7 quintals of pods per hectare under irrigated and good management conditions. The yield under rainfed conditions is about 10 quintals of leaves and 4 quintals of pods. The produce should contain about 2.5% of active principles, calculated as anthraquinones. Alamgir et al. (2004) reported the increased level of biomass and secondary metabolites production in different members of the Archichlamydeae under rich NPK fertilization.

(iii) Cultivation of *Atropa belladonna*

Belladonna (*A. belladonna* L. of Solanaceae) is European species, presently grown on a small scale in Kashmir, India. The plant is a small perennial herb which grows up to 1.5 m in height. It branched freely and produces a large tapering root. The leaves and roots of belladonna constitute the commercial drug which contains atropine, hyoscyamine. and hyoscine, used in pharmacy for their mydriatic, analgesic, and antispasmodic properties; the roots are used for external application only. The leaves and roots should contain not less than 0.3 and 0.4% of the total alkaloids calculated as hyoscyamine. *A. acuminata* Royel is a closely related Indian species, found at altitudes between 1800 and 3000 m in the western Himalayas; its leaves and roots contain similar alkaloids. A part of belladonna alkaloids and their products used in India is imported.

Climate

The crop prefers a well-drained slightly acidic, silty-loam to clayey-loam soil, rich in humus. It cannot stand water-logged conditions. It is a crop of the temperate climate prefers a sunny location and clear weather, particularly preceding and during the harvesting of the crop; continuous dampness or high humidity favors root rot.

Development of Seedlings in Nursery Bed

The crop shows a wide variation in growth and alkaloid content in its plants population. Seeds from selected plants with high alkaloid contents should be used for raising a plantation. Propagation through seed is the easiest and least expensive method, although vegetative methods, such as shoot, root, and root–shoot cuttings are also used. The seeds are very small, weighing about 700 per g. They should be treated with ethyl alcohol for 3 min or with petroleum either for 6 min for improving germination. The treated seeds should be washed in running water for a few hours to remove the adhering chemicals. The seeds are sown in rows in the nursery bed during early spring. Germination takes place within 10–21 days and is 15–40%. Therefore, 4 kg of seed gives enough seedlings to plants in one hectare.

Transplantation

The seedling bearing 1–3 leaves is planted in the field during August at 45 × 60 cm or 60 × 60 cm spacing. Ridge planting is preferred in localities receiving heavy monsoon rains.

Fertilization, Irrigation, and Management

The land is given about 40 tonnes of farmyard manure, besides 100 kg of diammonium phosphate and 30 g of K_2O per hectare before planting, and 20 kg of N is given at the time of branching and each time the crop is picked. The crop is irrigated after every 10–15 days during summer. The plantation is given to 2–3 weddings and hoeings before the first leaf crop is obtained and then one or two hoeings are usually given before each leaf picking.

Plant Protection

Cutworms (*Agrotis flammatra*) attack the tender growing seedling during yearly summer. The application of 5% Aldrin dust at 20–25 g/m^2 of the nursery bed before sowing protects the crop. The beds may be drenched with 1:19 wettable solutions of chlordane, 2–3 times after every 10 days during the attack of the pace. Sometimes damping-off of seedlings is caused by Pythium sp., and chloropicrin is recommended as a fumigant. Root rot also damages the crop; the affected plants along with the adhering soil are removed and burnt. Seeds treated with the Agrosan generally protect the seedlings from soil-borne diseases.

Harvest and Post-harvest Management

The first picking of leaves is obtained in October; in subsequent years, 3–4 leaf crops are obtained for the next three years. Harvesting is done on bright sunny days by cutting the plants 20–25 cm above the ground, except at the time of the autumn harvest when the plants are cut 3 cm above the ground. The stumps put forth fresh growth during the succeeding spring and bear flowers during June–August, and the berries are produced in October. The alkaloids are synthesized in the root and are translocated through the stem to the leaves.

The harvested crop is dried rapidly in the sun for 2–3 days, and the leafstalks are detached only after the produce is dried. The plants are uprooted after three or four years; the thicker are sliced into 3–4-cm-long pieces and dried. The crop loses 70–80% of its weight during drying. A well-dried leaf crop retains its green color.

Yield

The average crop yield in the first year is 300 kg of leaves and thereafter, 750 kg of leaves per hectare annually. An additional root crop of 200–300 kg per hectare is obtained when the plants are finally uprooted. Higher average yield of 1–1.2 tonnes per hectare is reported from European countries. The produce should be stored in cool dry place away from light.

(iv) Cultivation of *Catharanthus roseus*

C. roseus, commonly known as the Madagascar periwinkle, is native and endemic to Madagascar. It is naturalized in subtropical and tropical areas of the world. Numerous cultivars are known for variation in flower color (white, mauve, peach, scarlet, and reddish orange), and also for tolerance of cooler growing conditions in temperate regions. It thrives in hot and humid environments, in full sun or partial shade and flowers all year round in hot climates. But it is sensitive to over-watering and cannot withstand frosts (not below 5–7 °C). It is best grown indoors in temperate climates. In the wild, plant is an endangered species mainly due to habitat destruction by slash and burn agriculture.

Madagascar periwinkle is widely cultivated for herbal medicine and as an ornamental plant. It is easy to cultivate and can be easily propagated by seed or by apical semi-ripe cuttings in light, free-draining compost. The best results are obtained when bottom heat and high humidity are provided. Seeds set for germination should be maintained at 22–25 °C in the dark until the seeds germinate. Full sun and well-drained soil are preferred and because of its hardiness, and it can

tolerate dry and nutritionally deficient conditions. It is noted for its long-flowering period, throughout the year in tropical conditions, and from spring to late autumn, in warm-temperate climates.

It is an oral poison, but in Ayurveda and Traditional Chinese Medicine (TCM), the extracts of roots and shoots are used against several diseases, including diabetes, malaria, and Hodgkin's lymphoma. The alkaloids vinblastine and vincristine extracted from the plant are used in the treatment of leukemia and Hodgkin's lymphoma in modern medicine.

9.2 Biotechnology and In Vitro Production of Secondary Metabolites

The cultivation of medicinal plants at field level often becomes problematic due to following reasons:

(i) Field production is dependent on season and climate and affected by diseases and pests;
(ii) Natural sources are becoming extremely scarce;
(iii) There may be technical and economic problems in production;
(iv) Production is labor intensive and therefore costly;
(v) Political instability in the country of production; and
(vi) Diplomatic relation with the country of origin.

The above problems have led to make a new approach to get drug substances through biotechnological means from cell, tissue, and organ cultures of medicinal plants.

Advantages of Biotechnology

Biotechnologists have special interest in plant cell tissue and organ cultures for the large-scale production of commercially important compounds including pharmaceuticals, flavors, fragrances, cosmetics, food additives, feedstocks, and antimicrobials. The advantages of biotechnology for the production of industrially important secondary metabolic compounds over the field level of medicinal plants for active drug principles include the following:

(i) Plant cell cultures are independent from environmental factors, seasonal variations, pest and microbial diseases, and geographical constraints as well as political interference;
(ii) Compounds can be produced under controlled conditions as per market demands;
(iii) Cell growth can be controlled to facilitate improved product formation, a more consistent product quality and yield can be maintained;
(iv) New routes of synthesis can be recovered from mutant cell lines which may lead to the development of novel products of commercial importance, which are not normally found in plants;

(v) Culture of cells will reduce the pressure on already over-exploited medicinal and other economically important plants as well as the production time is less and labor costs are minimal;
(vi) Plant cell tissue and cultures are particularly useful in case of plants which are difficult or expensive to be grown in the fields; and
(vii) Many of the commercially high valuable chemicals may be produced including drugs, flavors, perfumes, pigments, and agrochemicals.
(viii) Biotransformation reactions (converting specific substrates to valuable products) can be carried out with certain cultured cells.

Tissue culture production of useful secondary metabolites is cheaper compared to synthetic production, and at present, about 25–30% of medicines for human use and the various chemical materials for industrial purposes are obtained from plant tissue cultures.

Disadvantages of Biotechnology

(i) In general, in vitro production of secondary metabolites is lower when compared to intact plants;
(ii) Many a times, secondary metabolites are formed in differentiated tissues or organs and in such cases, non-differentiated culture cells (callus) can produce little;
(iii) Cultured cells are genetically unstable and may undergo mutation and under such circumstances, the production of secondary metabolite may be drastically reduced, as the culture ages;
(iv) Vigorous stirring is necessary to prevent aggregation of cultured cells, and this may often lead to cell damage; and
(v) Strict aseptic conditions have to be maintained during culture technique, and any infection to the culture may severely affect product formation.

9.2.1 Principles of Biotechnology and Laboratory Techniques

Biotechnology is the use of living systems and organisms to develop or make useful products or any technological application that uses biological systems, living organisms, or derivatives thereof, to make or modify products or processes for specific use. Similar to biotechnology, plant biotechnology may be defined as generation of useful products or services from plant cells, tissues and, often, organs (very small organ explants). Such cells, tissues, and organs are either continuously maintained in vitro or they pass through a variable phase to enable regeneration from them of complete plants which are ultimately transferred to the field.

Research in the area of plant tissue and organ culture technology has resulted in the production of many bioactive metabolites (alkaloids, terpenoids, steroids, saponins, phenolics, flavonoids, and amino acids, etc.) for new therapeutics under defined conditions; however, there are some inherent limitations including low yield of bioactive metabolites. Recombinant DNA techniques can be used to manipulate metabolic pathways for high yield and to produce protein pharmaceuticals such as antibodies, and protein hormones. The new disciplines of bioinformatics and genomics can find application in drug discovery from plant-based products, and biotechnological procedures can enhance and advance the studies of medicinal plants. Therefore, in vitro culture of plant cell, tissue and organs form an integral part of any plant biotechnology activity.

The various objectives achieved by plant biotechnology may be summarized as follows:

(i) Useful biochemical production (large-scale cell cultures); (ii) Rapid clonal multiplication (e.g., adventitious shoot/bulb/protocorm); (iii) Virus elimination (e.g., thermo-, cryo-, or chemo-therapy coupled with meristem culture); (iv) Rapid development of homozygous lines by producing haploids (e.g., anther culture, ovary culture, interspecific hybridization); (v) Production/recovery of difficult to produce hybrids (e.g., embryo rescue, in vitro pollination); (vi) Germplasm conservation of vegetatively reproducing plants or those producing recalcitrant seeds (cryopreservation, slow growth cultures, DNA clones); (vii) Genetic modification of plants (e.g., somaclonal variation, somatic hybridization, cybridization, and gene transfer), and (viii) Creation of genome maps and use of molecular markers to assist conventional breeding efforts.

The secondary metabolite production process comprises of several aspects including (i) selection of cell lines for high yield of secondary metabolites, (ii) large-scale cultivation of plant cells, (iii) medium composition and effect of nutrients, (iv) elicitor-induced production of secondary metabolites, (v) effect of environmental factors, (vi) biotransformation using plant cell cultures, and (vii) secondary metabolite release and analysis.

Culture Media and Other Factors for Optimum Production of Secondary Metabolites

A wide variety of culture media is available, and the choice of culture media is dependent on the purpose and requirements of the experiment. Selection of appropriate growth medium is important for the in vitro cultivation of cell tissue and organ. A culture medium is a liquid or gel designed to support the growth of the selected explants. It generally contains of an appropriate source of energy, structural material, growth factors, etc., which support and regulate the metabolic activities and also cell cycle. A typical culture medium is composed of a complement of amino acids (nitrogen source), vitamins, hormones (growth factors), inorganic salts, glucose (carbon and energy source), elicitors, and attachment factors. In addition to nutrients, the medium also helps maintain pH and osmolality. Most commonly used

culture media include White's medium, Murashige and Skoog (MS) medium, Gamborg or B5 medium, Chu or N6 medium, Nitsch's medium, etc., are some common media used for cell tissue culture from different explant sources and Eagle's minimum essential medium (EMEM), Dulbecco's modified Eagle's medium (DMEM), RPMI-1640 (developed at Roswell Park Memorial Institute, RPMI, in Buffalo, New York), Ham's nutrient mixtures, etc., for some of the widely used media used for animal cell culture.

(i) Media Components

One of the most important factors governing the growth and morphogenesis of plant tissues in culture is the composition of the culture medium. The basic nutrient requirements of cultured plant cells are very similar to those of whole plants. Plant tissue and cell culture media are generally made up of some or all of the following components: macronutrients, micronutrients, vitamins, amino acids, or other nitrogen supplements, sugar(s), other undefined organic supplements, solidifying agents or support systems, and growth regulators. Several media formulations are commonly used for the majority of all cell and tissue culture work. These media formulations include those described by White, Murashige and Skoog, Gamborg et al., Schenk and Hilderbrandt, Nitsch and Nitsch, and Lloyd and McCown. Murashige and Skoog's MS medium, Schenk and Hildebrand's SH medium, and Gamborg's B-5 medium are all high in macronutrients, while the other media formulations contain considerably less of the macronutrients.

(ii) Macronutrients

The macronutrients provide the six major elements: nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), and sulfur (S), required for plant cell or tissue growth. The optimum concentration of each nutrient for achieving maximum growth rates varies considerably among species. Culture media should contain at least 25–60 mM of inorganic nitrogen for adequate plant cell growth. Plant cells may grow on nitrates alone, but considerably better results are obtained when the medium contains both a nitrate and ammonium nitrogen source. Certain species require ammonium or another source of reduced nitrogen for cell growth to occur. Nitrates are usually supplied in the range of 25–20 mM; typical ammonium concentrations range between 2 and 20 mM. However, ammonium concentrations in excess of 8 mM may be deleterious to cell growth of certain species. Cells can grow on a culture medium containing ammonium as the sole nitrogen source if one or more of the TCA cycle acids (e.g., citrate, succinate, or malate) are also included in the culture medium at concentrations of approximately 10 mM. When nitrate and ammonium sources of nitrogen are utilized together in the culture medium, the ammonium ions will be utilized together in the culture medium, the ammonium ions will be utilized more rapidly and before the nitrate ions. Potassium is required for cell growth of most plant species. Most media contain K, in the nitrate or chloride form, at concentrations of 20–30 mM. The optimum concentrations of P, Mg, S, and Ca range from 1 to 3 mM when all other requirements for cell growth

are satisfied. Higher concentrations of these nutrients may be required if deficiencies in other nutrients exist.

(iii) Micronutrients

The essential micronutrients for plant cell and tissue growth include iron (Fe), manganese (Mn), zinc (Zn), boron (B), copper (Cu), and molybdenum (Mo). Chelated forms of iron and zinc are commonly used in preparing culture media. Iron may be the most critical of all the micronutrients. Iron citrate and tartrate may be used in culture media, but these compounds are difficult to dissolve and frequently precipitate after media are prepared. Murashige and Skoog used an ethylene diaminetetraacetic acid (EDTA)-iron chelate to bypass this problem. Cobalt (Co) and iodine (I) may also be added to certain media, but strict cell growth requirements for these elements have not been established. Sodium (Na) and chlorine (Cl) are also used in some media but are not essential for cell growth. Copper and Cobalt are normally added to culture media at concentrations of 0.1 μM, Fe and Mo at 1 μM, I at 5 μM, Zn at 5–30 μM, Mn at 20–90 μM, and B at 25–100 μM.

(iv) Carbon and energy source

The preferred carbohydrate in plant cell culture media is sucrose. Glucose and fructose may be substituted in some cases, glucose being as effective as sucrose and fructose being somewhat less effective. Other carbohydrates that have been tested include lactose, galactose, raffinose, maltose, and starch. Sucrose concentrations of culture media normally range between 2 and 3%. Use of autoclaved fructose can be detrimental to cell growth. Carbohydrates must be supplied to the culture medium because few plant cell lines have been isolated that are fully autotrophic, e.g., capable of supplying their own carbohydrate needs by CO_2 assimilation during photosynthesis.

