Chapter

# Natural Products in Drug Discovery

*Akshada Amit Koparde, Rajendra Chandrashekar Doijad and Chandrakant Shripal Magdum*

## Abstract

Drug discovery using natural products is a challenging task for designing new leads. It describe the bioactive compounds derived from natural resources, its phytochemical analysis, characterization and pharmacological investigation. It focuses on the success of these resources in the process of finding and discovering new and effective drug compounds that can be useful for human resources. From many years, natural products have been acting as a source of therapeutic agents and have shown beneficial uses. Only natural product drug discovery plays an important role to develop the scientific evidence of these natural resources. Research in drug discovery needs to develop robust and viable lead molecules, which step forward from a screening hit to a drug candidate through structural elucidation and structure identification through GC–MS, NMR, IR, HPLC, and HPTLC. The development of new technologies has revolutionized the screening of natural products in discovering new drugs. Utilizing these technologies gives us an opportunity to perform research in screening new molecules using a software and database to establish natural products as a major source for drug discovery. It finally leads to lead structure discovery. Powerful new technologies are revolutionizing natural herbal drug discovery.

**Keywords:** natural products, herbal, drug discovery, phytochemicals, bioactive

## 1. Introduction

Natural products and traditional medicines are of great importance. Natural products and their derivatives have been recognized for many years as a source of therapeutic agents and structural diversity. Natural products have a wide range of diversity of multidimensional chemical structures; in the meantime, the utility of natural products as biological function modifiers has also won considerable attention [1].

Drug discovery is leading to be a challenging scientific task to find robust and viable lead candidates, which is nothing but the process flow from a screening of natural product to a new isolate that requires expertise and experience. However, in addition to their chemical structure diversity and their biodiversity, the development of new technologies has revolutionized the screening of natural products in discovering new drugs [2]. Applying these technologies offers a unique opportunity to reestablish natural products as a major source for drug discovery. The present

article attempts to describe the process of isolation, characterization, and utilization of bioactive compounds derived from natural products as drug candidates called as lead, which focus on the success of pharmacological activity in the process of finding new and effective drug compounds; this process is commonly referred to as "natural product in drug discovery."

Natural products played a vital role on this earth, so man's existence has been made possible. The outstanding phenomenon of nature always stands as golden mark for achieving the herbal drug discovery [3].

From earlier decades medicinal plants existed on earth. Thus, medicinal herbs are of global and paramount importance. The world is decorated with medicinal herbs, which is a rich wealth of endurance. Every plant is identified by its own different therapeutic properties due to active bioactive molecule. In the modern system of medicine, natural drug substances are reported to be vital and have appreciable roles. Their therapeutic role was justified by the presence of their bioactive molecules. Due to disease-inhibiting capabilities, they are extremely useful as natural drugs, provide basic bioactive compounds that are less toxic and more effective, and bring biological and chemical means of modification and extraction of natural products into potent drug.

The raw materials for Ayurvedic medicines were mostly obtained from plant sources in the form of crude drugs such as dried herbal powders or their extracts or mixture of products. Apart from these systems, there has been a rich heritage of ethnobotanical usage of herbs by various colorful tribal communities in the country [4].

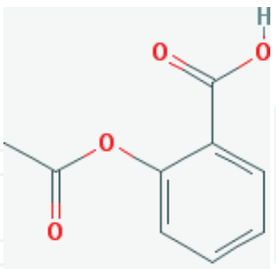
It has been estimated that nearly 75,000 species of higher plants exist on the earth, and only 10% have been used in traditional medicine. Only 1 to 5% have been studied scientifically and are known to have therapeutic value [5].

Around the globe, herbal medicine is based on traditional medicine. As per the oral survey made in many regions of the world, it has been said that traditional medicines have their own importance and basic philosophy. So exploration of the chemical constituents of the plants and their pharmacological screening may provide us the basis for developing a lead molecule through herbal drug discovery. The very important life-saving drugs have been provided by herbs in modern medicine. But among the estimated 4-lakh plant species, only 6% have been studied for their activity and very less not more than of 20% have been investigated phytochemically [6]. Thus, there is a need of investigating the various bioactive fractions and the phytoanalysis and phytopharmacological evaluation of herbal drugs for achieving the dreams of herbal drug discovery.

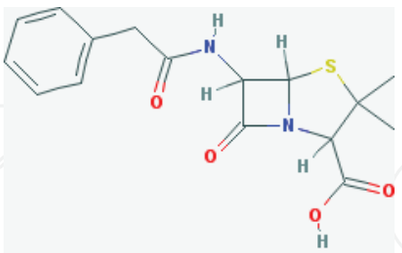
Working role of every green herbal drugs from plant source and synthesis of bioactive products in their own way as God's gift and preserve them within which are extractable and used raw material as and when required through various scientific process for various scientific investigations and study of herbal drug discovery. Many pharmaceutical compounds contain secondary metabolites of plants that are of vital importance in drug designing. However, in order to have a good supply of the source material, some factors like environmental changes, diverse geographical distribution, labor cost, and selection of the superior plant should be taken care of by green plant developers so that good plants will be beneficial to pharmaceutical industry to develop good-quality herbal drugs [7].

Natural products have played, and will continue to play, a key role in drug discovery and are therefore traditionally claimed as the cornerstones of drug discovery and development. Many drugs that are available in market today were discovered from natural sources [8]. An important example is the analgesic activity of aspirin [22],

which is so far the world's best known and most universally used medicinal agent. Its origin is from the plant genera *Salix* spp. and *Populus* spp. and it is related to salicin. A good example is serendipitous discovery of the antibiotic penicillin [22] in the laboratory from the fungus *Penicillium notatum*.

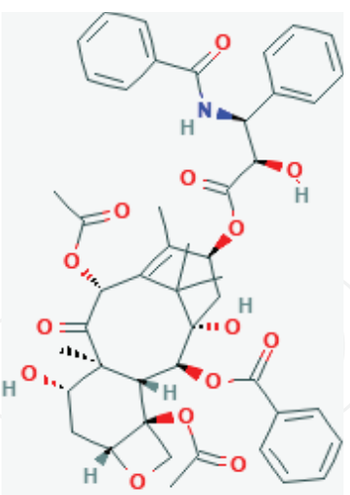


Aspirin [22]



Penicillin [22]

Many other examples show the value and importance of natural products from plants and microorganisms in modern days. Paclitaxel (Taxol [22]), which was first isolated from the bark of the Pacific yew tree *Taxus brevifolia* (Taxaceae), is the most recent example of an important natural product that has made an impact in medicine. Activity against a variety of retroviruses, including HIV, two compounds isolated from *Hypericum perforatum* (Guttiferae) are hypericin and pseudohypericin. They are of paramount importance due to inhibition of release of reverse transcriptase by stabilizing the structure of the HIV capsid and thus preventing the uncoating process [9, 10].



