Chapter 11
Harbouring the Potential of Medicinal and Aromatic Plants of India: Novel Biotechnological Approach and Extraction Technologies

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Abstract The chemical diversity of medicinal and aromatic plants is used in multiple ways. The biochemicals and metabolites produced by plants are used in medicines, as flavouring agents, as agricultural chemicals and in cosmetic industry. Mostly plants harvested from their wild habitats are utilised. Recently, the usage and demand has doubled causing the over-exploitation of natural habitats. The extent of wild-crafting is also influenced by factors like cultivation, cost of production and the utilization rate of resources. In such cases, various biotechnological tools come handy in maintaining the potential of the so valuable medicinal and aromatic plants. This chapter deals, in brief, with the recent biotechnological approaches used and elucidates their core concepts and advantages. These techniques can be used either as a sole component or in combination with the other techniques to derive the maximum beneficial potential of the medicinal plants.

Keywords Medicinal plants · Aromatic plants · Biotechnological tools · Extraction techniques · Approaches

Abbreviations

AFLP	Amplified Fragment Length Polymorphism
ATPS	Aqueous Two-Phase System
CCC	Counter Current Chromatography
cDNA	Complementary Deoxyribonucleic acid
CPC	Centrifugal Partition Chromatography
CT	Critical Temperature

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DAF	DNA Amplification Fingerprinting
DES	Deep Eutectic Solvents
DNA	Deoxyribonucleic acid
GHz	Giga Hertz
HPLC-MS	High Performance Liquid Chromatography – Mass Spectrometry
IUCN	International Union for Conservation of Nature
MAE	Microwave Assisted Extraction
MAPs	Medicinal and Aromatic Plants
MHz	Mega Hertz
NADES	Natural Deep Eutectic Solvents
NGS	Next Generation Sequencing
PC	Critical Pressure
RFLP	Restriction Fragment Length Polymorphism
SFE	Supercritical Fluid Extraction
SNPs	Single Nucleotide Polymorphisms
SSR	Simple Sequence Repeats
UAE	Ultrasound Assisted Extraction
WHO	World Health Organization

11.1 Introduction

A medicinal plant by definition is any plant which, in one or more of its parts/organs, contain substances that can be used for therapeutic purposes or which is a precursor for the synthesis of useful drugs (Sofowora 2008; Evans 2008; Sofowora et al. 2013). One can differentiate between plants that are already established as medicinal plants with the requisite properties and those which need farther studies, although are already considered as medicinal plants (Sofowora et al. 2013), e.g. bark of Cascara; plants used for extraction of pure substances and metabolites either for immediate medicinal use or for the indirect production of medicinal compounds through intermediate metabolites; food, spice, and perfumery plants used medicinally, e.g. ginger.

Medicinal plants have been a resource for healing in local communities around the world since centuries. Still it remains of contemporary importance as a primary healthcare mode for approximately 85% of the world's population (Pešić 2015), and as a resource for drug discovery, with 80% of all synthetic drugs deriving from them (Bauer and Brönstrup 2014). Aromatic plants (also known as herbs and spices) come in Medicinal plants category (collectively they are known as Medicinal and Aromatic Plants) are the prime source of many therapeutics which are responsible for preservation of health and also used in multiple ways in many facets of our life (e.g., cosmetics, medicinal products, food and feed additives).

11.1.1 Medicinal and Aromatic Plants of India

In India, as much as 7500 species are utilised in ethnomedicines (Badola and Aitken 2003) which is half of country's Indian native plant species. Approximately 6000 species of plants that have medicinal properties are employed in China (Khan 2014) and over 5000 plant species are used for medicinal purpose in Africa (Iwu 1993).

Out of all the 250,000 higher plant species on earth, more than 80,000 species are reported to have at least some medicinal value and around 5000 species have specific therapeutic value (Marinelli 2004; IUCN 2007). Besides the usual botanical classification, they are also classified based on the plant organ used, their habit and habitat, therapeutic value etc. (Joy et al. 2001). There is a huge number of species to be reported out of which, based on therapeutic value, *Cinchona* sp., *Azadirachta* sp., *Acacia* sp., *Digitalis* sp., *Phyllanthus* sp., *Terminalia* sp., *Silybum* sp. etc., are few of the many notable medicinal plants used in day-to-day life by the world population.