(v) Vitamins

Normal plants synthesize the vitamins required for their growth and development. Vitamins are required by plants as catalysts in various metabolic processes. When plant cells and tissues are grown in vitro, some vitamins may become limiting factors for cell growth. The vitamins most frequently used in cell and tissue culture media include thiamin (B1), nicotinic acid, pyridoxine (B6), and myo-inositol. Thiamin is the one vitamin that is basically required by all cells for growth. Thiamin is normally used at concentrations ranging from 0.1 to 10.0 mg/l. Nicotinic acid and pyridoxine are often added to culture media but are not essential for cell growth in many species. Nicotinic acid is normally used at concentrations of 0.1–5.0 mg/l; pyridoxine is used at 0.1–10.0 mg/l. Myo-inositol is commonly included in many vitamin stock solutions. Although it is a carbohydrate not a vitamin, it has been shown to stimulate growth in certain cell cultures. Its presence in the culture medium is not essential, but in small quantities myo-inositol stimulates cell growth in most species. Myo-inositol is generally used in plant cell and tissue culture media at concentrations of 50–5000 mg/l.

Other vitamins such as biotin, folic acid, ascorbic acid, pantothenic acid, vitamin E (tocopherol), riboflavin, and *p*-aminobenzoic acid have been included in some cell culture media. The requirement for these vitamins by plant cell cultures is generally negligible, and they are not considered growth-limiting factors. These vitamins are generally added to the culture medium only when the concentration of thiamin is below the desired level or when it is desirable to grow cells at very low population densities.

(vi) Amino acids or other nitrogen supplements

Although cultured cells are normally capable of synthesizing all of the required amino acids, the addition of certain amino acids or amino acid mixtures may be used to further stimulate cell growth. The use of amino acids is particularly important for establishing cell cultures and protoplast cultures. Amino acids provide plant cells with an immediately available source of nitrogen, which generally can be taken up by the cells more rapidly than inorganic nitrogen. The most common sources of organic nitrogen used in culture media are amino acid mixtures (e.g., casein hydrolysate), L-glutamine, L-asparagine, and adenine. Casein hydrolysate is generally used at concentrations between 0.05 and 0.1%. When amino acids are added alone, care must be taken, as they can be inhibitory to cell growth. Examples of amino acids included in culture media to enhance cell growth are glycine at 2 mg/l, glutamine up to 8 mM, asparagine at 100 mg/l, L-arginine and cysteine at 10 mg/l, and L-tyrosine at 100 mg/l. Tyrosine has been used to stimulate morphogenesis in cell cultures but should only be used in an agar medium. Supplementation of the culture medium with adenine sulfate can stimulate cell growth and greatly enhance shoot formation.

(viii) Undefined organic supplements

Addition of a wide variety of organic extracts to culture media often results in favorable tissue responses. Supplements that have been tested include protein hydrolysates, coconut milk, yeast extracts, malt extracts, ground banana, orange juice, and tomato juice. However, undefined organic supplements should only be used as a last resort, and only coconut milk and protein hydrolysates are used to any extent today. Protein (casein) hydrolysates are generally added to culture media at a concentration of 0.05–0.1%, while coconut milk is commonly used at 5–20% (v/v). The addition of activated charcoal (AC) to culture media may have a beneficial effect. The effect of AC is generally attributed to one of three factors: absorption of inhibitory compounds, absorption of growth regulators from the culture medium, or darkening of the medium. The inhibition of growth in the presence of AC is generally attributed to the absorption of phytohormones to AC. 1-Napthaleneacetic acid (NAA), kinetin, 6-benzylaminopurine (BAP), indole-3-acetic acid (IAA), and 6-γ-γ-dimethylallylaminopurine (2iP) all bind to AC, with the latter two growth regulators binding quite rapidly. The stimulation of cell growth by AC is generally attributed to its ability to bind to toxic phenolic compounds produced during

culture. Activated charcoal is generally acid-washed prior to addition to the culture medium at a concentration of 0.5–3.0%.

(ix) Solidifying agents or support systems

Agar is the most commonly used gelling agent for preparing semisolid and solid plant tissue culture media. Agar has several advantages over other gelling agents. First, when agar is mixed with water, it forms a gel that melts at approximately 60–100 °C and solidifies at approximately 45 °C; thus, agar gels are stable at all feasible incubation temperatures. Additionally, agar gels do not react with media constituents and are not digested by plant enzymes. The firmness of an agar gel is controlled by the concentration and brand of agar used in the culture medium and the pH of the medium. The agar concentrations commonly used in plant cell culture media range between 0.5 and 1.0%; these concentrations give a firm gel at the pH's typical of plant cell culture media. Another gelling agent commonly used for commercial as well as research purposes is Gelrite. This product is synthetic and should be used at 1.25–2.5 g/l, resulting in a clear gel which aids in detecting contamination. Alternative methods of support have included use of perforated cellophane, filter paper bridges, filter paper wicks, polyurethane foam, and polyester fleece. Whether explants grow best on agar or on other supporting agents varies from one species of plant to the next.

Growth Regulators

Four broad classes of growth regulators are important in plant tissue culture; the auxins, cytokinins, gibberellins, and abscisic acid. Skoog and Miller were the first to report that the ration of auxin to cytokinin determined the type and extent of organogenesis in plant cell cultures. Both an auxin and cytokinin are usually added to culture media in order to obtain morphogenesis, although the ratio of hormones required for root and shoot induction is not universally the same. Considerable variability exists among genera, species, and even cultivars in the type and amount of auxin and cytokinin required for induction of morphogenesis. The auxins commonly used in plant tissue culture media are 1H-indole-3-acetic acid (IAA), 1H-indole-3-butyric acid (IBA), (2,4-dichlorophenoxy) acetic acid (2,4-D), and 1-napthaleneacetic acid (NAA). The only naturally occurring auxin found in plant tissues is IAA. Other synthetic auxins that have been used in plant cell culture include 4-chlorophenoxyacetic acid or *p*-chlorophenoxyacetic acid (4-CPA, PCPA), (2,4,5-trichlorophenoxy)acetic acid (2,4,5-T), 3,6-dichloro-2-methoxybenzoic acid (Dicamba), and 4-amino-3,5,6-trichloropicolinic acid (Picloram). The various auxins differ in their physiological activity and in the extent to which they move through tissue, are bound to the cells, or metabolized. Naturally occurring IAA has been shown to have less physiological activity than synthetic auxins. Based on stem curvature assays, 2,4-D has eight to twelve times the activity, 2,4,5-T has four times the activity, PCPA and Picloram have two to four times the activity, and NAA has two times the activity of IAA. Although 2,4-D, 2,4,5-T, PCPA, and Picloram are often used to induce rapid cell proliferation, exposure to high levels or prolonged exposure to these auxins, particularly 2,4-D, results in suppressed morphogenetic

activity. Auxins are generally included in a culture medium to stimulate callus production and cell growth, to initiate shoots, particularly roots, and to induce somatic embryogenesis and stimulate growth from shoot apices and shoot tip cultures. The cytokinins commonly used in the culture media include 6-benzylaminopurine or 6-benzyladenine (BAP, BA), 6-γ-γ-dimethylaminopurine (2iP), *N*-(2-furanylmethyl)-1H-purine-6-amine (kinetin), and 6-(4-hydroxy-3-methyl-trans-2-butenylamino)purine (zeatin). Zeatin and 2iP are considered to be naturally occurring cytokinins, while BA and kinetin are synthetically derived cytokinins. Adenine, another naturally occurring compound, has a base structure similar to that of the cytokinins and has shown cytokinin-like activity in some cases. Many plant tissues have an absolute requirement for a specific cytokinin for morphogenesis to occur, whereas some tissues are considered to be cytokinin independent, i.e., no cytokinin or a specific cytokinin may be required for organogenesis. The cytokinins are generally added to a culture medium to stimulate cell division, to induce shoot formation and axillary shoot proliferation, and to inhibit root formation. The type of morphogenesis that occurs in a plant tissue culture largely depends upon the ratio and concentrations of auxins and cytokinins present in the medium. Root initiation of plantlets, embryogenesis, and callus initiation all generally occur when the ration of auxin to cytokinin is high, whereas adventitious and axillary shoot proliferation occurs when the ration is low. The concentrations of auxins and cytokinins are equally as important as their ratio. Gibberellins (GA3) and abscisic acid (ABA) are two other growth regulators occasionally used in culture media. Plant tissue cultures can usually be induced to grow without either GA3 or ABA, although, certain species may require these hormones for enhanced growth. Generally, GA3 is added to culture media to promote the growth of low-density cell cultures, to enhance callus growth, and to elongate dwarfed or stunted plantlets. Abscisic acid is generally added to culture media to either inhibit or stimulate callus growth (depending upon the species), to enhance, inhibit, or stimulate callus growth (depending upon the species), to enhance shoot or bud proliferation, and to inhibit latter stages of embryo development.

Preparation of Stock Solutions

The use of stock solutions reduces the number of repetitive operations involved in media preparation and, hence, the chance of human or experimental error. Moreover, direct weighing of media components (e.g., micronutrients and hormones) that are required only in milligram or microgram quantities in the final formulation cannot be performed with sufficient accuracy for tissue culture work. For these components, preparation of concentrated stock solutions and subsequent dilution into the final media is standard procedure. In addition, concentrated solutions of some materials are more stable and can be stored for longer periods than more dilute solutions. To prepare a stock solution, weigh out the required amount of the compound and place it in a clean flask. It is common practice to make a stock solution 10× or 100×, depending upon the solubility of the compound. Once the chemical is in the flask, dissolve it in a small amount of water, ethyl

alcohol, 1 N NaOH, or 1 N HCL. Next, slowly add double-distilled water to the flask, while agitating. Continue this until the proper volume is reached. Label the flask with the name of the solution, preparation and expiration dates, and the name of the person who prepared the solution. Certain items, e.g., IAA, must be prepared and stored in amber bottles to prevent photodecomposition.

Macronutrients in Stock Solutions

Stock solutions of macronutrients can be prepared at 10 times the concentration of the final medium. A separate stock solution for calcium salts may be required to prevent precipitation. Stock solution of macronutrients can be stored safely for several weeks in a refrigerator at 2–4 °C.

Micronutrients in stock solutions

Micronutrient stock solutions are generally made up at 100 times their final strength. It is recommended that micronutrient stocks be stored in either a refrigerator or freezer until needed. Micronutrient stock solutions could be stored in a refrigerator for up to 1 year without appreciable deterioration. Iron stock solutions should be prepared and stored separately from other micronutrients in an amber storage bottle. Formulations for preparing stock solutions of iron are presented later.

Vitamins in stock solutions

Vitamins are prepared as 100× or 1000× stock solutions and stored in a freezer (−20 °C) until used. Vitamin stock solutions should be made up each time media is prepared if a refrigerator or freezer is not available. Vitamin stock solutions should be made up each time media is prepared if a refrigerator or freezer is not available. Vitamin stock solutions can be stored safely in a refrigerator for 2–3 months but should be discarded after that time.

Growth Regulators

The auxins NAA and 2,4-D are considered to be stable and can be stored at 4 °C for several months; IAA should be stored at −20 °C. Auxin stock solutions are generally prepared at 100–1000 times the final desired concentrations. Solution of NAA and 2,4-D can be stored for several months in a refriger at or indefinitely at −20 °C. Generally, IAA and 2,4-D are dissolved in a small volume of 95% ethyl alcohol or KOH and then brought to volume with double-distilled water; NAA can be dissolved in a small amount of 1 N NaOH or KOH, which also can be used to dissolve 2,4-D and IAA. The cytokinins are considered to be stable and can be stored at −20 °C. Cytokinin stock solutions are generally prepared at 100× to 1000× concentrations. Many of the cytokinins are difficult to dissolve, and a few drops of either 1 N HCL, 1 N NaOH, in KOH or DMSO, is required to bring them into solution.

Storage of Stock Solutions

Storage conditions for most stock solutions have already been pointed out; however, some additional points can be made. For convenience, many laboratories prepare stock solutions and then divide them into aliquots sufficient to prepare from 1 to 10 l of medium; these aliquots are stored in small vials or plastic bags in a

freezer. This procedure removes the inconvenience of having to unthaw a large volume of frozen stock each time medium is prepared. Some have found that heating in a microwave oven is a satisfactory and quick method of thawing concentrated medium (PhytoTechnology Laboratories, Inc. 2003; www.phytotechlab.com).

The medium which limits rapid cell division and early cessation of exponential growth is best for production of secondary metabolites. Growth regulators play an important role in determining the potential productivity of a given culture. For stimulating alkaloid synthesis in suspension culture of *Papaver bracteatum*, IAA has been found to be better than other auxins. At high concentrations, kinetin inhibits the production of alkaloids in *Datura tabula*. In *Solanum aviculare*, reduction in the level of auxin and cytokinin in the medium results in increase of steroid spectrum. Increase in the levels of nitrate, potassium, ammonium, and phosphate supports rapid cell growth but decrease of any of these nutrients limits cell growth and production of secondary metabolites. Increase in sucrose level in the medium increases the yield of secondary metabolites.

(a) Light

Light stimulates biosynthesis of secondary metabolites in cultures. For example, 'cool white' light stimulates biosynthesis of diosgenin in tuber-derived callus and cell suspensions of Dioscorea, solasodine, and solamargine in *Catharanthus roseus* cell cultures.

(b) Temperature

Temperature greatly affects secondary metabolite production in cultures. In Peganum, optimal growth of the callus occurred a 130 °C but maximum alkaloid production was attained at 25 °C. The production of alkaloid decreases at higher temperatures.

(c) Rotation speed of shaker

In tobacco, increase in the rotation speed of shaker (150 r min^{-1}) induces nicotine production, but normal agitation (110 r min^{-1}) results in slight inhibition of nicotine synthesis.

(d) pH of medium

pH of the medium controls the biosynthesis of secondary metabolites. In Ipomoea when cells are cultured at pH 6.3, the production of tryptophol becomes double. The synthesis of tryptophol becomes completely inhibited If pH of the medium drops to 4.8.

9.2.2 *In Vitro Production of Secondary Metabolites*

Medicinal plants constitute a highly potential source for the synthesis of secondary metabolites which are economically important as drugs, flavor and fragrances, dye and pigments, pesticides, and food additives. Plant cells are biosynthetically totipotent, which means that each cell in culture retains complete genetic information and hence is able to produce the range of chemicals found generally in the parent plant. Over 80% of the approximately 30,000 known natural products are of plant origin (Balandrin and Klocke 1988; Fowler and Scragg 1988; Phillipson 1990).

Many of the pharmaceutically important bioactive secondary metabolites are derived from natural sources, and many of them are unique to the plant kingdom and not produced by microbes or animals. Besides, with the help of transgenic technology, it is now possible to modify the cellular biosynthetic routes to produce new bioactive compounds, which were not originally synthesized in plants. Biotechnology offers an opportunity to exploit the cell, tissue, organ, or entire organism by growing them in vitro and to genetically manipulate them to get desired compounds in one hand, and on other, to minimize the extra pressure on arable land (mostly used for food production) for cultivation MAPs. Many facets of biotechnological approaches can be considered and can be used for the production of pharmaceutically important secondary metabolites from plants such as (i) Plant cell tissue and organ cultures (e.g., cell culture, shoot culture, root culture, and scale-up of cultures), (ii) Transgenic plants/organisms (e.g., metabolic engineering, heterologous expression, and molecular farming), (iii) Micropropagation of medicinal plants (e.g., endangered plants, high-yielding varieties, and metabolically engineered plants), (iv) Newer sources (e.g., algae and other photosynthetic marine forms). Recent advances in the molecular biology, enzymology, and fermentation technology suggest that the secondary metabolic plant products can be extracted from the aseptic culture of plant cell, tissue, and organ.

