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Review Article

A review on extraction and phytochemical screening methods

Anuj Agarwal*, Ashish Kumar, Bhuvnesh Kumar Singh, Neelanchal Trivedi, K. K. Jha

Teerthanker Mahaveer College of Pharmacy, Teerthanker Mahaveer University, Moradabad, UP, India

Abstract:

Plants are the source of different drugs belonging to various therapeutic cateogries like antidiabetics, antispasmodics, antihypertensives, anticancers, antidepressants, antimicrobials, etc. Plants are used to treat various ailments and these plants have been used by different individuals and tribals worldwide. Use of plants to treat various ailments have also been mentioned in Ayurveda. Along these lines, various researchers are involved in isolating and assessing different bioactive molecules, to be isolated from various plant sources. Isolation of bioactive molecules is not a easy task for researchers.This review gives a focus on extraction and phytochemical screening methodsalongwith their merits and demerits.

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***Correspondence to:**
Mr. Anuj Agarwal
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INTRODUCTION:

Since plants-derived substances have broad applications worldwide so they are of great interest. Therapeutic plants are the wealthiest bioasset of traditional medicines, present day medicines, nourishment supplements, pharmaceutical intermediates and substance elements for manufactured medications. [1]

Extraction is the isolation of active pharmaceutical ingredient from the plant or animal tissues utilizing specific solvents. The substances got from the plants are generally blends of metabolic substances that can be in fluid/semisolid/ solid dry powder state (after evacuating the solvent), and are expected for oral or outer uses. These comprise a number of preparations such as liquid concentrates, infusions, decoctions, tinctures, powdered or semisolid extracts. These products are also known as galenicals. [2]

Extraction techniques utilize the isolation of active pharmaceutical ingredient from the dormant/latent parts of the plants by utilizing specific solvents. During the process of extraction, solvents penetrate into the solid plant material and soubise the substances that have similar polarity. [1]

Aim of the standard extraction methodology for rough medications is to take out the pharmaceutically active ingredients and to remove unwanted material by treatment with a particular dissolvable known as menstrum. Concentrate obtained in this way, may be used as therapeutic agent after standardization. Concentrate may be used in various forms like tinctures or liquid concentrates or may be further processed into any dosage forms. Concentrates are mixture of various plant metabolites like alkaloids, glycosides, flavonoids, lignans, terpenoids, etc. [3]

Decoction, digestion, infusion, alcoholic extraction by fermentation, hot continuous extraction (by using Soxhlet apparatus), microwave-assisted, supercritical fluid, counter-current and ultrasound extraction etc. are the most common techniques of medicinal plant extraction. Some other hydro-distillation techniques such as steam and/or water distillation, distillation preceded by hydrolytic maceration, cold fat extraction etc. may be the useful techniques for extraction of plants containing aromatic substances.

Headspace trapping, micro-distillation, protoplast extraction, solid phase micro-extraction, micro-distillation by heat, solid phase micro-distillation, molecular distillation etc. are the most recent extraction techniques for aromatic plants.[3] The fundamental parameters impacting the characteristics of an extract are as follows [1]:

1. Plant specimen used

2. Solvent system used for extraction

3. Method of extraction

The impact of extracted bioactive molecules depends on:[1]:

1. Nature of plant specimen

2. Original source

3. Level of preparing

4. Moisture content

5. Size reduction of the plant specimen

Different extraction methods will cause variation in amount of concentrate as well as its metabolite content. Factors affecting different extraction methods, are[1]:

1. Type of extraction

2. Duration of extraction

3. Temperature employed during extraction

4. Solvent system used

Nature of plant specimen

Plants are the source of phytopharmaceuticals and it is accepted from a long time. A number of substances can be obtained from any part of the plant such as bark, blossoms, leaves, roots, seeds, plant exudates etc. Therapeutically active ingredient can be obtained from any part of the plant**.** In a number of research laboratories there occurs screening of a number of plant species to find out new therapeutically active compounds and it is a routine exercise. The investigation of plants components with the experiments follow a consistent path. Plants are compiled randomly or collected by the suppliers in the geographical areas where the plant is grown. [5]For the extraction of secondary plant components either fresh or dried plant component can be used. A number of scientists had reported about the extraction from the fresh part of the plant. The fact behind itarised from the ethno therapeutic application of fresh part of the plant among the customary people. The plants are used either in dried or in aqueous extract form but there may be different water content in different plant tissues. So it is preferable to dry the plants in air before extraction. Some analysts utilize the plant by drying it for 72 hours at 400c in the oven. Out of the proclaimed works, a number of underground parts such as bulb, roots, rhizome, tuber etc. of a plant were utilized widely for the bioactive mixes having the activities against microbes. [1, 4]

