

Qualitative Phytochemical Analysis

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Abstract

Preliminary screening of phytochemicals is a valuable step in the detection of bioactive principles present in medicinal plants and may lead to novel environmentally friendly bioherbicides and drug. Plants are a source of large number of secondary metabolites comprising to different groups such as alkaloids, flavonoids, tannins, terpenoids, steroids etc. A large number of the plants are claimed to possess the antibiotic properties in the traditional system and are also used extensively by the tribal people worldwide. It is now believed that nature has given the cure of every disease in one way or another. Plants have been known to relieve various diseases in Ayurveda. Extraction of the bioactive plant constituents has always been a challenging task for the researchers. In this present review, an attempt has been made to give an overview of certain extractants and extraction processes.

Keywords: Secondary metabolites, alcoholic extraction, organic solvents, phytochemical screening

Introduction

There is ample literature on preliminary phytochemical surveys and the knowledge of the chemical constituents of plants is desirable to understand herbal drugs and their preparations. Most importantly, these studies will be helpful to isolate and characterize the chemical constituents present in those plant extracts. In addition, the knowledge of the chemical constituents of plants would further be valuable in discovering the actual value of folkloric remedies (Parray *et al.*, 2012).

Extraction is the separation of medicinally active portions of plant or animal tissues using selective solvents through standard procedures. The products so obtained from plants are relatively complex mixtures of metabolites, in liquid or semisolid state or (after removing the solvent) in dry powder form, and are intended for oral or external use. These include classes of preparations known as decoctions, infusions, fluid extracts, tinctures, pilular (semisolid) extracts or powdered. extracts. Such preparations have been popularly called galenicals, named after Galen, the second century Greek physician (Harborne, 1984). Extraction methods involves the separation of medicinally active portions of plant tissues from the inactive/inert components by using selective solvents. During extraction, solvents diffuse into the solid plant material and solubilize compounds with similar polarity (Harbrone, 1984).

The general techniques of medicinal plant extraction include maceration, infusion, percolation, digestion, decoction, hot continuous extraction (Soxhlet), aqueous-alcoholic extraction by fermentation, countercurrent extraction, microwave-assisted extraction, ultrasound extraction (sonication), supercritical fluid extraction, and phytonic extraction (with hydrofluorocarbon solvents). For aromatic plants, hydrodistillation techniques (water distillation, steam distillation, water and steam distillation), hydrolytic maceration followed by distillation, expression and enfl eurage (cold fat extraction) may be employed. Some of the latest extraction methods for

aromatic plants include headspace trapping, solid phase microextraction, protoplast extraction, microdistillation, thermomicrodistillation and molecular distillation (Handa *et al.*, 2008).

The basic parameters influencing the quality of an extract are (Parray *et al.*, 2012):

1. Plant part used as starting material
2. Solvent used for extraction
3. Extraction procedure

Effect of extracted plant phytochemicals depends on (Parray *et al.*, 2012):

1. The nature of the plant material
2. Its origin
3. Degree of processing
4. Moisture content
5. Particle size

The variations in different extraction methods that will affect quantity and secondary metabolite composition of an extract depends upon (Harbrone, 1984):

1. Type of extraction
2. Time of extraction
3. Temperature
4. Nature of solvent
5. Solvent concentration
6. Polarity

Plant material

Plants are potent biochemists and have been components of phytomedicine since times of immemorial; man is able to obtain from them a wondrous assortment of industrial chemicals. Plant based natural constituents can be derived from any part of the plant like bark, leaves, flowers, roots, fruits, seeds, etc i.e. any part of the plant may contain active components. The systematic screening of plant species with the purpose of discovering new bioactive compounds is a routine activity in many laboratories. Scientific analysis of plant components follows a logical pathway. Plants are collected either randomly or by following leads supplied by local healers in geographical areas where the plants are found (Parekh *et al.*, 2008). Fresh or dried plant materials can be used as a source for the extraction of secondary plant components. Many authors have reported about plant extract preparation from the fresh plant tissues. The logic behind this came from the ethno medicinal use of fresh plant materials among the traditional and tribal people. But as many plants are used in the dry form (or as an aqueous extract) by traditional healers and due to differences in water content within different plant tissues, plants are usually air dried to a constant weight before extraction. Most researchers dry the plants in the oven at about 40°C for 72 h (Salie *et al.*, 1996 and Gurjar *et al.*, 20102). In most of the reported works, underground parts (roots, tuber, rhizome, bulb etc.) of a plant were used extensively compared with other above ground parts in search for bioactive compounds possessing antimicrobial properties.

