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RESEARCH ARTICLE

METHODS FOR PREPARING MEDICINALPLANTEXTRACTS: A REVIEW

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ABSTRACT

Extraction of phytochemicals from medicinal plants is an important first step in preparing different formulations. Various modern methods have now come to the forefront which preserve the phytochemicals and prevent their degradation as compared to the ancient methods. This article focusses on elaborating the principles of different extraction processes with their pro's and con's and different factors influencing the process. It is of utmost importance for researchers working with plant extracts to have thorough knowledge of different processes and select the most suitable one to ensure high quality of extracts.

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INTRODUCTION

Plants and their parts such as stems, leaves, flowers, fruits,etc have been used in our country for decades in Unani, Ayurveda, homeopathy medicine. Millions of plants and trees possessing antimicrobial properties are reported in literature, but whose role in the field of medicine have not been explored. Studies are required to assess the potential of various plants present around us and put their novel bioactive compounds for use in the prevention and cure of diseases. Phytochemicals are organic constituents extracted from plants and their parts containing various biologically active compounds and can be considered an excellent natural alternative to synthetic chemicals for anti-cariogenic purpose (Ramakrishna, 2011). Numerous active phytoconstituents such as Flavanoids, Phenols etc pharmaceutically derived from plants have been associated with antioxidant activities are components of various life-saving drugs. Due to its benefits, there has been a shift towards natural products in pharmaceutical industry, the research on medicinal plants are as important as that of conventional drugs.

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Extraction is the process of separation of medicinally active portions of plants by using selective solvents through various standard procedures (Handa *et al*., 2008).

Extraction process: Extraction is a crucial first step because it is important to extract the desired phytochemical compounds for further isolation and characterization.

The basic steps are: Identification of the plant sample, its bulk collection, pre-washing, air- drying or freeze drying, crushing and grinding the plant material to obtain a homogeneousand uniform sample. This improves the kinetics of extraction and increases the contact area of the sample's surface with the solvent. Proper precautions must betaken so that phytoconstituents are not lost, distorted or destroyed while preparing the extract. The selection of solvent system depends on the specific nature of the bioactive compound (non-polar or polar and thermally labile) to be isolated. Various solvent systems are available. Hydrophilic compound extraction requires polar solvents such as methanol, ethanol, etc and for lipophilic compounds dichloromethane or a mixture of dichloromethane/methanol in a ratio of 1:1. Sometimes

extraction with hexane is required to remove chlorophyll (Sasidharan *et al*., 2011).

What makes extraction Difficult?: The aim of preparing a plant extract is to obtain certain bioactive phytoconstituents while getting rid of the rest of the plant material. To isolate individual constituents an elaborate and technique-sensitive procedure is required. Another issue remains requirement of bulk amount of plant material. Proper cleaning and drying of the plant part is an essential but neglected step. Step by step process in the extraction of plant material results in successful results (https://biohackacademy. github.io/bha3/ class/3/ pdf/Plant-Extraction.pdf).

Pre-extraction preparation of plant samples: Pre-preparation procedures of plant materials such as grinding and drying influence the preservation of phytoconstituents in the plant extracts and is a crucial step.

Fresh vs dried Samples: While both the types as used for preparing plant extracts, dried samples are preferred due to the limited time span in which the samples remain fresh. Sulaiman *et al* suggested a maximum period of 3 hours was available between the harvest of the fresh plant sample and its use for experiments. Also fresh samples tend to be fragile and deteriorate fast (Azwanida, 2015).

Different methods of drying plant material

- **Air-drying**: Takes a few days (Sasidharan, 2011; https://biohackacademy.github.io/bha3/class/3/pdf/Plant-Extraction.pdf; Azwanida, 2015; Fani, 2012; De Silva, 2017) to months to a year, depending on the part of the plant being dried (eg. leaves or seed). Plant samples, usually stem along with leavescan be hung after being tied together so that the plant is exposed to air at room temperature. This method preserves the heat-labile constituents of the plant materials from high temperature, but takes longer time compared to other methods and can be contaminated at unstable temperature condition (Azwanida, 2015).
- **Microwave-drying**: Uses the electromagnetic radiation for drying. The electric field causes simultaneous heating through dipolar rotation and ionic induction, which oscillates molecules resulting in faster heating of the samples simultaneously. This method shortens the drying time, but may cause the degradation of phytochemicals (Azwanida, 2015).
- **Oven-drying**: Uses thermal energy for removing moisture from the samples. This is an easy and fast method of thermal processing that can preserve phytochemicals. Oven-drying is done at 44.5°C for 4 hours using 80% methanol to produce optimized extracts (Azwanida, 2015).
- **Freeze-drying**is: Based on the principle of sublimation. The Sample freezes at -80 degree C to -20 degree C before lyophilisation to freeze any liquid (eg. solvent, moisture) within the samples. After freezing overnight (12hours), the sample is immediately lyophilized.Freeze-drying provides higher level of phenolic contents compared to other methods and preserves most of the phytochemicals. However, it is a complex and expensive method of drying (Azwanida, 2015).