Stockigt et al. (1995) enumerated a number of secondary metabolites that were isolated from tissue and suspension cultures of higher plants including (i) phenylpropanoids (anthocyanins, coumarins, flavonoids, hydroxycinnamoyl derivatives, isoflavonoids, lignans, phenalenones, proanthocyanidins, stilbenes, tannins, etc.); (ii) alkaloids (acridines, betalaines, quinolizidines, furonoquinones, harringtonines, isoquinolines, indoles, purines, pyridines, tropane alkaloids, etc.); (iii) terpenoids (carotenes, monoterpenes, sesquiterpenes, diterpenes, triterpenes, etc.); (iv) quinones (anthroquinones, benzoquinones, naphthoquinones, etc.); and (v) steroids (cardiac glycosides, pregnenolone, etc.). Plant produces innumerable number of secondary metabolites of several categories like (i) flavonoids and allied phenolic and polyphenolic compounds, (ii) terpenoids, (iii) nitrogen-containing alkaloids and sulfur-containing compounds. (Ravishankar and Rao 2000; Rao and Ravishankar 2002; Crozier et al. 2007). The complex structural features of many of the plant-derived compounds are difficult to synthesize. So the synthesis complex bioactive compounds as plants' secondary metabolites paved the way to get desired

Table 9.2 Secondary metabolites produced at higher levels by in vitro plant cell/suspension culture compared to whole plant

Compound	Plant species	Yields (% D.wt.)		Ratio in vitro culture/whole plant	Culture type, C/S[a]
		Tissue culture	Whole plant		
(i) Shikonin	*Lithospermum erythrorhizon*	20	1.5	13.33	S
(ii) Ginsenoside	*Panax ginseng*	27	4.5	6.0	C
(iii) Anthraquinones	*Morinda citrifolia*	18	0.3	60	S
(iv) Ajmalicine	*Catharanthus roseus*	1.0	0.3	3.33	S
(v) Terpentine	*Catharanthus roseus*	1.8	0.5	3.6	C
(vi) Rosmarinic acid	*Coleus blumeii*	15	3	5.0	S
(vii) Ubiquinone-10	*Nicotiana tabacum*	0.036	0.003	12.0	S
(viii) Diosgenin	*Dioscorea deltoides*	2	2	1,0	S
(ix) Benzylisoquinoline alkaloids	*Coptis japonica*	11	5–10	2.2–1.1	S
(x) Berberine	*Thalictrum minor*	10	0.01	1000.0	S
(xi) Berberine	*Coptis japonica*	10	2–4	5–2.5	S
(xii) Anthraquinones	*Galium verum*	5.4	1.2	4.5	S
(xiii) Anthraquinones	*Galium aparine*	3.8	0.2	19.0	S
(xiv) Nicotine	*Nicotiana tabacum*	3.4	2.0	1.7	C
(xv) Glutathione	*Nicotiana tabacum*	5.0	2.1	2.38	C
(xvi) Bisoclaurine	*Stephania cepharantha*	2.3	0.8	2.87	S
(xvii) Tripdiolide	*Tripteryqium wilfordii*	0.05	0.001	50.0	S

[a]*C* Callus culture, *S* Suspension culture

pharmaceuticals, and many of them may now be produced in cell culture in higher proportions compared to whole plant at commercial (Table 9.2).

One of the most exciting aspects of cell culture technology is the potential for producing novel structures not observed in the parent plant, e.g., rutacultin by cultures of *Ruta graveolens* and sesquiterpene lactones by cultures of *A. paniculata*.

Large-scale plant tissue culture is found to be an attractive alternative approach to traditional methods of cultivation of drug plants. Use of liquid medium in suspension cultures allows easy and extensive scaling up by employing bioreactors, although a limited scaling up can be achieved by using larger culture flasks (usually 250-ml flasks containing 50 ml culture medium) or bioreactor—a large volume (1 to >1000 L) culture vessel with provisions for (i) aeration, (ii) stirring to achieve

medium and cell mixing, (iii) contamination control, and (iv) replacement of used medium and/or used medium plus cells.

Plant cell tissue and organ culture

Plant cells are biosynthetically totipotent, each cell in culture retains complete genetic information and hence each cell is able to produce the range of chemicals found generally in the parent plant. Biochemical production by cultured cells can be increased chiefly by the following approaches (i) development of high-producing cultures, (ii) devising a suitable culture medium and conditions, (iii) use of elicitors, and (iv) use of organ cultures.

Strategies for Enhanced Production of Secondary Metabolites in Plant Cell Cultures

Proper Selection of Cell Lines

Development of high-producing clones is a must for high yield of the desired biochemicals. In general, high-producing plants yield high-producing cultures. Therefore, cell cultures must be started from the highest producing plants of the species in question.

Optimization of Medium and Culture Conditions

The constituents of culture medium, like nutrients, phytohormones and also the culture conditions, like temperature, pH, light, inoculum size, etc., influence the production of secondary metabolites. In case of rosamarinic acid, increase of sucrose concentration from 3 to 5%, production increases by five times; IAA enhances the yield of shikonin production, while 2, 4-D and NAA are inhibitory; and NH_4^+ (only 3% of the total N of medium) inhibit shikonin production, while a 30-fold increase in Cu^{2+} concentration caused a threefold increase in shikonin production. Physical factors like white and blue light strongly inhibit shikonin production, while sucrose (5%) is essential for the production of this pigment. It is, therefore, necessary to optimize the factors involved in the regulation of biosynthesis of the desired biochemical by cells in culture.

Addition of Elicitors

Some molecules and physical factors, so-called elicitors, stimulate the production of secondary metabolites in plants or cell culture, and the phenomenon is known as elicitation (induction by applied stresses). Elicitors produced within plant cells are termed as endogenous elicitors, while those produced by microorganisms are called exogenous elicitors. Elicitors of plant origin are cell-wall-derived polysaccharides, e.g., pectin, pectic acid, cellulose, and of microorganism origin are fungal hyphae, carbohydrates, yeast extract, MJ (methyl jasmonate), cell wall components like chitin, chitosan, or glucans; some glycoproteins and low-molecular weight organic acids also cause elicitation. Elicitors of biological origin are called biotic elicitors. Abiotic elicitors are UV, low or high temperature, salts of heavy metals, and

chemicals that disturb membrane integrity. Addition of these elicitors to the medium in low concentration (50–250 mg/1) enhances the production of secondary metabolites. Several abiotic elicitors enhance growth and ginseng saponin biosynthesis in the hairy roots of *Panax ginseng*.

Addition of Precursors

Precursors are the compounds, whether exogenous or endogenous, that can be converted by living system into useful compounds or secondary metabolites. It has been possible to enhance the biosynthesis of specific secondary metabolites by feeding precursors to cell cultures, e.g., amino acids have been added to suspension culture media for production of tropane alkaloids, indole alkaloids. Phenylalanine acts as a precursor of rosmarinic acid; addition of phenylalanine to *Salvia officinalis* suspension cultures stimulated the production of rosmarinic acid and also decreased the production time. Phenylalanine also acts as precursor of the *N*-benzoylphenylisoserine side chain of taxol; supplementation of *Taxus cuspidata* cultures with phenylalanine resulted in increased yields of taxol. The timing of precursor addition is critical for an optimum effect.

Permeabilization

Secondary metabolites produced in cells are often blocked in the vacuole. By manipulating the permeability of cell membrane, they can be secreted out to the media. Permeabilization can be achieved by electric pulse, UV, pressure, sonication, heat, etc. Even charcoal can be added to medium to absorb secondary metabolites.

Immobilization

Cell cultures encapsulated in agarose and calcium alginate gels or entrapped in membranes are called immobilised plant cell cultures. Immobilization of plant cells allows better cell to cell contact and the cells are also protected from high shear stresses. These immobilized systems can effectively increase the productivity of secondary metabolites in a number of species. Elicitors can also be added to these systems to stimulate secondary metabolism.

Procedure for the Production of Secondary Metabolites

The main research program for the production of secondary metabolites from plant cell culture as represented in Fig. 9.1 consists of following essential steps.

Selection of Explant from HYV Plant Species (Cell Line)

At first, the pharmacognosist needs to identify the desired active principles from the test plant. Once it is done, in the next step, investigator needs to screen out the hyper-producing explant that present the most valuable secondary metabolites from the available genetic pool of plants as outlined in Fig. 9.1.

Establishment of In Vitro Cell Lines

After choosing the most promising individual plants, begins the real work of in vitro culture with callus initiation. This work consists mainly in determining the

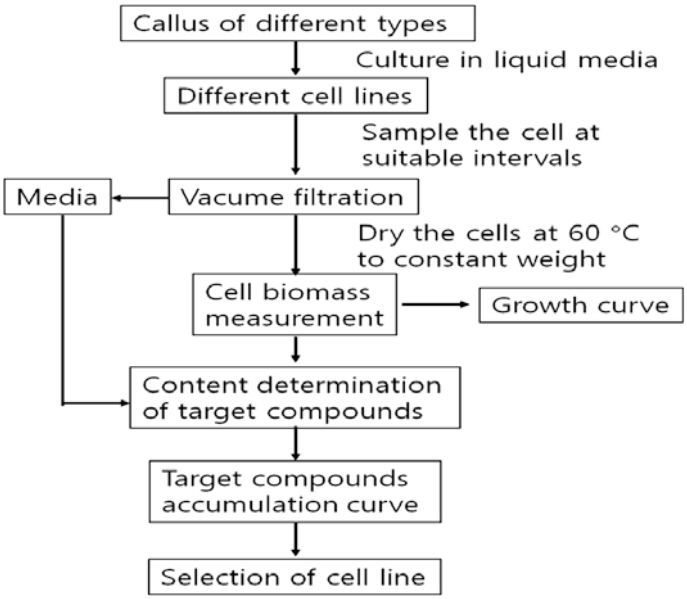

Fig. 9.1 Selection of explant from HYV plant species (cell line)

medium that will be best adapted for cultivation. This medium optimization includes mineral composition and organic constituents with special attention to hormonal balances that govern differentiation mechanisms. This work is now facilitated by the use of incomplete factorial experiments or surface response methods. Once calli are obtained, it is well known that they can undergo soma-clonal variation, usually during several subculture cycles (from several weeks to several years) until genetic stability occurs when each callus can be considered as homogeneous cell aggregate. For stability, growth parameters such as length of lag, of log phases, and growth speed during the log phase may be taken into consideration.

Cell Suspension Cultures

When genetic stability is reached, it is necessary to screen the different callus lines according to their aptitudes to provide an efficient metabolite production (Fig. 9.2). Hence, each callus must be assessed separately for its growth speed as well as intracellular and extracellular metabolite concentrations. This allows an evaluation of the productivity of each cell line (mg of products g^{-1} of cell day^{-1} or mg of products l^{-1} day^{-1}) so that only the best ones will be taken to cell suspensions and reactor studies. Compared to cell growth kinetics, which is usually an exponential curve, most secondary metabolites are produced during the plateau phase. However, some secondary plant products are known to be growth-associated with undifferentiated cells, such as betalains and carotenoids.

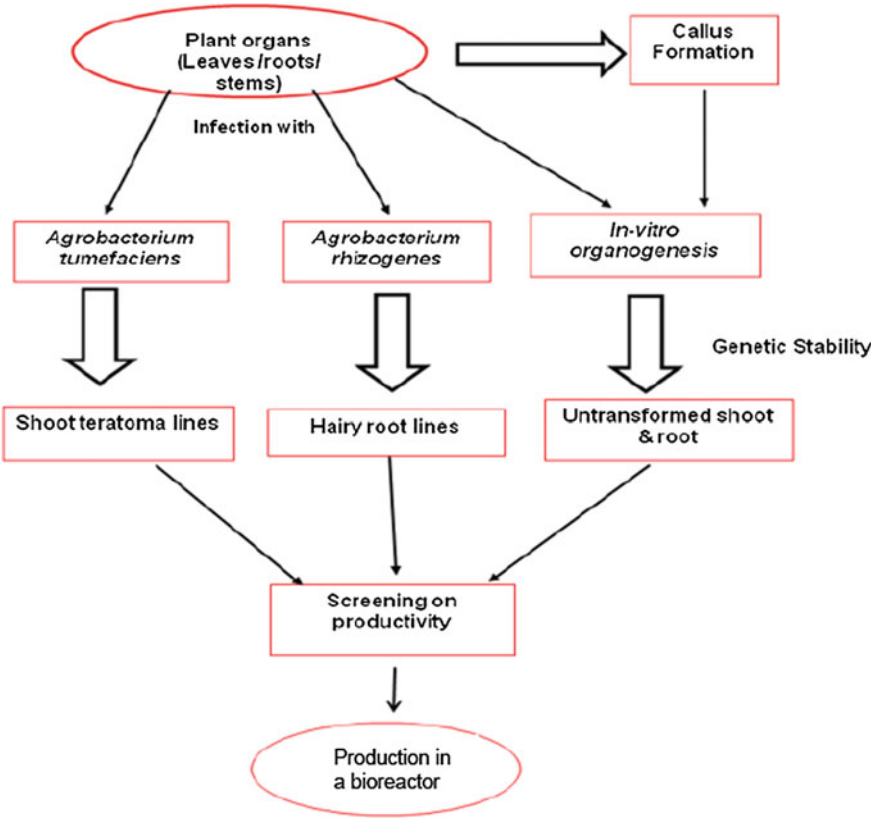

Fig. 9.2 Guidelines for the production of secondary metabolites from plant organ culture

Elicitors

Elicitation by biotic or abiotic elicitors may increase the production of secondary metabolites. It consists in applying by applying stresses by different elicitors to the cell suspension cultures that will trigger the production of secondary metabolites (induction by applied stresses) that are normally not produced. This elicitation can be very efficient at increasing secondary metabolite production as shown in Table 9.3. Addition of biotic and abiotic elicitors like SA and US into the cell suspension culture media of *Taxa baccata* enhanced taxol production independently and when in combination they produced synergic effect (Rezaei et al. 2011).

Bioreactor Cultures

The bioreactors used for culture of plant cells may be of following 4 types: (1) batch bioreactors, (2) continuous bioreactors, (3) multistage bioreactors, and (4) immobilized cell bioreactors; all bioreactors, except the last one, are commonly called stirred tank reactors since the vessel has a device for stirring. Bioreactor studies represent the final step for scaling up the work that leads to commercial production of secondary metabolites from plant cell cultures.

Table 9.3 Effect of salicylic acid (SA) and ultrasound (US) on taxol, a novel anticancer diterpene amide, (mg/l) content of cell suspension culture of *Taxa baccata*

Taxol (mg/l)						
Elicitators		Extracellular	Cell-associated	Total	Release (%)	Specific yield, mg/g cell
SA (mg/l)	US					
0	–	1.54 ± 0.23	2.52 ± 0.65	4.06 ± 0.88	37.93	0.29
	US	5.17 ± 0.89	3.15 ± 0.59	8.32 ± 1.48	62.13	0.62
25	–	10.24 ± 1.56	6.65 ± 1.04	16.89 ± 2.60	60.62	1.60
	US	21.44 ± 3.28	5.34 ± 0.82	26.78 ± 4.10	80.05	2.87
50	–	19.44 ± 2.45	7.54 ± 0.69	26.98 ± 3.14	72.05	3.25
	US	28.76 ± 1.78	4.24 ± 0.43	32.9 ± 2.21	87.11	4.88

SA and US were treated on days 8 and 16 post-inoculation, respectively. Data are mean ± SD, $n = 3$ (*Source* Rezaei et al. 2011)

In batch bioreactors, the medium and inoculum are loaded in the beginning, and the cells are allowed to grow. There is no addition/ replacement of medium, and the entire cell mass is harvested at the end of incubation period. The characteristic features of such bioreactor systems are as follows: (i) continuous depiction of medium, (ii) accumulation of cellular wastes, (iii) alterations in growth rate, and (iv) continuous change in the composition of cells.

In continuous bioreactors, there is continuous inflow of fresh medium and outflow of used medium (with or without cells) during the entire incubation period. A spin-filter bioreactor is a good example of continuous flow, bioreactor; it has the following features: (i) The central shaft of bioreactor houses a spinning filter which enables the removal of used medium, free of cells, through the shaft, (ii) A stirrer plate magnetically coupled to the central shaft provides continuous stirring; the spinning filter also stirs the culture, (iii) The culture is aerated by a sparger which allows a wide range of aeration rates, (iv) A port is provided for addition of fresh medium, while (v) another port enables removal of the culture (used medium + cells) as per need. This bioreactor provides a highly versatile system for control on medium change rate and on cell density; this becomes possible due to the two routes for medium removal while only one of them allows the removal of cells. A continuous flow bioreactor is used to grow cells at a specified cell density in an active growth phase; such cultures may either provide inocula for further culture or may serve as a continuous source of biomass yields.

In case of multistage bioreactors, culture systems use two or more bioreactors in a specified sequence each of which carries out a specific step of the total production process. The simplest situation would involve 2 bioreactors. The commercial production of a biochemical like red pigment shikonin by cultured cells of *Lithospermum erythrorhiza* in Japan is a two-phase process. Both the bioreactors are batch type: the first bioreactor provides conditions for rapid cell proliferation

and favors biomass production in bulk, while the second bioreactor has medium and conditions conducive for shikonin biosynthesis and accumulation.