The preference of the solvents:

The kind of solvent utilized in extraction determine the fruitful determination of therapeutically active ingredients from a plant. An ideal solvent in plant extraction should be non-toxic/ low toxic, easily vaporized at low heat, boost the absorption of the extract. The selection on a solvent depends on the amount of the phytopharmaceuticals to be removed, extraction rate, differences of different blends extricated, easiness of the extract handling etc. [6] The preference of the solvent is affected by what is to be done with the extract. Subsequent to the end product will contain hints of remaining solvent, the solvent must be non-poisonous and must not meddle with the bioassay. The decision of selecting a suitable solvent will likewise rely on upon the intended compound to be separated. [1, 4]

A number of solvents used in extraction process are as follows:

(1). Water:

Water is the most common solvent which is very useful to extract the substances with antimicrobial activity. However, the organic solvents have been found to be more useful to show predictable antimicrobial activity than that extracted with water. Moreover water dissolvable flavonoids (such as anthocyanins) have no antimicrobial importance and water dissolvable phenolics is just essential as an antioxidant substance. [4]

(2). Alcohol:

Since the alcoholic extract has more amount of polyphenols than the aqueous extract, so it has more activity. It is more productive in the degradation of cell walls and the seeds having non-polar character and it cause polyphenols to be discharged from the cells. In water the chemical, polyphenol oxidase cause degeneration of polyphenols whereas in alcohol (methanol, ethanol) it remain inactive. Besides, water is a good medium for the growth of microbes when contrasted with ethanol. [7] The higher strength of more bioactive flavonoids were distinguished with ethanol (70%) because of its higher polarity than absolute ethanol. By adding water to the unadulterated ethanol up to 30% for planning ethanol 70% the polarity of dissolvable was enhanced. [8]It is also easy to enter the cell membrane by the ethanol to extricate the intracellular substances from plant material. [9] Almost all the distinguished parts of plants effective against microbes are aromatic or saturated organic compounds and they are effectively extracted by ethanol or methanol.[10] Methanol has more polarity than ethanol but it is cytotoxic in nature so it is unsatisfactory for extraction in certain sort of studies as it may yield false results.

(3). Acetone:

Acetone disintegrates various hydrophilic and lipophilic portions of plants. It shows miscibility with water and has volatile nature, also it has low poisonous nature. Because of these qualities, it is a very important extractant, especially for antimicrobial studies. According to a study aqueous acetone shows better extraction of tannins and phenolics as compared to aqueous methanol.[4, 6]Both acetone and methanol can extract saponins having antimicrobial effectiveness. [1]

(4). Ether:

It is used mainly for the extraction of fatty acids and coumarins. [10]

(5). Chloroform:

It is used mainly for the extraction of tannins and terpenoids. [10]

(6). Dichloromethanol:

It is another dissolvable utilized for completing the extraction methods. Sometimes, it is used for the extraction of terpenoids.[

Table 1: Solvents used for extraction of therapeutically active component [10]

S. No.	Name of the solvent	Therapeutic active component
1).	Water	Anthocyanins, Lectins, Polypeptides, Saponins, Starch, Tannins, Terpenoids
2).	Ethanol	Alkaloids, Flavonol, Polyacetylenes, Polyphenols, Sterols, Tannins, Terpenoids
3).	Methanol	Anthocyanins, Flavones, Lactones, Phenones, Polyphenols, Quassinoids, Saponins, Tannins, Totarol,Terpenoids, Xanthoxylline,
4).	Chloroform	Flavanoids, Terpenoids
5).	Ether	Alkaloids, Coumarins, Fatty acids, Terpenoids
6).	Acetone	Flavonols, Phenol

Different Approaches of Extraction:

The variety in the extraction techniques generally relies on:

(1). Duration of extraction

(2). The solvent utilized

(3). Temperature

(4). pH of the solvent used

(5). Size reduction of the plant specimen

(6). Solvent:sample ratio

Depending upon the plant specimen used, it should be either shade dried (e.g. leaves, flowers) or sun dried (e.g. bark, root). Then appropriate size reduction should be done to increase the surface area. This will further increase the rate of extraction. Solvent: sample ratio should be ideally 10:1 (v/w)[4].

