CHAPTER

30

Herbal medicine and biotechnology for the benefit of human health

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Summary

Since ancient times, plants are known to have tremendous ethnopharmacological significance and are recognized as valuable resource for healthcare. India is the vast repository of these medicinal plants, and despite their innumerous medicinal uses, most of them remained unexplored using biotechnological tools. These plants naturally synthesize organic compounds called "secondary metabolites" by central metabolism of primary metabolites. These metabolites not only play major roles in adaptation of plants to the environment by interacting with the ecosystem but also are prominently used in pharmaceuticals. This chapter discusses the commercial utilization of plants using alternate biotechnological strategies but without affecting their natural population. Furthermore, it highlights the procedures involved in processing of plants and their derivatives for the production of herbal medicine and in drug discovery.

What you can expect to know

This chapter provides useful information on uses and beneficial effects of herbal medicines over conventional drugs. The methods on processing of plants and their parts for production of herbal medicines are described in details. Besides, alternative strategies of plant biomass production using in vitro tools and techniques and know-how of recent analytical techniques for herbal medicine production are also elaborately mentioned in this chapter.

Introduction

Herbal medicines refer to the use of plant seeds, berries, roots, leaves, bark, or flowers for medicinal purposes (Fig. 30.1). These plants are important for pharmacological research and drug development, not only when their constituents are used directly as therapeutic agents but also as starting materials for the synthesis of drugs or as models for pharmacologically active compounds (Wachtel-Galor and Benzie, 2011). Medicinal plants have been a major source of drugs for thousands of years, and even today they are the basis of systematic traditional medicines in almost all countries of the world. Unani and Ayurveda systems of medicine are two of the classic and oldest examples of this category. Around 80% of the population in developing countries is completely dependent on plants for their primary healthcare (Bannerman, 1983). Even in developed countries, which are enormously advanced in terms of medicinal chemistry, over one-fourth of all prescribed pharmaceuticals originate directly or indirectly from plants (Newman et al., 2000). Furthermore, of 252 drugs considered as indispensable by the World Health Organization (WHO), 11% are mainly derived from flowering plants and 28% of synthetic drugs are obtained from natural precursors (Namdeo, 2007). Few important compounds of plant origin used as drug or drug precursors are listed in Fig. 30.2.

Herbal medicines are secondary metabolites (Kubmarawa et al., 2007) derived from plants. Understandably, these pharmaceuticals are produced solely from massive quantities of whole plant parts,

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FIGURE 30.1 Herbal medicines. *Source: Courtesy Google.*

which have certain limitations. One limitation is that excessive harvesting can diminish local plant populations and erode genetic diversity. Second, it causes inconsistency in the production of compounds in terms of quality and quantity. The latter can spell trouble in terms of safety, supply, and economic feasibility of these herbal products on a commercial scale. To overcome these bottlenecks, domestication and use of good agricultural practices are crucial, especially for revival of diminishing plant populations. However, the conventional methods of plant propagation are lengthy and time consuming. The long cultivation periods between planting and harvesting make the entire process cumbersome and uneconomical, which in turn leads to the high cost of drugs. Moreover, wild populations are susceptible to problems of disease, drought, environmental fluctuations, low rate of fruit set, and poor seed yield, germination, and viability. Genetic variability also poses a concern in out-breeding plants, and owing to all these vulnerabilities batch-to-batch consistency of derived metabolites becomes questionable. Clearly, there is an urgent need of alternative and complementary methods for uniform qualitative and quantitative production of herbal medicine. An assured consistency of the metabolite could be achieved if the same plant is grown under controlled conditions of in vitro culture. In this context, tools and techniques of biotechnology, like in vitro plant, cell, tissue, and organ culture, offer solutions by maximizing the number of plantations and thus restoration of natural plant stock in a short time span. It also favors uniform metabolite production all year round, irrespective of seasons and vagaries of climatic conditions.

Traditional medicine

The WHO defines traditional medicine as being the "sum total of knowledge, skills, and practices based on the theories, beliefs and experiences that are indigenous to different cultures, which are used to maintain health, as well as to prevent, diagnose, improve, or treat physical and mental illnesses." Every early civilization used plants as their main source of medicine, and most of the world's population still relies on them. The first recorded literature on medicinal plants can be traced back to early human history, the Atharvaveda (2000 BCE) in India. With time, the original population of an area gained knowledge with which plants could be used for certain diseases or states of illness. In addition, they also gained knowledge of the harmful and poisonous plants. It is evident that the modern drug industry has been developed to a considerable degree as a result of plant-based traditional medicines.

There are a few closely related terms in use today, the meanings of which should be understood clearly. *Traditional medicine* refers to the following components: acupuncture (China), Ayurveda (India), Unani (Arabic countries), traditional birth attendant's medicines, mental healer's medicines, herbal medicines, and various forms of indigenous medicines. *Complementary or alternative medicine* refers to a broad set of healthcare practices that do not form the part of country's own tradition and are not integrated into the dominant health care system. Traditional medicine has maintained its popularity in all regions of the developing world, and its use is rapidly spreading in industrialized countries (Liu, 2011).

Ancient system of medicine

Ayurveda and traditional Chinese medicines are perhaps the most ancient of all medicinal traditions. Ayurveda means the "science of life" and is derived from "Ayur" meaning "life" and "Veda" meaning "knowledge." It takes a holistic view of human beings, their health, and illness. It aims at positive health, which has been defined as a well-balanced metabolism coupled with a healthy state of being. According to Ayurveda, disease can arise from the body and/or mind due to external factors or intrinsic causes. The origin of Ayurveda is lost in prehistoric antiquity, but its characteristic concepts appear to have matured between 2500 and 500 BCE. in ancient India. The earliest references to drugs and diseases can be found in the Rigveda and Atharvaveda.

Ayurvedic drugs have been found to perform very well against chronic ailments. Today, they are also attracting attention for diseases for which there are no or inadequate drugs for treatment in modern

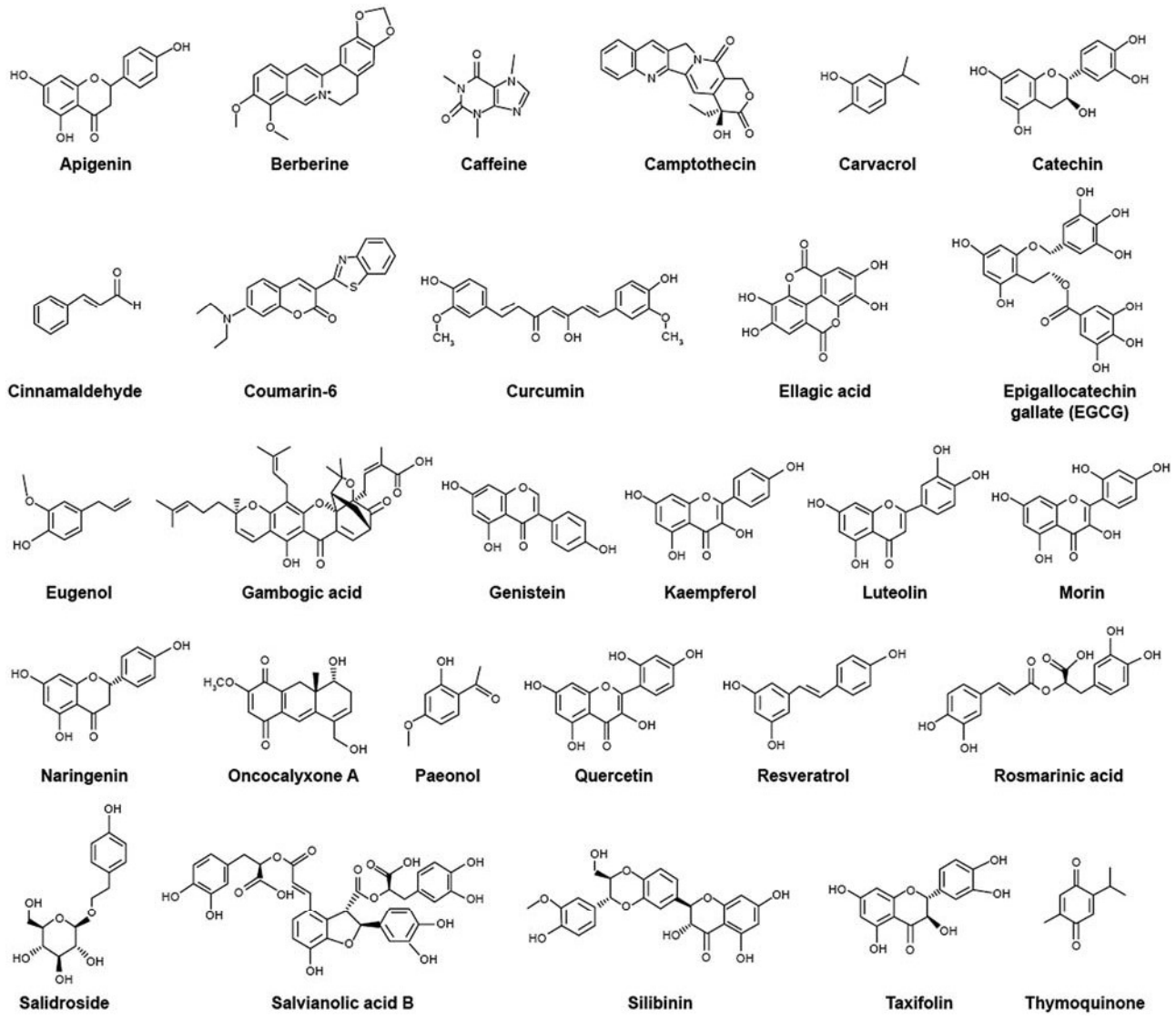

FIGURE 30.2 Chemical structures of some plant origin compounds used as drug or drug precursors. *Source: Adapted from Watkins, R., Wu, L., Zhang, C., Davis, R.M., Xu, B., 2015. Natural product-based nanomedicine: recent advances and issues. Int. J. Nanomedicine 10, 6055–6074.*