Immobilized cell bioreactors are based on cells entrapped either in gels, such as, agarose, agar, chitosan, gelatine, gellan, polyacrylamide, and calcium alginate, to produce beads, or in a membrane or metal (stainless steel) screen compartment or cylinder. The membrane/screen cylinder containing cells is kept in a chamber through which the medium is circulated from a recycle chamber. The medium flows parallel to the screen cylinder and diffuses across the screen into the cell mass. Similarly, products from cells diffuse into the medium and out of the screen cylinder. The membrane/screen compartment housing the cells may be cylindrical or flat, and medium movement may so adjust as to flow across the screen compartment rather than parallel to it. The technology is being refined for commercialization. Fresh medium is regularly added, and equivalent volume of used medium is withdrawn from the recycling chamber to maintain its nutrient status.

Cell immobilization changes the physiology of cells as compared to that of cells in suspension. This technique is useful where the biochemical of interest is excreted by the cells into the medium. Product excretion may also be brought about by immobilization itself, or by certain treatments like altered pH, use of DMSO (dimethyl sulfoxide) as a permealizing agent, changed ionic strength of medium, an elicitor. Immobilized cell reactors have the following advantages: (i) no risk of cell wash out, (ii) low contamination risk, (iii) protection of cells from liquid shear, (iv) better control on cell aggregate size, (v) separation of growth phase (in a batch/continuous bioreactor) from production stage (in an immobilized cell bioreactor), and (vi) cellular wastes regularly removed from the system, and (vii) cultures at high cell densities.

Harvest

When secondary products are produced at the end of the growth phase, then two-step reactor is adopted where a first reactor is used for building up the biomass, and a second one for metabolite production. A single-step reactor is sufficient to grow the cells and recover the molecules at the same time when the production of the metabolite is growth-associated. If the metabolites remain intracellular, it is usually necessary to kill the biomass, so that the chemicals can be extracted from the cells following a batch or fed-batch process while extracellular production avoids destruction of the biomass for the extraction of the compounds as they can be directly recovered from the medium following a continuous system with an improved productivity compared to a standard batch. Excretion of intracellular compounds is made possible with permeabilization methods (sonication, pH shock, addition of detergents, oligosaccharides, etc.) without impairing cell viability. Perfusion systems have also been designed with encapsulated cells.

Yield

Some metabolites, especially alkaloids excrete from the cells in the media; therefore, both qualitative and quantitative analyses of secondary metabolites are done on culture media and also on cells. Yields of metabolites are compared on a product

weight per unit weight of cells or on volume of medium basis (Mantell and Smith 1984).

Problems in Large-Scale Plant Cell Culture

Large-scale culture of plant cells for commercial biochemical production presents several problems such as:

(i) Plant cells have much slower growth rates than bacteria and fungi; therefore, larger reactors and longer fermentation times are necessary;
(ii) The long fermentation time increases the risk of contamination;
(iii) Plant cells are rather sensitive to shear, fermenters with conventional mechanical stirring are not suitable for their culture, bioreactors having specially designed mechanical stirrers, or airlift fermenters are far more suitable;
(iv) Plant cells show cytogenetic, genetic, and epigenetic variations during culture, the characteristics of a cell population may change during culture, and prolonged continuous cultures may not be desirable.
(v) In case of biochemical production, often conditions favoring rapid growth may suppress biochemical formation and vice versa, and organized cell masses and cells under stress are more likely to produce useful biochemicals. In all such cases, therefore, a multistage bioreactor system should be employed.

Organ Culture

A wide range of valuable secondary phytochemicals production requires more differentiated micro plant or organ cultures, which becomes essential when the desired metabolite is only produced in specialized plant tissues or glands in the parent plant. Some of the examples are (i) in vitro root culture of ginseng (*Panax ginseng*) is required because saponin and other valuable metabolites are specifically produced in ginseng roots, (ii) hypericins and hyperforins are accumulated only in foliar glands of *Hypericum perforatum* (St. John's-wort) and not in undifferentiated cells, (iii) biosynthesis of lysine to anabasine occurs in tobacco (*N. tabacum*) roots, followed by the conversion of anabasine to nicotine in leaves, and (iv) at least some degree of differentiation in a cell culture must occur before vincristine or vinblastine are synthesized in *Catharanthus roseus*. *Fritillaria unibracteata* can be rapidly propagated, directly from small cuttings of the bulb by the technique of organ culture. The growth rate was about 30–50 times higher than that under natural wild growth conditions, and content of alkaloid and beneficial microelements in the cultured bulbs was higher than found in the wild bulb.

Hairy Root Cultures

Hairy roots (HRs) are obtained after the successful transformation of a plant with *Agrobacterium rhizogenes*. *A. rhizogenes* may be used to transform leaf disks, other organs or even protoplasts. The roots are excised and used to initiate root cultures; usually a culture flask is inoculated with 3–4 roots of 2–3 cm in length. Hairy root cultures are easily developed in most dicot plants. It has received considerable

attention from plant biotechnologists in the two last decades as a method of producing secondary metabolites synthesized in plant roots. The hairy root phenotype is characterized by hormone-independent fast growth, lack of geotropism, profuse lateral roots, and genetic stability. The secondary metabolites produced by HRs are the same as those usually synthesized in intact parent roots, with similar or higher yields. This feature, together with long term genetic stability and generally rapid growth in simple media lacking phytohormones, makes them especially suitable for biochemical studies not easily undertaken with root cultures of an intact plant. A major characteristic of HRs is that they are able to produce secondary metabolites concomitantly with growth. Hence, it is possible to get a continuous source of secondary compounds from actively growing HRs, unlike the usual results obtained with cell suspension cultures. Metabolite production rate may be enhanced by modifying the nutrient composition of the medium or applying elicitors as in case of cell culture.

The hairy roots are normally induced on aseptic, wounded parts of plants by inoculating them with *A. rhizogenes*. The hairy roots (HRs) are differentiated cultures of transformed roots generated by the infection of wounded higher plants with *A. rhizogenes*. This pathogen causes the HR disease leading to the neoplastic growth of roots that are characterized by high growth rate in hormone free media and genetic stability. During the infection process, *A. rhizogenes* transfers a part of the DNA (transfer DNA, T-DNA) located in the root-inducing plasmid Ri to plant cells, and the genes contained in this segment are expressed in the same way as the endogenous genes of the plant cells. Some *A. rhizogenes*, such as strain A4, have the T-DNA divided into two sections: the TR-DNA (right) and TL-DNA (left), each of which can be incorporated separately into the plant genome. Two sets of pRi genes are involved in the root induction process: the *aux* genes located in the TR region of the pRi T-DNA and the *rol* (root loci) genes of the TL region. High stability and productivity features allow the exploitation of HRs as valuable biotechnological tool for the production of plant secondary metabolites. HRs can be also utilized as biological farm for the production of recombinant proteins, hence holding additional potential for industrial use.

Genetic Manipulation in Hairy Root Culture for Secondary Metabolite Production

Transformed roots provide a promising alternative for the biotechnological exploitation of plant cells. *A. rhizogenes*-mediated transformation of plants may be used in a manner analogous to the well-known procedure employing *A. tumefaciens*. *A. rhizogenes*-mediated transformation has also been used to produce transgenic hairy root cultures, and plantlets have been regenerated. None of the other T-DNA sequences are required for the transfer with the exception of the border sequences. The rest of the T-DNA can be replaced with the foreign DNA and introduced into cells from which whole plants can be regenerated. These foreign DNA sequences are stably inherited in a Mendelian manner. The *A. rhizogenes*-mediated transformation has the advantage of being able to transfer any foreign gene of interest placed in binary vector to the transformed hairy root clone,

e.g., the 6-hydroxylase gene of *Hyoscyamus muticus* introduced to hyocyamin-rich *A. belladonna* by a binary vector system. Engineered roots showed an increased amount of enzyme activity and a fivefold higher concentration of scopolamine.

Shoot Cultures

As with roots, it is possible to cultivate plant aerial parts (shoots) for the production of secondary metabolites (Fig. 9.2). Shoot cultures can be transgenic, the so-called shooty teratomas, if they are obtained after infection with *Agrobacterium tumefaciens*, or non-transgenic through the simple use of appropriate hormonal balance. Shoots exhibit genetic stability, good capacities for secondary metabolite production, and a link between growth and the production of secondary compounds, some of the comparable properties to hairy roots.

Organ Cultures in Bioreactors

Compared to cell suspension cultures, organ cultures generally display a lower sensitivity to shear stress with some exceptions. *Catharanthus roseus* hairy roots need to be cultivated in an air-sparged bioreactor. Immobilization of hairy roots into a polymer matrix is a well-known technique, and it is also possible to protect the roots from agitation by using screens or wire meshes. Other less sensitive organs can be cultivated in stirred bioreactors. One of the major problems encountered with organ cultures in bioreactors is due to the inhomogeneous character of the biomass compared to thin cell suspensions. Hairy roots in liquid systems grow in approximately spherical clumps but display a high degree of spatial heterogeneity. This heterogeneity can be partially attributed to inhomogeneous and limiting mass transfers to the roots, regarding oxygen and nutrients.

Due to their genetic stability, organs are less submitted to erratic metabolite production than undifferentiated cells except a spatial heterogeneity along the growing organ. The total root biomass is always composed of young (root tip) and older tissues, and these young and old tissues present various possibilities for the synthesis of secondary compounds. Young tips of *Psoralea* roots were more capable of synthesizing isoflavones (daidzein), whereas old roots accumulated isoflavone-derivatives like coumestrol.

In most organ cultures, the production of secondary plant products is usually concomitant with growth. As a consequence, it is possible to use a single-stage bioreactor for both growing the biomass and producing the compounds; most of the secondary metabolites tend to remain intracellular, especially when growth is still active.

Specific bioreactors have been designed for hairy root cultures in order to overcome the limiting factors existing for biomass and secondary metabolite production. Submerged cultures have successfully been replaced by dispersed liquid systems such as nutrient mist reactors or drip-tube techniques. Two-phase systems have also been used to facilitate the release and recovery of the secondary compounds in the medium. This technology helps to continuously remove the compounds from the medium and helps to prevent the feedback repression of the synthesis. Despite all the improvements that have been made to reach a better understanding of plant organ cultures in bioreactors, this technology has led to even

fewer commercial successes than cell suspension cultures for the production of secondary metabolites.

9.2.3 Industrial Application

9.2.3.1 Commercial Production of Shikonin

Shikonin (dye) was the first commercial product from cell cultures. This became possible due to the following:

Development of media for (i) biomass production, (ii) biochemical production, (iii) isolation of stable high-producing cell clones, and (iv) use of a two-stage production system. The high-producing clone cells to be used as inoculum. The inoculum is first added to a 200-l fermenter (first stage) containing the MG-5 medium for culture growth. After 9 days, the cells are filtered out and inoculated into a 750-l fermenter (second stage) containing M-9 shikonin production medium and incubated for 14 days. The cells are harvested by simple filtration, and shikonin and shikonin derivatives are extracted from the cells.

A 750-l bioreactor with 600 l medium would yield 1.2 kg of shikonin in 2 weeks. In contrast, Lithospermum roots from 1 ha land would yield about 9 kg shikonin after 4 years. Thus, 8 runs of 2 weeks of the bioreactor become equivalent to 4 years of a 1 ha field of *Lithospermum erythrorhizon* in terms of shikonin yields. Cell-culture-derived shikonin has been used in Japan since 1984 in the manufacture of cosmetics, lotion, and soap.

9.2.3.2 Biotransformation of Drug Precursors

Plant cells have the potential to produce, either by de novo synthesis or by biotransformation of specific precursors, an extensive range of secondary metabolites in culture. Modification of an exogenous compound by plant cells (or other biological entities) is called biotransformation or by conversion. The bioconversion reactions are catalyzed by enzymes present in plant cells. These reactions include esterification, oxidation, reduction, hydroxylation, and glycosylation. In all cases, the stereo—and regioselectivity expressed by the in vivo process is of enormous advantage. However, the low rates of biotransformation have prevented commercial exploitation of the very large number of bioconversions known for plant cells. The interest in bioconversion is mainly because the product of the process is more useful or valuable than the precursor used.

A relatively high rate of bioconversion (0.8 g/1 medium over 7-day period) of cardiac glycosides is affected by *Digitalis lantana* cell cultures. Digitalis cells hydroxylate the C-12 position of *p*-methyldigitoxin to convert it into *p*-methyldigoxin, which is more valuable than the former. Cell cultures of several species, i.e., *Datura innoxia*, *Catharanthus roseus*, *Rauwolfia serpentina*, biotransform

hydroquinone into its β-D-glucoside called arbutin. Arbutin is an efficient suppressor of melanin biosynthesis in human skin and is used in cosmetics. Catharanthus cells biotransform hydroquinone into arbutin at the rate of 9.2 g/1 of medium over 4 days, while Rauwolfia cultures give arbutin yield of 18 g/1 medium in 7 days. Arbutin is at present prepared chemically in a 3-step procedure. But it is expected that refinements may enable the single-step biotransformation process to outcompete the chemical procedure.

There is a wide scope for the industrial application of secondary metabolites from plant sources as indicated in Table 9.4.

Most of the above and many other secondary metabolites may be derived from plant cell/organ culture in vitro. However, these cultures exhibit relatively slow rates of growth, and the biosynthesis of the desired compounds is often at a much lower level than in the intact plant. In order for cell cultures to be used as commercial sources of these compounds, the in vitro production must be comparable to or to exceed the amount produced by the intact plant (Table 9.2).

Vanisree et al. (2004) noted the recent advances in tissue culture technology for the production of a wide variety of plant pharmaceuticals like alkaloids, terpenoids, steroids, saponins, phenolics, flavonoids, and amino acids, especially taxol, morphine and codeine, ginsenosides, L-DOPA, berberine, diosgenin, capsaicin, camptothecin, vinblastine and vincristine, tanshinones, Podophyllotoxin. In tissue culture technology, transcription factors are considered efficient new molecular tools for plant metabolic engineering to increase the production of valuable compounds (Gantet and Memelink 2002). The anticancer agent paclitaxel yield is low in nature, and plant cell culture technology is an amenable attractive alternative to scale-up production (Kolewe et al. 2008). Several published reports also indicated the in vitro culture yields exceeded that of the whole plant. Undoubtedly, the

Table 9.4 Secondary metabolites from plants and their associated industries

Industry	Plant product	Plant species	Industrial uses
(a) Pharmaceuticals	Codeine (alkaloid)	*Papaver somniferum*	Analgesic
	Diosgenin (steroid)	*Dioscorea deltoidea*	Antifertility agents
	Quinine (alkaloid)	*Cinchona ledgeriana*	Antimalarial
	Digoxin (cardiac glycoside)	*Digitalis lanata*	Cardiotonic
	Scopolamine (alkaloid)	*Datura stramonium*	Antihypertensive
	Vincristine (alkaloid)	*Catharanthus roseus*	Antileukemic
(b) Agrochemicals	Pyrethrin	*Chrysanthemum cinerariaefolium*	Insecticide
(c) Food and drink	Quinine (alkaloid)	*Cinchona ledgeriana*	Bittering agent
	Thaumatin (chalcone)	*Thaumatococcus danielli*	Non-nutritive sweetener
(d) Cosmetics	Jasmine	*Jasminum* sp.	Perfume

biosynthesis of compounds in vitro has many possibilities, although a number of the problems associated with it still need extensive research before this new technique can be applied on a large scale.

9.2.4 Animal Tissue Culture

Animal tissue culture generally involves the removal of cells tissues, or organs from an animal and their subsequent placement into an artificial environment (in vitro culture medium) conducive to growth.

Animal Cell Cultures
The in vitro cultivation of cell tissue and organ of animal origin is collectively known as animal tissue culture and is now used in many areas of science. The partial list of different cell types which can be grown in culture includes connective elements such as fibroblasts, skeletal tissue (bone and cartilage), skeletal, cardiac and smooth muscle, epithelial tissue (liver, lung, breast, skin, bladder, and kidney), neural cells (glial cells and neurons, although neurons do not proliferate in vitro), endocrine cells (adrenal, pituitary, pancreatic islet cells), melanocytes, and many different types of tumor cells. Tissue culture can be subdivided into three major categories such as cell culture, explant culture, and organ culture.