Taxol [22]

In four different ways, medicinal plants having good therapeutic properties are valuable for modern system of herbal and natural drug discovery.

1. They are used as direct sources of therapeutic and bioactive agents.

2. Bioactive fractions serve as raw material base for the elaboration and development of herbal-based more complex semisynthetic chemical compounds.

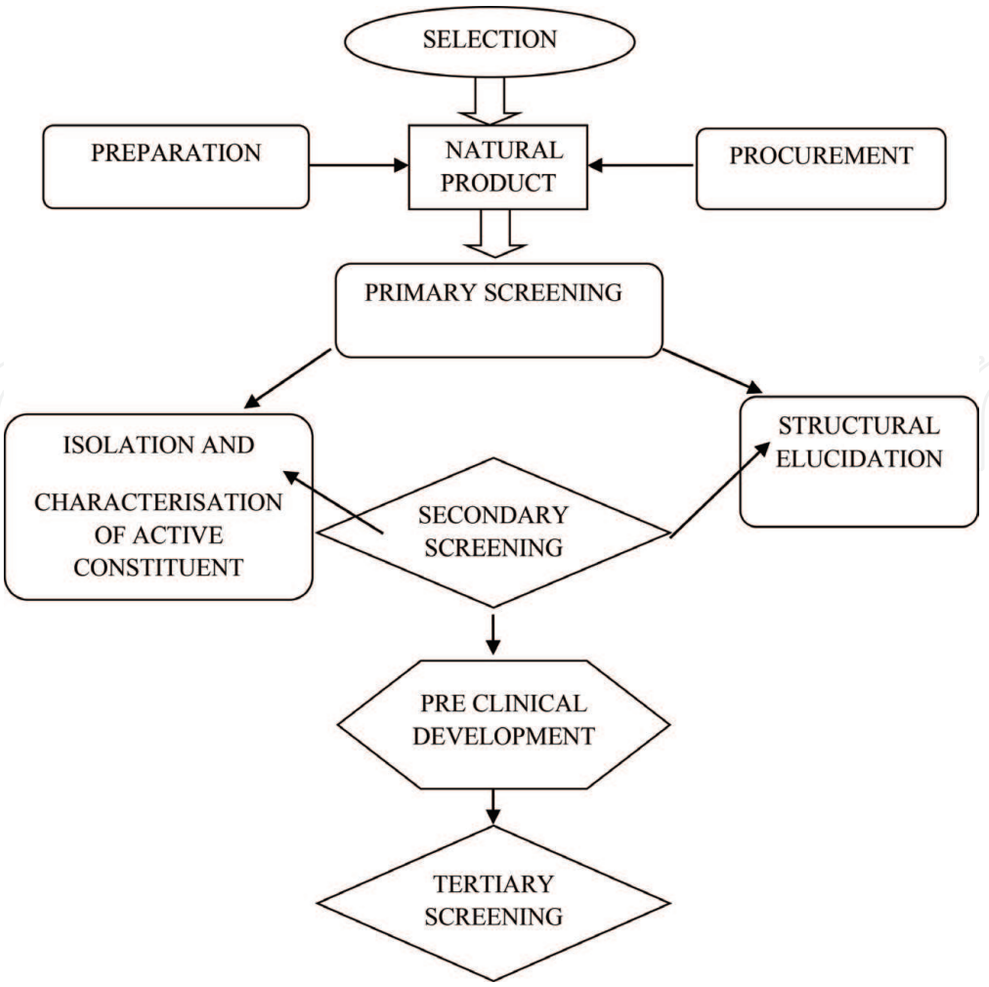



**Figure 1.**
*Various strategies for the discovery of drugs from natural resources.*

3. The isolated structures derived from herbal plant species can be used as lead for new drug discovery in developing herbal compounds.

4. Lastly, plants can be used as bioactive markers for the spectroscopic and chromatographic analysis along with the discovery of new compounds.

Various strategies for the discovery of drugs from natural resources can be seen in **Figure 1**.

## 2. Important steps for successful completion of natural drug discovery

Phytochemistry or phytoanalysis of natural product in chemistry research is the backbone and pillar of herbal pharmaceutical as well as food industry. To achieve success in natural drug discovery and use of herbals in modern medicine, the steps to be followed are listed below [11]:

1. Extraction, isolation with chromatographic separation, purification, and characterization of new phytoconstituents having good bioactivity

2. Use of newly isolated phytoconstituents as "lead" compound for designing of new analogues with either improved therapeutic activity or reduced toxicity

3. Conversation of lead phytoconstituents into medicinally important drugs by herbal drug discovery and herbal drugs used by common people showing socioeconomic benefit

## 3. Practical outlook of herbal drug discovery

The following facets represent outlook of the stages involved in the development of bioactive molecule as pure drug from a plant source [12].

1. Collection and identification of the plant, authentication, and deposition of sample in herbarium like the botanical survey of India

2. Literature survey and analysis on the plant species along with the activity present in the selected plants for studies

3. Extraction of nonpolar to polar solvent and preparation of extracts for phytochemical analysis and their biological testing [13]

4. Evaluation of plant extracts by judging of different biological test methods

5. Chromatographic analysis by activity-guided fractionation of the extract, monitoring each chromatographic fraction, its isolation calculating Rf values,area as per the computer based software's and comparison with available bioactive markers which leads to the investigation

6. Structure elucidation using spectroscopic techniques of bioactive isolates using chemical methods

7. Testing of each bioactive compound in all in vitro and in vivo phytopharmacological test methods, in order to determine potency and selectivity of the herbal extract or isolates for the discovery of herbal drugs

8. Performing molecular modeling studies and preparing derivatives of the active compound of interest

9. When total synthesis is not practical, carrying out large-scale reisolation of interesting active compounds for toxicological and pharmacological studies

10. Clinical trials (phase I–III).

First of all, in order to study medicinal plants, selection of plant and which type of pharmacological activity is to be studied should be clear to the researcher. Five principles of selection of plants are very important to know which are the random, the taxonomic, the phytochemical, the ethno-medical and the information-managed approach (**Figure 2**) [14].

