**European Journal of Biotechnology and Bioscience**
**www.biosciencejournals.com**
**ISSN: 2321-9122**
**Received: 29-11-2022, Accepted: 15-12-2022, Published: 02-01-2023**
**Volume 11, Issue 1, 2023, Page No. 1-5**

# Different extraction methods and various screening strategies for plants

**Sonal**
Research Scholar, Department of Centre for Medical Biotechnology (CMBT) MDU, Rohtak, Haryana, India

**Abstract**
Preparation of medicinal plants for experimental purposes is an initial step and key in achieving quality research outcome. It involves extraction and determination of quality and quantity of bioactive constituents before proceeding with the intended biological testing. The primary objective of this study was to evaluate various methods used in the preparation and screening of medicinal plants in our daily research. Although the extracts, bioactive fractions, or compounds obtained from medicinal plants are used for different purposes, the techniques involved in producing them are generally the same irrespective of the intended biological testing. The major stages included in acquiring quality bioactive molecule are the selection of an appropriate solvent, extraction methods, phytochemical screening procedures, fractionation methods, and identification techniques. The nitty-gritty of these methods and the exact road map followed solely depends on the research design. Solvents commonly used in extraction of medicinal plants are polar solvent (e.g., water, alcohols), intermediate polar (e.g., acetone, dichloromethane), and nonpolar (e.g., n-hexane, ether, chloroform). In general, extraction procedures include maceration, digestion, decoction, infusion, percolation, Soxhlet extraction, superficial extraction, ultrasound-assisted, and microwave-assisted extractions.

**Keywords:** chromatography, extraction, fractionation, isolation, medicinal plants

## Introduction

Medicinal plants are extracted and processed for direct consumption as herbal or traditional medicine or prepared for experimental purposes. The concept of preparation of medicinal plant for experimental purposes involves the proper and timely collection of the plant, authentication by an expert, adequate drying, and grinding. This is followed by extraction, fractionation, and isolation of the bioactive compound where applicable. In addition, it comprises determination of quantity and quality of bioactive compounds. Recently, plant as a source of medicine is gaining international popularity because of its natural origin, availability in local communities, cheaper to purchase, ease of administration, and perhaps less troublesome. Also, herbal medicine may be useful alternative treatment in case of numerous side effects and drug resistance. Extraction of medicinal plants is a process of separating active plant materials or secondary metabolites such as alkaloids, flavonoids, terpenes, saponins, steroids, and glycosides from inert or inactive material using an appropriate solvent and standard extraction procedure. Plant materials with high content of phenolic compounds and flavonoids were found to possess antioxidant properties, and hence are used to treat age-related diseases such as Alzheimer's disease, Parkinsonism, anxiety, and depression. Several methods were used in the extraction of medicinal plants such as maceration, infusion, decoction, percolation, digestion and Soxhlet extraction, superficial extraction, ultrasound-assisted, and microwave-assisted extraction. In addition, thin-layer chromatography (TLC), high-performance liquid chromatography (HPLC), paper chromatography (PC), and gas chromatography (GC) were used in separation and purification of the secondary metabolites. The choice of an appropriate extraction method depends on the nature of the plant material, solvent used, pH of the solvent, temperature, and solvent to sample ration. It also depends on the intended use of the final products. This study aimed to assess various solvents of extractions, methods of extraction, fractionation, purification, phytochemical screening, and identification of bioactive compounds in medicinal plants.