medicine, such as metabolic and degenerative disorders. Most of these diseases have multifactorial causation, and there is a growing awareness that in such circumstances, a combination of drugs, acting at a number of targets concurrently, is likely to be more effective than drugs acting at one target. Ayurvedic drugs, which are often multicomponent, have a promising impact on such conditions. Detailed chemical characterization in terms of composition and concentration of each ingredient in the formulation and studies of the biological activity of multicomponent Ayurvedic drugs will bring Ayurveda into the mainstream of scientific investigations. Recently there have been efforts to realize this objective (Fig. 30.3).

Methodology

Investigation of medicinal plants

Medicinal plants have formed the basis of healthcare throughout the world since the earliest days of civilization. They are still widely used and have noteworthy significance in the international trade. Recognition of their clinical, pharmaceutical, and economic value is still growing, although this varies widely between countries.

Each plant species has its own specific set of secondary metabolites. Apart from the family Poaceae, which harbors the world's worst weeds but is low in medicinal plants, many of the top 12 weed families are

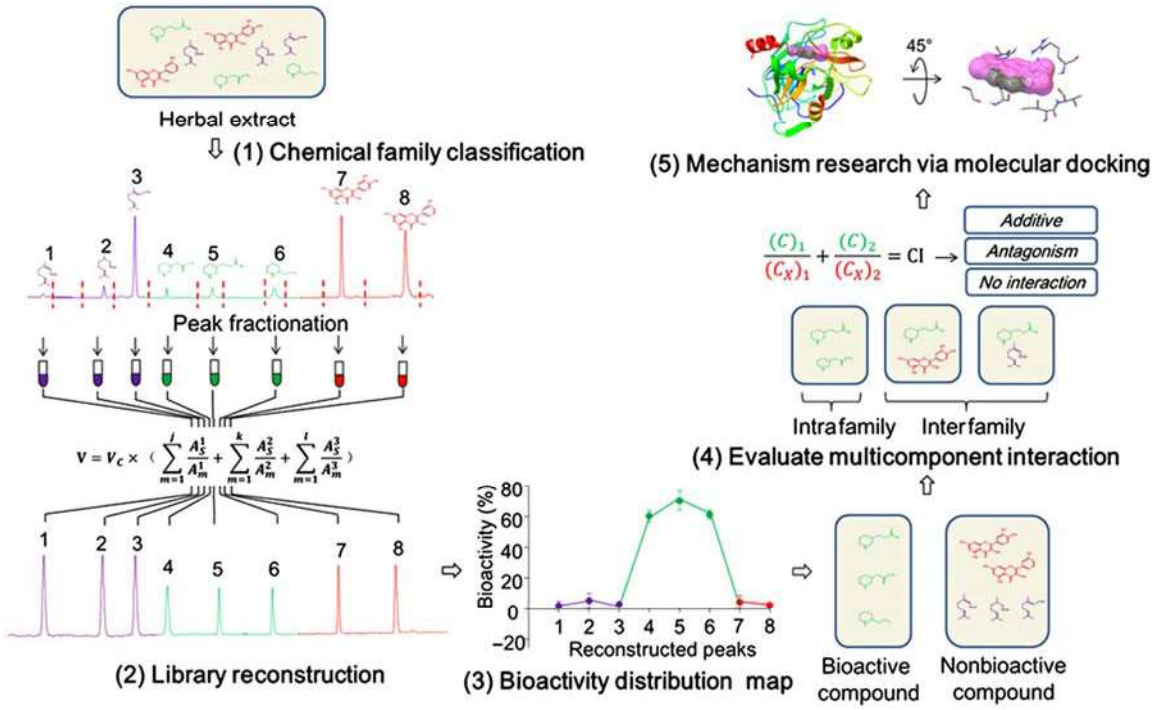

FIGURE 30.3 Diagram of the chemical family-based strategy for uncovering hidden bioactive molecules and multicomponent interactions in herbal medicines. *Source: Adapted from Song, H.P., Wu, S.-Q., Hao, H., Chen, J., Lu, J., Xu, X., et al., 2016. A chemical family-based strategy for uncovering hidden bioactive molecules and multicomponent interactions in herbal medicines. Sci. Rep. 6.*

also the ones that are important for medicines. The ecological and biochemical evidence suggest the preponderance of weeds in medicinal floras. Secondary compounds in plants are involved in the interaction of the plant with its environment and are important for ecological functions, such as allelopathy, insect and animal attractants for pollination, seed dispersal, and for chemical defense against microbes, insects, and herbivory (Bourgaud et al., 2001). These compounds do not participate in the vital metabolic processes of the plant system, but are the ones that exhibit bioactivity and can serve as medicine for humans. The spectrum of chemical structures synthesized by the plant kingdom is broader than that of perhaps any other group of organisms (Rao and Ravishankar, 2002).

In the present scenario, a large proportion of the drugs used in modern medicine are either directly isolated from plants or synthetically modified from a lead compound of natural origin. However, rarely is the drug isolated in the pure, usable form. What is initially obtained is the crude extract, which requires stepwise purification to obtain the finished product. The finished product as herbal medicine most of the time is a mixture of several compounds. When each and every component in the mixture is characterized qualitatively and quantitatively, it is called "characterized extract," which is understandably more desirable than the "uncharacterized extract." Plant extracts are known to consist of many chemicals, and among them, a few compounds could be acting synergistically. Sometimes, isolation of compounds from the extract may cause a decrease in desired activity, which underlines the importance of extract screening (Orhan et al., 2009).

Evidence-based studies on the efficacy and safety of traditional Indian medicines are limited. The essential ingredients in most formulations are not precisely defined. This is one of the most important challenges to scientists attempting to identify a single bioactive compound. Therefore in-depth studies and more stringent conditions should be followed to make a herbal formulation, so that the role of each and every component is known.

Drug discovery is the process by which drugs are discovered or designed. Plants have long been a very important source of drugs, and many plant species have been analyzed to see if they contain substances with therapeutic activity. Many plant drugs of folklore were investigated to determine the active ingredient in the mixture. Several reviews are available in the literature pertaining to approaches for selecting plants as candidates for drug discovery programs.

Today, many new chemotherapeutic agents are obtained synthetically based on "rational" drug design. The study of natural products has many rewards over synthetic drug design. The former leads to materials having new structural features with novel

