Phytochemical Screening and Extraction: A Mini Review

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Abstract
Plants are a source of large number of drugs comprising of different groups such as antispasmodics, anti-cancer, antimicrobials 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. Therefore, the researchers today are focusing on evaluation and characterization of various plants and plant constituents against a number of diseases based on their traditional claims of the plants given 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: Medicinal plants, phytochemicals, extraction, solvent, screening

Introduction
Plant-derived substances have recently become of great interest owing to their versatile applications. Medicinal plants are the richest bio-resource of drugs of traditional systems of medicine, modern medicines, nutraceuticals, food supplements, folk medicines, pharmaceutical intermediates and chemical entities for synthetic drugs (Ncube *et al*., 2008). The plants that possess therapeutic properties or exert beneficial pharmacological effects on the human body are generally designated as medicinal plants. In most of the traditional systems of treatment, the use of medicinal plant include the fresh or dried part, whole, chopped, powdered or an advanced form of the plant usually made through extraction with different solvents play a major role and constitute the backbone of the traditional medicine (Mukherjee *et al.,* 1986). Botanical medicines or phytomedicines refer to the use of seeds, berries, leaves, bark, root or flowers of any plant for medicinal purposes by significant number of people (Barret *et al.,* 1999). Although there are no apparent morphological characteristics in the medicinal plants growing with them, yet they possess some special qualities or virtues that make them medicinally important. It has now been established that the plants which naturally synthesize and accumulate some secondary metabolites, like alkaloids, glycosides, tannins, volatiles oils and contain minerals and vitamins, possess medicinal properties (Ghani, 2003). Accordingly, the World Health Organization (WHO) consultative group on medicinal plants has formulated a definition of medicinal plants in the following way: "A medicinal plant is any plant which, in one or more of its organ, contains substance that can be used for therapeutic purpose or which is a precursor for synthesis of useful drugs" (Goldstein *et al.,* 1974). Medicinal plants constitute an important natural wealth of a country. They play a significant role in providing primary health care services to rural people. They serve as therapeutic agents as well

as important raw materials for the manufacture of traditional and modern medicine. Substantial amount of foreign exchange can be earned by exporting medicinal plants to other countries. In this way indigenous medicinal plants play significant role of an economy of a country (Ghani, 2003).

Extraction (as the term is pharmaceutically used) is the separation of medicinally active portions of plant 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 galenicals, named after Galen, the second century Greek physician (Remington and Beringer, 2006).

Extraction methods used pharmaceutically 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 (Ncube *et al*., 2008).

The purpose of standardized extraction procedures for crude drugs (medicinal plant parts) is to attain the therapeutically desired portions and to eliminate unwanted material by treatment with a selective solvent known as menstrum. The extract thus obtained, after standardization, may be used as medicinal agent as such in the form of fluid extracts or further processed to be incorporated in any dosage form such as tablets and capsules. These products contains complex mixture of many medicinal plant metabolites, such as alkaloids, glycosides, terpenoids, flavonoids and lignin's (Handa *et al*., 2008).

The general techniques of medicinal plant extraction include maceration, infusion, percolation, digestion,decoction, hot continuous extraction (Soxhlet), aqueous-alcoholic extraction by fermentation, counter-current extraction, microwave-assisted extraction, ultrasound extraction (sonication), supercritical fluid extraction, and phytonic extraction (with hydro fluorocarbon solvents). For aromatic plants, hydro distillation techniques (water distillation, steam distillation, water and steam distillation), hydrolytic maceration followed by distillation, expression and enfleurage (cold fat extraction) may be employed.

Some of the latest extraction methods for aromatic plants include headspace trapping, solid phase micro-extraction, protoplast extraction, micro distillation, thermo-micro distillation and molecular distillation (Handa *et al*., 2008).

The basic parameters influencing the quality of an extract are (Ncube *et al*., 2008):

a. Plant part used as starting material
b. Solvent used for extraction
c. Extraction procedure

Effect of extracted plant phytochemicals depends on (Ncube *et al*., 2008):

a. The nature of the plant material
b. Its origin
c. Degree of processing
d. Moisture content
e. Particle size

The variations in different extraction methods that will affect quantity and secondary metabolite composition of an extract depend upon (Ncube *et al*., 2008):

a. Type of extraction
b. Time of extraction
c. Temperature
d. Nature of solvent
e. Solvent concentration
f. Polarity

1. Plant material

Plants are potent biochemists and have been components of phytomedicine since times 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 research 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*., 2006). Fresh or dried plant materials can be used as a source for the extraction of secondary plant components. 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. The plant material is dried in the oven at about 40°C for 72 h. 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, antifungal and antioxidant properties (Ncube *et al*., 2008; Das *et al*., 2010).