## Solvents of Extraction

The solvent used for the extraction of medicinal plants is also known as the menstruum. The choice of solvent depends on the type of plant, part of plant to be extracted, nature of the bioactive compounds, and the availability of solvent. In general, polar solvents such as water, methanol, and ethanol are used in extraction of polar compound, whereas nonpolar solvents such as hexane and dichloromethane are used in extraction of nonpolar compounds. During liquid–liquid extraction, the conventional way is to select two miscible solvents such as water–dichloromethane, water–ether, and water–hexane. In all the combinations, water is present because of its high polarity and miscibility with organic solvent. The compound to be extracted using liquid–liquid extraction should be soluble in organic solvent but not in water to ease separation. Furthermore, solvent used in extraction is classified according to their polarity, from *n*-hexane which is the least polar to water the most polar. The following are various solvents of extractions arranged according to the order of increasing polarity:

During fractionation, the selected solvent is added according to the order of increasing polarity, starting from *n*-hexane, the least polar to water with the highest polarity. If a researcher wishes to select five solvents during fractionation, the usual practice is to choose two solvents with low polarity (*n*-hexane, chloroform), two with medium

polarity (dichloromethane, *n*-butanol), and one with the highest polarity (water).

Various factors enumerated below should be taken into consideration when choosing a solvent of extraction. (i) *Selectivity*. The ability of a chosen solvent to extract the active constituent and leave the inert material. (ii) *Safety*. Ideal solvent of extraction should be nontoxic and nonflammable. (iii) *Cost*. It should be as cheap as possible. (iv) *Reactivity*. Suitable solvent of extraction should not react with the extract. (v) *Recovery*. The solvent of extraction should be quickly recovered and separated from the extract. (vi) *Viscosity*. Should be of low viscosity to allow ease of penetration. (vii) *Boiling temperature*. Solvent boiling temperature should be as low as possible to prevent degradation by heat.

**Methods used in Extraction of Medicinal Plants**

Quite numbers of procedures were technically used in the extraction of medicinal plants. Some newer methods are still evolving, whereas the existing ones are undergoing modifications. The choice of an appropriate way of extraction is very vital, which in some cases depends on the intended use of an extract.

**Factors to be considered in choosing extraction method**

(a) *Stability to heat*. Heat-stable plant material is extracted using Soxhlet extraction or microwave-assisted extraction, whereas plant materials that are not heat stable are extracted using maceration or percolation. (b) *Nature of solvent*. If the solvent of extraction is water, maceration is a suitable method but for volatile solvent percolation and Soxhlet extraction are more appropriate. (c) *Cost of the drug*. Cheap drugs are extracted using maceration, whereas costly drugs are preferably extracted using percolation. (d) *Duration of extraction*. Maceration is suitable for plant material requiring long exposure to the menstruum, whereas techniques such as microwave- or ultrasound-assisted extraction are used for a shorter duration. (e) *Final volume required*. Large volume products such as tinctures are prepared by maceration, whereas concentrated products are produced by percolation or Soxhlet extraction. (f) *Intended use*. Extracts intended for consumption by human are usually prepared by maceration, whereas products intended for experimental testing are prepared using other methods in addition to maceration.

**Phytochemical Screening Mwethods**

Phytochemical screenings are preliminary tests conducted to detect the presence of both primary and secondary metabolites in an extract. Several qualitative analyses described below have been used to detect the presence of alkaloids, flavonoids, tannins, saponins, flavones, sterols, terpenes, cardiac glycosides, protein, carbohydrates, and fats.

**Test for alkaloids**

(a) *Dragendorff's test*. 1mL of extract was taken and placed into a test tube. Then 1mL of potassium bismuth iodide solution (Dragendorff's reagent) was added and shaken. An orange red precipitate formed indicates the presence of alkaloids. (b) *Wagner's test*. 1mL of extract was taken and placed into a test tube. Then 1mL of potassium iodide (Wagner's reagent) was added and shaken. Appearance of reddish brown precipitate signifies the existence of alkaloids.

(c) *Mayer's test*. 1mL of extract was taken and placed into a test tube. Then 1mL of potassium mercuric iodide solution (Mayer's reagent) was added and shaken. Emergence of whitish or cream precipitate implies the presence of alkaloids. (d) *Hager's test*. 1mL of solution of an extract was taken and placed into a test tube. Then 1mL of saturated ferric solution (Hager's reagent) was added and shaken. Formation of yellow-colored precipitate indicates the existence of alkaloids.