- In the random selection, collection of all available plants in the area, which is to be studied, is collected based only on visualization and observation without having knowledge and experience about the selected plants.

- In the taxonomic approach, prior knowledge about the plants of interest with their specific genus or family and their different locations should be known.

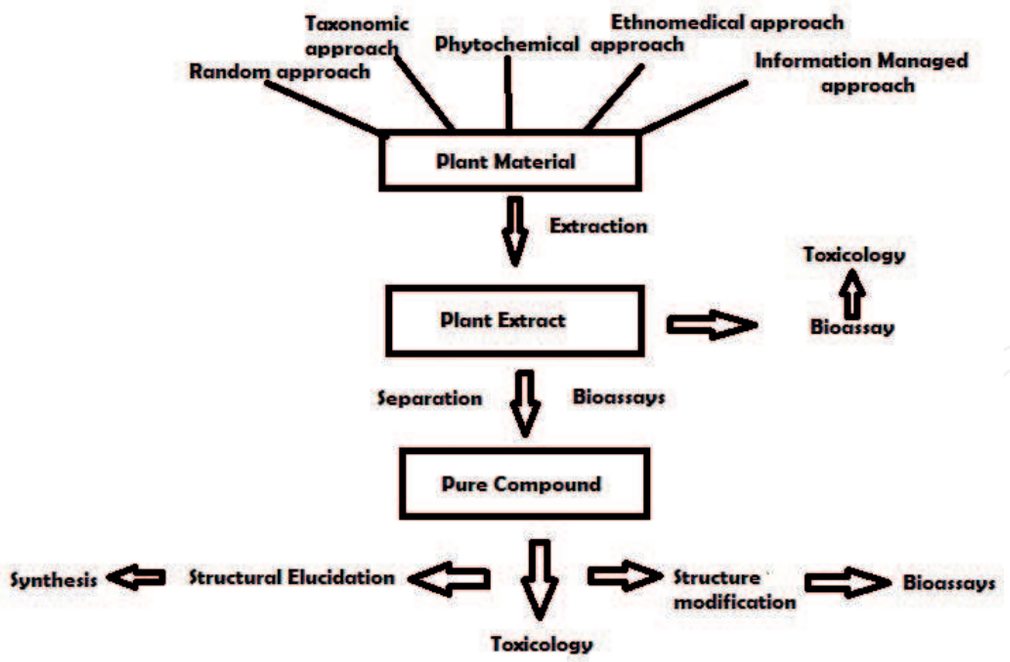



**Figure 2.**
*General procedure for obtaining active principles from plants.*

- The phytochemical (chemotaxonomic) approach is based on the knowledge of bioactive chemical type for treating particular disease of interest should be known and are collected. Taxonomic and the phytochemical approaches are interrelated.

- In the ethnomedical approach, selection is totally based on the information of the medicinal use of that particular plant in various areas.

- Lastly, information managed approach is basically collection of plants based on survey and use of plants from their local area that gives prior idea about their usage and activity and then their evaluation scientifically.

## 4. Position of herbal drugs

In the current era, new and newer diseases are causing threat to common people around the world. Thus, disease percentage differs in every part of the world, but diseases are not new; due to global warming, they are detected newly. Prevention is better than cure, so WHO had taken the vouch of providing "Health for all" by 2000 AD.

Multidisciplinary research on plants has led to many new drugs, as well as prototype active molecules and biological tools; for examples, see [14].

## 5. Natural drugs available in market: anti-inflammatory

### 5.1 Himalaya *Boswellia*

Himalaya herbals are developed herbal product from Boswellia, which are a pure herb extract. The bioactive molecule constituent in the gum resin of Shallaki or *Boswellia serrata* was boswellic acid. Pyrazoline as a lead molecule is present in boswellic acid. It acts through the mechanism of supporting the body's natural

immune response and preventing inflammation and providing healthy joints and muscle. *Boswellia* is a natural and safe herb for joint health, as it gently cares for it. *Boswellia* is a good promoter of healthy cholesterol and triglyceride levels and provides broad health and immune-modulating benefits. *Boswellia* has been used extensively in Ayurveda for arthritis and to provide an overall sense of well-being.

### 5.2 Ginger

From long years ago, herbal medicine has paid hats off to ginger due to its ability to boost the immune system. It is believed that ginger is used in day-to-day life because it plays an important role in warming the body. It can help to clean our body from accumulated toxins by its break down in your body. It's also known to cleanse the lymphatic system, our body's sewage system. Ginger prevents the accumulation of toxins and a person's body is highly safe guarded from viral, fungal, and bacterial infections. Medicinal plant ginger also shows many health benefits. It is specially used as natural remedy for nausea and pain alleviation and for its anti-inflammatory properties and inhibiting diabetes.

### 5.3 Licorice root

Licorice is becoming evident and lighten up in various researches for treatment and prevention of diseases like hepatitis C, HIV, and influenza. From a study, it confirms the antiviral activity of licorice root due to its triterpenoid content. It notes that licorice's antioxidant, free radical-scavenging and immuno-stimulating effects. Licorice root benefits also include pain relief.

### 5.4 Olive leaf

The olive leaf has antiviral properties, giving it the ability to treat the common cold and dangerous viruses.

### 5.5 Oregano

Oregano oil benefits are lightening up to be more superior to some antibiotics, with no harmful side effects on health, and can be used in day-to-day life. Carvacrol and thymol are the bioactive molecules isolated and studied and reported to have powerful properties and uses. They act upon viral infections, as well as allergies, tumors, parasites and disease-causing inflammation.

## 6. Future avenues in herbal drug discovery

In the current era, in many developed countries, priorities has been given to scientific research on medicinal plants is growing need of an hour in various research institutes, universities and pharmaceutical laboratories as well as in the clinics thereof. This research is put forward in mainly two directions: first, bioactive molecule of plants that have long been known and used for their healing properties based on the prior knowledge of the survey and literature. The second phase of basic research has led to the discovery of new medicinal plants with new bioactive molecules, new bioactivity, and new drugs from the more remote regions of the world [15].