The list of all the medicinal and aromatic plants are huge and the mere mentioning of the same is beyond the scope of this chapter.

11.1.2 Processing of Medicinal and Aromatic Plants: Approaches

The medicinal principles (biologically active substances) of plants are present in the different organs of the plant like root, stem, bark, heartwood, leaf, flower, fruit or plant exudates. These medicinal principles can be isolated by several processes, out of which extraction is the most frequently used. It implies the isolation of the required constituents from plant materials by using a solvent (Paroda 1993).

In many developing countries, there has been an increased awareness to promote and develop the production and use of medicinal and aromatic plants. Due to the general resurgence of interest towards plant based products, both researchers and producers are focussing on strategies to harness low cost and purpose oriented technologies which go hand in hand with the green consumerism, liberalised and free market economy, ultimately aiming at biodiversity conservation and sustainable use of natural resources (Joy et al. 2001).

For the majority of the world's population, Medicinal and Aromatic Plants (MAPs) tend to be an everlasting source of life saving drugs in primary healthcare. The issues put forward by growing human population, in conjunction with depletion in renewable resources, indirectly causes an increase in the global demand for medicinal plants. Under these circumstances, the ever-increasing demand for therapeutic molecules, triggered by the "green processes", and the depleting quantity of wastes have also come to the forefront of concepts for the viable production of phyto-pharmaceuticals from plants and plants waste. Industrial biotechnology seems to be a promising tool serving this purpose. In addition to this, the isolation and optimization of high value phytoconstituents through development of

alternative techniques have additional social and economic importance (WHO 2019; Fierascu et al. 2020).

11.2 Biotechnological Approaches

The following chapter summarizes the notable major biotechnological tools employed in plant industry to produce derive the secondary metabolites for both small scale and industrial purposes. Some of the conventional approaches, like extraction and chromatographic techniques are excluded. Focus is placed on the prime techniques, which are much more promising and "greener" in the near future. The various tools used in India are described in a brief manner without getting much deeper into the aspects of methods involved.

11.2.1 Micropropagation

Micropropagation also known as plant tissue culture is defined as aseptic asexual plant propagation on a defined culture medium, in culture vessels, under controlled conditions of light and temperature. Here, the isolated plant cells, tissues, organs or whole plants are grown on semi-solid or in liquid synthetic nutrient media, under aseptic conditions. The term micropropagation refers to the miniature size of shoots/plantlets initially produced in culture vessels (*in vitro*). Micropropagation allows to capture maximum genetic gains from the genetic variability of natural populations. Micropropagation of economically important plants can be feasible when relevant selection methods are adopted (Kane et al. 2008; Ganguli 2009; Kumar and Loh 2012; Máthé et al. 2015).

The conventional propagation methods are frequently time consuming, less efficient and occasionally even unsuccessful. Aseptic *in vitro* propagation techniques allow to achieve the multiplication of disease-free plants, on a large scale, in short period of time and throughout the year (Rout et al. 2000a, b; Rath and Puhan 2009; Lakshimi and Reddy 2009; Yaadwinder 2010). Plant regeneration from shoot meristems has yielded encouraging results in numerous medicinal plants: selected examples are: *Aegle marmelos* (Arumugam et al. 2003); *Aloe vera* syn barbadensis Mill. (Baksha et al. 2005; Ujjwala 2007), *Astragalus cicer* (Basalma et al. 2008), *Centella asiatica* L. (Tiwari et al. 2000), *Rhodiola rosea* (Tasheva and Kosturkova 2012) etc. Direct micropropagtion without callus phase has been described in medicinal plants like *Leucojum aestivum*, *Eryngium foetidum* and *Lilium rhodopaeum Catharanthus roseus*, *Cinchona ledgeriana* Digitalis spp., *Rauvolfia serpentina* and *Isoplexis canariensis* and *Eryngium foetidum* (Arockiasamy et al. 2002). Mass multiplication by tissue culture mode was targeted and achieved in several threatened and endemic

medicinal plants; Mass clonal multiplication has been successfully achieved in several Himalayan medicinal plants including *Potentilla fulgens* using axillary buds (Thangavel et al. 2014; Sambyal et al. 2006). Another *in vitro* technique using somatic embryogenesis can rapidly produce uniform plants. It is a type of vegetative propagation based on plant cell totipotency. It has established itself as a powerful substitute to other vegetative propagation methods (Thangavel et al. 2014).