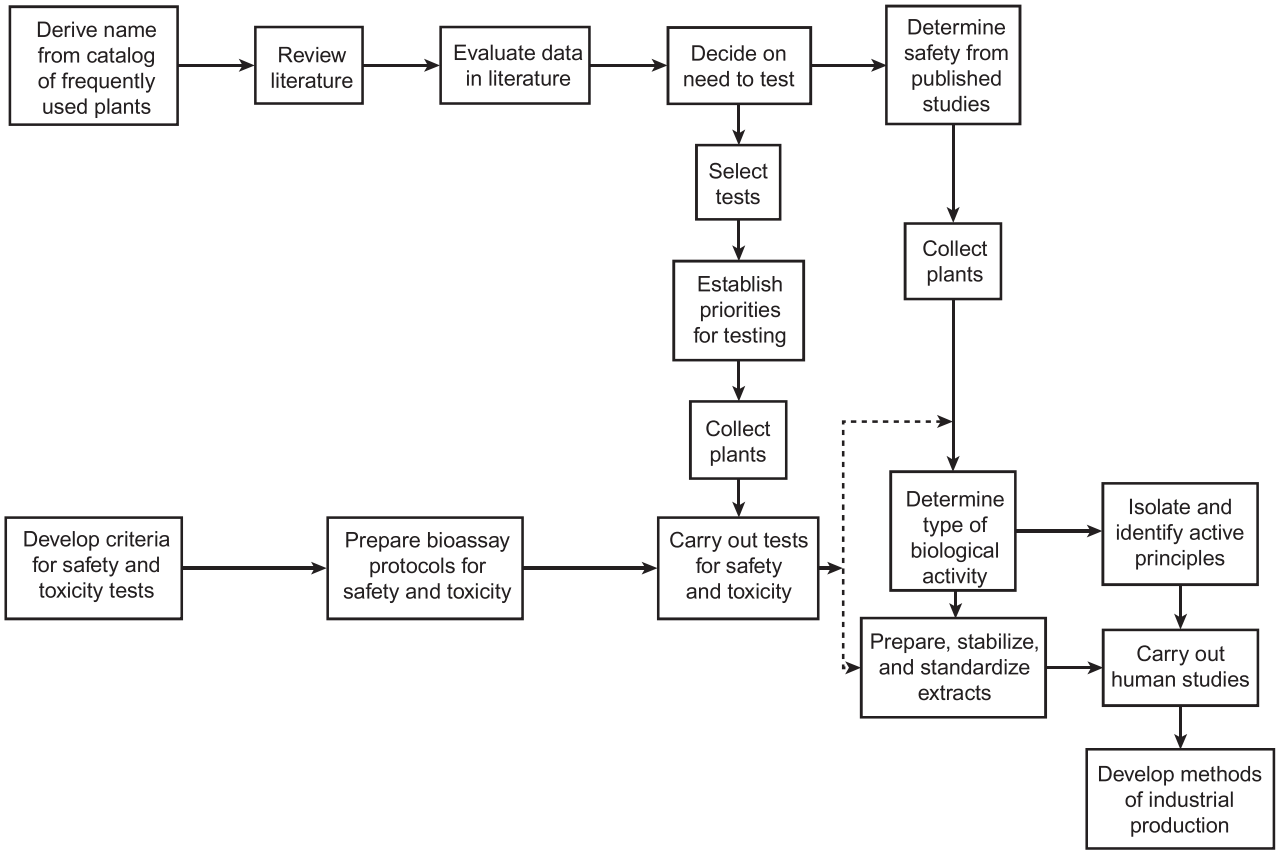

FIGURE 30.4 Flow chart of sequence for the study of plants used in traditional medicine. *Source: Adapted from Fabricant, D.S., Farnsworth, N.R., 2001. The value of plants used in traditional medicine for drug discovery. Environ. Health Perspect. 109, 69–75.*

biological activity. In this context not only do plants continue to serve as possible sources for new drugs but also chemicals derived from the various parts of these plants can also be extremely useful as lead structures for synthetic modification and optimization of bioactivity. The starting materials for about one-half of the medicines come from natural sources. There is no doubt that the future of plants as source of medicinal agents for use in investigation, prevention, and treatment of diseases is very promising.

Drug discovery from natural resources is a very tedious process. It involves identification of plant material, extraction, preliminary phytochemical screening of the crude extract, evaluation of biological activity, isolation of various bioactive compounds, and finally elucidation of structures. If the molecule is appealing, with strong pharmacological properties, then further preclinical studies are conducted on the molecules, such as toxicity, stability, and solubility studies. After undertaking these studies, if it is found that a molecule is substantially more active than the currently used drug, only then processes are developed for its economical and easy isolation from the source so that it can be readily available for therapeutic use.

In context of isolation and screening of chemicals from plants that possess medicinal properties, different approaches can be used. The process of obtaining bioactive substances and their chemical characterization are schematically represented in Fig. 30.4.

Extraction

Extraction involves the separation of medicinally active fractions of plant from inactive or inert components by using selective solvents through extraction procedures. The products so obtained from plants are relatively complex mixtures of metabolites in a liquid, a semisolid, or (after removing the solvent) a dry powder form. This is the critical first step in the investigation of medicinal plants.

The selection of a solvent system mainly depends on the exact nature of the bioactive compounds being targeted because during the extraction process, solvents diffuse into the solid plant material and solubilize compounds of similar polarity. The extraction of hydrophilic compounds uses polar solvents, such as methanol, ethanol, or ethyl acetate. For extraction of more lipophilic compounds, dichloromethane is used.

In a few cases, extraction with hexane is used to eliminate chlorophyll and oil.

As the target compounds may be nonpolar to polar and thermally labile, the suitability of the methods of extraction must be well thought out. Different methods, such as sonication, heating under reflux, soxhlet extraction, and others, are commonly used for plant sample extraction. In addition, plant extracts are also prepared by maceration or percolation of fresh green plants or dried powdered plant material in water and/or organic solvent systems.

Other modern extraction techniques include solid-phase microextraction, supercritical-fluid extraction, phytonics process, pressurized-liquid extraction, microwave-assisted extraction, counter-current extraction, solid-phase extraction, and surfactant-mediated techniques, which possess certain advantages (Handa et al., 2008; Patil and Shettigar, 2010).

Chemical screening

This technique is also known as phytochemical screening. In this method, aqueous and organic extracts are prepared from those plant samples that are the reservoir of secondary metabolites, such as leaves, stems, roots, or bark. The plant extracts are then analyzed for the presence of secondary metabolites like alkaloids, terpenes, and flavonoids. Standard tests are available in the literature for each class of compounds to be analyzed. Following this, a simple separation technique like thin-layer chromatography (TLC) is generally used to analyze the number and type of components present in the mixture. In TLC, the extracts are loaded on a glass coated with silica gel or other adsorbent, which is then kept in a chromatographic chamber containing a suitable running solvent. This technique mainly consists of a mobile phase and a stationary phase, whereby the compounds are separated based on their polarity. Sometimes a developing solvent might also be used after the plate has been taken out of the chromatographic chamber to detect chemicals. This approach has been used in the past and is still being used in developing countries. Since the isolation of pure bioactive components is a long and tedious process, this procedure enables the early recognition of known metabolites in the extracts and is thus economically viable. The tests are simple to perform; however, it is not suitable for the efficient separation of metabolites and has low selectivity and sensitivity of detection, which makes it difficult to detect traces of components in the sample.

Biological assays

Plant extracts have served as an important source of bioactive compounds for many drug discovery programs, and several important drugs have been isolated and identified from plants. In any isolation program in which the end product is a drug or lead compound, some type of bioassay screening or pharmacological evaluation must be necessarily used to guide the isolation process toward the pure bioactive component.

The selection of the biological assay to be adopted usually depends on the target syndrome and on the available information about the plant to be studied. For instance, if a plant has an ethnopharmacological history of use against a particular disease, then one would rationally use a specific bioassay technique that can predict the reputed therapeutic activity to isolate the lead that is responsible for that biological activity.

Bioassays can be categorized into primary and secondary assays. Primary bioassays, such as antimicrobial, antiviral and cytotoxic, anthelmintic activity, hepatotoxicity, antiinflammatory are performed when a large number of samples (plants or extracts) are to be screened for bioactivity. Primary bioassays, most of the time qualitative and not quantitative, provide reproducible results and offers potential tolerance against extract impurities. These assays are usually low cost and provide the results quickly. Secondary bioassays are performed after screening of lead compounds and are usually low capacity, slow, and costly assays (Mukherjee, 2019). It involves more exhaustive and comprehensive investigation of lead compounds on a number of model systems to select compounds for clinical trials. Model systems can be lower organisms, isolated subcellular systems, isolated intact cells of human or animal, and isolated organs of humans.

The major limitations of bioassay techniques are the use of biological organisms, particularly mice and rats, which are most often have to be sacrificed. Moreover, the phytochemical extracts are highly heterogeneous due to the presence of a mixture of different bioactive components. A desired biological response may not be due to a single bioactive compound, but due to a mixture of several bioactive compounds. Finally, isolation, screening, and quantification of a specific bioactive compound are difficult.

Identification, quantification, and characterization of bioactive compounds

Due to the fact that plant extracts usually contain various types of compounds with different polarities, their separation still remains a big challenge for the process of identification, quantification, and characterization of bioactive compounds. Apart from this, there are always chances of wide variations with respect to their chemical content in crude drugs/raw materials of plant origin due to varied reasons, such as climatic conditions, geographical distribution, source and

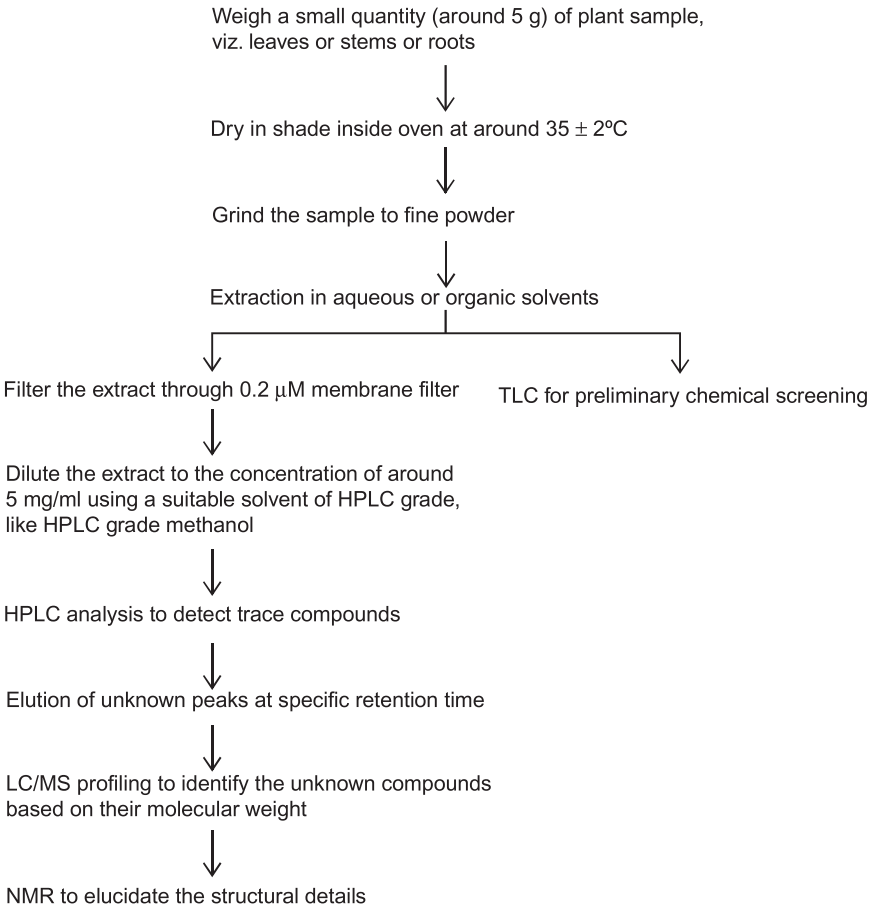

FIGURE 30.5 Schematic representation showing the process of chemical screening, isolation, and characterization of bioactive substances from plants.

season of collection, and lack of scientific methods of postharvest processing, storage, and preservation. Therefore identification, quantification, and characterization of bioactive compounds are essential prerequisites for herbal drug development (Fig. 30.5).