Choice of solvents

Successful determination of biologically active compounds from plant material is largely dependent on the type of solvent used in the extraction procedure. Properties of a good solvent in plant extractions includes, low toxicity, ease of evaporation at low heat, promotion of rapid physiologic absorption of the extract, preservative action, inability to cause the extract to complex or dissociate. The factors affecting the choice of solvent are quantity of phytochemicals to be extracted, rate of extraction, diversity of different compounds extracted, diversity of inhibitory compounds extracted, ease of subsequent handling of the extracts, toxicity of the solvent in the bioassay process, potential health hazard of the extractants (Eloff, 1998). The choice of solvent is influenced by what is

intended with the extract. Since the end product will contain traces of residual solvent, the solvent should be nontoxic and should not interfere with the bioassay. The choice will also depend on the targeted compounds to be extracted (Parray *et al.*, 2012).

The various solvents that are used in the extraction procedures are:

Water: Water is universal solvent, used to extract plant products with antimicrobial activity. Though traditional healers use primarily water but plant extracts from organic solvents have been found to give more consistent antimicrobial activity compared to water extract. Also water soluble flavonoids (mostly anthocyanins) have no antimicrobial significance and water soluble phenolics only important as antioxidant compound (Das *et al.*, 2010).

Acetone: Acetone dissolves many hydrophilic and lipophilic components from the two plants used, is miscible with water, is volatile and has a low toxicity to the bioassay used, it is a very useful extractant, especially for antimicrobial studies where more phenolic compounds are required to be extracted. A study reported that extraction of tannins and other phenolics was better in aqueous acetone than in aqueous methanol. Both acetone and methanol were found to extract saponins which have antimicrobial activity (Eloff, 1998).

Alcohol: The higher activity of the ethanolic extracts as compared to the aqueous extract can be attributed to the presence of higher amounts of polyphenols as compared to aqueous extracts. It means that they are more efficient in cell walls and seeds degradation which have unpolar character and cause polyphenols to be released from cells. More useful explanation for the decrease in activity of aqueous extract can be ascribed to the enzyme polyphenol oxidase, which degrade polyphenols in water extracts, whereas in methanol and ethanol they are inactive. Moreover, water is a better medium for the occurrence of the micro-organisms as compared to ethanol (Lapornik *et al.*, 2005). The higher concentrations of more bioactive flavonoid compounds were detected with ethanol 70% due to its higher polarity than pure ethanol. By adding water to the pure ethanol up to 30% for preparing ethanol 70% the polarity of solvent was increased (Bimakr, 2010). Additionally, ethanol was found easier to penetrate the cellular membrane to extract the intracellular ingredients from the plant material. Since nearly all of the identified components from plants active against microorganisms are aromatic or saturated organic compounds, they are most often obtained through initial ethanol or methanol extraction. Methanol is more polar than ethanol but due to its cytotoxic nature, it is unsuitable for extraction in certain kind of studies as it may lead to incorrect results (Wang, 2010).

Chloroform: Terpenoid lactones have been obtained by successive extractions of dried barks with hexane, chloroform and methanol with activity concentrating in chloroform fraction. Occasionally tannins and terpenoids will be found in the aqueous phase, but they are more often obtained by treatment with less polar solvents (Cowan, 1999).

Ether: Ether is commonly used selectively for the extraction of coumarins and fatty acids [Cowan, 1999].

Methods of extraction:

Variation in extraction methods usually depends upon:

1. Length of the extraction period
2. Solvent used
3. pH of the solvent
4. Temperature
5. Particle size of the plant tissues
6. The solvent-to-sample ratio

The basic principle is to grind the plant material (dry or wet) finer, which increases the surface area for extraction thereby increasing the rate of extraction. Earlier studies reported that solvent to sample ratio of 10:1 (v/w) solvent to dry weight ratio has been used as ideal.

Extraction procedures

Plant tissue homogenization: Plant tissue homogenization in solvent has been widely used by researchers. Dried or wet, fresh plant parts are grinded in a blender to fine particles, put in a certain quantity of solvent and shaken vigorously for 5 - 10 min or left for 24 h after which the extract is filtered. The filtrate then may be dried under reduced pressure and redissolved in the solvent to determine the concentration. Some researchers however centrifuged the filtrate for clarification of the extract (Parray *et al.*, 2012).

Serial exhaustive extraction: It is another common method of extraction which involves successive extraction with solvents of increasing polarity from a non polar (hexane) to a more polar solvent (methanol) to ensure that a wide polarity range of compound could be extracted. Some researchers employ soxhlet extraction of dried plant material using organic solvent. This method cannot be used for thermolabile compounds as prolonged heating may lead to degradation of compounds (Nikhil *et al.*, 2010).

Soxhlet extraction: Soxhlet extraction is only required where the desired compound has a limited solubility in a solvent, and the impurity is insoluble in that solvent. If the desired compound has a high solubility in a solvent then a simple filtration can be used to separate the compound from the insoluble substance. The advantage of this system is that instead of many portions of warm solvent being passed through the sample, just one batch of solvent is recycled. This method cannot be used for thermolabile compounds as prolonged heating may lead to degradation of compounds (Nikhil *et al.*, 2010).

Maceration: In maceration (for fluid extract), whole or coarsely powdered plantdrug is kept in contact with the solvent in a stoppered container for a defined period with frequent agitation until soluble matter is dissolved. This method is best suitable for use in case of the thermolabile drugs (Harbrone, 1984).