2. 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 the various solvents that are used in the extraction procedures are (Table-1):

1. *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).

2. *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 unipolar 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 *et al*., 2010). Additionally, ethanol was found easier to penetrate the cellular membrane to extract the intracellular ingredients from the plant material (Wang *et al*., 2010). 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 (Cowan *et al*.,

1999). 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.

3. *Acetone:* Acetone dissolves many hydrophilic and lipophilic components from the plants used, it 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 (Das *et al*., 2010; Eloff, 1998). Both acetone and methanol were found to extract saponins which have antimicrobial activity (Ncube *et al*., 2008).

4. *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 *et al*., 1999).

5. *Dichloromethane:* It is specially used for the selective extraction of only terpenoids (Cowan *et al*., 1999).

6. *Ether:* Ether is commonly used selectively for the extraction of coumarins and fatty acids (Cowan *et al*., 1999).

Structural features and activities of various phytochemicals from plants are given in table-2.

Table 1. Solvents used for active component extraction

Water	**Ethanol**	**Methanol**	**Chloroform**	**Ether**	**Acetone**
Anthocyanins	Tannins	Anthocyanins	Terpenoids	Alkaloids	Phenol
Starches	Polyphenols	Terpenoids	Flavonoids	Terpenoids	Flavonols
Tannins	Polyacetylenes	Saponins		Coumarins	
Saponins	Flavonol	Tannins		Fatty acids	
Terpenoids	Terpenoids	Xanthoxyllines			
Polypepdies	Sterols	Totarol			
Lectins	Alkaloids	Quassinoids			
		Lactones			
		Flavones			
		Phenones			
		Polyphenols			

Table 2. Structural features and activities of various phytochemicals from plants.

Phytochemicals	Structural features	Example(s)	Activities
Phenols and Polyphenols	C3 side chain, - OH groups, phenol ring	Catechol, Epicatechin, Cinnamic acid	Antimicrobial, Anthelmintic, Antidiarrhoeal
Quinones	Aromatic rings, two ketone substitutions	Hypericin	Antimicrobial
Flavones Flavonoids Flavonols	Phenolic structure, one carbonyl group Hydroxylated phenols, C6-C3 unit linked to an aromatic ring Flavones + 3-hydroxyl group	Abyssinone Chrysin, Quercetin, Rutin Totarol	Antimicrobial Antidiarrhoeal
Tannins	Polymeric phenols (Mol. Wt. 500-3000)	Ellagitannin	Antimicrobial, Anthelmintic, Antidiarrhoeal
Coumarins	Phenols made of fused benzene and α-pyrone rings	Warfarin	Antimicrobial
Terpenoids and essential oils	Acetate units + fatty acids, extensive branching and cyclized	Capsaicin	Antimicrobial Antidiarrhoeal
Alkaloids	Heterocyclic nitrogen compounds	Berberine, Piperine, Palmatine, Tetrahydropalmatine	Antimicrobial, Anthelmintic, Antidiarrhoeal
Lectins and Polypeptides	Proteins	Mannose-specific agglutinin, Fabatin	Antimicrobial
Glycosides	Sugar + non carbohydrate moiety	Amygdalin	Antidiarrhoeal
Saponins	Amphipathic glycosides	Vina-ginsenosides-R5 and -R6	Antidiarrhoeal

3. Methods of extraction

Variation in extraction methods usually depends upon:

a. Length of the extraction period,
b. Solvent used
c. pH of the solvent
d. Temperature
e. Particle size of the plant tissues
f. The solvent-to-sample ratio (Das *et al*., 2010)

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 (Das *et al*., 2010).

3.1. Extraction procedures

3.1.1. Plant tissue homogenization: Plant tissue homogenization in solvent has been widely used. 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 dissolved in the solvent to determine the concentration. Some researchers however centrifuged the filtrate for clarification of the extract [Das *et al*., 2010].After drying, crude extracts were stored in stock vials and kept in refrigerator for further use. Percent of Yield **(**Patel *et al*., 2010) was calculated as follows:

Extract yield % = $W_1/W_2 \times 100$

Where, W_1= Net weight of powder in grams after extraction and W_2= Total weight of sample powder in grams taken for extraction.

3.1.2. 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. Sometimes Soxhlet extraction has been used for dried plant material using organic solvent. This method cannot be used for thermo labile compounds as prolonged heating may lead to degradation of compounds (Das *et al*., 2010).

3.1.3. 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 thermo labile compounds as prolonged heating may lead to degradation of compounds (Nikhal *et al*., 2010).

3.1.4. Maceration: In maceration (for fluid extract), whole or coarsely powdered plant 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 thermo labile drugs (Ncube *et al*., 2008).