Various spectrophotometric, chromatographic, and chromatospectrophotometric methods are in use to obtain precise results and to identify, quantify, and characterize the phytoconstituents present in the plants. Chromatographic techniques, such as TLC, high-performance thin-layer chromatography (HPTLC), and high-performance liquid chromatography (HPLC), are used for the identification and quantification of known compounds. With the advancement in technology, superior hyphenated chromatographic and spectrophotometric techniques like Liquid chromatography coupled to mass spectrometry (LC-MS), liquid chromatography coupled with high field nuclear magnetic resonance (LC-NMR), liquid chromatography coupled with high field nuclear magnetic resonance and mass spectrometry (LC-NMR-MS), liquid-chromatography-Fourier transform infrared spectroscopy (LC-FTIR), and gas chromatography/mass spectrometry (GC-MS) are in practice. These hyphenated techniques are used with biological screening methods to circumvent reisolation of known compounds as well as for structural elucidation of the promising novel compounds (Philipson, 2007; Patel et al., 2010).

TLC is a rapid and simple analytical tool extensively used in herbal medicine analysis due to the fact that it requires minimum sample clean-up. Moreover, it gives qualitative and semiquantitative information of the separated compounds (Doughari, 2012). HPTLC, an extension of TLC, is one of the most versatile, reliable, rapid, and cost-efficient analytical tool in the quantitative analysis of compounds. This separation technique is based on TLC, but with the increased resolution, reproducibility, and accuracy of the compounds to be separated (Attimarad et al., 2011).

High-pressure liquid chromatography, also called as HPLC, is an important analytical tool for detection, separation, quantification, and qualitative assessment of bioactive compounds. It involves the injection of a small volume of liquid sample into a tube packed with porous particles (stationary phase), and the individual components of the sample are pulled along the packed tube (column) by a solvent (mobile phase). A pump forces the liquid through the column at a specific flow rate and generates high pressure. The column packing separates the components from the sample by various

physical and chemical interactions between the molecules and the packing material. The separated compounds collected at the exit column are detected by several techniques, like ultraviolet, fluorescence detection, diode array detection, etc. Data are generated in the form of chromatograms, where individual components show peaks at specific retention times (RTs) at which the component was eluted. Since HPLC has a high resolution and is very sensitive, this technique is suitable for the detection of trace components whose concentration in the sample is very low (Doughari, 2012).

The processing of a plant crude extract to provide a sample suitable for HPLC analysis, as well as the selection of solvent for sample reconstitution, can have a significant bearing on the overall success of natural product isolation and identification. The source material (e.g., dried powdered plant) will initially need to be treated in such a way as to ensure that the compound of interest is efficiently liberated into solution. This is where an efficient extraction protocol becomes important. An organic solvent may be used for extraction, and then solid material is removed by centrifugation and filtration of the extract. The filtrate is then concentrated and injected into an HPLC instrument for separation. The use of guard columns is necessary in the analysis of crude extract. Many natural product materials contain significant levels of strongly binding components, such as chlorophyll and other endogenous materials, that may in the long term compromise the performance of analytical columns.

LC-MS is a newer technique and is one of the most sensitive methods of molecular analysis. It yields information on the molecular weight and structure of the analytes and is suitable for the analysis of large, polar, ionic, thermally unstable, and nonvolatile compounds. In LC-MS, LC separates the different components of crude extracts, which are analyzed by mass spectrometry (MS) to selectively detect and confirm molecular identity. An MS detector senses a compound eluting from the LC column first by ionizing it using electro spray, thermo spray, and ion spray ionization techniques, and then by measuring its mass or by fragmenting the molecule into smaller pieces through collision-induced dissociation that are unique to the compound. The MS detector can sometimes directly identify the compound since every compound has its own unique mass spectrum and acts as a fingerprint for that particular compound. LC-MS in combination with biological assays have been proved to be very effective for the rapid analysis of herbal drugs (Herderich et al., 1997; Patel et al., 2010).

LC-NMR combines HPLC, and nuclear magnetic resonance (NMR) spectrometer is one of the most effective chromatospectrophotometer techniques for the separation and structural elucidation of unknown compounds and mixtures (Wann, 2005). The recent advancement in NMR devices has improvised LC-NMR sensitivity, like use of high magnetic field magnets and sensitive probes, employment of detection cells with smaller volumes, and automatic measurement software most appropriate for multicomponent analysis. Introduction of pulsed field gradient technique in high-resolution NMR has further improved the NMR techniques for structural elucidations and molecular weight information of usually light and oxygen-sensitive compounds (Wolfender et al., 1998; Doughari, 2012). It is useful for the identification and separation of chiral and isomer compounds. This equipment is usually supplemented with a parallel MS detector (LC-NMR-MS) to provide additional chromatographic traces (total ion current, extracted ion chromatogram, etc.) and complementary structural data (molecular ions and their fragments) (Burton et al., 1997).

LC-FTIR is a hyphenated technique coupling LC with FTIR. FTIR spectroscopic technique is used for the quantitative measurement of the interaction between IR radiation and organic materials. It is generally used when specific detection and identification of bioactive compounds (e.g., complex mixture of isomers) is required. It gives information related to functional groups (e.g., -OH, -COOH) that are present in the molecule (Patel et al., 2010).

GC-MS is based on the partitioning of compounds between a liquid and a gas phase. This technique is widely used for the qualitative and quantitative analyses of a large number of herbal drugs because it has high sensitivity, reproducibility, and speed of resolution. It has proved to be most valuable for the separation of volatile, nonpolar, and semipolar bioactive compounds. In GC-MS, the sample is injected into a long tubular column, the chromatography column, which has a high boiling point stationary phase, such as silicon grease. The basis of the separation is the difference in the partition coefficients of volatilized compounds between the liquid and gas phase as the plant metabolites are carried through the column by the inert carrier gas (e.g., nitrogen, helium, hydrogen, or argon). The time taken by the sample to pass through the length of the column is referred to as its RT. The RT for a given sample is an identifying characteristic. The detector for the GC is the MS detector. As a sample exits the end of the GC column, it is ionized into gaseous ions in the ionization source and then enter into the mass analyzer. The major principle of ionization is electron impact and chemical ionization techniques. Fragmented ions with different mass-to-charge ratio are sequentially sorted, and an electron multiplier finally multiplies the fragmented ions signal to

generate electric signal (Philipson, 2007; Patel et al., 2010; Mukherjee, 2019).

Supercritical fluid chromatography (SFC), a new analytical tool, combines the best feature of gas and liquid chromatography. It is an important chromatography that is beginning to find use in herbal medicine. It is mainly used for the separation and detection of compounds that are nonvolatile and thermolabile and contain no functional groups (Henry and Yonkar, 2006).

Biotechnological approaches for herbal drug production

Intact plants in the field or wild habitats produce high-value bioactive compounds. However, as mentioned in the forgoing sections, the quantity and availability of these economic products from natural resources restrict their maximized uses for the benefit of humankind. As the demand for bioactive compounds has increased in the last few years, exploitation of medicinal plants has also increased. Hence, there is an urgent need to develop alternative methods for large-scale production of metabolites and quality plants. In this respect, biotechnology puts forward an attractive alternative to whole-plant extraction for homogeneous, controlled production, especially, when we take the commercial demand into picture. It also results in more consistent yield and quality of the products, irrespective of the seasons and the regions. Biotechnology offers an opportunity to exploit plant cells, tissues, organs, or entire organisms by growing them in vitro and genetically manipulating them to get desired compounds (Rao and Ravishankar, 2002). Many biotechnological strategies, such as embryogenesis, organogenesis, screening of cell lines, media optimization, and elicitation, can be carried out for the enhanced production of secondary metabolites from medicinal plants. The subsequent sections briefly discuss various in vitro culture techniques that can be used for herbal drug production.