Cell Culture
Cell culture refers to cultures derived from dissociated cells taken from the original tissue ('primary cell culture'). Cells are dispersed (mechanically and/or enzymatically) into a cell suspension which may then be cultured as a monolayer on a solid substrate, or as a suspension in the culture medium.

Explant (or Organotypic) Culture
In explant culture, small pieces of the tissue of interest are simply allowed to attach to an appropriate substrate, usually one that has been coated with collagen, and are cultured in a rich medium, usually one containing serum.

Organ Culture
Organ culture refers to a three-dimensional culture of tissue retaining some or all of the histological features of the tissue in vivo. The whole organ or part of the organ is maintained in a way that allows differentiation and preservation of architecture.

Basic Equipment and Facilities Requirement in Animal Cell Culture
Some of the specific equipment and techniques are required for the maintenance of cell cultures. A rule of thumb is that the more equipments are available, the more efficient cell culturing be performed.

Sterile Work Area
A separate clean room equipped with an airflow cabinet, e.g., HEPA (High Efficiency Particle Air Filter) for filtered air supply around the work surface should be made available for clean cell culture work. A laminar flow hood offers the best

sterile protection available. All work surfaces, benches and shelves, and the base of the airflow cabinets must be kept clean by frequent swabbing with 70% ethanol or an alternative disinfectant.

Incubation Facilities

In addition to an airflow cabinet and benching which can be easily cleaned, the cell culture laboratory will need to be furnished with an incubator or hot room to maintain the cells at 30–40 °C. The incubation temperature will depend on the type of cells being cultivated. It may be necessary to use an incubator which has been designed to allow CO_2 to be supplied from a main supply or gas cylinder so that an atmosphere of between 2 and 5% CO_2 is maintained in the incubator. In general, many cell lines can be maintained in an atmosphere of 5% CO_2:95% air and at 99% relative humidity.

Refrigerators and Freezer (−20 °C)

Both items are very important for storage of liquid media at 4 °C and for enzymes (e.g., trypsin) and some media components (e.g., glutamine and serum) at −20 °C. A refrigerator or cold room is required to store medium and buffers. A freezer will be needed for keeping pre-aliquoted stocks of serum, nutrients, and antibiotics. Reagents may be stored at a temperature of −20 °C but if cells are to be preserved it may be necessary to provide liquid nitrogen or a −70 °C freezer.

Microscopes

A simple inverted microscope is essential so that cultures can be examined in flasks and dishes. It is vital to be able to recognize morphological changes in cultures since these may be the first indication of deterioration of a culture. A very simple light microscope with 100× magnification will suffice for routine cell counts in a hemocytometer. A camera, CCD video camera, adapter and attachments, and UV facility may also be required for some purposes.

Tissue culture ware

A variety of tissue culture plasticware is available (specially treated polystyrene). Cells can be maintained in petri dishes or flasks (25 or 75 cm^2) which have the added advantage that the flasks can be gassed and then sealed so that a CO_2 incubator need not be used.

Washing Up and Sterilizing Facilities

Glassware such as pipettes should be soaked in a suitable detergent and then passed through a stringent washing procedure with thorough soaking in distilled water prior to drying and sterilizing. Glassware, such as pipettes, conical flasks, beakers (covered with aluminum foil), are sterilized in a hot air oven at 160 °C for 1 h. All other equipment, such as automatic pipette tips and bottles (lids loosely attached), are autoclaved at 121 °C for 20 min. Sterilizing indicators such as sterile test strip are necessary for each sterilizing batch to ensure that the machine is operating effectively. Autoclave bags are available for loose items. Aluminum foil also makes good packaging material.

Liquid N_2/Deep Freezer

Invariably for continuous and finite cell lines, samples of cultures will need to be frozen down for storage. It is important to maintain continuity in cells to prevent genetic drift and to guard against loss of the cell line through contamination and other disasters. The procedure for freezing cells is general for all cells in culture. They should be frozen in exponential phase of growth with a suitable preservative, usually dimethylsulfoxide (DMSO). The cells are frozen slowly at 1 °C/min to −50 °C and then kept either at −196 °C immersed in liquid N_2 (in sealed glass ampoules) or above the liquid surface in the gas phase (screw top ampoules). Deterioration of frozen cells has been observed at −70 °C, therefore, −196 °C (liquid N_2) seems to be necessary.

Water Still or Reverse Osmosis Apparatus

A double-distilled (glass distilled) or reverse osmosis water supply is essential for preparation of media, and rinsing glassware. The pH of the double-distilled water should be regularly checked.

Water is sterilized by autoclaving at 121 °C for 20 min. The distilled water must be stored in glass if it is to be used for the preparation of media.

Filter Sterilization

Media that cannot be autoclaved must be sterilized through a 0.22-μm-pore-size membrane filter. These are obtainable in various designs to allow a wide range of volumes to be filtered (e.g., Millipore, Gelman).

Facilities for Counting Cells

It is possible to monitor cell growth by eyes (looking for confluency). More accurate cell counts are required for most experimental purposes, and the most commonly used device is the improved Neubauer hemocytometer originally designed for counting blood cells.

General Small Items of Equipment

A number of small items of equipment are useful for performing cell culture, e.g., water bath and centrifuge with sealed buckets, vacuum pump, graduated pipettes of various sizes, centrifuge tubes and universal containers, disposable Pasteur pipettes, rubber bulbs for use with Pasteur pipettes, glass or plastic pipettes for large volume, precisely calibrated automatic pipettes (small volumes of 1–1000 μl) with disposable, autoclavable, plastic tips, suction aid such as ‘Pipet-aid’ or ‘pi-pump’, etc., are necessary.

Culture Media

The tissue culture media essentially consists of metabolites (e.g., carbohydrates, amino acids, vitamins, proteins, and peptides), inorganic ions, hormones and extracellular matrix, and also the biological fluid for culturing cells, serum. The environment is regulated with regard to the temperature, osmotic pressure, pH, etc., which closely simulate the situation in vivo.

A wide variety of culture media is currently available. The choice of culture media is dependent on the requirements of cells. The components of suitable culture media include:

Basic Media
The most basic media are balanced salt solutions (BSS), e.g., phosphate-buffered saline (PBS), which may be used for washing cells and for short incubations in suspension. More complex defined media are used for growth and maintenance. Defined media can also vary in complexity, by the addition of a number of constituents, e.g., from Eagle's minimum essential medium (MEM) which contains essential amino acids, vitamins, and salts, to McCoy's medium, which contains a larger number of different amino acids, vitamins, minerals, and other extra metabolites (such as nucleosides).

Buffering Capacity
Cell cultures have an optimum pH for growth, generally between pH 7.4 and 7.7. The type of buffering that is used for the media depends on the growth conditions. When cells are incubated in a CO_2 atmosphere, an equilibrium is maintained between the medium and the gas phase. A bicarbonate–CO_2 buffering system is most often used due to its low toxicity toward the cells.

Glutamine and Amino Acids
In addition to buffering the medium, there are other growth requirements including amino acids, the requirement for which may vary with cell culture type. Commonly the necessary amino acids include cysteine and tyrosine, but some non-essential amino acids may be needed. Glutamine is also required by most cell lines, and it has been suggested that cultured cells use glutamine as an energy and carbon source in preference to glucose, although glucose is present in most defined media. Glutamine is usually added at a final concentration of 2 mM; however, once added to the medium, the glutamine is only stable for about 3 weeks at 4 °C.

Serum
Various sources of serum may be used such as calf, fetal calf, and horse. Many continuous cultures utilize calf serum, but often fetal calf serum provides the best growing conditions. The level of serum used depends on the particular cell line and should be determined empirically.

Antibiotics and Antimycotics
In absence of good sterile conditions, it is necessary to incorporate antibiotics and antimycotics into the media like penicillin/streptomycin solutions, or broader spectrum antibacterial/antimycotic agents such as kanamycin or amphotericin B. The antibiotics chosen should clearly not to be toxic to the cells in culture.

Supply and Preparation of Culture Media
The choice of culture media used will depend on the type of primary cell, cell line, and the incubation conditions. However, it is best to start with the medium recommended by the original supplier of the cells.

Culturing Animal Cells
Selecting Sources of Tissue for Culture

Adult or embryonic tissue
Cultures can be derived from adult tissue or from embryonic tissue. Cultures derived from embryonic tissue generally survive and grow better than those taken from adult tissue. Tissues from almost all parts of the embryo are easy to culture, whereas tissues from adult are often difficult or even impossible to culture.

9.2.5 Animal Products in Therapeutic Use

The WHO estimates that as many as 80% of the world's more than six billion people rely primarily on animal- and plant-based medicines. Ingredients sourced from wild plants and animals are used in traditional medicines and as raw materials also in the preparation of modern medicines and herbal preparations (Kang and Phipps 2003).

Animals and products derived from different organs of their bodies have constituted part of medicinal substances in different cultures since ancient times (Adeola 1992; Agrimi et al. 1992; Lev 2003); such uses still exist in traditional medicine. The healing of human ailments by using therapeutics based on medicines obtained from animals is known as zootherapy (Costa-Neto 2005) In modern societies, zootherapy constitutes an important alternative therapy and wild and domestic animals, and their by-products (e.g., hooves, skins, bones, feathers, tusks) form important ingredients in the preparation of curative, protective, and preventive medicine (Adeola 1992, Agrimie et al. 1992). Traditional Chinese Medicine (TCM) contains more than 1500 animal species (Anonymous 1995); in India, nearly 15–20% of the Ayurvedic medicine is based on animal-derived substances (Unnikrishnan 1998) and in Bahia State, in the northeast of Brazil, over 180 medicinal animals have been recorded (Neto 2004).

Animal metabolites of therapeutic application include a wide-spectrum product such as: Carmine (red dye) is made from crushed female cochineal insects. It is also known as natural red 4. Gelatin is a protein that is obtained by boiling the skin, ligaments, tendons, and bones from cows and pigs in water. The capsule cover of modern drug is made from gelatin. Glycerin may be obtained from cow or pig fat. Heparin anticoagulant medication is derived from cows (lungs) and pigs (intestines). Insulin—much of the insulin on the market is made from hog pancreas, diabetics can also get synthetic insulin. Lactose is extremely common and is milk sugar from mammal milk. Lanolin is a product of the oil glands of sheep. It is an ingredient in some ophthalmic drugs (found in eye drops because it has antibacterial properties) and is used as a carrier in certain drugs that are given by injection. Magnesium stearate—the stearate portion of magnesium stearate is a form of stearic acid, which is a saturated fat, found in cows, coconut oil, cocoa butter, and other foods and depending on the origin, it may be of animal or plant product. Premarin is

a conjugated estrogen product comes from horse urine. Vaccines for both children and adults, including the flu vaccine, contain animal by-products or are prepared with them, including gelatin, chicken embryo, guinea pig embryo cells, and serums. If some of the animal-derived products are synthesized by biotechnological methods, then the pressure on animals would be minimized proportionately. At present, about 40% of all prescription drugs are substances originally extracted from plants, animals, fungi, and microorganisms (Wilson 1995). The use of animals in popular medicine certainly provokes pressure on natural resources exploited through traditional forms of collection, mainly due to general acceptance of popular medicine (Almeida and Albuquerque 2002). The world is facing potentially massive loss of wildlife due to over-hunting (Robinson and Bennett 2000; Bennett et al. 2002).

Nowadays, animal cell culture becomes a reasonable alternative for animal experiments in the process of drug discovery and development. Overall, an aspect of pharmaceutical research which promisingly employs cell culture models is the study of in vitro drug transport/absorption and metabolism. More recently, cultured animal cells have been preferred for the expression of human genes encoding pharmaceutically valuable proteins. Some of these proteins have already been approved for therapeutic use, e.g., hGH (for dwarfism), tissue plasminogen activator (tPA; thrombolysis) crythropoietin (anemia), and blood clotting factor VIII (hemophilia); the last two of the proteins have been approved for marketing in India.

9.2.6 Fermentation and Production of Microbial Primary and Secondary Metabolites

Any of a group of chemical reactions induced by microorganisms or enzymes (ferments) that split complex organic compounds like sugar into relatively simple substances such as acetic acid, citric acid, ethanol, CO_2, and energy. Industrial fermentation is the intentional use of fermentation by microorganisms such as bacteria and fungi are used intentionally in industrial fermentation to make useful products. Fermented products have applications as food as well as in general industry. Some commodity chemicals are made by fermentation.

Fermentations (or industrial fermentations) based on the end-product application can be divided into four types (Stanbury et al. 1999):

(a) Biomass production—the end product is viable cellular material single cell protein, baker's yeast, probiotic cultures;
(b) Production of extracellular metabolites—Chemical compound intermediates of microbial biochemical pathways are produced and can be divided into two groups:

(i) Primary metabolites (produced during the growth phase of the organism, e.g., ethanol, citric acid, glutamic acid, lysine, vitamins, and polysaccharides)
(ii) Secondary metabolites (produced during the stationary phase, e.g., penicillin, cyclosporin A, gibberellin, and lovastatin);

(c) Production of intracellular components—enzymes and other proteins production, followed cell lysis at the end and purification of the products; and
(d) Transformation of substrate—raw material is biologically transformed into a finished product and generally used for steroid transformations, food fermentations, and sewage treatment.

Single cell protein, bakers yeast, lactobacillus, *E. coli*, etc., are the intended microbial cells or biomass products of fermentation.

Microbial metabolites are divided into two broad groups: (i) primary metabolites produced during the growth phase of the organism (e.g., ethanol, citric acid, glutamic acid, lysine, vitamins, and polysaccharides) and (ii) secondary metabolites produced during the stationary phase (e.g., penicillin, cyclosporin A, gibberellin, and lovastatin).

Intracellular components of microbial origin include microbial enzymes (catalase, amylase, protease, pectinase, glucose isomerase, cellulase, hemicellulase, lipase, lactase, streptokinase) and many others and recombinant proteins (insulin, hepatitis B vaccine, interferon, granulocyte colony-stimulating factor), etc. The cells are ruptured (lysed) at the end of fermentation to release the products.

Substrate transformation involves the transformation of a specific compound into another, such as in the case of phenylacetylcarbinol, and steroid biotransformation, or the transformation of a raw material into a finished product, in the case of food fermentations and sewage treatment. Ancient fermented food processes, such as making bread, wine, cheese, curds, idli, dosa, etc., also constitute biotechnology. Marmite (made from yeast extract) is a by-product of beer brewing. Other products similar to marmite are: Australian Vegemite, Swiss Cenovis, and German Vitam-R.

In the process of sewage treatment, sewage is digested by enzymes secreted by bacteria into harmless, soluble substances, carbon dioxide, methane, etc. Digested solids is dried and used as fertilizer and gaseous by-products (methane) can be utilized as biogas. Liquids that result are disinfected before being discharged into rivers or the sea or can be used as liquid fertilizers.

Bacterial metabolism can be classified into three major categories viz. (i) the kind of energy used for growth, (ii) the carbon source, and (iii) the electron donors used for growth. Metabolites, the metabolic intermediates and products, are typically characterized by small molecules with various functions. Metabolites can be categorized into (i) primary and (ii) secondary metabolites. These metabolites can be used in industrial microbiology to get various types of chemicals such as amino acids, vaccines, antibiotics, and other chemicals necessary for organic synthesis. They are produced in two different phases of the life cycle of the organism (Fig. 9.3).

