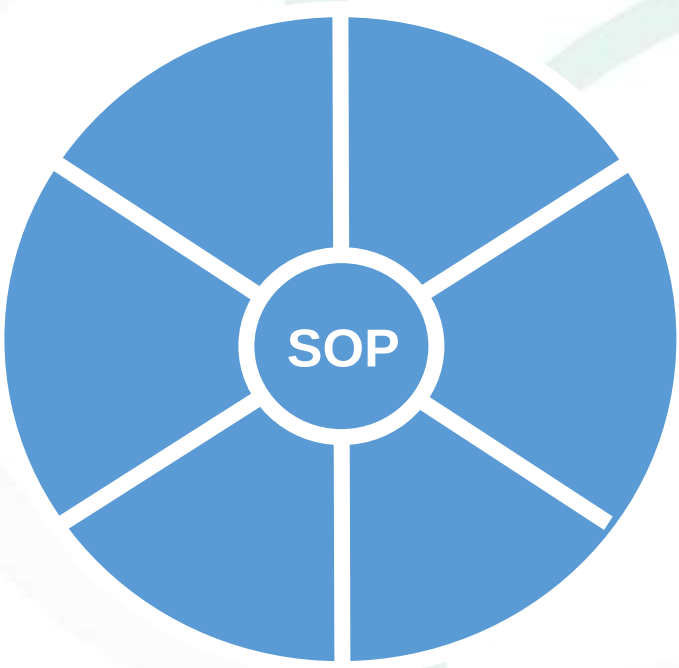
آزمایشگاه جامع تحقیقات
دانشگاه علوم پزشکی تبریز



Techniques for extraction of Medicinal Plants

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Extraction is the first step to separate the desired natural products from the raw materials. Extraction methods include solvent extraction, distillation method, pressing and sublimation according to the extraction principle. Solvent extraction is the most widely used method. The extraction of natural products progresses through the following stages:

(1) the solvent penetrates into the solid matrix;
(2) the solute dissolves in the solvents;
(3) the solute is diffused out of the solid matrix;
(4) the extracted solutes are collected.

Any factor enhancing the diffusivity and solubility in the above steps will facilitate the extraction. The properties of the extraction solvent, the particle size of the raw materials, the solvent-to-solid ration, the extraction temperature and the extraction duration will affect the extraction efficiency.

The general techniques of medicinal plant extraction:

a. Maceration,
b. Infusion
c. Percolation
d. Digestion
e. Decoction
f. Hot continuous extraction (Soxhlet)
g. Aqueous-alcoholic extraction by fermentation
h. Counter-current extraction
i. Microwave-assisted extraction,
j. Ultrasound extraction (sonication)
k. Supercritical fluid extraction,
l. Phytonic extraction (with hydrofluorocarbon solvents)

The selection of the solvent is crucial for solvent extraction. Selectivity, solubility, cost and safety should be considered in selection of solvents. Based on the law of similarity and intermiscibility (like dissolves like), solvents with a polarity value near to the polarity of the solute are likely to perform better and vice versa. Alcohols (EtOH and MeOH) are universal solvents in solvent extraction for phytochemical investigation.

Properties of a good solvent in plant extractions

1. low toxicity,
2. ease of evaporation at low heat
3. promotion of rapid physiologic absorption of the extract
4. preservative action
5. inability to cause the extract to complex or dissociate

Solvents:

Water:
It is the most polar solvent and is used in the extraction of a wide range of polar compounds.

Advantages. It dissolves a wide range of substances; it is cheap, nontoxic, nonflammable, and highly polar.

Disadvantages. It promotes bacterial and mold growth; it may cause hydrolysis, and a large amount of heat is required to concentrate the extract.

Alcohol:
It is also polar in nature, miscible with water, and could extract polar secondary metabolites.

Advantages. It is self-preservative at a concentration above 20%. It is nontoxic at low -concentration, and as small amount of heat is required for concentrating the extract.

Disadvantages. It does not dissolve fats, gums, and wax; it is flammable and volatile.

Chloroform:
It is a nonpolar solvent and is useful in the extraction of compounds such as terpenoids, flavonoids, fats, and oils.

Advantages. It is colorless, has a sweet smell, and is soluble in alcohols. It is also well absorbed and metabolized in the body.

Disadvantages. It has sedative and carcinogenic property.

Ether:
It is a nonpolar solvent and is useful in the extraction of compounds such as alkaloids, terpenoids, coumarins, and fatty acids.

Advantages. It is miscible with water, has low boiling point, and is tasteless in nature. It is also a very stable compound and does not react with acids, bases, and metals.

Disadvantages. It is highly volatile and flammable in nature.

Ionic liquid (green solvent).:
This is a unique solvent of extraction and is highly polar and extremely heat stable. It can remain in a liquid state even at 3,000oC and usable where high temperature is applicable. It has extreme miscibility with water and other solvent and is very suitable in the extraction of polar compounds.
Advantages. It has excellent solvent that attracts and transmit microwave, and hence it is suitable for microwave-

assisted extraction. It is nonflammable and is useful for liquid-liquid extraction and highly polar.