Drugs of Ayurveda, Unani, and Siddha need scientific investigation of each and every traditional medicine, which should be put forward for testing and validation. Many government and private companies like CSIR, New Delhi, are already

involved in this filed and have validated about thousands of formulations for different activities. This is a welcome trend and it plays a vital role to correlate the traditional practice with modern knowledge for the betterment of health. WHO has emphasized the need to ensure the quality control of herbs and herbal formulations by using modern techniques. Almost many countries have their own herbal pharmacopeias and make time to time amendments for new monographs and procedures to maintain their quality of herbal products that are benefited by common man. Example Ayurvedic pharmacopeia of India includes many basic quality parameters, isolation techniques, separation, and spectroscopic identification for more than hundred common herbal drugs.

## 6.1 Analytical methodologies

It plays an eminent role in herbal natural drug discovery, and without analytical methodologies, it is hardly impossible. Spectroscopic characterization is the backbone and pillar of herbal drug discovery. The knowledge of this plays an important role in developing the new lead, which can be used for designing new molecules with short modification. The important steps are the extraction, isolation, and characterization of active ingredients from herbal plants [16]. Different techniques of extraction are well known as extraction is the most important step toward the analysis of bioactive constituents. Microwave-assisted extraction and conventional extraction should be studied specifically, which give the ideas about the yield obtained. Further, it highlights the isolation of active molecules by chromatographic techniques like TLC, column chromatography. The most important step toward analysis of bioactive compounds present in the plant extracts is characterization, which includes phytochemical screening assays that give ideas about the presence of secondary metabolites used to cure the health problems. Highly sophisticated techniques for structure identification of lead molecule bioactive fraction are high-performance liquid chromatography (HPLC), high-performance thin-layer chromatography (HPTLC), Fourier-transform infrared spectroscopy (FTIR), nuclear magnetic resonance (NMR), and gas chromatography–mass spectrometry (GC–MS). These techniques are the heart and key challenges in research of natural drug discovery giving rise to natural products in drug discovery.

## 6.2 Isolation, identification, and characterization of phytochemicals

The combination of various types of bioactive compound or phytochemicals is usually present in different plant extracts. The different bioactive compounds have different polarities. Separation, identification, and characterization of bioactive compounds are a big challenging job in the herbal drug development process.

## 6.3 Phytochemical screening assay

Phytochemical screening assay is a simple, quick, and inexpensive procedure that tells about various types of phytochemicals in a mixture and an important tool in bioactive compound analyses. Phytochemical examinations are carried out for all extracts as per the standard methods [15].

### *6.3.1 Tests for carbohydrates*

Preparation of test solution: the test extract was prepared by dissolving with water. Add 1 volume of 2 N HCl so that it gets hydrolyzed and is further subjected to the following chemical tests:

- Molisch's test (general test): take 2 ml of extract, add two drops of α-naphthol solution in the alcohol and shake, and then add five drops of concentrated $H_2SO_4$ to the sides of the test tube to observe violet ring at the junction of two liquids.

- Fehling's test: in a test tube, add 1 ml Fehling's A and 1 ml Fehling's B solutions and mix and boil for 1 min. Add equal volume (2 ml) of test solution. Heat in boiling water bath for 5 min. Observe for yellow and then brick red precipitate.

- Benedict's test: add 1 ml of Benedict's reagent and 1 ml of test solution in a test tube and then mix well. Heat in boiling water bath for 5 min. The solution may appear green, yellow, or red depending on the amount of reducing sugar present in test solution.

- Barfoed's test: add 1 ml of Barfoed's reagent and 1 ml of test solution in the test tube. Heat for 1–2 min, in boiling water bath, and cool. Observe for red precipitate.

*6.3.2 Tests for proteins*

- Biuret test (general test): for a 2 ml test solution, add two drops of 4% NaOH and two drops of 1% $CuSO_4$ solution, and observe for violet or pink color.

- Millon's test (for proteins): add 2 ml of TS and mix with 4 ml of Millon's reagent; observe for white precipitate. Precipitate if warm, turns brick red or precipitate dissolves giving red color.

- Xanthoproteic test (for protein containing tyrosine or tryptophan): mix 2 ml TS with 0.5 ml concentrated $H_2SO_4$, and observe for white precipitate.

*6.3.3 Tests for amino acids*

- Ninhydrin test (general test): add 2 ml TS and two drops of 5% ninhydrin solution, and heat in boiling water bath for 5 min. Observe for purple or bluish color.

- Test for tyrosine: add 2 ml TS and two drops of Millon's reagent. Heat the solution and observe for dark red color.

- Test for tryptophan: add 2 ml of TS and 2 drops of glyoxylic acid and concentrated $H_2SO_4$ and observe for reddish violet ring at the junction of the two layers.

- Test for cysteine: add 2 ml of TS and few drops of 40% sodium hydroxide and 10% lead acetate solution. Boil. Black ppt. of lead sulfate is formed.

*6.3.4 Tests for steroid and triterpenoid*

- Salkowski reaction: add 3 ml of extract, 3 ml of chloroform, and 3 ml of concentrated $H_2SO_4$ in a test tube; shake well; and observe whether chloroform layer appeared red and acid layer showed greenish yellow fluorescence.

- Liebermann-Burchard reaction: add 2 ml extract with 2 ml of chloroform and add 1–2 ml acetic anhydride and 2 drops of concentration $H_2SO_4$ from the side of test tube. Observe for first red, then blue, and finally green color.

- Liebermann's reaction: add 3 ml of extract with 3 ml acetic anhydride. Heat and cool. Add few drops of concentrated $H_2SO_4$ and observe for blue color.

*6.3.5 Tests for glycosides*

Preparation of test solution: the test solution was prepared by dissolving extract in the alcohol or hydro-alcoholic solution.

a. Tests for cardiac glycosides:

- Baljet's test: add a test solution with 1 ml of sodium picrate and observe for yellow to orange color.
- Legal's test (for cardenolides): to 1 ml of test solution, add 1 ml pyridine and 1 ml sodium nitroprusside. Observe for pink to red color.
- Test for deoxysugars (Keller-Kiliani test): to 2 ml extract, add 0.5 ml glacial acetic acid, one drop of 5% $FeCl_3$, and concentrated $H_2SO_4$. Observe for reddish brown color at the junction of the two liquid and upper layers bluish green.
- Liebermann's test (for bufadienolides): add 2 ml extract to 2 ml acetic anhydride. Heat and cool. Add few drops of concentrated $H_2SO_4$ and observe for blue color.

b. Tests for saponin glycosides:

- Foam test: the extract was mixed with water and shaken vigorously. Persistent foam was observed.
- Hemolytic test: add test solution to one drop of blood placed on the glass slide. Hemolytic zone appears.

c. Tests for anthraquinone glycosides:

- Borntrager's test: to 3 ml extract, add dil. H2SO4. Boil and filter. To cold filtrate, add equal volume benzene or chloroform. Shake well. Separate the organic solvent. Add ammonia. Ammoniacal layer turns pink or red.
- Modified Borntrager's test: to 3 ml extract, add 3 ml 5% FeCl3 and 3 ml dil. HCl. Heat for 5 min in boiling water bath. Cool and add benzene, shake well, and separate organic layer. Add equal volume dil. ammonia in organic layer. Ammoniacal layer shows pinkish red color.