To date, micropropagation appears to be the most efficient and practical plant propagation technology used commercially. The success of *in vitro* culturing of plants depends on various factors viz., selection of the starting material, composition of the nutrient media, incorporation of the specific growth regulators and environmental factors. Direct organogenesis is generally considered the safer route for micropropagation of clonal, true-to-type plants (Sandhu et al. 2018); this involves synergistic interactions between physical and chemical factors (Chand et al. 1997) and is started within the shoot meristem of the explant (Altman and Loberant 1998). The advances made in the field of plant cell culture techniques could pave way even for manufacture of rare plants and their cells which would be capable of producing essential chemicals (Lemma et al. 2020).

11.2.2 Somaclonal Variation

Secondary metabolite production is often observed during the *in vitro* culturing of medicinal plants, especially in cell suspension and callus cultures compared to untreated plants (Ngezahayo 2018). An important feature of *in vitro* cultures is the occurrence of somaclonal variation as a result of gene mutation or epigenetic alterations (Larkin and Scowcroft 1981; Gould 1986; Kaeppler et al. 2000).

Sources of variations detected in plant tissue culture include: the type of explant, its source, process of regeneration, extent of culture period, the number of subculture cycles, environment maintained for the culture, genotype, and ploidy, etc. Highly differentiated tissues (roots, leaves, and stems) generally produce more somaclonal variations than axillary buds and shoot tips (reviewed in Krishna et al. 2016).

It has been observed that callus, shoot tip cultures, and somatic embryogenesis are accompanied by microRNA gene expression in which microRNAs accomplish different roles such as target gene regulation, stress response, revitalization in micropropagated plants, formation of embryogenic callus and somatic embryogenesis, embryogenesis and postembryonic development, downregulation of target genes, response to light photoperiod (Qiao and Xiang 2013; Chávez-Hernández et al. 2015; Szyrajew et al. 2017; Ngezahayo 2018). MicroRNAs expression was observed in the callus culture of Taxus trees (Zhang et al. 2015). Other epigenetic variations, like histone modifications, are also bound to occur.

11.2.3 Synseed Technology

The process involves encapsulation of plant material by using explants, such as shoot tips, nodal segments, hairy roots, calli, protocorm like bodies along with encapsulating agent and matrix for the sustainability of the synthetic seed (Gantait et al. 2015). It is absolutely necessary that the artificial seed coat should be able to shield the explants, possess the efficiency to include nutrients as well as other growth and biological factors, protect the developed artificial seed through the entire process of storage and handling. It should be capable of elucidating mode of action for activating 'germination', should be edible, maintain a good affinity with the biological and chemical systems; and biodegradable (Khor and Loh 2005). The types of explants, the concentration of encapsulating agents used as well as matrix have prominent roles in the production of synthetic seeds in medicinal plants. The above three factors for the most part govern the success of synthetic seed production (Gantait et al. 2015).

In the recent past, increasing importance has been shown towards the usage of synthetic seeds produced via encapsulation technology. This is considered to be an exemplary route for safer conservation and exchange of the species. Synseed technology has shown promising results with unsteady genotypes, and in the preservation, as well as large scale micropropagation of hybrids of rare varieties. Similarly, the method has been described to yield positive results with genetically modified plants that require mycorrhizal-fungal association or do not produce viable seeds (Chaudhury and Malik 2003; Ara et al. 2000; Gantait et al. 2015).

In this method, seeds are, generally, produced as the outcome of a sexual procedure: as a result, in cross-pollinating species, the naturally produced seeds are genetically different from the individual parents (Senaratna 1992). Conservation of seeds can be useful in several tropical and subtropical plants with pharmaceutical values that are difficult to propagate owing to their its discreteness. A large number of medicinal plant species bear desiccation-sensitive or recalcitrant seeds that limit confine the storage duration only up to few weeks or months (Gantait et al. 2015).