**Test for glycosides**

(a) *Bontrager's test (modified)*. One gram of the crude extract was first weighed, placed into a test tube, and dissolved in 5mL of dilute hydrochloric acid. Then 5mL of ferric chloride (5%) solution was added. The mixture was shaken and placed over water bath. Then the mixture was allowed to boil for 10min, cooled, and filtered. Afterward, the mixture was then extracted again with benzene. Finally, equal volume of ammonia solution was added to benzene layer. Appearance of pink color indicates the presence of anthraquinone glycosides.

(b) *Legals test*. 1mL of an extract was taken, and then an equal volume of sodium nitroprusside was added followed by few quantity of sodium hydroxide solution and shaken. Formation of pink-to-blood-red precipitate signifies the existence of cardiac glycoside.

(c) *Keller–Killiani test*. 2mL of the extract was taken and diluted with equal volume of water. Then 0.5mL of lead acetate was added, shaken, and filtered. Again, the mixture was extracted with equal volume of chloroform, evaporated, and dissolved the residue in glacial acetic acid. Then few drops of ferric chloride was added. Again, the whole mixture was placed into a test tube containing 2mL of sulfuric acid. Emergence of reddish brown layer that turns bluish green implies the presence of digitoxose.

**Test for steroids and triterpenoids**

(a) *Libermann Burchard's test*. This method is utilized for an alcoholic extract. Extract need to dry out first through evaporation, then extracted again with chloroform.Add few drops of acetic anhydrites followed by sulfuric acid from the side of the test tube. Formation of violet to blue-colored ring at the junction of the two liquids indicated the presence of steroids.

(b) *Salkowski's test*. 1mL solution of the extract was taken and 2mL of chloroform was added, shaken, and filtered. Few drops of concentrated sulfuric acid were added to filtrate, shaken, and allowed to stand. Development of golden-yellow precipitate indicates the presence of triterpenes.

**Test for tannins**

(a) *Gold Beater's skin test*. A Gold Beater's Skin was obtained from Ox skin. The Gold Beater's Skin was soaked in 2% hydrochloric acid and washed with distilled water. Then it was placed in a solution of an extract for 5min and washed with distilled water. Finally, it was placed in 1% ferrous sulfate solution. If the Gold Beater's Skin changed to brown or black tannins is present. (b) *Gelatin's test*. 1mL of extract was taken and placed in a test tube. Then 1% gelatin solution containing sodium chloride added and

shaken. Appearance of white precipitate indicates the presence of tannins.

**Test for flavonoids**

(a) *Shinoda's test*. 1mL of extract was taken and placed into a test tube. Then, few drops of concentrated hydrochloric acid was added followed by 0.5mg of mRimandoium turnings and shaken. Emergence of pink coloration indicates the presence of flavonoids. (b) *Lead acetate test*. To detect the presence of flavonoids, 1mL of extract was taken and placed into a test tube. Then few drops of lead acetate added and shaken. Formation of yellow precipitate signifies the presence of flavonoids. (c) *Alkaline reagent test*. 1mL of extract was taken and placed into a test tube. Then few drops of sodium hydroxide solution were added and shaken. Emergence of intense yellow color that turns to colorless after adding dilute acid implies the existence of flavonoids.

**Test for phenols**

(a) *Ferric chloride test*. 1mL solution of an extract was taken and placed into a test tube. Then 1% gelatin solution containing sodium chloride was added and shaken. Formation of bluish-black color indicates the presence of phenols. (b) *Lead acetate test*. 1mL solution of an extract was taken and placed into a test tube. Then 1mL of alcoholic solution was added, followed by dilution with 20% sulfuric acid. Finally, solution of sodium hydroxide was added. Formation of red-to-blue color signifies the occurrence of phenols. (c) *Gelatin test*. A solution of plant extract was placed into test tube followed by 2mL of 1% gelatin solution and shaken. Appearance of white precipitate indicates the presence of phenols. (d) *Mayer's reagent test* (potassium mercuric iodide test). To a solution of plant extract, 1mL of Mayer's reagent was added in an acidic solution. Manifestation of white precipitate shows the existence of phenolic compounds.