Table 2: Mechanism of action of some phytopharmaceuticals[10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23]

S. No.	Phytopharmaceuticals	Therapeutic Activity	Mechanism of action
1).	Alkaloids	Antidiarrhoeal, Antimicrobial, Anthelmintic	Introduce in to the parasitic cell wall and DNA, restrict the discharge of autacoids and prostaglandins, have antioxidating impact, lesson the formation of nitrate which is required for the synthesis of protein, put down the transmission of sucrose to small intestine from the stomach, decrease the backing of glucose to the helminthes, effecting CNS to cause paralysis
2).	Coumarins	Antiviral	Interaction of DNA of Eukaryotes
3).	Flavonoids	Antidiarrhoeal, Antimicrobial	Complex with the cell wall and ties to adhesins, restrict the discharge of autacoids, prevent contractions brought on by spasmogens, constrain GI discharge of acetylcholine
4).	Glycosides	Antidiarrhoeal	Restrict the discharge of autocoids and prostaglandins
5).	Quinones	Antimicrobial	Causes cell wall lysis
6).	Polypeptides	Antiviral	Bar viral combination and forms disulfide bonds.
7).	Saponins	Anticancer, Antidiarrhoeal Anthelmintic	Represses histamine discharge in vitro, have membrane permeabilizing properties
8).	Steroids	Antidiarrhoeal	Increase the absorption of Na+ and water from the intestine
9).	Tannins & Polyphenols	Antidiarrhoeal Anthelmintic Antimicrobial	Ties to adhesins, restrict the enzymes, complex with cell wall, splitting of membrane, metal particle complexation, makes mucosa of intestinesafer and diminishes discharge.
10).	Terpenoids and essential oils	Antidiarrhoeal, Antimicrobial	Splitting of membrane, restrict the discharge of autacoids and prostaglandins.

Extraction methodology:

(A). Homogenization of the plant tissues:

This technique has been generally applied by scientists. In this technique,appropriate size reduction of plant specimens are done in a blender.Fine particles of plant specimens are put in a specific amount of dissolvable and shaken vigorously for 5 - 10 min and then left for 24 h after which the concentrateis separated. The filtrate then might be dried under low pressure.[4]

(B). Extraction by soxhlet apparatus:

This method is required when the intended compound has a constrained dissolvability in a dissolvable, and the contaminant is insoluble in that dissolvable. In case the intended compound has more dissolvability in a dissolvable then a straight forward filtration can be utilized to discrete the compound from the insoluble substance. The merit of this is that rather than numerous segments of warm dissolvable being gone through the example, only one group of dissolvable is reused. This technique can't be

utilized for thermolabile substances because heating for longer duration may cause degeneration or denaturation of the compounds. [24]

(C). Decoction:

This technique is utilized for the extraction of those substances (obtained from crude drug) that are soluble in water and are thermostable. The crude drug is boiled in water for about 15 minutes then cooled, filtered and adequate quantity of water is added to it to make the required volume. [2]

(D). Maceration:

In this process either entire plant or its some part or its roughly powdered specimen is kept in a solvent, which has ability of dissolving a number of active ingredients, in a closed container for a specific period of time with successive stirring. This strategy is best suitable for use if there should be an occurrence of the thermolabile medications. [1]

(E). Digestion:

It is a type of maceration where heat is given to the maceration process. It is utilized when respectably lifted temperature does not affect the active ingredient of the plant material and the dissolvable proficiency of the menstrum is expanded in this manner.[2]

(F). Percolation:

This method is mostly utilized for the preparation of tinctures and liquid concentrates. In this technique, ingredients in the solid form are kept in contact with the specific solvent and permitted to remain for around 4 hours in a properly closed container, then the mass is stuffed and the highest point of the percolator is shut. Extra menstruum is added to shape a shallow layer over the mass, and the blend is permitted to macerate in the shut percolator for 24 h. Outlet of percolator is then disclosed and the fluid contained in that is permitted to dribble gradually. Extra menstruum is included as desired, until the percolate measures around three quarters of the required volume of the final product. Now marc is squeezed and obtained fluid is then added to the percolate. Adequate menstruum is mixed to deliver the exact volume, and the blended fluid is filtered or decanted for the purpose of clarification. [3]

(G). Successive extraction:

In this technique, solvents of increasing polarity are successively used so that a number of compounds of different polarity could be removed. A few scientists utilize soxhletapparatus for the extraction of dried plant specimen using natural solvent. This strategy can't be utilized for thermolabile substances as delayed warming might cause degeneration of substances. [4]

(H). Sonication:

In this technique, ultrasounds of frequency range 20 kHz to 2000 kHz is used, which increases the porosity of cell wall and makes cavity in it. In spite of the fact that the procedure is valuable now and again, its extensive use is constrained because of its cost.Demerit of this technique is the production of free radicals, which may deteriorate bioactive molecules of plants.[3]

(I). Infusion:

In it the readily soluble parts of the crude drug are dissolved in a solvent and then diluted. The preparation of fresh infusions is by maceration of the crude drug for a brief timeframe with either boiled or cold water.

Phytochemical screening: These are done as per the methods described below:

1. Detection of alkaloids: Alkaloids are detected by a number of tests (as mentioned below). Firstly the extracts are solubilized in dilute hydrochloric acid and then clarified by filtration to remove impurities.