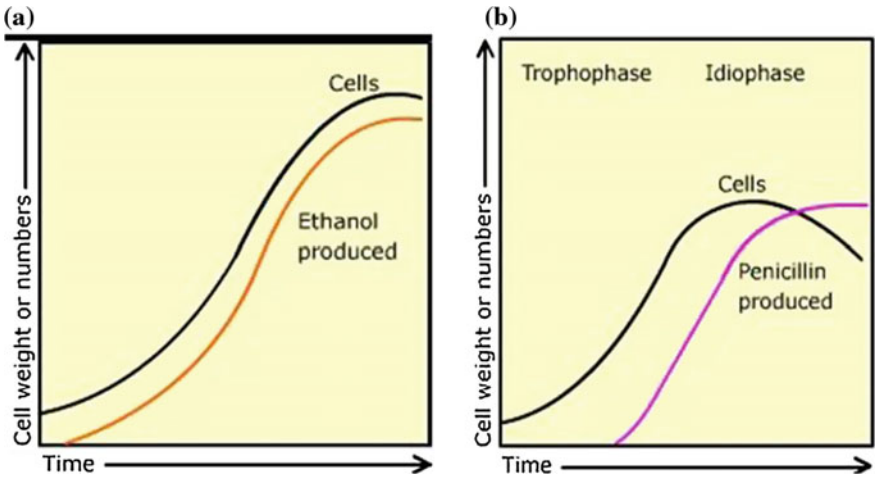

Fig. 9.3 **a** A primary metabolite ethanol from yeast has a production curve that lags only slightly behind the line showing cell growth. **b** Secondary metabolite penicillin from mold begins to be produced only after the logarithmic growth phase of the cell (trophophase) is completed. The main production of the secondary metabolite occurs during the stationary phase of cell growth (idiophase)

9.2.6.1 Primary Metabolites

Primary metabolites of microorganisms are involved in growth, development, and reproduction of the organism. The primary metabolite, often referred to as a central metabolite, is typically a key component in maintaining normal physiological processes of the organism. They are essential for proper growth, and they are typically synthesized during the growth phase as a result of energy metabolism and include alcohols (ethanol), lactic acid, citric acid, amino acids (glutamic acid, lysine), vitamins, polysaccharides. In industrial microbiology, alcohol fermentation by *Saccharum officinarum* fungus is most common primary metabolite product used for large-scale production of beer and wine, citric acid is produced by *Aspergillus niger*, glutamate, lysine, threonine, tryptophan, and other amino acids are produced by some *Micrococcus* sp. and *Corynebacterium* sp. (e.g., *Corynebacteria glutamicum*). These products are most widely used ingredients in food, pharmaceutical and cosmetic industries.

9.2.6.2 Secondary Metabolites

Secondary metabolites are produced through the modification of primary metabolites, and they do not play a role in growth, development, and reproduction or other vital processes of the organisms but in defense and in similar ecological functions. The secondary metabolites are produced during the stationary phase of cell growth

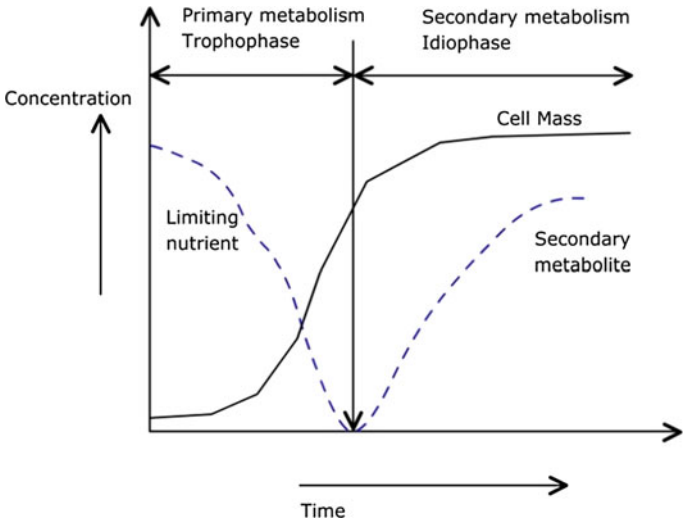

Fig. 9.4 Primary and secondary metabolism. During the trophophase, the cell mass increases logarithmically but as the resources become limiting, growth rate drops and production stops. Idiolites are special metabolites usually possessing bizarre chemical structures, and although not essential for the producing organism's growth in pure culture, they have survival functions in nature

(idiophase). Penicillin, cyclosporin A, erythromycin, lovastatin, bacitracin, etc., are good examples of secondary metabolites. Penicillin is synthesized by *Penicillium* molds that prevents bacterial growth, bacteria *Bacillus subtilis,* and other *Lactobacillus* sp. are able to produce bacteriocins (non-ribosomal peptides) which prevent the growth of bacteria either as antibiotics or as antiseptics (gramicidin S) is commonly used a topical drug. Erythromycin, derived from *Saccharopolyspora erythraea*, is a commonly used antibiotic with a wide antimicrobial spectrum. Griseofulvin fungicides are also produced as secondary metabolites. Typically, secondary metabolites are not produced in the presence of glucose or other carbon sources which would encourage growth, and like primary metabolites are released into the surrounding medium without rupture of the cell membrane (Fig. 9.4).

9.3 Some High-Value Medicinal Plants Including Spices, Beverage, Aromatic Plants

High-value medicinal plants

There are hundreds of herbs, and their parts (leaf, flower, fruit, root, rhizome, etc.) serve all kinds of important medicinal and health purposes ranging from anti-inflammatory, antifungal, insect repellent, antiseptic, remedies for injuries, scrapes and bites, expectorant, antibacterial, detoxification, fever reduction,

antihistamine to pain relief. They grow in nature, some are cultivated and some are available in local market for purchase. These plants are valuable for medicinal use, culinary purposes, and also valuable because they provide a means of earning the livelihood of a group of rural people. They are also valuable in different traditional systems of indigenous medicines and source of lead compounds and drug principles of modern medicine. Hundreds of medicinal plants (including spices, culinary, and aromatic herbs) are traded worldwide, and many of them are considered to be of very high-value items per weight among the traded plants and plant products. These pharmaceutical cash crops have a huge potential for rural communities that practice subsistence agriculture with insignificant access to main stream economy (Dubey et al. 2004; Chauhan et al. 2013; Sher et al. 2014). These minor crops have relatively small contribution to total agricultural output of a country, but their value in global trade is significant; it was about US$60 billion in 2006 (Adhikari 2001; Hamilton 2006). Collection, cultivation, and trade (internal and world trade) of medicinal plants in many Asian countries constitute an age-old practice (Ali-Shtayeh et al. 2000; Lev and Amar 2002; Ghorbani 2005; Al-Quran 2008). Europe imports about US$ 1 billion in MAPs from Africa and Asia annually (Ghimire et al. 2002; Sher and Hussain 2009), and it is expected to expand substantially by the year 2050 because of the increasing trend in herbal medicine use (Lange 1999; Al-Quran 2008; Khan et al. 2011).

High-value medicinal plants may be from cultivated crops, forest grown, or wild source plants. Some of the examples of high-value medicinal plants include *A. moschatus*, *Abies spectabilis*, *Abrus precatorius*, *Achyranthes aspera*, *Aconitum ferox*, *A. heterophyllum*, *Acorus calamus*, *Acacia catechu*, *A. sinuate*, *A. marmelos*, *Aesculus hippocastanum*, *Adhatoda zeylanica*, *Adiantum capillus-veneris*, *Albizzia amara*, *Aloe barbedensis*, *A. vera*, *Alpinia calcarata*, *Alstonia scholaris*, *A. paniculata*, *Angelica sinensis*, *Anogeissus latifolia*, *A. annua* and other *Artemisia* sp., *Asparagus adscendens*, *A. racemosus*, *Astragalus membranaceus*, *Aerva lanata*, *A. indica*, *B. monnieri*, *Baliospermum montanum*, *B. aristata*, *B. vulgaris*, *B. erythroclada*, *Bergenia ciliate*, *Boerhavia diffusa*, *Boswellia serrata*, *Bunium persicum*, *Butea monosperma*, *Caesalpinia sappan*, *Cardiospermum halicacabum*, *Cassia absus*, *C. angustifolia*, *C*, *fistula*, *C. tora*, *Catharanthus roseus*, *Cedrus deodara*, *C. paniculatus Centella asiatica*, *Centratherum anthelminticum*, *Cephaelis peruviana*, *Citrullus colocynthis*, *Careya arborea*, *Chlorophytum borivillanum*, *C. tuberosum*, *Cinnamomum sulphuratum*, *C. tamala*, *Cichorium intybus*, *Chlorophytum borivilianum*, *Chrysopogon zizanioides*, *Clerodendrum phlomides*, *Colchicum luteum*, *Commiphora caudate*, *C. mukul*, *C. wightii*, *Convolvulus pluricaulis*, *Coptis chinensis*, *Coscinium fenestratum*, *Crataegus* spp., *Croton tiglium*, *Curculigo orchioides*, *Curcuma angustifolia*, *C. zerumbet*, *Cymbopogon cytratus*, *Cyperus esculentus*, *C. rotundus*, *Cyclea peltata*, *Datura metel*, *Decalepis hamiltonii*, *Desmodium gangeticum*, *D. deltoidea*, *Diospyros lotus*, *Echinacea* spp., *Eclipta prostrate*, *E. prostate*, *Eleutherococcus senticosus*, *E. officinalis*, *Embelia ribes*, *E. tsjerium-cottam*, *Ephedra gerardiana*, *Ficus benghalensis*, *Ficus religiosa*, *Fumaria indica*, *Garcinia indica*, *G. glabra*, *G. sylvestre*, *Gardenia resinifera*, *Geranium wallichianum*, *Gingko biloba*, *Gloriosa superba*,

Gmelina arborea, *G. sylvestre*, *Gynura procambens*, *Hedyotis corymbosa*, *Helicteres isora*, *Hemidesmus indicus*, *Holarrhena pubescens*, *Holoptelea integrifolia*, *Holostemma ada-kodien*, *Hydnocarpus kurzii*, *Hydrastis canadensis*, *Hypericum perforatum*, *Hygrophylla schulli*, *Indigofera tinctoria*, *Inula racemosa*, *Ipomoea mauritiana*, *Ipomoea nil*, *Ixora coccinea*, *Jatropha curcas*, *Juniperus communis*, *Jurinea himalaica*, *J. macrocephala*, *Kaempferia galangal*, *Lannea coromandelica*, *Lawsonia inermis*, *Lepidium sativum*, *Litsea glutinosa*, *Lobelia nicotianaefolia*, *Madhuca indica*, *Mecanopsis grandis*, *M. peniculata*, *Mentha arvensis*, *Merremia tridentate*, *Messua ferrea*, *Mimusops elengi*, *Morchella esculenta*, *Morinda pubescens*, *Mucuna puriens*, *Nardostachys grandiflora*, *N. jatamansi*, *Neopicrorhiza scrophulariiflora*, *Nilgirianthus ciliates*, *Ocimum americanum*, *O. basilicum*, *O. sanctum*, *O. tenuiflorum*, *Onosma hispidum*, *Operculina turpethum*, *Oroxylum indicum*, *Paeonia emodi*, *Panax ginseng*, *P. somniferum*, *Paris polyphylla*, *Parmelia perlata*, *Peganum harmala*, *Persicaria amplexicaulis*, *Phyllanthus amarus*, *Picrorhiza kurroa*, *P. longum*, *P. nigrum*, *Picrohiza kurroa*, *Pistacia chinensis*, *P. integerrima*, *Plantago ovata*, *P. psyllium*, *Pluchea lanceolata*, *Plumbago zeylanica*, *Plantago ovate*, *Plectranthus barbatus*, *P. peltatum*, *Pogostemon cablin*, *Pongamiapinnata*, *Polygonatum multiflorum*, *Potentilla peduncularis*, *Prunus armeniaca*, *Pseudarthia viscida Psoralea corylifolia*, *Premna serratifolia*, *Pterocarpus marsupium*, *P. santalinus*, *R. serpentina*, *Rhamnus purshianus*, *Rheum austral*, *Rhododendron anthopogon*, *Rubia cordifolia*, *S. album*, *Sapindus mukorossi*, *Saraca asoca*, *Saussurea costus*, *S. lappa*, *Serenoa repens*, *Silybum marianum*, *Simmondsi chinensis*, *Schrebera swietenioides*, *Semecarpus anacardium*, *Sida rhombifolia*, *Sinopodophyllum hexandrum*, *Sisymbrium irio*, *Solanum anguivi*, *S. nigrum*, *S. virginianum*, *Smilax glabra*, *Soymida febrifuga*, *Sphaeranthus indicus*, *Sterculia urens*, *Stereospermum chelonoides*, *Strychnos nux-vomica*, *S. potatorum*, *Symplocos racemosus*, *Swertia chirayita*, *T. wallichiana*, *Tephrosia purpurea*, *Terminalia arjuna T. bellirica*, *T. chebula*, *T. cordifolia*, *Trachyspermum ammi*, *Tragia involucrate*, *Tribulus terrestris*, *Trichosanthes cucumerina*, *Trillium govanianum*, *Urtica dioca*, *Uncaria* sp., *Valeriana jatamansii*, *Vateria indica*, *Viola pilosa*, *Vetiveria zizanioides*, *Vitex negundo*, *Withania coagulens*, *W. somnifera*, *Woodfordia fruticosa Wrightia tinctoria*, *Zanthoxylum nepalense*, *Ziziphus jujube*, *Z. xylocarpus*, etc. (Gupta and Chadha 1995; Ayensu 1996; Grünwald and Büttel 1996; Laird 1999).

9.4 Trade of Herbal Drugs

Medicinal and Aromatic Plants in Trade

Medicinal and aromatic plants have been offering in a wide variety of products on the market. At least every fourth flowering plant is used for the purpose. The enormous demand in botanicals results in a huge trade from local to international level. In the 1990s, the reported annual worldwide importation of pharmaceutical

Table 9.5 The world's top 12 leading countries of import and export of pharmaceutical plants, according to average quantities and values for the period 1991–2000

Country of import	Quantity (t)	Value (USD)	Country of export	Quantity (t)	Value (USD)
Hong Kong	67,000	291,200,000	China	147,000	281,800,000
Japan	51,350	136,000,000	Hong Kong	63,150	228,800,000
USA	49,600	135,500,000	India	33,900	56,650,000
Germany	45,350	110,200,000	Germany	15,100	70,050,000
Rep. Korea	32,250	52,300,000	USA	13,500	115,500,000
France	21,350	52,000,000	Mexico	13,000	11,250,000
China	13,650	41,600,000	Egypt	11,750	13,850,000
Italy	11,700	42,850,000	Chile	11,600	28,200,000
Pakistan	11,050	11,150,000	Bulgaria	10,050	14,500,000
Spain	9100	27,650,000	Singapore	9600	56,600,000
UK	7650	27,000,000	Morocco	8000	13,300,000
Singapore	6300	50,600,000	Pakistan	7800	4950,000
Total	326,300	978,150,000	Total	344,400	893,400,000

Figures based on commodity group pharmaceutical plants (SITC.3: 292.4 = HS 1211). Export figures include re-export. The quantities are given in tonnes (t). The main trade centers are underlined gray. *Source* UNCTAD COMTRADE database, United Nations Statistics Division, New York (*Source* Lange 2004)

plants amounted on average to 400,000t valued at USD 1224 million. The international trade is dominated by only few countries. About 80% of the worldwide imports and exports are allotted to only 12 countries with the dominance of temperate Asian and European countries. Japan and the Republic of Korea are the main consumers of pharmaceutical plants, and China and India are the world's leading producing nations; Hong Kong, the USA, and Germany stand out as important trade centers. Until now, the production of botanicals relies to a large degree on wild collection (Table 9.5).

Traded forms of botanicals

The plant raw material in trade consists of mainly dried plant parts, roots, leaves, bark, wood, flowers, or seeds, or sometimes, of several plant parts or even the whole plant if the demanded constituents are concentrated in several plant organs or even in the whole plant body. To a small extent, the plant material is traded fresh or preserved in alcohol (Lange 1996). The bulk of traded plant material is not or only little processed, which is in general cheaper than that which has been further processed, i.e., cut, rubbed, powdered, or even extracted.

Major traded botanicals

There are no or only few reliable trade data available for single botanicals (Lange 1996, 1998). The commodity group pharmaceutical plants include those used only in small quantities as well as bulk material with great industrial importance. In Germany, the most used medicinal plant is Gingko (*Gingko biloba*), followed by i.

a. Horse-chestnut (*Aesculus hippocastanum*), Hawthorn (*Crataegus* spp.), St John's-wort (*Hypericum perforatum*), Nettle (*Urtica dioca*), Echinacea (*Echinacea* spp.), Saw Palmetto (*Serenoa repens*), and Milk Thistle (*Silybum marianum*) (Grünwald and Büttel 1996). Some of these plants are also highly used in the USA, like Echinacea, St John's-wort, and Saw Palmetto, but the preferences are somewhat different: Siberian Ginseng (*Eleutherococcus senticosus*), Goldenseal (*Hydrastis canadensis*), Cat's claw (*Uncaria species*), Astragalus membranaceus, Dong Quai (*Angelica sinensis*), and Cascara Sagrada (*Rhamnus purshianus*) are listed among the top-selling botanicals (Laird 1999).