*6.3.6 Tests for flavonoids*

Flavonoids are present in hydrolyzed plant extracts. Its presence is maximum in parts of the leaves and they are highly soluble in methanol. The flavonoids are all derived structurally from the important substance called flavone. The flavonoids occur in the free form as well as bound to sugars as glycosides. Flavonoids are found maximum in herbal plants and have good phytopharmacological activities.

Preparation of test solution:

i. To 1 ml of extract, equal volume of 2 M HCl was added and heated in a test tube for 30 to 40 min. at 100°C.

ii. The cooled extract was filtered, and extracted with ethyl acetate.

iii. The ethyl acetate extract was concentrated to dryness and used to test for flavonoids.

- Shinoda test: to 2 ml of extract, add 5 ml of 95% ethanol, 5 drops of concentrated HCl, and 0.5 g magnesium turnings. Pink color was observed. To small quantity of residue, acetate solution was added and observed for yellow colored precipitate. Addition of sodium hydroxide to the residue showed yellow coloration, which was decolorized after addition of dilute hydrochloric acid.

- Ferric chloride test: to 2 ml of test solution, add few drops of ferric chloride solution, which shows intense green color.

- Alkaline reagent test: 2 ml of test solution was treated with 2 ml of sodium hydroxide solution, which showed intense yellow color that became colorless on addition of few drops of dilute hydrochloric acid.

- Lead acetate solution test: 2 ml of test solution with few drops of lead acetate solution (10%) gives yellow precipitates.

*6.3.7 Tests for alkaloids*

- Mayer's test: test solution treated with Mayer's reagent (potassium mercuric iodide); cream colored precipitate was not obtained.

- Wagner's reagent: the test solution treated with Wagner's reagent (iodine in potassium iodide); brown precipitate was not obtained.

- Hager's test: the test solution treated with Hager's reagent (saturated picric acid solution); gives yellow precipitate.

- Dragendorff's test: the test solution treated with Dragendorff's reagent (potassium bismuth iodide); reddish brown precipitate was not obtained.

*6.3.8 Tests for tannins and phenolic compounds*

To 2–3 ml of extract, add few drops of the following reagents:
5% $FeCl_3$ solution shows deep blue-black coloration.
Addition of lead acetate solution shows white precipitate.
Addition of gelatin solution shows white precipitate.
Addition of bromine water shows decoloration of bromine water.
Addition of acetic acid solution shows red colored solution.
Addition of dilute iodine solution shows transient red color.
Addition of dilute $HNO_3$ shows reddish to yellow color.
Addition of dilute $KMnO_4$ shows disappearance of Pink color.

## 7. Chromatography techniques

Chromatography is a technique where the molecules are separated based on their shape, size, and charge. In any extract, there are hundreds of unknown components and many of them are in very low amount. During chromatography, analyte in

solvent and move through solid phase that acts as a sieving material. As molecule proceeds further through molecular sieve, it gets separated. Moreover, there usually exists variability within the same herbal materials. Hence, it is very important to obtain reliable chromatographic fingerprints that represent pharmacologically active and chemically characteristic components of the herbal medicine. Thin layer chromatography is a chromatographic technique that readily provides qualitative information and through which it becomes possible to obtain quantitative data [17].

### 7.1 Thin layer chromatography (TLC)

Stahl gave the first practical application of thin layer chromatography. TLC is a most versatile technique and it shows its separation with good speed. Advantage of TLC is its sensitivity. TLC works on the principle of an adsorption chromatography in which samples were separated. Separation is based on the interaction between a thin layer of adsorbent attached on the plate and solvent system. The technique is mostly used for the separation of low molecular weight compounds. Many different adsorbents are used in TLC like silica gel, aluminum, cellulose powder, starch, etc. and can be used to separate various compounds like amino acids, alkaloids, phenols, steroids, vitamins, etc.

It is being implemented extensively due to the following reasons:

1. It carries out good speedy separation and rapid analysis of herbal extracts.

2. It shows with minimum sample clean-up requirement.

3. It has the ability for calculating qualitative and semiquantitative information of the separated compounds with Rf values.

4. It enables the quantification of chemical constituents (**Table 1**).

### 7.2 High performance thin layer chromatography (HPTLC)

HPTLC is a more powerful separation tool for quantitative analysis and it uses the technique in a more optimized way. High performance thin layer chromatography (HPTLC) is based on the principle of planar chromatography where separation of sample components is achieved on high performance layers with detection and data evaluation. These high performance layers on TLC plates are precoated with an adsorbent of 6 micron particle size and a 160 microns layer thickness. The lesser the thickness of layer and particle size results in increased plate efficiency as well as nature of separation. HPTLC has an ability to show its performance on graphical representation in the form of chromatogram. Separation can be easily visualized by pictorial representation, which is possible only in case of HPTLC. The procedure used is as follows: silica gel 60 F254 precoated plates (20 × 10 cm) are used with any developed solvent system. Different extracts are to be spotted on precoated HPTLC plates. Spots of different concentration (l μL) was applied on HPTLC plates to study the exact separation of spots. Saturation time will be 20 min and room temperature 25°C ± 2°C. TLC plates were developed up to 8 cm. After air drying, a plate was heated at 110°C for 2–3 min. In TLC fingerprinting analysis, the information can be stored and recorded using specific highly sophisticated instruments like high performance TLC scanner. It gives information about the chromatogram, retardation factor (Rf) values, the color of the separated bands, their absorption spectra, and λ max. After derivatization and using different visualization reagents, snaps of TLC plates can be obtained and saved for further process. Thus, this represents TLC fingerprint profile of the provided sample. The information so generated has