3.1.5. 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 (Remington and Beringer, 2006).

3.1.6. 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 (Remington and Beringer, 2006).

3.1.7. 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 men strum is increased thereby (Remington and Beringer, 2006).

3.1.8. 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 menstruum 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 *et al*., 2008).

4. Phytochemical screening

Primary phytochemical screening was carried out for all the extracts as per the standard methods.

4.1. Detection of alkaloids: Extracts were dissolved individually in dilute Hydrochloric acid and filtered.

Mayer's Test: Filtrates were treated with Mayer's reagent (Potassium Mercuric Iodide). Formation of a yellow colored precipitate indicates the presence of alkaloids.

Wagner's Test: Filtrates were treated with Wagner's reagent (iodine in potassium iodide). Formation of brown/reddish precipitate indicates the presence of alkaloids.

Dragendroff's Test: Filtrates were treated with Dragendroff's reagent (solution of potassium bismuth iodide). Formation of red precipitate indicates the presence of alkaloids.

Hager's Test: Filtrates were treated with Hager's reagent (saturated picric acid solution). Presence of alkaloids confirmed by the formation of yellow coloured precipitate.

4.2. Detection of carbohydrates: Extracts were dissolved individually in 5 ml distilled water and filtered. The filtrates were used to test for the presence of carbohydrates.

Molisch's Test: Filtrates were 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.

Benedict's test: Filtrates were treated with Benedict's reagent and heated gently. Orange red precipitate indicates the presence of reducing sugars.

Fehling's Test: Filtrates were hydrolysed with dil. HCl, neutralized with alkali and heated with Fehling's A & B solutions. Formation of red precipitate indicates the presence of reducing sugars.

4.3 Detection of glycosides: Extracts were hydrolysed with dil. HCl, and then subjected to test for glycosides.

Modified Borntrager's Test: Extracts were treated with ferric chloride solution and immersed in boiling water for about 5 minutes. The mixture was cooled and extracted with equal volumes of benzene. The benzene layer was separated and treated with ammonia solution. Formation of rose-pink colour in the ammonical layer indicates the presence of anthranol glycosides.

Legal's Test: Extracts were treated with sodium nitropruside in pyridine and sodium hydroxide. Formation of pink to blood red colour indicates the presence of cardiac glycosides.

4.4 Detection of saponins

Froth Test: Extracts were diluted with distilled water to 20 ml and this was shaken in a graduated cylinder for 15 minutes. Formation of 1 cm layer of foam indicates the presence of saponins.

Foam Test: 0.5 gm of extract was shaken with 2 ml of water. If foam produced persists for ten minutes it indicates the presence of saponins.

4.5. Detection of phytosterols

Salkowski's Test: Extracts were treated with chloroform and filtered. The filtrates were treated with few drops of conc. sulphuric acid, shaken and allowed to stand.

Appearance of golden yellow colour indicates the presence of triterpenes.

Libermann Burchard's test: Extracts were treated with chloroform and filtered. The filtrates were treated with few drops of acetic anhydride, boiled and cooled. Conc. sulphuric acid was added. Formation of brown ring at the junction indicates the presence of phytosterols.

4.6 Detection of phenols

Ferric Chloride Test: Extracts were treated with 3-4 drops of ferric chloride solution. Formation of bluish black colour indicates the presence of phenols.

4.7. Detection of tannins

Gelatin Test: To the extract, 1% gelatin solution containing sodium chloride was added. Formation of white precipitate indicates the presence of tannins.

4.8. Detection of flavonoids

Alkaline Reagent Test: Extracts were treated with few drops of sodium hydroxide solution. Formation of intense yellow colour, which becomes colourless on addition of dilute acid, indicates the presence of flavonoids.

Lead acetate Test: Extracts were treated with few drops of lead acetate solution. Formation of yellow colour precipitate indicates the presence of flavonoids.

4.9. Detection of proteins and aminoacids

Xanthoproteic Test: The extracts were treated with few drops of conc. Nitric acid. Formation of yellow colour indicates the presence of proteins.

Ninhydrin Test: 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.

4.10 Detection of diterpenes

Copper acetate Test: Extracts were dissolved in water and treated with 3-4 drops of copper acetate solution. Formation of emerald green colour indicates the presence of diterpenes (Roopashree *et al*., 2008; Obasi *et al*., 2010; Audu *et al*., 2007).

Test for phlobatannins: Plant powder sample was mixed with distill water in a test tube, then shaked it well, and filtered to take plant extract. Then to each plant extract, 1% aqueous hydrochloric acid was added and each plant sample was then boiled with the help of Hot plate stirrer. Formation of red colored precipitate confirmed a positive result.

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 and to develop and follow the best extraction processes.

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