**Test for protein**

(a) *Biuret test*. Some quantity of an extract was taken and 4% sodium hydroxide solution of the drug was produced. This is followed by the addition of 1 % copper sulfite. Appearance of violet color implies the existence of peptide linkage. (b) *Ninhydrin test*. 1mL of an extract was taken and placed into a test tube. Then 0.25% of ninhydrin reagent was added and shaken. The mixture was then boiled for few minutes. Formation of blue color signifies the presence of protein. (c) *Xanthoproteic test*. 1mL of the extract was taken and placed it into a test tube. Then few drops of nitric acid were added and shaken. Emergence of yellow-color indicates presence of protein.

**Modern Screening Techniques for Plant Extracts**

Over centuries, world population exclusively used medicinal plants as therapeutic agents. Today, in spite of the exponential development of synthetic pharmaceutical chemistry, including combinatorial chemistry and microbial fermentation, 25% of prescribed medicines in industrialised countries are of plant origin. This percentage can reach 50% for the over-the-counter (OTC) market (drugs for self-medication). In fact, it is also estimated that natural products are implicated in the development of 44% of all new drugs, generally as leads for the preparation of semi-synthetic derivatives. Over the last decathe de, there has been a renewed interest in plants; the pharmaceutical industry now fully considers plants as a viable option for the discovery of new leads. Among the estimated 350,000 plant species on the earth, only a small percentage has been phytochemically investigated; the fraction subjected to biological or pharmacological screening is even smaller. Moreover, a plant extract may contain several thousand different secondary metabolites but any phytochemical analysis will reveal only a narrow spectrum of its constituents. The plant kingdom thus represents an enormous reservoir of pharmacologically valuable molecules to be discovered. Searching for new drugs in plants implies screening of the extracts for the presence of novel compounds and an investigation of their biological activities. Suspected novel or bioactive compounds are generally isolated in order to elucidate the structure and to perform further biological and toxicological testing. The path that leads from an intact plant to its pure constituents is long.

**Bioactivity-guided isolation strategy**

The standard procedure of searching for active plant metabolites involves biological screening followed by activityguided fractionation. Simple and inexpensive bioassays have been introduced in phytochemical laboratories for rapid screening of crude plant extracts. Bioassays also serve as a guide during the isolation process. Thus, all fractions are biologically evaluated and those continuing to exhibit activity are carried through further isolation and purification until pure active principles are obtained. In this way, different properties and effectiveness against different types of ailments, including microbial afflictions and parasitic diseases, can be investigated. However, the number of available targets is limited. The principal targets for biological tests can be divided into six groups: – lower organisms: microorganisms (bacteria, fungi, viruses). – invertebrates: insects, crustaceans, molluscs. – isolated subcellular systems: enzymes, receptors. – animal or human cell cultures. – isolated organs of vertebrates. – whole animals. One major drawback of the bioassay-guided fractionation strategy is the frequent isolation of previously known metabolites. The chemical screening of crude extracts therefore constitutes an efficient complementary approach allowing localization and targeted isolation of new types of constituents with potential activities. This procedure also enables recognition of known metabolites at the earliest stage of separation, thus avoiding time consuming and expensive isolation of common constituents.