Organ cultures

The selection of an appropriate technique depends on the results. In plants where molecules of interest are localized in specialized cells, dedifferentiated (callus) cultures are not desirable but establishment of organogenic cultures would be advantageous. Under in vitro conditions, redifferentiation is generally associated with an improved synthesis of secondary metabolites (Collin, 2001). This is probably due to the appearance of complex cells and tissues that are metabolically more proficient. In all redifferentiated cell lines, along with the shoot-forming nodules, nonmorphogenic cell masses are also present. Although nonmorphogenic it might have a certain degree of differentiation at the cellular stage and, due to coevolution, imitate the biochemistry of redifferentiated cells (Brown and Charlwood, 1986). The reports on *Artemisia annua* and *Azadirachta indica* stated that artemisinin and azadirachtin production, respectively, were very poor in dedifferentiated callus cultures, and a certain degree of redifferentiation was obligatory for compound production (Singh and Chaturvedi, 2013). Organogenesis was also found to be an essential prerequisite for steroidal saponin production in *Ruscus aculeatus*. Similar observations were made for the biosynthesis of picroside in *Picrorhiza kurroa*, wherein the metabolite did not accumulate in the dedifferentiated callus cultures, but occurred specifically in the redifferentiated cultures. Berkov et al. (2010) also demonstrated that alkaloid synthesis in *Pancratium maritimum* is closely related to tissue differentiation.

Since it was observed that the production of bioactive compounds is generally higher in organized plant tissues, there are attempts to regenerate whole plant organs (i.e., shoots or roots) under in vitro conditions, either directly from explants or indirectly via an intervening callus phase (Fig. 30.6). As expected, such regenerating cultures produce patterns of secondary metabolites that are similar to the field-grown parent plant, with the added advantage of improved production of metabolites. Another advantage of using the

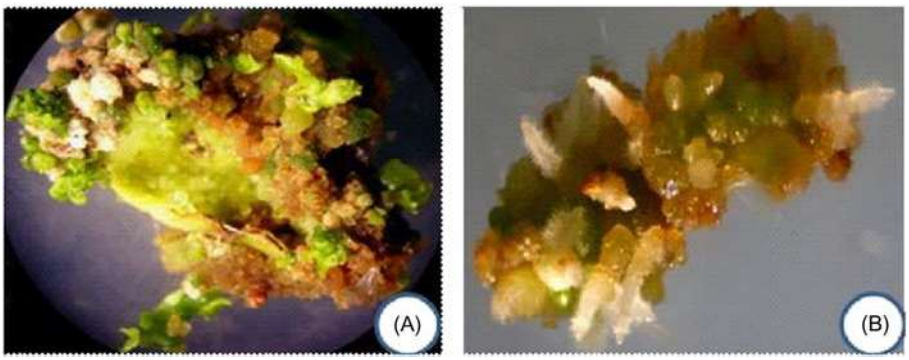

FIGURE 30.6 Neem organogenesis from leaf explants indirectly via callusing. (A) Shoot differentiation. (B) Root differentiation.

organized cultures is that they are relatively more stable in the production of secondary metabolites than cultures of undifferentiated cells, such as cells in callus or suspension cultures (Rao and Ravishankar, 2002).

Callus cultures

Callus cultures are considered as dedifferentiated mass of cells obtained from explants inoculated in vitro on the medium consisting of relatively higher auxin concentrations or a combination of equal concentration of both auxin and cytokinin. In plants, where sought after metabolites are present in leaves, establishing in vitro cultures from leaves and using them for the extraction of compounds would be an ideal alternative. Callus cultures containing the bioactive substances are collected at a specific stage (usually during the stationary phase of their growth cycle, since secondary metabolite production is greater during the stationary phase), dried, extracted, and the extract then taken for identification and quantification of the desired medicinal compound using HPLC, LC-MS, etc. The further scale-up and yield enhancement studies of the compound are performed by raising the callus in suspension, first in a shake-flask culture, and then in a suitably designed bioreactor, to maximize its production.

Suspension cultures

A breakthrough in cell-culture methodology occurred with the successful establishment of cell lines capable of producing high yields of secondary compounds in cell suspension cultures (Zenk, 1978). During the past decades, this approach of metabolite production has attracted much academic and industrial interest. The technique of using plant cell suspension cultures for secondary metabolite production is based on the concept of biosynthetic totipotency of plant cells, which means that each cell in the culture retains the complete genetic information for the production of range of compounds found in the whole plant. Cell suspension cultures are initiated from established callus cultures by inoculating them into liquid media. The cultures are then kept in glass flasks under continual agitation on horizontal or rotating shakers; they can be eventually transferred to a specialized bioreactor. Cells in suspension cultures grow much better than in semisolid media because of better mixing of oxygen and nutrients during shaking conditions.

Productivity of suspension cultures is critical to the practical application of this cell technology for bioactive compound production. To improve the production of secondary metabolites in in vitro cultures, various strategies such as the manipulation of parameters of the environment and medium, selection of high-yielding cell clones, precursor feeding, and elicitation can be opted for.

Case study: *Lantana camara* L

Owing to the tremendous relevant ethnopharmcological significance and demand of the genus, *L. camara L*, an alternate strategy is explored in this case study to maximize the cell biomass utilization and, thereby, restoration of natural plant resources. The plant tissue culture technique offers to generate the cell biomass in a shorter duration, throughout the year, irrespective of the seasons and regions with a provision of tunability of the cells to increase the metabolite production. *L. camara* L. [Sage (in English) or Caturang (in Hindi)] is an aromatic, evergreen shrub belonging to the family Verbenaceae. It is a reservoir of several important bioactive molecules. It has been listed as one of the important medicinal plants in the world. For many years, natural products from *Lantana* have been used in the prevention and cure of many serious diseases, including cancers. The most significant bioactive molecules of this plant are presented in Fig. 30.7.

For establishing tissue cultures, the first prerequisite is the selection of healthy plant material. Thus, for this study, leaves from *Lantana* plants bearing pink-yellow flowers were picked. Leaves were disinfected using 1% (v/v) Tween-20 and 0.1% (w/v) mercuric chloride, followed by three rinses in sterile distilled water after each step. The leaf disk explants were prepared using a cork borer of 5 mm diameter. The basal media used in all the experiments related to callus induction and proliferation consisted of MS (Murashige and Skoog, 1962) medium enriched with 30 g/L sucrose and solidified with 0.8% agar (HiMedia Laboratories, Mumbai, India). The pH of the media was adjusted to 5.8 before autoclaving at 1.06 kg/cm^2 and 121°C for 15 minutes. The media was supplemented with different plant growth regulators (auxins and cytokinins) at defined concentrations. Remaining steps are explicitly described in Fig. 30.8.

Opportunities and challenges

The consumption of herbal medicines and the importance of the herbal medical industry are fast growing and widespread. According to estimates of the World Health Organization, more than 80% of the world's population depends primarily on herbal medicines. The ancient art of herbal medicine is fast developing today and is undergoing something of a

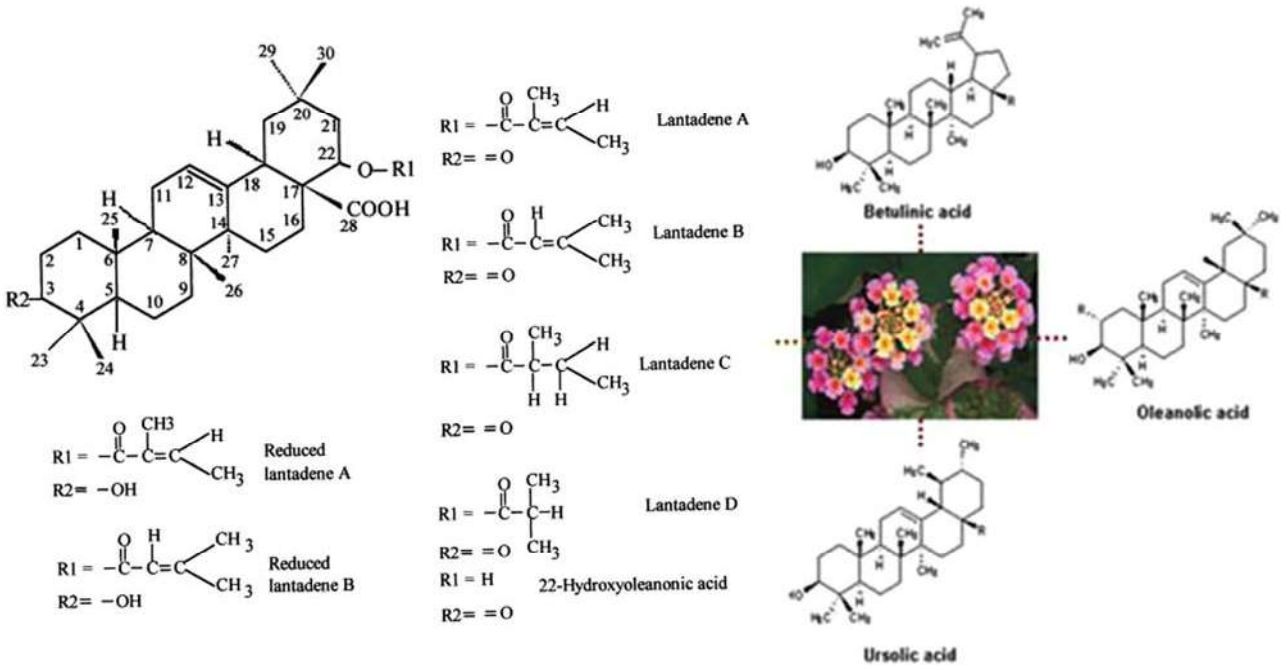

FIGURE 30.7 Bioactive compounds of *Lantana*.

renaissance all over the world, particularly in developed countries. Most of the ingredients used in herbal medicines are taken from wild plants, and the increasing demand for medicinal plants, along with habitat loss and climate change, is putting pressure on many species. Indiscriminate harvesting from the wild has led to loss of genetic diversity, diminishing populations, local extinctions, and habitat destruction. This has raised the ire of plant conservationists.