11.2.4 Protoplast Culture

Protoplasts are living cells without their cell walls (Riazunnisa et al. 2007; Sinha and Caligari 2009; Yang et al. 2009). Usage of protoplasts for the manufacture of useful metabolites is that the metabolites are liberated readily into the culture medium. This has double benefits: they increase overall productivity and facilitate downstream processing in cases where the cell wall limits the secretion of useful products. Protoplast cultures represent a sustainable and relatively clean source of enzymes and useful secondary metabolites (Aoyagi 2011). However, the main constraint is that they cannot be used for long term production, as they are very fragile.

This can be overcome by usage of immobilization matrix and Alginate, as a common elicitor (Aoyagi 2011).

11.2.5 Development of Novel Transgenics

Transgenic crops are better defined as the genetically engineered crops. Those characters and traits which are not possible of introduction by conventional / usual streamlined approaches can be tailored via transgenics. It involves the introduction of agronomic, pathological, entomological, nutritional, therapeutic-, and vaccine-related characters/traits in plants (Khan and Malik 2018). There are multiple ways and means to transplant and introduce genes into the plant genome: these are based on the choice of explant to be used in transformation experiments, for example through *Agrobacterium*-mediated gene transfer (which tends to be the commonly used method), through gene gun, agro-infiltration method, sonication and treatment by polyethylene glycol. Of these, the *Agrobacterium* mediated and the so called "gene gun" methods are the most commonly used approaches (Saito et al. 1992). Nevertheless, this method involves tailoring of plants for desired traits, and also plays a role in the production of secondary metabolites, the transfer and expression of artificially manipulated foreign genes (Marchev et al. 2020).

11.2.6 Molecular Markers and Maps

Molecular markers provide information on diversity at the nucleotide level (SNPs) to gene and allele frequencies (genotype information), extent and distribution of genetic diversity, and population structure (Sarwat et al. 2012).

They can be used for germplasm cataloguing and are essential in formulating both *in-situ* and *ex-situ* germplasm conservation programs. They also aid in solving taxonomic problems and help in assigning plants to their correct taxonomic hierarchies which are critical in phylogenetic studies. Such information can be ultimately utilized for devising a proper conservation strategy, management of gene-bank, and germplasm collections (Sarwat et al. 2012).

11.2.6.1 Crop Profiling

Crop profiles are descriptions of crop production and pest management recommendations compiled by the state and commodity. They are considered as living documents. They provide agricultural statistics for the crop; here in this case, the entire information about the medicinal and aromatic plants on regions within the state; an inventory of pests and strategies used for management (e.g., cultural practices,

biological control, and pesticides); and lists of key contacts, references, and online resources are made available.

11.2.6.2 Genetic Fingerprinting

The concept of fingerprinting has been increasingly applied in the past few decades to determine the ancestry of plants. Genotypic characterization of plant species and strains is useful as most plants, though belonging to the same genus and species, may show considerable variation between strains (Henry 2001; Breithaupt 2003; Vasudevan 2009).

In the case of medicinal plants, their content of active principles may vary from plant to plant (Vasudevan 2009). This has been a problem in the production of standardized medicines. Climatic factors and adaptability dictate the viability of a particular species and subsequently the content of active principle. In such cases, variations can be observed in the genetic composition of the plants, in addition to varying amounts of the active principle (Henry 2001; Breithaupt 2003; Vasudevan 2009).

A recent offshoot of this method is the use of **biomarkers**. When using these, the chemical marker compound possesses an intrinsic biological activity. For this purpose, DNA fingerprinting is successfully employed for profiling. The various techniques used are microsatellites (Simple Sequence Repeats – SSR), Restriction Fragment Length Polymorphisms (RFLP), Amplified Fragment Length Polymorphism (AFLP) and Random Amplified Polymorphic DNA (RAPD). Further techniques used are Single Nucleotide Polymorphisms (SNPs), DNA Amplification Fingerprinting (DAF) (Henry 2001; Vasudevan 2009).