Extraction Methods

Maceration, infusion, percolation and decoction: Maceration is a technique commonly used in wine-making and to make plant extract. Whole or coarsely powdered crude drug is placed in a container which is stoppered and contains the solvent and is allowed to stand at room temperature for a period of 3days with constant agitation until the soluble matter has completely dissolved. This process dissolves the plant cell wall, releasing the soluble phytochemicals. The mixture isthen strained, the Marc (the damp solid material) is pressed, and the combined liquids are then clarified by the method of filtrationor decantation after standing for sometime. (Handa, 2008; Azwanida, 2015; Fani, 2012). The commonly used solvents are methanol, ethanol or a mixture of water and alcohol (Sasidharan *et al*., 2011) Maceration is an old technique as not all active phytoconstituents are extracted (De Silva, 2017). Infusion **s**can be freshly prepared by macerating the crude drug for a short period of time with either cold or boiling water. These forms dilute solutions of the easily soluble components of crude extract (Handa *et al*., 2008; Azwanida, 2015).

Decoction is similar to infusions, here the crude drug is macerated and boiled in a specified volume of water (1:4 or 1:16) for a defined period of time. Boiling reduces the volume to one-fourth the original volume. The extract obtained is cooled, followed by straining or filtering. This procedure is used for extracting water-soluble, heat stable components, hard plants materials (e.g. roots and barks) (Handa, 2008; Azwanida, 2015; Fani, 2012; Majekodunmi, 2015). Percolationis frequently used to extract active components in the preparation of tinctures and fluid extracts. A percolator is a narrow, cone-shaped vessel open at both ends. The solid ingredients are moistened with a specified menstruum and isallowed to stand for around 4 hours in a closed container to obtain a packed mass. Extra amounts of menstruum is added above the mass to form a shallow layer. The mixture is allowed to macerate within the closed percolator for 1 day. The percolator's outlet is opened and the liquid contained inside is allowed to slowly drip (6 drops/min). Additional menstruum is again added as required, until the volume of the percolate measures about three-quarters of the required volume of the final extract. The marc is pressed and the liquid which is expressed is added to the percolate. The mixed liquid is clarified by either filtration or by allowing it to stand followed by decanting. (Handa *et al*., 2008; Azwanida, 2015; Fani, 2012; Majekodunmi, 2015)

Advantages and Disadvantage: Simple and easy method. Since large volume of solvents is used, proper organic waste management is required. Changes in temperature as well as correct selection of solvents reduces the volume needed for extraction. (Azwanida, 2015)

Soxhlet extraction or hot continuous extraction: Soxhlet extraction is a common and well- established technique. Here, the finely ground crude sample is placed inside a "thimble" (porous bag) which is made of strong filter paper or cellulose, placed in the thimble chamber of the Soxhlet apparatus. The extracting solvent in the bottom flask is heated, and its vapors are allowed to condense in the condenser. The condensed extract drips slowly into the thimble where the crude sample is present, and extracts it via contact. When the level of liquid rises to the peak of arm of siphon tube, the liquid contents of

chamber are emptied into bottom flask and the process continues again until a single drop of solvent from thesiphon tube does not leave behind a residue when evaporated. (Azwanida, 2015; Fani, 2012; Majekodunmi, 2015). The commonly used solvents for this procedure are methanol, ethanol or a mixture of water and alcohol (Sasidharan, 2011).

Advantages and Disadvantages: The advantage of this method, is that large amounts of extract can be obtained with a small quantity of solvent. Filtration of the extract obtained is not required, saving large amount of time, energy and financial inputs. It has disadvantage of exposure to flammable and hazardous solvents, with toxic emissions during the process, thus not eco-friendly. High-purity solvents need to be used Agitation of the content is not possible in this apparatus.