Decoction: This method is used for the extraction of the water soluble and heat stable constituents from crude drug by boiling it in water for 15 minutes, cooling, straining and passing sufficient cold water through the drug to produce the required volume (Mute, 2009).

Infusion: It is a dilute solution of the readily soluble components of the crude drugs. Fresh infusions are prepared by macerating the solids for a short period of time with either cold or boiling water (Roy, 2010).

Digestion: This is a kind of maceration in which gentle heat is applied during the maceration extraction process. It is used when moderately elevated temperature is not objectionable and the solvent efficiency of the menstrum is increased thereby (Audu *et al.*, 2007).

Sonication: The procedure involves the use of ultrasound with frequencies ranging from 20 kHz to 2000 kHz; this increases the permeability of cell walls and produces cavitation. Although the process is useful in some cases, like extraction of *Rauwolfia* root, its large-scale application is limited due to the higher costs. One disadvantage of the procedure is the occasional but known deleterious effect of ultrasound energy (more than 20 kHz) on the active constituents of medicinal plants through formation of free radicals and consequently undesirable changes in the drug molecules (Handa, 2008).

Percolation: This is the procedure used most frequently to extract active ingredients in the preparation of tinctures and fluid extracts. A percolator (a narrow, cone-shaped vessel open at both ends) is generally used. The solid ingredients are moistened with an appropriate amount of the specified menstrum and allowed to stand for approximately 4 h in a well closed container, after which the mass is packed and the top of the percolator is closed. Additional menstrum is added to form a shallow layer above the mass, and the mixture is allowed to macerate in the closed percolator for 24 h. The outlet of the percolator then is opened and the liquid contained therein is allowed to drip slowly. Additional menstrum is added as required, until the percolate measures about three quarters of the required volume of the finished product. The marc is then pressed and the expressed liquid is added to the percolate. Sufficient menstrum is added to produce the required volume, and the mixed liquid is clarified by filtration or by standing followed by decanting (Handa, 2008).

Phytochemical screening:

Phytochemical examinations were carried out for all the extracts as per the standard methods.

Proteins (Xanthoproteic Test)

The extracts were treated with few drops of conc. Nitric acid. Formation of yellow colour indicates the presence of proteins (Jigna and Sumitra, 2007).

Aminoacids

To the extract, 0.25% (w/v) ninhydrin reagent was added and boiled for few minutes. Formation of blue colour indicates the presence of amino acid (Harborne, 1984).

Tannins

About 0.5 g of the dried powdered samples is boiled in 20 ml of water in a test tube and then filtered. A few drops of 0.1% ferric chloride is added and observed for brownish green or a blue- black coloration (Jigna and Sumitra, 2007).

Saponins

About 2 g of the powdered sample is boiled in 20 ml of distilled water in a water bath and filtered. 10ml of the filtrate was mixed with 5 ml of distilled water and shaken vigorously for a stable persistent froth. The frothing was mixed constantly with 3 drops of olive oil and shaken vigorously, then observed for the formation of emulsion (Harborne, 1984).

Flavonoids

5 ml of dilute ammonia solution is added to a portion of the aqueous filtrate of each plant extract followed by addition of concentrated H₂SO₄. A yellow colouration observed in each extract indicated the presence of flavonoids (Harbrone, 1984).

Steroids

Two ml of acetic anhydride is added to 0.5 g methonlic extract of each sample with 2 ml H₂SO₄. The colour changed from violet to blue or green in some samples indicating the presence of steroids (Sofowara, 1993).

Cardiac glycosides (Keller-Killani test)

Five ml of extracts was treated with 2 ml of glacial acetic acid containing one drop of ferric chloride solution. This was underlayed with 1 ml of concentrated sulphuric acid. A brown ring of the interface indicates a deoxysugar characteristic of cardenolides. A violet ring may appear below the brown ring, while in the acetic acid layer, a greenish ring may form just gradually throughout thin layer (Harborne, 1984).

Phenols (Ferric Chloride Test)

Extracts are treated with 3-4 drops of ferric chloride solution. Formation of bluish black colour indicates the presence of phenols (Sofowara, 1993).

Carbohydrates (Molisch's Test)

Extracts are treated with 2 drops of alcoholic α -naphthol solution in a test tube. Formation of the violet ring at the junction indicates the presence of carbohydrates (Harborne, 1984).

Alkaloids (Dragendroff's Test)

Plant extracts is treated with dragendroff's reagent (solution of Potassium Bismuth Iodide). Formation of red precipitate indicates the presence of alkaloids (Harborne, 1984).

Conclusion

Non standardized procedures of extraction may lead to the degradation of the phytochemicals present in the plants and may lead to the variations thus leading to the lack of reproducibility. Efforts should be made to produce batches with quality as consistent as possible (within the narrowest possible range) and to develop and follow the best extraction processes.

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