9.5 Herbal Wealth and Its Role in National Economy

Medicinal and aromatic plants, culinary herbs, etc., constitute an important natural wealth, the herbal wealth, of a country. It is estimated that up to 70,000 species are used in folk medicine (Farnsworth and Soejarto 1991), and over 21,000 plant taxa used for medicinal purposes (Groombridge 1992). According to the World Health Organization (WHO), about 25% of modern medicines are developed from plants sources used traditionally; and research on traditional medicinal herbal plants leads to discovery of 75% of herbal drugs (Mian-Ying et al. 2002). Traditional medicines derived from medicinal plants are used by about 60% of the world's population (Modak et al. 2007). They are important because they (a) play a significant role in providing primary health-care services to rural people; (b) serve as therapeutic agents as well as important raw materials for the manufacture of traditional and modern medicine; (c) serve as an income generating source of the rural people; and (d) substantial amount of foreign exchange can be earned by exporting medicinal herbs to other countries. In this way, indigenous medicinal herbs play significant role in health sector as well as in economy of a country. Bioactive compounds from medicinal plants can be directly used as healing agent and their phytochemicals also serve as lead compound for developing potential drugs to cure various diseases in human (Kamboj 2000; Verma and Singh 2008). Herbal preparations at present are known as alternative medicines. It has attracted increased attention of national media, policy makers, medical communities, government as well as non-government organizations all over the world largely because of the iatrogenic effects (adverse effects or complications) of the modern medicine. The World Health Organization also encouraged developing countries to use traditional plant medicines to fulfill a need unmet by modern system. The use of botanical raw material is in many cases much cheaper than to use chemical alternative substances. As a consequence, there is an enormous demand in botanicals in national and international arena resulting in a huge trade, on local, regional, national, and international levels. Diversity, flexibility, easy accessibility, broad continuing acceptance in developing countries and increasing popularity in developed countries, relative low cost, low levels of technological input, relative low side effects and growing economic importance are some of the positive features of traditional

medicine (WHO 2002). In the recent past and also in this first decade of the 21st century, there has been a growing interest in Traditional/Complementary and Alternative Medicine (TCAM) and their relevance to public health both in developed and developing countries. Traditional medicine refers to health practices, approaches, knowledge and beliefs incorporating plant-, animal-, and mineral-based medicines, spiritual therapies, manual techniques and exercises, applied singularly or in combination to treat, diagnose, and prevent illnesses or maintain well-being and the term 'complementary' and 'alternative' medicine (non-conventional or parallel) are used to refer to a broad set of healthcare practices that are not part of country's own tradition, or not integrated into the dominant healthcare system Traditional medicine refers to health practices, approaches, knowledge and beliefs incorporating plant-, animal-, and mineral-based medicines, spiritual therapies, manual techniques and exercises, applied singularly or in combination to treat, diagnose and prevent illnesses or maintain well-being. TCAM is practice almost worldwide, and often it is called in various ways such as traditional medicine, alternative medicine, complementary medicine, natural medicine, herbal medicine, phytomedicine, non-conventional medicine, indigenous medicine, folk medicine, ethno medicine, etc. Chinese medicine, Ayurveda, Herbal medicine, Siddha, Unani, Kampo, Jamu, Thai, Homeopathy, Acupuncture, Chiropractic, Osteopathy, bone-setting, spiritual therapies, are some of the popular, established systems. However, there is no homogenous body of medical thought and practice which can be put under one name (Van der geest 1997; Patwardhan 2005).

Herbs includes entire plant, plant parts (leaves, flowers, fruits, seeds, stems, wood, bark, roots, rhizomes, or other plant parts in entire, fragmented, or powdered form), fresh juices, gums, fixed oils, essential oils, resins, etc. Herbal-finished products include comminuted or powdered herbal materials, or extracts, tinctures and fatty oils of herbal materials and finished herbal products consist of herbal preparations made from one or more herbs (mixed herbal product). Finished herbal products and mixed herbal products may contain excipients in addition to the active ingredients. In some countries, herbal medicines may contain, by tradition, natural organic or inorganic active ingredients that are not of plant origin (e.g., animal materials and mineral materials).

Phytopharmaceuticals, herbal remedies, dietary supplements, homeopathics, herbal teas, liqueurs, spirits, sweets, aromas, perfumes, cosmetics, coloring agents, varnishes, detergents, etc., constitute herbal products. They are produced by extraction, fractionation, purification, concentration, and by other physical or biological processes. They also include preparations made by steaming, roasting, or stir baking with honey, alcoholic beverages, or other materials. Finished herbal products consist of herbal preparations made from one or more herbs. If more herbs are used, the term mixed herbal product be used. Finished herbal products and mixed herbal products may contain excipients in addition to the active ingredients. The bitter taste of Campari is based on the common centaury (*Centaurium erythraea*), and the fenugreek (*Trigonella foenum graecum*) contains steroid-saponins which are extracted for use in oral contraceptives.

Asia covers about 30% of the earth's land area and has some most biologically diverse countries like China, India, Indonesia, Malaysia, and the Philippines with rich traditional knowledge on the use of plants for therapeutic purposes (Anonymous 2010). Christophe Wiart working in the School of Biomedical Sciences of the University of Nottingham (Malaysia Campus) studied the medicinal plants of India, Southeast Asia, and China and collected, identified and classified 6000 medicinal plants species and he is regarded as the most prominent authority in the field of Asian ethnopharmacology, chemotaxonomy, and ethnobotany (Wiart 2007). Widely used Chinese herbs like *P. somniferum*, *A. annua* and *Taxus brevifolia* produce morphine, artemisinin, and paclitaxel, respectively (Cao and Kingston 2009).

Many of the Asian medicinal plant species that yield high-value products include *Catharanthus roseus*, *R. serpentina*, *Cephaelis peruviana* and other spp., *Coptis chinensis* and other spp., *P. somniferum*, *Dioscorea* spp., *Panax ginseng*, *P. peltatum*, *A. vera*, *Commiphora caudate* and other spp., *Mentha arvensis*, *Ocimum sanctum* and other spp., *Cymbopogon cytratus*, *Plantago psyllium*, *A. indica*, *A. annua* and other spp., *Cassia* spp., *Psoralea* spp., *Chlorophytum borivilianum*, *Pogostemon cablin* and other pp., *Piper nigrum* and other spp., *Chrysopogon zizanioides* (Gupta and Chadha 1995; Ayensu 1996).

Some commonly used medicinal (plants with therapeutic activity), medicinal and culinary (plants with therapeutic activity that enhance culinary dishes), and others are nutricicals (they have no therapeutic effects but health-promoting value) are *Actinidia sinensis* (fever, skin used fruits), *Adansonia digitata* (asthma, toothache as oil), *A. vera* (healing as balm, pills, oil), *Agrimonia eupatoria (*digestion, sore throat as infusion, decoction), *Allium sativum* (heart, anti bacterial in cooking), *A. cepa* (tension, colds as oignon cuit, potage), *A. schoenoprasum* (digestion whole plant, infusion), *Althaea officinalis* (anti-inflammator as infusion), A*nethum graveolens* (stimulant, stomachic as infusion, condiment), A*nthemis nobilis* (stress as infusion, balm), *Archangelica officinalis* (cough, colds), *Arctium lappa* (dermatosis as compresses), *Arnica montana* (contusions, ecchymoses as cream, decoction), *Artemisia absinthium (*intestinal problems as infusion), *Bambousa* (arthritis as cooking), *Borrago officinalis* (emollient, purgative as infusion, oil), *Bryophyllum pinnatum* (joint pains, leaves are used), *Butyrospermum* (healing, hydration as chocolate, baume), *Buxus chinensis* (dry skin as huiles), *B. sempervirens* (hair loss as infusion), *Calendula officinalis* (skin irritation as infusion, salad), *Camelia sinensis* (as infusion), *Calluna vulgaris* (urinary infection as powder, capsules), *Centaurea cyanus* (irritation of eyes and ears as infusion, eye drops), *Cereus grandiflorus* (hypertension as infusion), *Cinchomae cortex* (antibacterial, analgesic as écorce, poudre), *Cinnamomum verum* stomach ache as infusion), *Citrus aurantium* (tonic as infusion), *C.* limonum (sore throat, nausea as jus et fruit, gélule), *Colchicum* (neuralgia as infusion, vin), *Daucus carota* (cholesterol as légume cru), *Echium vulagre* (purgative as infusion), *Crataegus oxyacantha* (anxiety, palpitations as powder, capsules), *Elletaria caramomum* (loss of appetite seed is used), *Elytrigia repens* (kidney problems as decoction), *Ephedra sinica* (nasal decongestant as gélule), *Eucalyptus* globulus (respiratory problems as infusion, baume), *F. vulgare* (stomach

ache as cuisine, infusion), *Fraxinus excelsior* (rheumatism as feuilles), *Gentiana lutea* (loss of appetite, fatigue as tonique), *Ginkgo biloba* (antispasmodic as infusion), *G. glabra* (dermatosis as infusion, poudre), *Hamamelis virginiana* (circulatory troubles as décoction, crème), *Hibiscus* (fat elimination as infusion), *Humulus lupulus* (anxiety, insomnia as infusion), *Jasminum* (colds, cough, dermatosis as infusion), *Lavandula officinalis* (nervousness, insomnia as huiles), *Leontopodium alpinum* (cough, skin as infusion, serum), *Malva sylvestris* (respiratory, ENT as infusion, feuilles), *Matricaria recutita* (flu as infusion), *Melissa officinalis* (anxiety, insomnia as infusion), *Menyanthes trifoliate* (loss of appetite as compresse, *O. basilicum* (digestion as infusion, cooking), *Olea europaea* (tonic, hydrating as huile, fruits), *Panax* ginseng (stimulant, tonic as poudre, ampoule), *Passiflora incarnate* (insomnia as infusion), *P. crispum* (hypertension as persil frais), *Parietaria* officinalis (cystitis as infusion), *Papaver rhoeas* (insomnia, cough as infusion), *Pimpinella anisum* (antispasmodic, sedative as infusion), *Pinus sylvestris* (respiratory problems as poudre, infusion), *Pogostemon cablin* (colds, migraines as huiles), *Rosa canina* (tonic, anemia as infusion, decoction), *Rubus idaeus* (intestinal problems as feuilles), *R. fruticosus* (oral complaints as decoction), *Rosmarinus officinalis* (healing, stimulant as infusion), *Salvia officinalis* (asthma, menopause as poudre, infusion), *Sambucus* (flu, fever as infusion, baume), *Spirea ulmaria* (analgesic as poudre, infusion), *Taraxacum* (kidney problems as infusion, salade), *Tilia platyphylos* (insomnia, inflammation as infusion), *Vaccinium oxycoccos* (urinary infections as infusion), *Verbascum* (cough as powder, capsules), *Viola tricolor* (purgative as poudre, infusion), *Viscum album* (arteriosclerosis, gout as jus, feuilles), *Vitis vinifera (*glowing skin fruits are used), *Z. officinale* (aphrodisiac, tonic as infusion, cuisine), *Glycine max* (anemia as cuisine), *Mentha spicata* (antispasmodic as poudre, infusion), *Hypericum perforatum* (depression as gélule, infusion), *Ortica dioica* (diuretic as infusion, potage), *Arbutus unedo* (diuretic as sirup), *Helianthus annuus* (headache, fever as graines, huile), *Origanum marjorana* (nausea as condiment), *Thymus vulgaris* fortifying as infusion), *Lycopersicon* (sunburn used fruit), *V. officinalis* (insomnia, anxiety as infusion), *Lippia citriodora* spasms, insomnia as infusion, feuilles), *Triticum* (skin, hair yeast, oil), *Thymus serpyllum* (gastric infections as infusion).

Natural excipients like coloring (*Curma longa*, *C. sativus*, *Carthamus tinctorius*, *Calendula officinalis*) and flavoring agents (*Mentha arvensis*, *Cymbopogon flexuosus*, *C. martini*, *Cyperus scariosus*, *E. globules*), emulsifying and suspending agents (*Plantago ovata*), diluents, bulking agents or filler (plant cellulose as well as lactose, sucrose, glucose, mannitol, sorbitol, calcium carbonate, and magnesium stearate) and disintegrants (carboxymethyl cellulose), anesthetic aids (*Cannabis sativus*, *P. methysticum*), sweeteners (*G. glabra*, *Stevia rebaudiana*), carrier for targeted drug delivery (e.g., pectins, agar, gelatin, wax, fixed oils), binders (non-starch polysaccharides-pectins, alginates and proteins-gelatin), adhesives (guar gum, amylase, and karaya gum), solidifiers (beeswax, cocoa butter, or theobroma oil), material for surgical dressings (natural fibers, filtering agents—diatomite, support media) yielding plants are also included within the herbal spectrum as pharmaceutic necessities. Natural excipients are stable, biodegradable,

inexpensive, easily available, and safe in contrast to their many synthetic counterparts.

Beverages like tea and coffee (*Camellia sinensis*, *Coffea arabica*), spices, and condiments like Cinnamon (*Cinnamum zeylnicum*) bark, cardamom (*Amomum aromaticum* and *A. subulatum*) fruit and various fruits of Apiaceae such as fennel (*F. vulgare*), coriander (*C. sativum*), cumin (*Cuminum cyminum*), anise (*Pimpinella anisum*, etc.); seeds of mustard (*Brassica alba*, *B. juncea*, *B. nigra*), flower-bud of clove (*Syzygium aromaticum*), and rhizome of ginger (*Z. officinale*) are some typical examples; poisonous (e*Abrus precatorius*, *A. belladonna*, *Colchicum autumnale*), hallucinogenic or psychoactive (*Cannabis sativa*, *Datura stramonium*, *Ipomoea purpurea*, *Salvia divinorum*) and teratogenic (*Datura stramonium*, *Lupinusformosus*, *Nicotiana glauca*, *Coniummaculatum*) plants, raw materials for the production of oral contraceptives (*Dioscorea alata*, *D. villosa*), aphrodisiacs (*Epimedium*, *Glycoirrhiza glabra*, *Smilax ornate*, *Turnaria aphrodisiaca*, ginger, ginseng, *Ginkgo biloba*), allergens, enzymes, vitamins, antibiotics, herbicides, and insecticides yielding plants remain within the arena.

Lange (2004) gave an elaborate enumeration on the herbal wealth of different countries and continent of the world. UNESCO (1996) has observed that the use of traditional medicine and medicinal plants in most developing countries is a normative basis for the maintenance of good health. The World Health Organization has estimated conservatively that between 60 and 90% of the population of the non-industrialized/developing countries rely (fully or partially) on medicinal plants to cure variety of aliments. Different estimates announced number of medicinal plant species that are used for medicinal purposes worldwide. Out of the total 250,000–500,000 plant species on earth (Borris 1996), more than 80,000 are known as medicinal plants and about 35,000–70,000 species are used medicinally across the world. According to an estimation by Farnsworth and Soejarto (1991) that up to 70,000 species are used in folk medicine while an enumeration of WHO from the late 1970s listed 21,000 medicinal species (Penso 1980) and now it is assumed that, out of the global total 422,000 flowering plant species, the number of plant species used for medicinal purposes across the world is >50,000 (Govaert 2001; Bramwell 2002; Schippmann 2002). However, only 1–10% of them have been studied chemically and pharmacologically for their potential medicinal value (Verpoorte 2000). The World Health Organization reported the use of over 21,000 plant taxa for medicinal purposes around the world (Penso 1980, Groombridge 1992). But how many plant species are used worldwide in cosmetics, spirits, or aromas are yet not known exactly. However, only 1–10% of them have been studied chemically and pharmacologically for their potential medicinal value (Verpoorte 2000). From a calculation based upon the estimated total number of about 35,000–70,000 species are used medicinally across the world, it is apprehended that at least every fourth plant is in use. The use of the number of medicinal and aromatic plant species in some regions of the world is quite impressive.