| Plant constituents | Stationary phase | Mobile phase | Detection |
|---|---|---|---|
| Carbohydrates | Silica gel | Ethyl acetate:toluene(1:1) | 10% ethanolic sulfuric acid |
| Alkaloids/phenanthrenes | Silica gel | Toluene:ethyl acetate:diethylamine(7:2:1) | Dragendorff reagent |
| Flavonoids | Silica gel | Ethyl acetate:formic acid:glacial acetic acid:water(10:1.1:1.1:2.6) | UV 254 nm or 366 nm |
| Tannins | Silica gel | Ethyl acetate:formic acid:glacial acetic acid:water(7.5:0.3:0.2:2) | Vanillin sulfuric acid reagent |
| Saponin glycoside | Silica gel | Chloroform:glacial acetic acid:methanol:water(6.4:3.2:1.2:0.8) | Vanillin sulfuric acid reagent |
| **Specific Mobile phases** | | | |
| Betasitosterol | Silica gel | Benzene:ethylacetate(9:1) | Vanillin sulfuric acid reagent |
| Rutin | Silica gel | Ethyl acetate:formic acid:glacial acetic acid:water(10:1.1:1.1:2.6) | UV 254 nm or 366 nm |
| Curcumin | Silica gel | Chloroform:methanol(9.8:0.2) | Visible light |
| Gingerol | Silica gel | Toluene:ethylacetate(9.3:0.7) | Vanillin sulfuric acid reagent |
| Stigmasterol | Silica gel | Petroleum ether:ethyl acetate(7:3) | Vanillin sulfuric acid reagent |

**Table 1.**
*TLC mobile phase for important classes of phytoconstituents [15].*

a potential application in the identification of an authentic drug when compared with bioactive marker and it helps in maintaining the quality and consistency of the isolates or herbal drugs in natural drug discovery [18].

### 7.3 Column chromatography (CC)

Column chromatography works on the principle of ion exchange, molecular sieves, and adsorption phenomenon. CC is a most useful technique for separation of active constituents with larger concentration. Sometimes fractions require another step for concentration. Displacement chromatography is a newer method that contains elution of bioactive compounds that have great affinity for the adsorbent. Fractions of elute materials can be more concentrated than the original solution placed in column. The column was prepared using silica for column chromatography. The fraction was dissolved in smallest possible volume of solvent and it was mixed with 2 gms of silica for column chromatography. Wet column or dry column packing can be done. Packing of column plays an important role in good quality separation. The mixture was dried to obtain free flowing powder and it was added to column. Then, the column was eluted with solvent of various proportions. Every eluent was collected in properly cleaned test tube separately for further studies to be carried out.

### 7.4 High performance liquid chromatography (HPLC)

High performance liquid chromatography (HPLC) plays a mandatory role in isolation of natural products. It is a versatile and widely used technique for the isolation and identification. In the modern era, HPLC technique is becoming popular for studying separation, identification, and fingerprinting study for the quality control

of herbal plants. Currently, this technique works as the main choice for research scientists. The multicomponent samples on both an analytical and preparative scale can be separated and studied more easily by HPLC. Thus resolving power of HPLC is ideally used for the rapid processing of herbal extracts. HPLC instruments are designed in modular ways and they contain delivery pump for solvents and manual injection valve along with an auto-sampler. As sample is introduced in autosample, it carries toward the important part or heart of HPLC that is an analytical column, a guard column. Further, a detector, recorder, and printer are used to show a graphical representation on the software based or installed computer device. In every chemical separation, the working and result production differ due to the fact that certain compounds have different migration rates, which can be fulfilled using HPLC by utilizing a particular column and mobile phase as per the requirement for separations. Trial and error concept is applied for developing new mobile phase along with prior knowledge of separation, its structure, and required solvent polarity or nonpolarity. Thus, the extent or degree of separation is based upon the choice of stationary phase or mobile phase. Generally, the identification and separation of phytochemicals can be achieved by using isocratic system that is using single mobile phase. Gradient elution sometimes can be used in which the proportion is altered from organic solvent to water. It also depends on time, and it may be desirable if more than one sample component is studied. Or it also differs from each other significantly in retention of components with column as per the conditions achieved. Identification of compounds by HPLC is a crucial part of HPLC assay. Identification of any bioactive compound by HPLC selection of detector is again the next important step. Once the detector is selected and the setting is done, the assay may be developed by trial and error of solvent system. Once the sharpness of the peak of known sample is obtained, the solvent system can be selected. The important parameters of this assay is that a clean sharp peak of the known sample is observed from the chromatograph. The reasonable retention time of identifying peak should be there. The extraneous peaks at the detection levels should be well separated from the main sharp peak. At maximum time, UV detectors are popularly used in HPLC detection. UV detectors are used among all the detectors because they have high sensitivity and UV absorbance of majority of naturally occurring compounds is possible at low wavelengths of 200–210 nm. If bioactive compound needed to be isolated is only present in small amounts within the sample, then the high sensitivity of UV detection is a bonus in herbal natural drug discovery. Liquid chromatography coupled with mass spectrometry (LC/MS) is also a powerful technique for the analysis of complex botanical extracts. It offers accurate determination of molecular weight of proteins, peptides. Isotopes pattern can also be detected by this technique. A recent advance includes electrospray, thermospray, and ionspray ionization techniques, which offer unique advantages of high detection sensitivity and specificity [19].

HPLC when combined with mass spectrometry (MSn) gives lot of information for structural elucidation of the compounds because its ability of recognition increases and separation with structure identification becomes very easy. Therefore, when a biomarker which is of a pure standard is unavailable, fast and accurate identification of bioactive chemical compounds in medicinal herbs is possible due to the combination of HPLC and MS. In order to count the overall success of natural product in isolation and separation, the most important is processing of raw material further to provide a sample suitable for HPLC analysis. The significant bearing is on the choice of solvent for sample active compounds identification. The source material that is dried powdered herbal plant material should be studied very efficiently in earlier stages and steps: first, its dried form and second to learn about its chemical structural part that is the powder's ability to release the bioactive

compound of interest into the solution. In such cases, mobile phase development initially using TLC and having idea about the solvent system before applying to HPLC saves your time. Thus, in normal case of extraction, dried plant material is treated with an organic solvent methanol, chloroform. After extraction, extracts are dried over rotary evaporator and powdered extracts are concentrated and injected into HPLC for separation and analysis. HPLC is useful for compounds that cannot be vaporized or that decompose under high temperature, and it provides a good complement to gas chromatography for detection of compounds.