Large-scale cultivation of medicinal plants offers a viable conservation strategy and also eliminates the problems that are generally faced in herbal extracts, such as misidentification, genetic and phenotypic variability, extract variability and instability, toxic components, and contamination. Optimized yield and uniform high-quality product can also be achieved through cultivation. However, in a rapidly shifting and fashion-prone market, the cultivator has to make the difficult decision of which particular species to grow.

Therefore, the difficulty in predicting which extracts will remain marketable is another serious obstacle in bringing medicinal plants into successful commercial cultivation.

Although a large number of plant species used in herbal medicine are cultivated, a great majority of them are still utilized from the wild population. There are certain difficulties faced by growers in the cultivation of herbal plants because of low germination rates, unavailability of quality planting materials, or specific ecological requirements. Lack of knowledge about the specific requirements for pollination, seed germination, and growth are the main hindrances in the cultivation of herbal plants. Fungal infection or mechanical damage frequently results in low germination rates that can be easily overcome by improved seed treatments and by ensuring optimal storage conditions. Moreover, difficult-to-grow herbal plants can be easily cultivated on a commercial scale by using controlled environments, including hydroponic systems.

Another major challenge faced in the production of herbal medicines is that the main bioactive component, which is the major ingredient in the herbal medicine, is synthesized in a very small quantity in the specific plant. This is obvious, as the bioactive components are mainly produced as secondary metabolites in plant cells that are produced in small quantities. This leads to cutting down of a large number of herbal plants for producing a single drug. However, by the use of modern tissue culture techniques and genetic transformation that can alter the pathways for the biosynthesis of target metabolites, today this wasteful harvesting technique can be easily prevented.

Together with supporting the use of herbal medicines, it is high time for everyone, herbalist and conservationist alike, to reduce the overexploitation of the world's wild plants. In the modern world, the trade in medicinal plants is everincreasing, but largely unmonitored. At the moment, many harvesting practices are unsustainable, which is threatening population of medicinal plants and their habitats and also the

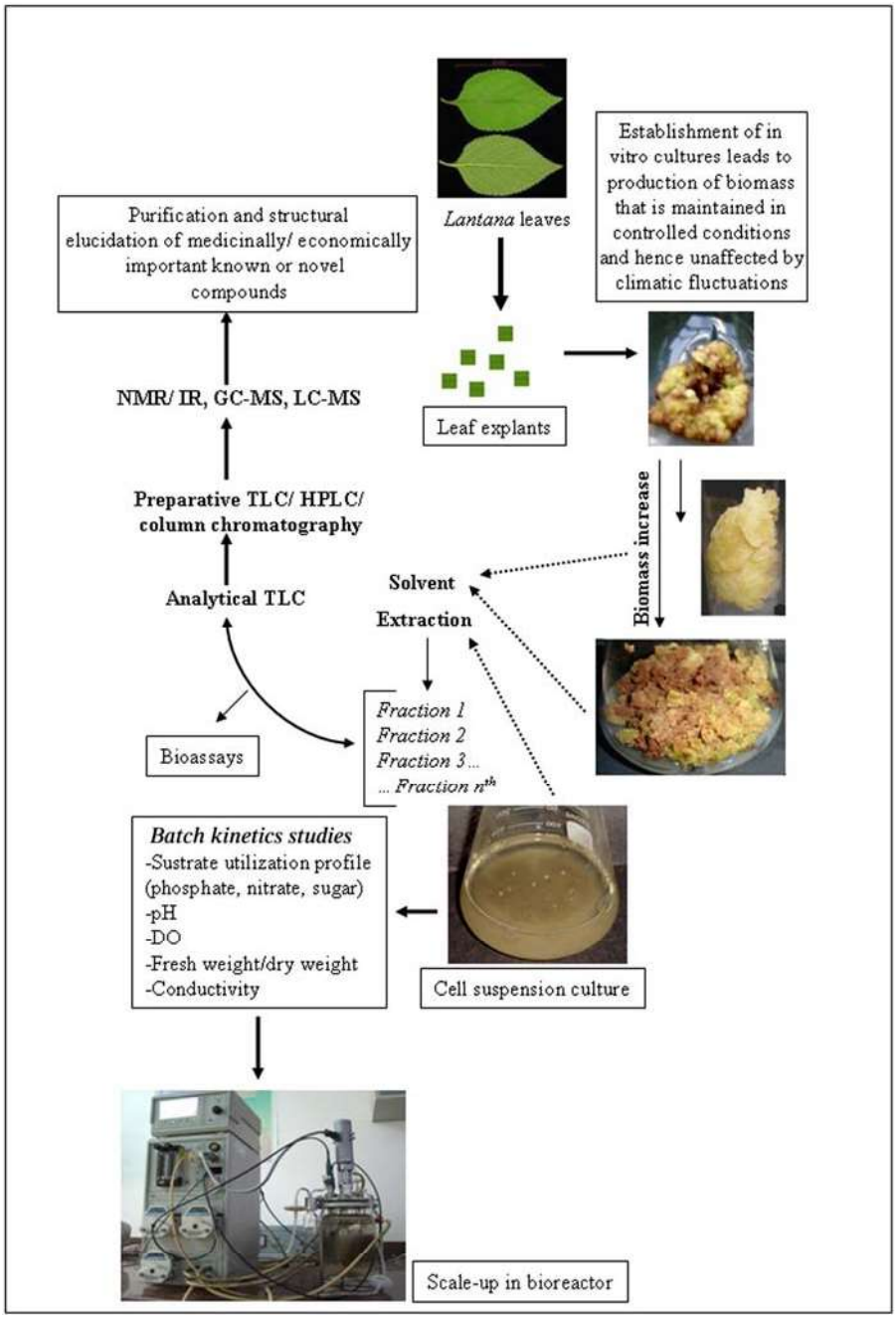

FIGURE 30.8 Isolation of bioactive compounds from *Lantana camara*, a medicinal plant.

livelihoods of those people engaged in their collection. It is time for the conservationists, the government, and each and every one of us to find workable global solutions.

Conclusions and outlook

Medicinal plants are widely used by the people living in both rural and urban areas. Globalization has greatly renewed the interest in herbal medicines, and today most people prefer to take herbal medicines as an alternative therapy. This resurgence in plant remedies has mainly resulted from the following factors:

1. Herbal medicines are found highly effective in curing diseases including chronic symptoms.
2. Most modern drugs have one or more side effects.
3. Development of science and technology.

In addition to these factors, economic benefits also contribute to their everincreasing popularity. Development of modern science and technology, and further studies into traditional plant medicines, conducted with modern theories and techniques have greatly enriched the use of herbal medicines by

absorbing new ideas, and concepts from traditional plant medicine are in use across the world. This has led to the tremendous expansion of herbal medicine industry and employment generation in a last few decades. Realizing these facts, it is understood that in not-too-distant future, traditional plant medicine will emerge as an area of prime importance in healthcare system. However, efforts are to be concentrated on sustainable harvesting of medicinal plants to avoid their overexploitation and extinction. Also, to utilize the available resources of medicinal plants to their fullest, social, cultural, and economic problems, lack of well-planned and integrated strategies, and poor access to scientific information must be dealt with first.

Ethical issues

Although approximately 80% of the people today depend on herbal medicine as a component of their primary healthcare according to the World Health Organization, there is still concern about the safety and efficacy of herbal drugs. Despite the fact that herbal medicine can potentially contribute to the improvement of healthcare, many major challenges must be overcome prior to the successful incorporation of herbal remedies into medicine. Beneficence, nonmalfeasance, patient autonomy, justice, and public accountability are the pillars of bioethical principles, which are religiously followed in conventional medicine. They guide the clinicians such that the patients' interests are best served. As the use of complementary medicine (including herbal medicines) becomes increasingly popular, it is becoming apparent that the same bioethical principles are applicable to these alternate forms of healthcare (Kemper and Cohen, 2004). Beneficence is the principle that says it is a clinician's responsibility to promote a patient's wellbeing; clinicians must take appropriate measures to ensure that some positive outcome will occur. Nonmalfeasance is the responsibility to not hurt others. This ethical principle is almost the same as beneficence, but with important distinctions, as one's duty to prevent harm is not the same as the duty to promote wellbeing (Beauchamp and Childress, 2009). Patient autonomy is a foundation of conventional medicine that is pertinent to the use of herbal medicines too. In most parts of the world, consumer access to herbal medicines is controlled by prescription, thus allowing for extensive use. With self-care as one component of patient autonomy, another key element is that the patient must consists of complete information to make an informed treatment decision (Ernst and Cohen, 2001). Time and again researchers come across cases where a patient has gathered information about herbal medicines from relatives, friends, magazines, and Internet (Gardiner and Riley, 2007; Khader et al., 2008; Low, 2009), all of which are perceived as less reputable than official sources.