11.2.6.3 Identification of Adulterations

Adulteration involves the deterioration, admixture, sophistication, substitution, inferiority and spoilage of the actual nutraceuticals (crude drug) obtained from the medicinal plants with variety of substances like heavy metals, organic pollutants, mycotoxins, endotoxins etc. (Al Lawati et al. 2017).

Preliminary identification of adulterants by exomorphic features like shape, size, colour, texture and odour of leaves, flowers, and fruits, leaf type, leaf margin, leaf tips, flowers and their characteristics, inflorescence etc. through the naked eye, should be done. This needs to be compared with the reference material in the form of herbarium or voucher specimens. Microscopic identification is using distinguished histological, cell morphological characters, cell type and cell contents. Advanced microscopic techniques, as the application of phase contrast microscopes, fluorescence and confocal microscopes, scanning electron microscope have greatly increased the accuracy and precision of identification (Sarvananda et al. 2019).

Identification can be implemented also through the study of organoleptic characters, the assessment of colour, odour, taste etc. The microscopic characters are

identified using research microscope. Analytical techniques like infrared, nuclear magnetic resonance spectroscopy, High Performance Liquid Chromatography coupled with mass spectrometry (HPLC–MS), tandem mass spectrometry is used and comparative studies are performed (Al Lawati et al. 2017; Sarvananda et al. 2019).

11.2.6.4 Marker Assisted Breeding

The problems faced in the conventional breeding of medicinal and aromatic plants have changed with the advancement of DNA based molecular markers and molecular breeding strategies. The uniqueness of the molecular markers' correlates to the plant's genotype. Molecular markers are unique and relate directly to the plant's genotype (Bhau 2012).

Except for *Artemisia annua*, only few reports exist for the improvement of medicinal plants through molecular marker based approaches (Graham et al. 2010).

Plant genome sequencing has progressed rapidly since the first genome (*Arabidopsis thaliana*) was completed in 2000 (Fraser 2000) followed by completion of 389- Mb rice genome in 2004 (Takuji 2005). DNA probes from one species can often be used to identify homologous sequences in another closely related species. There is a high degree of similarity in the DNA sequences of functional genes among the different plant species (Bhau 2012).

The aim of developing new breeding strategies lies with the usage of molecular markers wherein the objectives will be based on increasing the germplasm base increasing the number of traits which could be selected simultaneously. The above developments rely upon the technologies that offer cost effective screening of markers and high multiplexing capabilities (Bhau 2012).

11.2.7 Transcriptome Sequencing (Identification, Isolation and Cloning of Useful Genes)

Study of the entire pool of transcripts in an organism (or single cells), specifically the transcriptome, at certain physiological or pathological stage, is highly essential in unravelling the connection and regulation between DNA and protein. Our understanding of genomics in a faster, cost-effective, and tractable manner has been reformed by Next Generation Sequencing technology (NGS). Adopting NGS could lead to enhancement of elucidating genes responsible for the production of active compounds from the medicinal plants. This entire process involves stages right from the sampling of the plant material, preparation of cDNA library, deep sequencing and subsequently the processes involving bioinformatics to extract information (Han et al. 2016).

11.3 Novel Extraction Techniques of Medicinal and Aromatic Plants

The processing of bioactive compounds from medicinal and aromatic plants relies upon the important steps of pre-extraction and extraction procedures. Traditional methods such as maceration and Soxhlet extraction are commonly used at small scale. However, significant advances have been made in the processing of medicinal plants with the help of modern extraction methods like Microwave-Assisted Extraction (MAE), Ultrasound-Assisted Extraction (UAE) and Supercritical Fluid Extraction (SFE), wherein these advances are aimed to increase yield at lower cost. Moreover, the methods can be continuously improved and developed. With such wide choice of available methods, the selection of proper extraction method needs meticulous evaluation (Swami et al. 2008).

The pre-extraction method involves the preparation of plant samples to preserve the biomolecules prior to extraction. This requires proper and timely collection of the plant, authentication by an expert, adequate drying and grinding. Also, this comprises the determination of quantity and quality of bioactive compounds. Plants samples such as leaves, barks, roots, fruits and flowers can be extracted from fresh or dried plants material. Other methods like grinding and drying influences the preservation of phytochemicals in the final extracts (Swami et al. 2008; Azwanida 2015).