## 8. Methods of detection

### 8.1 Fourier-transform infrared spectroscopy (FTIR)

FTIR has proven to be a valuable tool for the characterization and identification of compounds or functional groups (chemical bonds) present in an unknown mixture of plant extracts. It helps for identification and structure determination of the molecule. In addition, FTIR spectra of bioactive compounds are usually so unique that they are called as a molecular "fingerprint." Once the isolation of bioactive compound is possible, then drying of extract and isolates using rotary evaporator is done. Dried powdered plant extract spectrum can be obtained from FTIR. FTIR software contains library of known compounds, and thus, the spectrum of an unknown compound can be identified by its comparison. Preparation of samples for FTIR analysis can be done in different ways. In earlier years, solid herbal plant extract powder was milled with potassium bromide (KBr) with good trituration techniques and then compressed into a thin pellet, which can be analyzed. Now due to new advancements, only solid or liquid sample is available and you only have to place one drop or one pinch of sample between two plates and the drop or sample forms a thin film between the plates. It is the easiest way for performing FTIR, and graphs and wave number are recorded by using computer-based software. The region in IR spectrum above 1200 $cm^{-1}$ shows spectral bands or peaks due to the vibrations of individual bonds or functional groups under examination. The region below 1200 $cm^{-1}$ is known as the 'fingerprint region.' It indicates bands due to the vibrations of the complete bioactive molecule. Complexity of compounds is seen in fingerprint region. Intensities of the various bands in FTIR are recorded specifically on a simple scale as strong (S), medium (M), or weak (W). And as per new techniques developed, the advanced instruments of company bruker, jasco has made easier by application of one drop or pinch of sample on the instruments and this software will give the results. Lastly, the advantage is that samples can be reused.

### 8.2 Mass spectrometry (MS)

Mass spectrometry plays a vital role and works as a powerful analytical technique. It is the only technique used for identification of unknown compounds for its molecular weight. Thus, the quantification of known compounds and elucidation of the structure and chemical properties of molecules are possible due to MS. The most powerful MS spectrum gives an idea about the molecular weight of sample, which can be determined. The value of the technique is that it requires only microgram amounts of material and that it can provide an accurate molecular weight and that it may yield a complex fragmentation pattern, which is often characteristic of that particular compound. This technique works successfully for the structural elucidation of herbal extracts and organic compounds, for peptide or oligonucleotide sequencing. MS helps in monitoring the characterization of compounds in

complex mixtures with a high specificity by defining both the molecular weight and a diagnostic fragment of the molecule simultaneously. Gas chromatography equipment can be directly coupled with rapid scan mass spectrometer (GCMS) of various types. High resolution analysis can be performed due to coupling of equipment.

Liquid chromatography–mass spectroscopy (LC–MS) offers accurate determination of molecular weight of proteins and peptides. Isotopes pattern can also be detected by this technique. Recent advances include electrospray, thermospray, and ionspray ionization techniques, which offer unique advantages of high detection sensitivity and specificity.

## 8.3 Nuclear magnetic resonance spectroscopy (NMR)

Nuclear magnetic resonance spectroscopy gives physical, chemical, and biological properties of matter. $C^{13}$ NMR is used to identify the types of carbon present in the compound. H1-NMR is used to find out the types of hydrogen present in the compound and to find out how the hydrogen atoms are connected. Proton NMR spectroscopy basically works on principle by measuring the magnetic moments of its hydrogen atoms and it provides a method for determining the structure of an organic compound. In almost all compounds, hydrogen atoms are present, which are attached to different groups such as -CH2-, -CH-, -CHO, -NH2, -CHOH-, etc. The graphical representation of proton NMR spectrum provides a record of the number of hydrogen atoms in these different situations. It gives information only about the number of hydrogen atoms in the compound but not the number of carbon atoms. However, direct information on the nature of the carbon skeleton of the molecule can only be obtained by carbon 13 NMR spectroscopy. 13C-NMR spectroscopy works hand in hand with proton NMR and thus the combination of the results of two methods provides very useful information for identification of unknown compound. It is a powerful means of structural elucidation for new terpenoids, alkaloids, or flavonoids. It is also useful in the identification or analysis of glycosides, in indicating the linkage between sugar moieties and their configurations. Many proteins or other macromolecules can be identified by both proton and 13C-NMR. For NMR analysis, very small amount of sample is needed for analysis and that sample can be reused for further analysis. For examples, NMR instrument cost much so there are many sophisticated analytical instrumentation technical analysis are available to perform your research work. Scientist handling NMR has good hands on working of NMR. In order to get high resolution, the new technique is liquid chromatography–nuclear magnetic resonance (LC-NMR). It is a combination of chromatographic separation technique for isolation of active fraction and its number of hydrogen or carbon atom identification with NMR spectroscopy. It is one of the most powerful and time-saving method for the separation and structural elucidation of unknown compounds and mixtures, especially for the structure elucidation of herbal plant extracts and their isolates in herbal natural drug discovery.

## 8.4 Development of new technologies in natural drug discovery research

Nature is a God's gift for finding new herbal drugs for carrying out research scientifically. A new driving force for screening of novel drugs, biologically active metabolites from these products derived from nature which leads to the success of drugs. The chemistry is a branch where the new technologies are emerging in which pharmaceutical chemistry is very important because it deals with the health of common people. Combinatorial chemistry, high-throughput screening, bioinformatics, proteomics, and genomics are newer techniques that have emerged widely in the field of pharmaceutical discovery research. All drug discovery research and

technologies have enormous potential to make use of the chemical and natural diversity of products. Newly developed techniques are growing rapidly with good outputs in natural drug discovery [20]. These include molecular diversity, compound-library design, protein 3D structures, NMR-based screening, 3D-QSAR in modern drug design, physicochemical concepts, and computer-aided drug design using different software, its prediction of drug toxicity, and metabolism [21]. New approaches to improve and accelerate the joint drug discovery and development processes are expected to take place mainly from the innovation in drug target elucidation and lead structure discovery. Powerful new technologies are revolutionizing drug discovery. Some software will be useful in performing studies, which are freely available such as Zinc.docking.org. It is used for calculating SWISSADME, drug properties, SMILES formula, drug likeness properties, and many more [8].

Technologies for drug discovery advanced and diversified greatly [22]. NPDD (natural product drug discovery) activities work hand in hand and make use strongly with HTS, combinatorial chemistry, and genomics. New approaches have proved to show improvement and accelerate the joint drug discovery and development processes. New techniques are emerging and take place mainly from the innovation in drug target elucidation. It finally leads to lead structure discovery. Powerful new technologies are revolutionizing natural herbal drug discovery.