Translational significance

Animal models are used in study on human diseases because both animals and humans are similar in genetics, anatomy, and physiological aspects. Also, animal models are often preferable because of their easy and abundant supply and ease of manipulation. Also, for statistical analysis, a sufficient number of specimens must be used for a particular experiment. Therefore, scientists cannot conduct research on just one animal or human, and it is easier for scientists to use sufficiently large numbers of animals instead of humans to get reliable results. Only in cases of advanced clinical trials, humans are used for investigations. Otherwise, animals, like mice, rats, monkeys, dogs, and several fungal, bacterial, and plant species, are used as model organisms for such studies. However, even with the evident similarities between animal models and humans, only about 1% of drugs reach the last phase of clinical trials. As far as herbal medicines are concerned, the chemical constituents present in them are a part of the physiological functions of living plants, and therefore, they have better compatibility with the human body. However, scientific proof of this statement is not sufficient, and this is, therefore, one major area where research can be carried out.

Clinical correlation

Unhealthy lifestyle, rise in environmental toxins due to pollution, and other factors increase the risk of diseases. In addition, continued usage and side effects of allopathic drugs are a cause of concern. In 2013, WHO developed and launched "WHO Traditional Medicine Strategy 2014–2023," which emphasized on integration of traditional and complementary medicine to promote universal healthcare while ensuring the quality, safety, and effectiveness of such medicines (Sen and Chakraborty, 2017).

In spite of familiarity and possible benefits, integration of herbal medicine faces a number of problems and challenges. Among many, incorrect identification of plants, adulteration, and/or incorrect formulation process are the main problems that incorporate inconsistency and reduce the effectiveness of herbal medicines. Besides these, lack of good manufacturing practices, quality control and regulatory measures,

ignorance on side effects are other bottlenecks that prevent integration of herbal medicines in mainstream. Due to similar reasons, clinical trials that are important for examining the efficacy and safety of these medicines on humans are sparse and limited. Even after all these shortfalls, the good news is that research and funding bodies like AYUSH are profoundly supporting studies on herbal and traditional forms of medicine. AYUSH research portal includes information on numerous clinical trials, preclinical researches, and drug researches related to plants used in these systems. AYUSH practitioners have the rights to prescribe herbal formulations to patients and have a wider reach in rural and remote parts of India (Roy, 2015). In a nutshell, although a lot still remains, but a pioneering step has already been taken, to integrate herbal medicines with contemporary forms of treatment.

Turning point

Although there is a long way to go, but the subject will certainly revolutionize the therapeutic industry. The human connection with the herbal drugs from nature is boundless since ancient times. Written documents, preserved plant materials, and original plant medicines are some of the evidences that elucidate the use of plants as medicines by human. Even today, most of the pharmacologists prefer to use only plant resources as medicine due to its less toxic side effects. About 80% of world population depends on herbal medicine to treat various kinds of ailments. However, the significant number of medicinal plants are either endemic or are on the verge of extinction. Therefore, the ultimate challenge is to increase the availability of plant biomass as a raw material and also to increase the production of valuable metabolites at an industrial scale. This can be achieved by adopting biotechnological methods, like plant tissue culture techniques, where cell biomass can be generated constantly throughout the year, irrespective of the seasons, and regions and environmental fluctuations, in large bioreactors. This cell biomass can serve as raw materials for the production of useful drugs. However, the plant cell cultures are not as commercially utilized as microbial cell cultures due to the increased size of plant cells that make it more sensitive to shear stress. The other major limitations are lack of availability of data, mostly the published work are dealt with plant cells at lab scale due to their comparatively large size and rigid cell wall than animal cells. Bioprocess parameters like, engineering considerations, optimization of process parameters, and process strategies can be applied to increase the production of medicinal metabolites from plant cells. Therefore, establishment of in vitro cultures and investigating their potential by using various in vitro assays is a route to conquer many life-threatening diseases.

World Wide Web resources

One of the first steps in the use of herbal medicine is to find out the best source for complete information about herbs and/or derivatives. At present the Web is the most prominent (and perhaps most familiar) tool, but the Internet, like other resources, has its own strengths and weaknesses. The major strength of Internet is that it is an especially valuable research tool when looking for information that is current and frequently updated. It is also quick to access.

As far as weaknesses are considered, the Internet is not the best place to find established viewpoints in their original form since it is often the case that information is changed from its original source. Information on the Internet is often second, third, or even fourth hand. Published books remain the safest place to get established facts and opinions, especially when looking for traditional ideas.

However, following websites do provide comprehensive information on herbal medicines:

http://ethnomedicinetomodern.blogspot.in/

http://www.umm.edu/altmed/articles/herbal-medicine-000351.htm

http://www.nlm.nih.gov/medlineplus/herbalmedicine.html

http://www.herbs.org/herbnews/

http://www.journals.elsevier.com/journal-of-herbal-medicine

http://www.who.int/bulletin/volumes/86/8/07-042820/en/

References

Attimarad, M., Ahmed, K.K.M., Aldhubaib, B.E., Harsha, S., 2011. High performance thin layer chromatography: a powerful analytical technique in pharmaceutical drug discovery. Pharmaceut. Meth. 2 (2), 71–75.

Bannerman, R.H., 1983. The Role of Traditional Medicine in Primary Health Care, Traditional Medicine and Health Care Coverage. World Health Organization, Geneva, pp. 318–327.

Beauchamp, T.L., Childress, J.F. (Eds.), 2009. Principles of Biomedical Ethics. 6th ed Oxford University Press, New York.

Berkov, S., Pavlov, A., Georgiev, V., Weber, J., Bley, T., Viladomat, F., et al., 2010. Changes in apolar metabolites during *in vitro* organogenesis of *Pancratium maritimum*. Plant Physiol. Biochem. 48, 827–835.

Bourgaud, F., Gravot, A., Milesi, S., Gontier, E., 2001. Production of plant secondary metabolites: a historical perspective. Plant Sci. 161, 839–851.

Brown, J.T., Charlwood, B.V., 1986. Differentiation and monoterpene biosynthesis in plant cell cultures. In: Morris, P., Scragg, A.H., Stafford, A., Fowler, M.W. (Eds.), Secondary Metabolism in Plant