The purposes of extraction procedures for crude drugs are to obtain the therapeutically desired portion of active principles. In addition, they are expected to eliminate inert materials by selective solvent (*menstruum*) treatment. It involves the separation of medicinally active principles of the plant using selective solvents wherein it is intended to soften and break the plant's cell wall to release the phytochemicals. Commonly used extraction methods include maceration, infusion, percolation, decoction. The extract obtained could be ready for use as a medicinal agent, in the form of tinctures and fluid extracts, or further processed to be incorporated in any of the dosage forms: e.g., tablets, capsules. It can be fractionated to isolate individual chemical entities. The volume of solvents used in these processes play a critical role (Azwanida 2015). The following processes offer a brief introduction to some of the important extraction methods in use and their advantages.

11.3.1 Liquid Liquid Extraction

The Aqueous Two-Phase System (ATPS) one step liquid-liquid extraction is the most classical approach to the recovery of biomolecules. ATPS offers a faster separation of phases, low interfacial tension mass transfer and critical separation of compounds with or without little denaturation. An ATPS is formed by two water soluble polymers (e.g., polyethylene glycol/dextran) or a polymer and a salt (e.g., polyethylene glycol/phosphate) with the presence of more than 80% of water, in both phases. This technique is highly suitable for separation and purification of proteins,

active secondary metabolites, enzymes and cell organelles (Aguilar 2017; Fierascu et al. 2020).

11.3.2 Natural Deep Eutectic Solvents

The initially the use of petrochemical solvents and volatile organic compounds was mostly a mostly flammable, volatile and toxic process. In the past decade, the Deep Eutectic Solvents (DES) and their natural equivalents, the Natural Deep Eutectic Solvents (NADES) are being used which deliver promising results. They are produced from plant based primary metabolites (Ivanović et al. 2020). As a routine, DES are usually a combination of two or more solid organic or inorganic compounds which under optimal temperature and stirring time liquefies and forms a stable eutectic (Choi et al. 2011). DES/NADES might be the most widely used solvents in the near future owing to its adjustable physical-chemical properties and its "green" character. The use of DES/NADES in combination with other avant-garde extraction techniques can lead to enhancement in terms of extracted yields of selected bioactive compounds, as well as to the benefits in terms of economic and environmental safety. They are used to extract polar and non-polar natural compounds. Two medicinal plants *Sideritis scardica* and *Plantago major* were extracted using NADES; simultaneous extraction of hydrophobic and hydrophilic bioactive compounds from leaves of *Ginkgo biloba*; phenolic compounds from *Carthamus tinctorius* in DES; extraction of two major flavonoids myricetin and amentoflavone from *Chamaecyparis obtusa* with polyalcohol-based DES have been carried out successfully. According to Ivanović et al. (2020) the recovery of target compounds from the extracts obtained and the recycling of the DES/NADES solvents used are still great disadvantages of the method.

11.3.3 Counter Current Chromatography and Centrifugal Partition Chromatography

This technique is being currently used for the preparative isolation and purification of natural products are Counter Current Chromatography (CCC) and Centrifugal Partition Chromatography (CPC). In both of the above techniques, separation usually occurs between two divergent phases (stationary and mobile), which in turn generates droplets or film. In CCC, the stationary phase is maintained by the gyratory motion in the polytetrafluoroethylene coil, while in CPC, the stationary phase is maintained in a constant gravity field aided by single axis rotation through rotary seals (Fierascu et al. 2020). They are helpful in efficiently separating, isolating, purifying milligrams to multigram with retention of virtually all biological activity and molecular integrity (Arige et al. 2017). Some of the plants extracted using this

method include purification of oridonin and ponicidin from *Rabdosia rubescens,* diterpene compounds from *Pseudolarix kaempferi*, Saponins from *Codonopsis lanceolata* roots and ginsenosides from *Panax ginseng*.

