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Concept of standardization, extraction and pre phytochemical screening strategies for herbal drug

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ABSTRACT

Standardization of drugs means confirmation of its identity and determination of its quality and purity. At present due to advancement in the chemical knowledge of crude drugs various methods like botanical, chemical, spectroscopic and biological methods are used for estimating active constituents present in the crude drugs in addition to its physical constants. Plants have been known to relieve various diseases in Ayurveda. Therefore, the researchers today are emphasizing 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 with their advantages and disadvantages.

Keywords: Standardization, quality, purity, herbal products, phytochemicals, extraction, solvent, screening.

1. Introduction

Standardization is defined as best technical application consensual wisdom inclusive of processes for selection in making appropriate choices for ratification coupled with consistent decisions for maintaining obtained *standards*. This view includes the case of "spontaneous standardization processes", to produce de facto standards. 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. World Health Organization (WHO) encourages, recommends and promotes traditional/herbal remedies in national health care programmes because these drugs are easily available at low cost, safe and people have faith in them. 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 solubilise compounds with similar polarity. Phytopharmaceutical and secondary plant product of medicinal importance such as alkaloids, glycosides, terpenoids, Flavonoids and lignans.

2. Pharmacopoeial Standards

The authenticity, quality and purity of herbal drugs are established by reference given in pharmacopoeia. The pharmacopoeia prescribes (numerical value) like structural, analytical, physical standards for the drugs. The important standards mentioned in pharmacopoeia are shown in figure 1. A critical examination and identification of crude drugs is required in manufacturing of herbal formulation because of great diversity and variability in their chemical characters. To overcome this problem all the pharmacopoeias have laid down certain standards. Specific tests for certain plant materials are given below. Volatile oil content haemolytic activity foaming index bitter value tannin content. Fat content Acid value Saponification value Iodine value Assay for Aluminium/Arsenic/Borate/Calcium.Camphor/Chloride/Copper/Gold/Iron.Lead/Magnesium/Mercury/Phosphate. Potassium/Silica/Silver/Sodium.Sulpher/Sulphate/Tin.

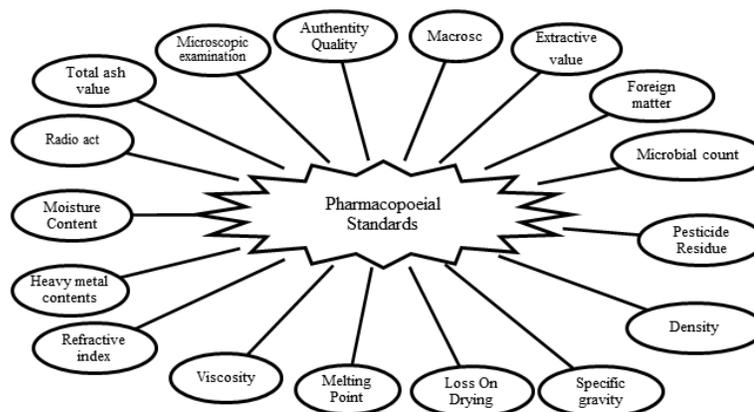


Fig 1: Standardization parameter for plant drugs

3. Extraction Procedure

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 distillation techniques (water distillation, steam distillation, phytonic extraction (with hydro fluorocarbon solvents). For aromatic plants, hydro water and steam distillation), hydrolytic maceration followed by distillation, expression and effleurage (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.

The basic parameters influencing the quality of an extract are:

- Plant part used as starting material
- Solvent used for extraction
- Extraction procedure

Effect of extracted plant phytochemical depends on:

- The nature of the plant material
- Its origin
- Degree of processing
- Moisture content
- Particle size

The variations in different extraction methods that will affect quantity and secondary metabolite composition of an extract depend upon:

- Type of extraction
- Time of extraction
- Temperature
- Nature of solvent
- Solvent concentration
- Polarity

Plant material

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.

Choice of solvents

For Successful determination of biologically active compounds from plant material is largely dependent on the type of solvent used in the extraction procedure.

A property 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 phytochemical 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

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.

Variation in extraction methods usually depends upon:

- Length of the extraction period,
- Solvent used,
- pH of the solvent,
- Temperature,
- Particle size of the plant tissues
- 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

Solvents used for active component extraction are: Water, Ethanol, Methanol, Chloroform, Ether, and Acetone.

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	Flavonols	Tannins		Fatty acids	
Terpenoids	Terpenoids	Xanthoxylines			
Polypeptides	Sterols	Totarol			
Lectins	Alkaloids	Quassinoids			
		Lactones			
		Flavones			
		Phenones			
		Polyphenols			

Extraction procedures

- a. **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 ^[4].
- b. **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 ^[4].
- c. **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 ^[18].
- d. **Maceration:** In maceration (for fluid extract), whole or coarsely powdered plant-drug 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 ^[1].
- e. **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 ^[2].
- f. **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 ^[2].
- g. **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 menstruum is increased thereby ^[2].
- h. **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 menstruum 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 menstruum 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 menstruum is added to produce the required volume, and the mixed liquid is clarified by filtration or by standing followed by decanting ^[3].
- i. **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 ^[3].

4. Phytochemical Assay

Most of the drugs have definite specific chemical constituents to which their biological or pharmacological activity is attributed. Qualitative and quantitative characterization of the active ingredient should be assayed using biomarkers. Defining of the biomarker has to be very specific and a lot of insight has to go into it before declaring any distinct molecule. Additionally the mixture should be analyzed to develop finger print profile. A general protocol followed for chemical assay for herbal drugs is shown in figure 2

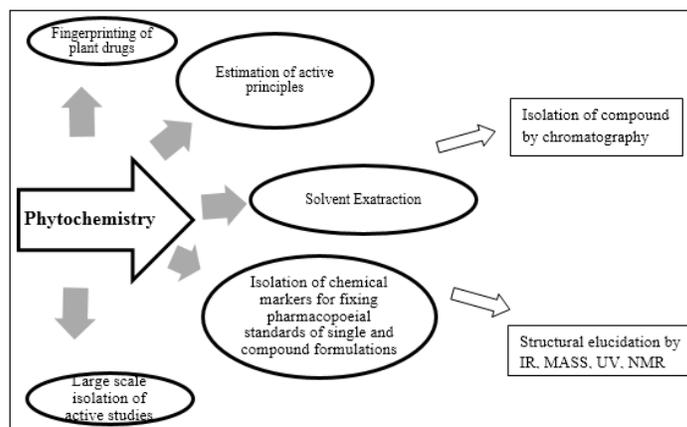


Fig 2: Phyto chemical evaluation of herbal drugs

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

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 coloured 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.

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.

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 ammoniacal layer indicates the presence of anthranol glycosides.

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

5. Detection of saponins

- **Froth Test:** Extracts were diluted with distilled water to 20ml 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.

6. 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.
- **Liebermann Burchard 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.

7. 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.

8. 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.

9. 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.

10. Detection of proteins and amino acids

- **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.

11. 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 [19, 21, 22].

5. Conclusion

The pharmacopoeial standards in Ayurvedic Pharmacopoeia of India are not adequate enough to ensure the quality of plant materials since the materials received in the manufacturing premises are not in a condition that effective microscopic examination can be done. Therefore chemical, methods, instrumental methods and then layer chromatographic analysis would determine the proper quality of plant material. Non standardized procedures of extraction may lead to the degradation of the phytochemical 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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