Cell Cultures. Press Syndicate of the University of Cambridge, Cambridge, pp. 68–74.
Burton, K.I., Everett, J.R., Newman, M.J., Pullen, F.S., Richards, D.S., Swanson, A.G., 1997. On-line liquid chromatography coupled with high field NMR and mass spectrometry (LC-NMR-MS): a new technique for drug metabolite structure elucidation. J. Pharmaceut. Biomed. Anal. 12, 1903–1012.
Collin, H.A., 2001. Secondary product formation in plant tissue cultures. Plant Growth Regul. 34, 119–134.
Doughari, J.H., 2012. Phytochemicals: extraction methods, basic structures and mode of action as potential chemotherapeutic agents. In: Rao, V. (Ed.), Phytochemicals—A Global Perspective of Their Role in Nutrition and Health. InTech, Rijeka, Croatia, pp. 1–33.
Ernst, E., Cohen, M.H., 2001. Informed consent in complementary and alternative medicine. Arch. Intern. Med. 161, 2288–2292.
Fabricant, D.S., Farnsworth, N.R., 2001. The value of plants used in traditional medicine for drug discovery. Environ. Health Perspect. 109, 69–75.
Gardiner, P., Riley, D.S., 2007. Herbs to homeopathy—medicinal products for children. Pediatr. Clin. N. Am. 54, 859–874.
Handa, S.S., Khanuja, S.P.S., Longo, G., Rakesh, D.D., 2008. Extraction Technologies for Medicinal and Aromatic Plants. International Centre for Science and High Technology, Trieste, pp. 21–25.
Henry, M.C., Yonkar, C.R., 2006. Supercritical fluid chromatography, pressurized liquid extraction, and supercritical fluid extraction. Anal. Chem. 78, 3909–3916.
Herderich, M., Richling, E., Roscher, R., Schneider, C., Schwab, W., Humpf, H.U., et al., 1997. Application of atmospheric pressure ionization HPLC-MS-MS for the analysis of natural products. Chromatographia 45, 127–132.
Kemper, K.J., Cohen, M., 2004. Ethics meet complementary and alternative medicine: New light on old principles. Contemp. Pediatr. 21, 61–67.
Khader, Y., Sawair, F.A., Ayoub, A., Ayoub, N., Burgan, S.Z., Amarin, Z., 2008. Knowledge and attitudes of lay public, pharmacists, and physicians toward the use of herbal products in north Jordan. J. Alternative Compl. Med. 14, 1186–1187.
Kubmarawa, D., Ajoku, G.A., Enwerem, N.M., Okorie, D.A., 2007. Preliminary phytochemical and antimicrobial screening of 50 medicinal plants from Nigeria. Afr. J. Biotechnol. 6, 1690–1696.
Liu, W.J.H., 2011. Traditional Herbal Medicine Research Methods: Identification, Analysis, Bioassay and Pharmaceutical and Clinical Studies. John Wiley & sons, Inc, Hoboken, NJ, p. 466.
Low, D.T., 2009. The use of botanicals during pregnancy and lactation. Alternative Ther. Health Med. 15, 54–58.
Mukherjee, P.K., 2019. Plant metabolomics and quality evaluation of herbal drugs. Quality Control and Evaluation of Herbal Drugs: Evaluating Natural Products and Traditional Medicines. Elsevier Radarweg, Amsterdam, Netherlands, pp. 629–653.
Murashige, T., Skoog, F., 1962. A revised medium for rapid growth and bioassays with tobacco cultures. Physiol. Plantarum 15, 473–497.
Namdeo, A.G., 2007. Plant cell elicitation for production of secondary metabolites: a review. Pharmacol. Rev. 1, 69–79.
Newman, D.J., Cragg, G.M., Snader, K.M., 2000. The influence of natural products upon drug discovery. Nat. Prod. Rep. 17, 215–234.
Orhan, I., Deliorman, O.D., Özçelik, B., 2009. Antiviral activity and cytotoxicity of the lipophilic extracts of various edible plants and their fatty acids. Food Chem. 115, 701–705.
Patel, K.N., Patel, J.K., Patel, M.P., Rajput, G.C., Patel, H.A., 2010. Introduction to hyphenated techniques and their applications in pharmacy. Pharmaceut. Meth. 1 (1), 2–13.
Patil, P.S., Shettigar, R., 2010. An advancement of analytical techniques in herbal research. J. Adv. Sci. Res. 1 (1), 8–14.
Philipson, J.D., 2007. Phytochemistry and pharmacognosy. Phytochemistry 68, 2960–2971.
Rao, R.S., Ravishankar, G.A., 2002. Plant cell cultures: chemical factories of secondary metabolites. Biotechnol. Adv. 20, 101–153.
Roy, V., 2015. Time to sensitize medical graduates to the Indian Systems of Medicine and Homeopathy. Ind. J. Pharmacol. 47, 1–3.
Sen, S., Chakraborty, R., 2017. Revival, modernization and integration of Indian traditional herbal medicine in clinical practice: importance, challenges and future. J. Tradit. Complementary Med. 7, 234–244.
Singh, M., Chaturvedi, R., 2013. Sustainable production of azadirachtin from differentiated in vitro cell lines of neem (*Azadirachta indica*). AoB Plants 5, plt 034.
Song, H.P., Wu, S.-Q., Hao, H., Chen, J., Lu, J., Xu, X., et al., 2016. A chemical family-based strategy for uncovering hidden bioactive molecules and multicomponent interactions in herbal medicines. Sci. Rep. 6. Available from: https://doi.org/10.1038/srep23840.
Wachtel-Galor, S., Benzie, I.F.F., 2011. Herbal medicine an introduction to its history, usage, regulation, current trends, and research needs. In: Benzie, I.F.F., Wachtel-Galor, S. (Eds.), Herbal Medicine: Biomolecular and Clinical Aspects, 2nd ed.) CRC Press/Taylor & Francis, Boca Raton, FL.
Wann, M.-H., 2005. Application of LC-NMR in pharmaceutical analysis. In: Ahuja, S., Dong, M.W. (Eds.), Handbook of Pharmaceutical Analysis by HPLC. Elsevier, Amsterdam, p. 569.
Watkins, R., Wu, L., Zhang, C., Davis, R.M., Xu, B., 2015. Natural product-based nanomedicine: recent advances and issues. Int. J. Nanomed. 10, 6055–6074.
Wolfender, J.L., Rodriguez, S., Hostettmann, K., 1998. Liquid chromatography coupled to mass spectrometry and nuclear magnetic resonance spectroscopy for the screening of plant constituents. J. Chrom. A 794, 299–316.
Zenk, M.H., 1978. The impact of plant cell culture on industry. In: Thorpe, T.A. (Ed.), Frontiers of Plant Tissue Culture. IAPTC, Calgary.

Further reading

Hostettmann, K., 1998. Strategy for the biological and chemical evaluation of plant extracts. Pure Appl. Chem. 70, 1–9.
Klefenz, H., 2002. Industrial Pharmaceutical Biotechnology. Business Horizons Pharmaceutical Publishers, New Delhi.
Kohli, J.P.S., 2009. Dictionary of Pharmaceuticals and Biotechnology. Business Horizons Pharmaceutical Publishers, New Delhi.
Makkar, H.P.S., Sidhuraju, P., Becker, K., 2010. Plant Secondary Metabolites. Humana Press, New York.
Sharma, R., 2004. Agro Techniques of Medicinal Plants. Business Horizons Pharmaceutical Publishers, New Delhi.
Verpoorte, R., 2000. Metabolic Engineering of Plant Secondary Metabolism. Springer., Verlag.

Glossary

Bioactivity Specific effect on, or a reaction in, living being upon exposure to a substance.
Biosynthetic totipotency The inherent potentiality of a plant cell to give rise to a whole plant.
Dedifferentiation The phenomenon of mature cell reverting to the meristematic state and forming undifferentiated callus tissue is termed dedifferentiation.
Plant metabolite *Plant metabolites* are the intermediates and products of *metabolism*. It is usually restricted to small molecules of plant.
Morphogenic The development of form and structure during growth.
Redifferentiation The phenomenon of whole plant formation from undifferentiated callus tissue.
Secondary metabolite Secondary metabolites are organic compounds that are not directly involved in the normal growth, development, or reproduction of the plant but often have an ecological role, such as attractant of pollinators and chemical defense against microorganisms. Humans use secondary metabolites as medicines, flavorings, and recreational drugs.
Traditional medicine Traditional medicine refers to the knowledge, skills, and practices based on the theories, beliefs, and experiences, used in the maintenance of health and in the prevention, diagnosis, improvement, or treatment of physical and mental illness.
Natural product Natural product is a chemical compound or substance produced by a living organism. Natural product often has pharmacological or biological activity for use in pharmaceutical drug discovery and drug design. A natural product can be considered as such even if it can be prepared by total synthesis.

Abbreviations

GC/MS Gas chromatography/mass spectrometry
HPLC High-performance liquid chromatography
LC/MS Liquid chromatography/mass spectrometry
MS Mass spectrometry
NMR Nuclear magnetic resonance
RT Retention time
TLC Thin-layer chromatography
WHO World Health Organization

Long answer questions

1. Write an essay on plant secondary metabolites. Discuss the preponderance of weeds in medicinal flora.
2. Elucidate various steps for study of plants in traditional medicine.
3. What is drug discovery? What are different ways for drug discovery from natural products?
4. Write a detailed account of the tools and techniques of plant tissue culture and highlight the importance of each.
5. Enlist and describe in detail important analytical techniques associated with characterization of medicinal metabolites.

Short answer questions

1. Define the term "secondary metabolites."
2. What is ethnobotany?
3. Differentiate between *characterized* and *uncharacterized* plant extracts.
4. Give the names of three solvents that can be used for the extraction of hydrophilic compounds?
5. Which analytical technique can be used for the separation and identification of volatile compounds? Which spectroscopic technique analyzes functional groups of the compounds?

Answers to short answer questions

1. Secondary metabolites are compounds that are not directly involved in primary metabolic processes of an organism. They generally defend the organisms from environmental stresses and predators.
2. Ethnobotany is the study of how people of a particular region relate to the plants of their environment.
3. Characterized extracts are ones where each component, its concentration, and function are known; for uncharacterized extracts, the entire components of the mixture and the role they play are not known.
4. Methanol, ethanol, and acetone.
5. GC-MS, FTIR

Yes/no type questions

1. In TLC, the extracts are loaded on a glass coated with silica gel or other adsorbent.
2. Primary bioassays are performed when less number of plant extracts are to be screened.
3. HPLC is used for the identification and quantification of unknown compounds.
4. LC-MS gives information on the molecular weight and structure of the analytes.
5. Pulsed field gradient technique is introduced in GC-MS to elucidate structure of oxygen-sensitive compounds.
6. LC-NMR is useful for the identification and separation of isomer compounds.
7. LC-MS is used to analyze thermally unstable compounds.
8. Nonvolatile compounds are separated in GC-MS.
9. SFC is mainly used for the separation and detection of thermolabile and nonvolatile compounds.

10. Production of bioactive compounds is generally higher in unorganized tissues.

Answers to yes/no type questions

1. Yes, silica gel is the most widely used adsorbent and remains the dominant stationary phase for TLC.
2. No, primary bioassays are performed when large number of plant extracts are to be screened because it is easy to perform and cost effective.
3. No, HPLC is used for the identification and quantification of known compounds for which standards are available.
4. Yes, LC-MS gives information on the molecular weight and structure of the analytes by detecting ions.
5. No, introduction of pulsed field gradient technique has improved the NMR to elucidate structure of oxygen sensitive compounds.
6. Yes, LC-NMR can separate and identify isomer compounds without reference compounds.
7. Yes, LC-MS is used to analyze thermally unstable compounds because soft ionization techniques are used in LC-MS.
8. No, GC-MS separate and detect volatile and thermally stable compounds as gas phase is use to elute analytes.
9. Yes, SFC can provide high resolution at low temperature and thus thermolabile compounds are separated and detected easily.
10. No, production of bioactive compounds is generally higher in organized tissues due to the appearance of complex cells and tissues that are metabolically more proficient.

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