(i). Dragendroff's Test:

In this test the filtrates are reacted with Dragendroff's reagent. If alkaloids are present, a red precipitate is obtained.

(ii). Hager's Test:

In this test the filtrate is reacted with Hager's reagent which is a concentrated solution of picric acid. If yellow coloured precipitate is obtained, it indicate the presence of alkaloids.

(iii). Mayer's Test:

In this test the filtrate is reacted with Mayer's reagent which is potassiomercuric iodide solution and obtained by dissolving mercuric chloride and potassium iodide. Development of precipitate having yellow colourconfirms the existence of alkaloids.

(iv). Wagner's Test:

In this test the filtrate is reacted with Wagner's reagent. The development of precipitate having brown/redcolourconfirms the existence of alkaloids.

2. Detection of carbohydrates:

For performing this test the extract is solubilized individually in 5 ml of purified water and then filtered. This filtrate is utilized for the detection of carbohydrates in the sample. A number of tests can be used for the detection of the carbohydrates as follows:

(i). Benedict's test:

The filtrate was reacted with Benedict's reagent and then heated delicately.

Development of precipitate having redcolourconfirms the existence of reducing sugars such as glucose, galactose, fructose, etc.

(ii). Fehling's test:

In this method the filtrate is reacted with dilute hydrochloric acid to make it hydrolysed, neutralized with an alkali and then warmed with Fehling's solutions A & B. Appearance of precipitate having red color specify the existence of reducing sugars.

3). Detection of flavonoids:

(a). Alkaline reagent test:

The extract is reacted with few drops of solution having sodium hydroxide. Development of extreme yellow shading demonstrate the existence of flavonoids.

b) Lead acetate Test:

The extract is reacted with some drops of solution of lead acetate. Development of precipitate having yellow colourdemonstrate the existence of flavonoids.

4). Detection of glycosides:

Firstly the extract is reacted with dilute HCl to hydrolyse it and afterward subjected to test for glycosides as follows:

(a). Legal test:

In this test the extract is reacted with sodium nitroprusside (present in a mixture of pyridine and sodium hydroxide). Development of pink to red colour demonstrates the existence of cardiac glycoside.

(b). Modified Borntrager's test:

The extract is reacted with a solution containing ferric chloride and then drenched in boiled water for around 5 minutes. The blend is cooled and removed with equivalent volumes of benzene. The benzene layer was isolated and reacted with ammonia solution. Development of rose-pink shading in the ammonical layer demonstrates the existence of anthranol glycosides.

5). Detection of phenols:

Ferric Chloride Test:

The extract is reacted with few drops of solution containing ferric chloride. Development of bluish black colour demonstrate the existence of phenol.

6). Detection of phytosterols:

(a). Libermann Burchard's test:

Extract is made to react with chloroform and then filtered. Filtrate is made to react with some drops of acetic anhydride and then after boiling it is cooled. Concentrated sulphuric acid is included to it. Development of brown ring at the intersection demonstrate the existence of phytosterols.

(b). Salkowski's Test:

The extract is reacted with chloroform and filtered. Filtrate is then made to react with some drops of conc. sulphuric acid, stirred and permitted to stand. Development of brilliant yellow colour demonstrate the existence of triterpenes.

7). Detection of proteins and aminoacids

a). Ninhydrin Test:

0.25% w/v ninhydrin reagent is made to react with the extract and then boiled for some minutes. Development of blue colour demonstrate the existence of amimo acid.

a) Xanthoproteic Test:

The extract is reacted with some drops of concentrated nitric acid. Development of yellow colour demonstrate the existence of proteins.

8). Detection of saponins:

(a). Foam Test:

The extract (0.5 gm) is taken with about 2 ml of water. In case the foam appears on shaking and remains for ten minutes, it demonstrate the existence of saponins.

(b). Froth Test:

In this method the extract is diluted to small quantity of water (20 ml) and then it is stirred in a graduated cylinder for about 15 minutes. Development of foam of 1 cm, demonstrate the existence of saponins.

8. Detection of tannins

Gelatin Test:

1% solution of gelatin was made to react with the extract. Development of a precipitate having white colordemonstrate the existence of tannins.

9). Detection of diterpenes:

Copper acetate Test:

The extract is solubilized in water and then made to react with some drops of copper acetate solution. Development of emerald greencolour demonstrate the existence of diterpenes. [25, 26,27]

Conclusion:

The non-standard methods of extraction might prompt the degeneration of the phytochemicals that are present in the plants and might prompt the varieties in this way prompting the absence of reproducibility. Endeavors ought to be made to create batches with quality and maintain consistency (as far as possible) and to establish and follow the best extraction method.

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