**LC/MS and LC/MSn analysis of saponins with molluscicidal properties**

Schistosomiasis, commonly known as bilharzia, is a parasitic disease that affects more than 250 million people throughout South America, Africa, and the Far East. The reproductive cycle of schistosomes involves a stage associated with aquatic snails of the genera Biomphalaria and Bulinus, in which the parasite multiplies into cercariae. These cercariae, after leaving the snails, can penetrate the skin of humans who come in contact with contaminated water. Once through the skin, they change gradually into the mature trematodes known as schistosomes. The schistosomes mate and lay eggs that are carried away with faeces or urine. As the eggs reach water, they produce miracidia, which locate snails of the appropriate species and the cycle begins again. One way to attack the problem of schistosomiasis is to disrupt the lifecycle of the parasite and

kill the intermediate host with molluscicides from plant sources. The use of molluscicidal plants growing abundantly in areas where schistosomiasis is endemic is a simple, inexpensive, and appropriate technology for local control of the snail vector and may become, in the near future, useful for the control of this disease. Among the plants of greatest interest are those containing large quantities of saponins. Saponins possess high toxicity toward cold-blooded organisms including snails; they are often present in large amounts in plants, and due to high solubility in water, their extraction and application is simple and does not require any sophisticated infrastructure. Many saponin-containing plants have been screened in our institute. LC/MS is the method of choice in order to rapidly obtain partial structural information on the saponin of interest. The LC/MS analysis of the such labile polar constituents, however, is delicate if correct information on both molecular weight and sugar sequence have to be obtained; great care in the choice of the ionization conditions has to be taken.

**Combination of LC/MS and LC/NMR with on-line assays for the dereplication of antioxidant compounds**

Oxidative stress and free radicals play an important role in biological systems. Thus, the search for antioxidants and radical scavengers may lead to the discovery of new preventive or therapeutic agents. Many antioxidants of natural origin belong to the polyphenols and, in those cases, bioac tivity-guided isolation often leads to known structures. In order to find antioxidants with original structure, dereplication is of prime importance. An example of dereplication of antioxidant agents is illustrated by the on-flow LC/1 H-NMR analysis of a fraction from the Indonesian plant *Orophea enneandra* (Annonaceae). In order to determine the nature of the active constituents of this fraction, an LC/UV/MS and onflow LC/NMR analysis was performed. The LC/UV chromatogram enabled the detection of five main constituents (5–9). For the determination of the compounds responsible for the antioxidant activity, fractions were directly collected every two minutes from an analytical HPLC separation and were subjected to autographic assays. LC/UV peaks 5 and 8 were thus assigned as the antioxidant compounds.

**Combination of LC/MS and LC/NMR for the screening and the structure elucidation of new antifungal agents**

The increasing incidence of mycoses associated with AIDS and arising after treatment by immunosuppressive drugs has given fresh impetus to the search for novel antifungal agents. There are few really effective antifungal preparations currently indicated for the treatment of systemic mycoses and their efficacy is rather limited. Another area which is badly in need of new lead compounds is agrochemicals. Consequently, the investigation of higher plants for antifungal properties is of great importance at the moment. For the isolation of active compounds by activity-guided fractionation, bioautography is the method of choice. This technique combines TLC with a bioassay in situ and allows localization of active constituents in a plant extract. producing fungi, such as Aspergillus, Penicillium, and Cladosporium spp., can all be employed as target organisms in direct bioautographic procedures. Zones of inhibition appear where fungal growth is prevented by the active components of the plant extract. Bioautography with *Cladosporium cucumerinum* has been used successfully in our laboratory for several years now, and a large number of fungicidal natural products of different chemical structures have been isolated. Since direct bioautography is not possible with yeasts such as Candida albicans, a simple and rapid agar overlay assay has been developed.

**Conclusion**

Several works have been done on medicinal plant either to investigate and prove a reported claim of biological activity or to mimic its traditional medicinal use based on ethno medicinal survey. Large numbers of medicinal plants have been extracted, fractionated, and compounds isolated successfully. In addition, compounds obtained were tested for biological or pharmacological activity, and in most cases, they were found to be active. Nonetheless, the rate of success and the authenticity of these findings depends on the accuracy in selection of solvents, selection and proper execution of extraction methods, phytochemical screening, fractionation, and identification techniques. Lastly, proper understanding and implementation of these techniques are indispensable. Advancement and modification of these methods periodically will ease research processes and improve the outcome.

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