Bangladesh is rich in herbal resources because of its geographical location, fertile soil, and favorable subtropical monsoon climate. Bangladesh shows richness in greeneries with significant species diversity. Geographically, Bangladesh falls

near the Indo-Burma region which is one of the ten global hot-spot areas that supposed to have 7000 endemic plant species (Mittermeier et al. 1998). Bangladesh has been the abode of more than 6000 higher plant species, of which about 300 are exotic and 8 are endemic. Of the total number of plant species, there are 5000 angiosperms, 4 gymnosperms, 250 Pteridophytes including 230 ferns, 250 Bryophytes. About 300 species and varieties of algae have been recorded from freshwater habitats alone and many more grow in brackish and sea water habitats (Banglapedia 2003). A total of 334 species of higher plant species (Spermatophytes and Pteridophytes) were identified from the Sundarbans forest and along the 710-km-long coastline of the Bay of Bengal. About 168 species of seaweeds from the Bay are also known. In Bangladesh, more than 5000 higher plant species grow (Mia 1990), and about 1000 plant species (20%) are considered to have medicinal properties. At least 177 species and a variety of orchids under 70 genera of the family Orchidaceae grow in Bangladesh (Huda 2007). Most of the identified 5000 angiosperms in Bangladesh grow wild in nature, and only 210 species are cultivated for flowers, food, fodder, fiber, beverage, medicine, and timber. About 455–747 plant species of Bangladesh have been described with their specific medicinal properties (Ghani 2003; Yusuf et al. 2009). Many of these medicinal plants grow in the wild but some of them are garden plants and some them are cultivated crop species. Most of this floral wealth of Bangladesh is used in the indigenous systems of medicine, particularly Ayurveda, Unani, and Homeopathy medicine as well as folk medicine.

In India, more than 45,000 different plant species grow and out of them, about 15,000–20,000 plants (3.3–4.4%) have good medicinal value. However, only 7000–7500 species are used by traditional communities for medicinal purposes (Joy et al. 1998). Earlier, Shankar and Majumdar (1997) reported that out of the 17,000 Indian native plant species, about 7500 species are used in ethnomedicines, and Grover et al. (2002) reported that many traditional medicines used in India are derived from medicinal plants, minerals, and organic matter. In India, the largest producer of medicinal herbs (so the botanical garden of the world), grows about 2500 species out of the world 21,000 enlisted medicinal plants of the World Health Organization and out of which 150 species are used commercially on a fairly large scale (Seth and Sharma 2004). Modak et al. (2007) prepared a list of Indian medicinal plants with proven antidiabetic and related beneficial effects including *Allium sativum*, *Eugenia jambolana*, *Momordica charantia*, *Ocimum sanctum*, *Phyllanthus amarus*, *Pterocarpus marsupium*, *T. cordifolia*, *Trigonella foenum graecum,* and *W. somnifera*. In Nepal, 6653 species of Angiospermic plants were documented among which 1792–2331 were recorded as potential medicinal and aromatic plants (Rokaya et al. 2010). The two ethnic groups Magar and Majhi of Nepal use ethnobotanically 132 plant species of 67 families and 99 genera against 12 human ailments. The herbs were the primary sources of medicine 87 (66%), followed by shrubs 26 (20%), trees 11 (8%), and climbers 8 (6%) in Parbat district of Nepal (Mallaa et al. 2015). Widely used medicinal plant species of Nepal include *Paris polyphylla*, *Bergenia ciliate*, *Swertia chirayita*, *Potentilla polyphylla*, *Zanthoxylum acanthopodium*, *Centella asiatica*, *Camellia kissi*, *Benincasa hispida*,

Valeriana hardwickii, *Cuscuta reflexa*, *B. aristata*, *Bryophyllum pinnatum*, *Tinospora sinensis*, *Dendrobium moschatum*, *Nephrolepis auriculata*, *Vitex negundo*, *Wikstroemia canescens*, *Acampe papillosa*, *Indigofera bracteata*, *T. wallichiana*, *Zizyphus mauritiana*, *Spiranthes sinensis*, *Sambucus adnata*, *Chlorophytum nepalense*, *Neolitsea pallens*, *Coelogyne corymbosa*, etc. (Mallaa et al. 2015).

The number of medicinal plant species used in China is about 6000 (Xiao 1991) but may be over ten thousand according to another source of estimation (He and Ning 1997). In China, 4941 of 26,092 native species (18.9%) are used as drugs in traditional medicine (Duke and Ayensu 1985). It is thought that approximately 1000 plant species are commonly used in Chinese medicine, and about half of these are considered as the main medicinal plants (He and Ning 1997). The traditional medicine of eastern Asia, the traditional Chinese medicine (TCM), relies in most cases on indigenous plant species. Following TCMT, the Japanese established Kampo medicine prepared following Kampo formulae. Under the Japanese law, products with nutritive function (e.g., vitamins, minerals) and without therapeutic activities are traditionally considered as foods (e.g., soybean, tea, psyllium, wheat, guava, coffee, Eucommunia bark, seaweed, sesame seed, broccoli, cabbage). Crude drugs must meet at least 13 Japanese pharmacopoeia (JP) criteria (e.g., name, origin, medicinal part, preparation process, content of specific constituents, description, identification, purity including heavy metals, arsenic, residual pesticides, loss of drying, total ash, acid-insoluble ash, extract content, assay) to qualify as medicine. In 2001, 121 crude drug species and their 52 powdered forms were listed in JP 14th edition. However, revisions and new advances from the non-JP crude drug standards (internal regulations for approval) after 2006 led to the addition of Supplement I in JP 15 in 2007 which expanded the list to 153 crude drugs and their 54 powdered forms. Medicinal plants in the Republic of Korea present concise monographs of 150 medicinal plant species that are most commonly used for medicinal purposes in traditional Korean medicine of the Republic of Korea.

The Japan Kampo medicines manufacturer association (JKMA) most frequently uses 150 crude drugs for medicine and food (Anonymous 2006). The most commonly used drugs are Ginger, Coix seed, Capsicum, turmeric, Glycyrrhiza, cinnamon bark, Cassia seed, safflower, Angelica, Astragalus, Phellodendron, Coptis, Rhubarb, Aconite, bamboo grass, Zedoary, Cnidium rhizome, Artemisia, Zanthoxylum fruit, etc. Japan, however, imports a bulk quantity of medicinal and non-medicinal crude drugs in 2002 (import—56,221t, domestic cultivation 1723t) from China (58%), Thailand (19%), India (13%), Sudan (2%), and Taiwan (1%) (Anonymous 2007). Wiart (2012) in his book 'Medicinal Plants of China, Korea, and Japan: Bioresources for Tomorrow's Drugs and Cosmetics' provides an elaborate conceptual tools and understanding of intercorrelated basics of botany, ethnopharmacology, biomolecular pharmacology, phytochemistry, and medicinal chemistry to guide researchers in appreciating, estimating, and forecasting the pharmacological or cosmetological value of Asian medicinal plants with emphasis on numerous patentable pharmaceutical and cosmetological leads. Detailing 200

medicinal plant species carefully selected for their potential importance in pharmacological and cosmetological importance, Wiart opined that the Asian medicinal plants have great promise in pharmaceutical and cosmetological development.

Medicinal herbs are used in Philippines as an economical alternative medicine to treat many ailments (e.g., from boils to body odor) which is beneficial to many Filipinos, especially in the economic crisis. Common practices of herbal healing in Philippines are boiling of fruits and flowers, extraction of juices from leaves, and poultices from barks and roots. For this, at least 75 medicinal plants including cultivated and wild plant species of Philippines are in use, e.g., *Abelmoschus esculentus*, *Allamandra cathartica*, *Allium sativum*, *ALoe barbadensis*, *Amaranthus spinosus*, *Ananas comosus*, *Andropogon citratus*, *Anona reticulate*, *Anona squamosal*, *Areca catechu*, *Artocarpus heterophylla*, *Auerrhoa carambola*, *Basella rubra*, *Biva orillan*, *Blumea balsamifera*, *Brassica oleracea*, *Cassia alata*, *Cassia fistula*, *Carica papaya*, *Centella asiatica*, *Chrysanthemum indicum*, *Chrysophyllum cainito*, *Coeus blumei*, *Croton tiglium*, *Curucuma domestica*, *D. metel*, *Daucus carota*, *Jatropha curcas*, *Nerium Indicum Mil*; *Premna Odorata*, *Portulaca olearacea L.*; *Tagetes erecta Linn*; *Momordica charantia*, *O. basilicum*, *Ficus stipulosa Miq. Linn.*; *Lagerstroemia speciosa*, *Psidium guajava*, *Piper beetle.*; *Theobroma cacao.*; *Symphytum officinale*; *Syszygium jambolanum*; *Kaempferia galanga*; *Entada phaseikaudes*; *Hibiscus rosa-sinensis*; *Mentha cordifolia*; *Leucaena glauca*; *Gliridia sepium*; *Plumeria acuminata*; *Impatens balsamina*; *Pithecolobium dulce.*; *Hedychium coronarium*; *Manihot esculenta*; *Ipomea aquatica*; *Lantana camara*, *Kalanchoe pinnata*; *Imperata cylindrica.*; *Vitex negundo*, *Raphanus sativus*, *Tinospora rumphii*, *Mimosa pudica*, *M. oleifera*, *Mangifera indica*, *Quisqualis indica*, *Coleus aromaticus*, *Tabernaemontana pandacaqui*, *Pandanus odoratissimus*, *Luffa acutangula*, *Ros marinus officinalis*, *Tamarindus Indica*, *Pachyrrhizus erosus*, *N. tabacum*, *Solanum melongena*, *Polymnia sanchifolia*; *Zea mays*, *Z. officinale*, *Below are links to certain kinds of illness with corresponding herbal treatments.* One or more these herbs in combination are used to prepare home remedy to treat many diseases such as boils, chronic cystitis, asthma, bee sting, bleeding from wound,, burns, chicken pox, constipation, cough, dandruff, diarrhea, eczema, fainting, fever, flatulence, fractures, sinusitis, hemorrhoids, herpes, hyper-acidity, indigestion, head lice, skin rashes, mosquito bites, mumps, acne, arthritis, scabies, ringworm, dermatitis, dry itchy skin, snake bites, throat sore, ankle sprain/wrist sprain, sunburn/prickly heat, stomatitis/gum sore, toothache, body odor, worm infestation.

Traditional healers in many African countries rely on local or at most regional plant material (Marshall 1998). Over 5000 plant species are known to be used for medicinal purposes in Africa (Iwu 1993), while about 2000 medicinal and aromatic plant species are used on a commercial basis in Europe as it has a long tradition in the use of botanicals (Lange 1998). The use of medicinal and aromatic plant species in Germany would be not less than 1500 (Lange 1996) and that of Spain would be 800 medicinal of which 450 species are associated with commercial use (Lange 1998). In Bulgaria, about 750 native plant species, 20% of the total flora, are used in folk medicine. Of these, 200–300 species are most commonly used (Hardalova 1997).

In Albania, 205 native plant species are used as sources of botanicals (Lange 1998). In Hungary, some 270 native medicinal and aromatic plant taxa are used, 180–200 of which are officially recognized by the Hungarian pharmacopoeia as cited by Lange (2004). Lange (1998) from the French pharmacopoeia and lists of medicines noted some 900 taxa, of which almost half are native to Europe.

Herbs used in a country can be either indigenous or native to other regions or even continents. The share of both plant groups depends on the country's cultural preferences, importance of traditional medicines, history, trade relations, and of course of the wealth or property of a country. Traditional medicines are playing an important role in many parts of the world. In south and Southeast Asia, the Ayurveda, Unani, and Siddha medicines are widely distributed and based on not less than 400, 500, respective 1800 native Indian plant species (Shankar and Majumdar 1997). The TCM, the traditional medicine of eastern Asia, relies in most cases on indigenous plant species. Traditional healers in many African countries rely on local or at most regional plant material (Marshall 1998). In Bulgaria, about 750 native plant species, or 20% of the total flora, are used in folk medicine. Of these, 200–300 species are most commonly used (Hardalova 1997). Further, in Albania, 205 native plant species are used as sources of botanicals (Lange 1998). In Hungary, some 270 native medicinal and aromatic plant taxa are used, 180–200 of which are officially recognized by the Hungarian pharmacopoeia (Lange and Schippmann 1999). In Turkey, about 337 native taxa have been commercially traded since at least 1990 (Lange 2001). From the French pharmacopoeia and lists of medicines (Lange 1998) noted some 900 taxa, of which almost half are native to Europe. This means, that many countries rely on a major part on their own plant diversity. Many of them cannot afford to import foreign botanicals, finished herbal products, or even phytopharmaceuticals, and the country's own 'biodiversity' is mainly offered in a crude form or at most as little processed products on the market. On the other side, there are the developed countries which use besides indigenous plant species a lot of non-native species and process them in their well-developed pharmaceutical, cosmetic, and extract-producing industry. Accordingly, the plant material is offered to the consumers as mainly packed and finished products, and the crude material plays a minor role in the retail trade. This features apply above all to the highly industrialized countries of temperate Asia (e.g., Japan and the Republic of Korea), of the Americas and of Europe. The geographical origin of the botanicals used in Germany may illustrate the kind of use of non-native and native species (Lange 1996). In general, the medicinal and aromatic plants are coming from all geographical regions of the world, and many of these species show a wide geographical range. The high figure of 849 species occurring in temperate Asia are increasingly used in Germany, and many of the 454 species grow in North America are used in homeopathy. Species with their distribution area restricted to Africa, South America, Australia, New Zealand, or the Pacific play a minor role in Germany's industry. A high number of not less than 605 species are native to Europe. The majority of them are distributed across several geographical units, e.g., Eurasia or even the northern hemisphere; only 16 species are limited to Europe. Many of the 605 species are growing in Mediterranean countries like Italy, France,

Spain, and Greece, and more than two-thirds of them occur in east and southeast Europe, in Romania (451 species), Bulgaria (421 species), Hungary (415 species), Albania (391 species), and in Poland (386 species). In general, both regions are rich suppliers for botanicals within Europe (Lange 2001).

Threats and Conservation Aspects

Threats facing medicinal and aromatic plants and their wild populations are (1) the intensive and increasing commercial collection, often concentrated in few areas, (2) the largely unmonitored trade, (3) destructive harvesting techniques, (4) trade structure changes in countries of the former Eastern Bloc, and (4) global habitat loss and alteration. During the 1990s, the demand in raw material increased due to the increasing demand in plant-based remedies and products—a result of the increasingly global nature of the trade, the worldwide population growth and the increasing popularity of herbs and herbal products in industrial nations and their effective marketing. This has resulted in some countries, above all in the USA and Japan, in explosive demand and consumption of medicinal plants (Laird 1999); in particular, the demand in St. John's-wort increased by several hundred percent in the USA in the middle of the 1990s. Additionally, more and more people in industrialized countries are increasingly interested in foreign traditional medicines like Ayurveda and TCM. In all, the imports of pharmaceutical plants increased from about 270,000t in 1991 to almost 400,000t in 2000. In general, on regional and country levels, the imports increased until 1996–1998, sometimes considerably as in the case of the temperate Asian countries. Between 1996 and 1998, the market in pharmaceutical plants broke down in particular in the USA and in temperate Asia, but has already started to recover. In many of the major supply countries of botanicals, the exports have increased significantly in the 1990s. In China, the exports of pharmaceutical plants doubled almost from 107,500t in 1991 to 186,450t in 2000, and the domestic demand for botanicals has grown at an annual rate of 9% over the past decades (He and Ning 1997). Also, Europe has doubled its export during this period, and Mexico has shown very high export increases since the mid-1990s.

9.5.1 Conservation Concepts and Management of Botanical Resources

In the case of medicinal and aromatic plants, conservation concepts and management measures have to meet both (1) future supply and (2) the provisions of species conservation. Measures to be taken on local, regional, national, or international ranging from resource management, cultivation, adequate species conservation programmes, and shifting processing from consumer to source countries, to trade restrictions or even trade bans. In the following, some selected conservation aspects

and resource management issues will be briefly discussed. According to IUCN, WHO, and WWF (1993), the cultivation of medicinal and 187 aromatic plants is the best and promising way to satisfy the market's expanding demand for these raw materials. But, up to now, cultivation has not proved to be profitable for the majority of taxa in trade (Lange 1998): (1) Many plants are difficult to cultivate, (2) to take a plant into cultivation, if possible, will often last many years, (3) many plants are only required in small quantities, (4) in some cases the quality of wild-harvested material is supposed to be superior, and (5) the costs for wild-crafted plant material are in general lower than for cultivated material (Lange 1997).

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