Microwave assisted extraction (MAE): MAE uses microwave energy to facilitate separation of analytes from the crude sample in to the solvent. Microwave radiation usually interacts with dipoles of polar and polarizable materials (e.g. solvents and sample) which heats the surface near the materials and the heat is transferred by conduction disrupting the hydrogen bonds by Dipolerotation, which promotes the penetration of solvent into the matrix. In non-polar solvents, there is poor heating, as the energy is transferred by dielectric absorption. Commercial microwave commonly uses the frequency 2450 MHz, which corresponds to an energy output of 600–700 Watts.MAE is a method that favours polar molecules and solvents with high dielectric constant (Azwanida, 2015; Majekodunmi, 2015; Al-Manhel, 2015).

Advantages and Disadvantages: MAE is an alternative to conventional methods because it reduces time as well as extraction solvent volume. The recoveries of analytesand reproducibility were improved, but proper caution needs to be maintained to avoid thermal degradation. In case Additional cycles of MAE were used, drastic decrease in the yield of phytoconstituents due to the oxidation of compounds is seen. Tannins and anthocyanins potentially degrade at high temperature of MAE. (Azwanida, 2015; Al-Manhel, 2015)

Ultrasound-assisted extraction (UAE) or sonication extraction: UAE uses ultrasound with frequencies ranging from 20 kHz to2000 kHz. The acoustic cavitation has a mechanical effect of the ultrasound, which increases the surface contact between the sample and the solvent as well as the permeability of cell walls, maintaining a high quality of compounds. There is an alteration of Physical and chemical properties of the materials treated by ultrasound and disruption of the plant cell wall which releases the compounds and enhances bulk transport of the solvents into the plant cells. Solvents commonly used for this process are similar to Soxhlet extraction. Here a crushed sample is placed into the ultrasonic bath after mixing it with a suitable solvent, while controlling the temperature and extraction time. (Handa, 2008; Sasidharan *et al*., 2011; Azwanida, 2015; Altemimi, 2007).

Advantages and Disadvantages: Extraction is achieved at low temperatures so it is suitable for extraction of thermally unstable compounds. This technique uses a less quantity of solvent and extraction time. This method is restricted to large scale use due to its high cost. Ultrasound also has deleterious effects on the active phytoconstituents due to formation of free radicals (Handa, 2008; Azwanida, 2015; De Silva, 2017; Altemimi, 2017).

Accelerated solvent extraction (ASE): ASE is an effective form of liquid solvent extraction as there is minimal amount of solvent used. Sample is packed in a stainless steel extraction cell with an inert material such as sand to prevent the sample from blocking the system tubing, with layers of sand-sample mixture in between sand layers and cellulose filter paper. This process is performed at increased temperatures, 500 -2000^{0}C, and at pressures between 10-15 MPa. Elevated temperature accelerates the kinetics of extraction and increased pressure keeps the solvent in the liquid state, thusextraction process is safe and rapid. Mostly organic solvents are used, but pressurized hot water may be used, thus its also called hot water extraction or sub-critical water extraction. (Azwanida, 2015; Fani, 2012; De Silva, 2017; Majekodunmi, 2015).

Advantages and Disadvantages

This technique controls temperature and pressure for individual samples and requires very little time as well as less amount of solvents. This method has a widehandling of acidic and alkaline matrices. As it is performed at high temperatures, it may degrade the thermo labile compounds. (Azwanida, 2015; Fani, 2012; De Silva, 2017; Majekodunmi, 2015).

Supercritical fluid extraction (SFE): Supercritical fluid (dense-gas) is an entity that shares the physical properties of both liquid and gas at its critical point.Temperature and pressure are factors that push a substance into its critical region. Supercritical gases such as carbon dioxide, nitrogen, ethane, methane, ethylene, sulfur dioxide, nitrous oxide, propane, propylene, ammonia and sulfur hexafluoride are used to extract active phytoconstituents. The sample is kept in a vessel filled with a gas under controlled conditions of temperature and pressure. The active phytoconstituents which dissolved in the gas get separated when both temperature and pressure are lowered. CO2 often has low solubility for polar components so methanol or ethanol or Argon can be added. (Handa *et al*., 2008; Azwanida, 2015; Fani, 2012; De Silva, 2017).

Advantages and Disadvantages: Co2 is inexpensive and safe extracting fluid. The process takes place at low temperatures, which avoids damage from heat as well as leaves no solvent residues behind. This is an eco-friendly method. SFE finds application in the pesticides, fragrances, essential oils, food etc. industry (Handa *et al*., 2008; Azwanida, 2015; De Silva, 2017).

Aqueous alcoholic extraction by fermentation: Ayurvedic preparations use this technique for extraction of phytoconstituents. Different types of solvents, such as methanol, hexane, and ethyl alcohol are used for antioxidant extraction of plant material.The crude sample is soaked in either a powder or a decoction form, for a specificperiod of time, during which fermentation takes place and generates alcohol in situ. The alcohol formed also serve as a preservative (Handa *et al*., 2008; Altemimi, 2017).