11.3.4 Ultrasound Assisted Extraction

It is worth noting that, when in high frequency Ultrasound Assisted Extraction (UAE) ultrasound is employed, the extraction yield did does not increase significantly, however, the degradation of the herb constituents was diminished. This becomes more important when alkaloids are extracted. This method could be employed as a tool to help in the extraction of medicinal compounds by using lower frequencies to assist in the degradation of toxic alkaloids during the process. This method can be used both on small- and large-scale processes (Vinatoru 2001). This technique has the major advantage of lesser energy consumption. In addition, it can be performed at a lower temperature in a short period of time. Moreover, the cells of plant material can be destroyed by hydrolytic enzymes. This method was proven to be useful in the extraction of rosmarinic acid from mycorrhizal hairy roots of *Ocimum basilicum* L., and in extraction of isoflavonoids from hairy root cultures of *Astragalus membranaceus.* Being a proper method, it can be used to extract polysaccharides from different materials (Guo et al. 2015), the release of the active compounds being obtained through the enzyme degraded cell walls (Jia et al. 2015; Fernando et al. 2017; Fierascu et al. 2020).

11.3.5 Supercritical Fluid Extraction (SFE)

The Supercritical Fluid Extraction (SFE) technique basically relies upon the fluid that is at supercritical condition: it is also referred to as a dense gas (a fluid above its Critical Temperature (TC) and Critical Pressure (PC)). The density of the supercritical fluids determines their properties when used as an extraction solvent. CO_2.is Till date, a high percentage of medicinal and aromatic plants have been scrutinized for possible extraction by supercritical CO_2, the only solvent used so far. Some of the plants extracted using SFE include *Taxus brevifolia, Taxus cuspidate, Hypericum perforatum, Echinacea purpurea, Serenoa repens* etc. Organic solvent-free products can be obtained and the low operating temperature makes it possible to preserve all their natural properties. The feasibility study on specific products can be performed rather easily at laboratory scale. However, accurate evaluation of production costs, including both capital and operating ones, must be done in order to exploit SFE at the industrial level (Chandrakant et al. 2011).

11.3.6 Microwave Assisted Extraction

Microwaves are part of electromagnetic spectrum of light with a range of 300 MHz to 300 GHz and wavelengths of theses waves range from 1 cm to 1 m (Mandal et al. 2007; Akhtar et al. 2019). Microwave Assisted Extraction (MAE) method is a method of low energy-high efficiency. Extraction in use is feasible, as it has the positive aspects of processes with reduced energy consumption, decreased quantity of raw material with increased yield of final biologically active compounds (Fierascu et al. 2020). Practically, the hydrogen bonding destruction is achieved by microwaves which induce dipole rotation in organic molecules along with heating. The increased kinetic energies of the ions and their friction results in a heating effect (Akhtar et al. 2019). Destruction of hydrogen bonding also increases the penetrating efficiency of the solvents into the plant matrix (Hudaib et al. 2003; Datta et al. 2005; Akhtar et al. 2019). Some of the herbs extracted through this method are *Artemisia annua*, *Cortex fraxini*, *Curcuma longa*, *Adathoda vesica* etc. (Akhtar et al. 2019).

These techniques can be used to extract active metabolites on an industrial scale. They have the advantage of offering "green" characteristics (shorter extraction time, no use of toxic chemicals, higher extraction yields with low solvent and energy consumption). By this technique, small amounts of essential oil components – that often form a hydrosol with condensate water – can be separated and collected (Fierascu et al. 2020).

11.4 Conclusions

Efficient usage of medicinal and aromatic plants and their metabolites assisted by the above-mentioned biotechnological tools, singly or in combination, are expected to yield positive results, in the near future. Both in small- and large-scale applications, they are economical, environment friendly and efficient. At the same time, they are capable of maintaining the integrity and purity of the bioactive compounds obtained. *In vitro* micropropagation tools, usually in combination with *Agrobacterium* transformation, can be regarded as most promising methods adopted for genetic transformation, also in the case of important medicinal species. To date, micropropagation and the *in vitro* production of secondary metabolites appear to be the most efficient and practical technology used commercially. DNA microarray has proved itself as a potential tool in drug discovery and development. This chapter also highlights some biotechnological methods used in India, that are still in progress of development/improvement. Apparently, they are likely to assume an important role in the industrial scale utilization, processing, conservation, etc. of medicinal and aromatic plants, in the foreseeable future.

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