### 8.5 High-throughput screening

High-throughput screening (HTS) is a specially deigned technique in herbal drug discovery that is a standard method for hit discovery based on identification through stored libraries. HTS is relevant to the fields of biology and chemistry and helps for scientific experimentation especially used in drug discovery.

HTS is using data processing and control software and sensitive detectors that help researchers to carry out the research scientifically for designing and developing new structure from herbal drug discovery. It is robotics and allows a researcher to quickly conduct various biochemical, genetic, or phytopharmacological tests. Through HTS, one can rapidly identify bioactive compounds that can be useful in a particular biomolecular pathway in inhibiting the diseased condition. Thus, biomolecular pathway provides information about the mechanism of drug as well as internal way of diseased condition person. The knowledge and the results of these experiments provide starting points for designing a drug and for understanding the interaction or role of a particular biochemical process in biology.

HTS is a relatively recent innovation and it requires high-speed computer technology. It works on the principle of high-throughput screening of large amount of natural compounds using computer-based technology, which is more easy and more time saving. Knowledge can be further utilized for new herbal drug discovery. Interest of research can be generated in natural drug discovery through all these newer techniques. Many well-developed countries have highly specialized and expensive screening labs to run an HTS operation. However, the countries having interest of working in research that cases a small-to-moderately sized research institution will use the existing HTS facility as per their convenience rather than full set up.

## 9. Conclusion

With growing interest in herbal drug development with minimum side effects, there are better opportunities to explore the medicinal and other biological properties of previously inaccessible natural products. To establish its usefulness, it is

mandatory to focus on visualization and identification of unused herbal plants over the world. Then, it is emphasized on extraction, its isolation, and characterization of phytochemicals, which is a gift of nature in a rational and scientific way. There is an unmet need for utilization of the natural products for the benefit of human kind and development of new lead for drug discovery. Once the phytochemical is obtained, this can be used for further exploration through QSAR studies, molecular modeling, and animal studies followed by clinical trial. The success of natural products in drug discovery essentially for pharmaceutical companies and research institutes is essentially related to their ability and benefits to common person that is socio-economic benefits for well-being of common person its health is important for the world rather than all coming come to your hands if health is top priority. Natural products contain complex chemical structures, which differ according to their various species in nature, and when the existing high technology methods that are available are applied, it can lead to new discovery of drugs, benefitting the whole world. Thus, the world is always gifted with nature, and man is gifted with brain, so let us make use of it to discover new entities that will be available to common people in economical rate and we will be happy to lead a life on this earth. Moreover, natural products have been, and will be, important sources of new pharmaceutical compounds. Many years ago life was made possible or was prolonged only due to natural herbs as per the references that can be obtained in literature. In the new era of twenty-first century, no life is possible on earth without herbal drugs or products that are obtained through natural herbal drug discovery. Hats off to it!

## Author details

Akshada Amit Koparde[1*], Rajendra Chandrashekar Doijad[2] and Chandrakant Shripal Magdum[3]

1 Department of Pharmaceutical Chemistry, Dean Academics, KIMSDTU's Krishna Institute of Pharmacy, Malkapur, Karad, India

2 DEAN, KIMSDTU's Krishna Institute of Pharmacy, Malkapur, Karad, India

3 Guide and principal, KES's Rajarambapu College of Pharmacy, Kasegaon, India

*Address all correspondence to: akshadakakade@yahoo.com

## References

[1] Sircar NN. Medicinal plants. The Eastern Pharmacist. 1982;**29**(291):49-52

[2] Tariq O, Siddiqi AJ. Vitamin C content of Indian medicinal plants- a literature review. Indian Drugs. 1985;**23**(2):72-83

[3] Panda H. Handbook on Medicinal Herbs With Uses. New Delhi: Asia pacific business press; 2004. p. 564

[4] Kar A. Pharmacognosy and Pharma Biotechnology. New Delhi: New age international Ltd.; 2006. pp. 5-11

[5] Rao AVR, Gurjar MK. Drugs from plant resources: An overview. Pharmatimes. 1990;**22**(5):19-20

[6] Handa SS. Plants as drugs. The Eastern Pharmacist. 1991;**34**(397):79-85

[7] Handa SS. Future trends of plants as drugs. Pharmatimes. 1991;**23**(4):13-23

[8] Farnsworth NR. A computerized data base for medicinal plants. The Eastern Pharmacist. 1985;**28**(326):53-55

[9] Available from: https://pubchem.ncbi.nlm.nih.gov/compound/36314#section=2D-Structure

[10] Mukherjee P. Quality Control of Herbal Drugs – An Approach to Evaluations of Botanicals. 5th ed. 2005. p. 2

[11] Vogel GH. Drug Discovery and Evaluation, Pharmacological Assays. 2003. p. 202

[12] Cragg GM, Newman DJ, Sander KM. Natural products in drug discovery and development. Journal of Natural Products. 1997;**60**:52-60

[13] Hemalatha S, Manda V, Mohan Y. Microwave assisted extraction – An innovative and promising extraction tool for medicinal plant research. Pharmacognosy Reviews. 2007;**1**(1):7-17

[14] Joy PP, Thomas J, Mathew S, Skaria BP. Medicinal Plants. Kerala Agricultural University, Aromatic and Medicinal Plants Research Station; 1998. pp. 3-5

[15] Mukherjee PK. Quality Control of Herbal Drugs. 1st ed. Vol. 2002. Business Horizons Pharmaceutical Publishers. p. 103

[16] Spreeman R, Gaedcke F. Herbal drugs. The Eastern Pharmacist. 2000;**23**(512):29-37

[17] Bhanu PS, Sagar ZR. Herbal drugs. The Indian Pharmacist. 2003;**2**(12):13-16

[18] Samanta MK, Mukherjee PK, Prasad MK, Suresh B. The Eastern Pharmacist. 2000;**23**(512):23-27

[19] Tyagi K, Bhanu PS, Sagar ZR. Failures and successes of herbal medicines. The Indian Pharmacist. 2003;**2**(12):17-23

[20] Gupta AK, Chitme HR. Herbal medicine for health. The Eastern Pharmacist. 2000;**23**(512):41-44

[21] Dobriyal RM, Narayana DBA. Ayurvedic raw material. The Eastern Pharmacist. 1998;**30**(484):31-35

[22] Littleton J. The future of plant drug discovery. Expert Opinion on Drug Discovery. 2007;**2**(5):673-683