Counter-current extraction (CCE): In this method, fine slurry is produced by pulverising wet raw material, using toothed disc disintegrators .The material to be extracted is moved within a cylindrical extractor in one direction, in the form of a fine slurry, where it comes in contact with extraction solvent. The further movement of the starting material, makes the material more concentrated.

Optimization of the quantities of solvent andmaterial and their flow rates completes the extraction process. The process is highly efficient and no risk from high temperature. The extraction time required is limited (Handa *et al*., 2008).

Phytonics process: A new solvent 1,1,2,2-tetrafluoroethane (hydrofluorocarbon-134a)is used to optimize the extraction of plant materials by offering significant environmental advantages and health and safety benefits. The boiling point of this solvent is -25° C. It isn't flammable/toxic, neither does it deplete the ozone layer. It has a vapor pressure of 5.6 bar. The processing sample is totally sealed to continually recycle the solvents and fully recover them at the end of each production cycle. Since the solvents don't escape the process is eco-friendly, with no harmful emissions. The process takes place in cool and gentle temperatures not harming the constituents (Handa *et al*., 2008).

Parameters for selecting an appropriate extraction method

- Plant material should be authenticated and all foreign material eliminated before the extraction.
- Record Age of plant, time, season and place of collection.
- Phytoconstituents to be extracted decide the drying conditions. Hot or cold blowing air flow is preferred for drying.
- Grinding methods should be specified.
- Uniform sized particles can be obtained by seiving the powdered plant material.
- Nature of constituents:
- Nonpolar solvents to be used, if non-polar constituents are required.
- If thermo labile constituents are required, cold maceration, CCE and percolation are preferred.
- For thermos table constituents, Decoction could be used when water is the mestrum or Soxhletextr action if nonaqueous solvents are used.
- Constituents that degrade in organic solvents, (flavonoids etc.) should be dealt with precautions.
- Higher than required temperatures to be avoided, as some constituents degrade.
- Standardization of extraction time is important . Longer extraction time, may extract unwanted constituents.
- F) Duration and number of extraction cycle is important for complete extraction.
- Control the quality of menstruum.
- Drying under reduced pressure (e.g. Rotavapor) and lyophilization is widely used.
- The design and material of the extractor should be considered.
- Document analytical parameters of the final extract, to monitor the quality of the extracts (Handa *et al*., 2008; Majekodunmi, 2015).

Factors affecting selection of an extraction process

- Physical nature of the drug
- The Cost of drug: Expensive drugs are extracted by percolation whereas cheap drugs may be extracted by maceration.
- Stability of drugs
- Therapeutic value of drug: Bitter drugs or drugs with flavouring agents may be extracted by maceration as they have less therapeutic value.
- Nature of solvent: maceration is used when the solvent is water.
- Concentration of the product: Dilute preparations may be prepared bymaceration and concentrated preparations by percolation or reserved percolation process. (8)

DISCUSSION

B Vongsaka *et a .,* 2013 saw that maceration of *Moringa oliefera* with 70% ethanol powdered dried samples at 1:40 w/v showed highest phenolic and flavonoid content when compared to percolation or soxhlet extraction with similar solvent. M A Hossain *et al.,* 2013 studied that numerous phytochemicals were retrieved with the Extraction of *Azadirachta indica* (Neem) leaf powder in methanol (~1:5 w:v) using Soxhlet extraction; mostly non-polar compounds. Li *et al.,* 2012 saw that extracts prepared using MAE presented more antioxidant activity and phenolic content than conventional methods using various solvents. P Puttarak *et al.,* 2012 saw that MAE extraction of triterpene from *Centella asiatica* showed twice the extract of Soxhlet extraction under similar conditions. Mulinacci *et al* ., 2004 compared the extraction of phenolic compounds from strawberries using UAE with other methods such as solid–liquid, subcritical water, and MAE saw that UAE had best results. Tan *et al.,* 2014 saw 94% recovery of flavonoids from *Rheum palmatun* using 80% aqueous methanol by ASE, proving the suitability of this method.

Conclusion

The process of extraction of phytoconstituents from different plant parts is highly technique sensitive and proper knowledge of all steps from pre-extraction to extraction is important for obtaining a high quality extract. There is not a single universalextraction method which is ideal. Each extraction procedures depends on the plant as well on various factors as mentioned in the study. The efficiency of the process depends on the meticulous way in which it is performed. Thus, selection extraction methods should be suitably done depending on the study objectives, samples, and target compounds for successful results.

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