Disadvantage. It is not ideal for preparation of tinctures.

There are 5 basic steps working on plant extraction:

1. **Size reduction:**
   To rupture plant organ, tissue & cell structures so that its medicinal ingredients are exposed to the extraction solvent.
   Size reduction maximizes the surface area, which in turn enhances the mass transfer of active principle from plant material to the solvent. The 30-40 mesh size is optimal. Hammer mill or a disc pulverizer which has built in sieves controlled by varying the speed of the rotor clearance b/w the hammers & the lining of the grinder.

2. **Extraction:**

   Medicinal plants:
   1. Cold aqueous percolation
   2. Hot aqueous extraction (decoction)
   3. Solvent extraction (cold / hot)

   Plant based natural constituents can be derived from any part of the plant like bark, leaves, flowers, roots, fruits, seeds, etc.

   - Plants are usually air dried to a constant weight before extraction.
   -oven drying: every part was cut into pieces
   -dried in an oven @ 60°C for 9 hrs. & pulverized.
   -Other method for drying the plants is the oven drying at about 40°C for 72 h.

3. **Filtration:**
   The extract so obtained is separated out from the marc (exhausted plant material) by allowing it to trickle into a holding tank through the builtin false bottom of the extractor, which is covered with a filter cloth.
   - The marc is retained at the false bottom, and the extract is received in the holding tank. • From the holding tank, the extract is pumped into a sparkler filter to remove fine or colloidal particles from the extract.

4. **Concentration:**
   The enriched extract from percolators or extractors, known as miscella, is fed into a wiped film evaporator where it is concentrated under vacuum to produce a thick concentrated extract.
   -The concentrated extract is further fed into a vacuum chamber dryer to produce a solid mass free from solvent.

   -The solvent recovered from the wiped film evaporator and vacuum chamber dryer is recycled back to the percolator or extractor for the next batch of plant material.

   -The solid mass thus obtained is pulverized and used directly for the desired pharmaceutical formulations

or further processed for isolation of its phytoconstituents.

5. Drying:

The filtered extract is subjected to spray drying with a high-pressure pump at a controlled feed rate and temperature to get dry powder.

-The desired particle size of the product is obtained by controlling the inside temperature of the chamber and by varying the pressure of the pump.

-The dry powder is mixed with suitable diluents or excipients and blended in a double cone mixer to obtain a homogeneous powder that can be straight away used (for example, for filling in capsules or making tablets).

Methods used in extraction of medicinal plants:

Factors to be considered in choosing extraction method:

a) Stability to heat. Heat-stable plant material is extracted using Soxhlet extraction or microwave-assisted extraction, whereas plant materials that are not heat stable are extracted using maceration or percolation.

(b) Nature of solvent. If the solvent of extraction is water, maceration is a suitable method but for volatile solvent percolation and Soxhlet extraction are more appropriate.

(c) Cost of the drug. Cheap drugs are extracted using maceration, whereas costly drugs are preferably extracted using percolation.

(d) Duration of extraction. Maceration is suitable for plant material requiring long exposure to the menstruum, whereas techniques such as microwave- or ultrasound-assisted extraction are used for a shorter duration.

(e) Final volume required. Large volume products such as tinctures are prepared by maceration, whereas concentrated products are produced by percolation or Soxhlet extraction.

(f) Intended use. Extracts intended for consumption by human are usually prepared by maceration, whereas products intended for experimental testing are prepared using other methods in addition to maceration.

The general techniques of medicinal plant extraction

Maceration:

This is an extraction procedure in which coarsely powdered drug material, either leaves or stem bark or root bark, is placed inside a container; the menstruum is poured on top until completely covered the drug material. The container is then closed and kept for at least three days. The content is stirred periodically, and if placed inside bottle it should be shaken time to time to ensure complete extraction. At the end of extraction, the micelle is separated from marc by filtration or decantation. Subsequently, the micelle is then separated from the menstruum by evaporation in an oven or on top of water bath. This method is convenient and very suitable for thermolabile plant material.

Infusion:

This is an extraction process such as maceration. The drug material is grinded into fine powder, and then placed inside a clean container. The extraction solvent hot or cold is then poured on top of the drug material, soaked, and kept for a short period of time. This method is suitable for extraction bioactive constituents that are readily soluble. In addition, it is an appropriate method for preparation of fresh extract before use. The solvent to sample ratio is usually 4:1 or 16:1 depending on the intended use.

Digestion:

This is an extraction method that involves the use of moderate heat during extraction process. The solvent of extraction is poured into a clean container followed by powdered drug material. The mixture is placed over water bath or in an oven at a temperature about 50^{o}C. Heat was applied throughout the extraction process to decrease the viscosity of extraction solvent and enhance the removal of secondary metabolites. This method is suitable for plant materials that are readily soluble.

Decoction:

This is a process that involves continuous hot extraction using specified volume of water as a solvent. A dried, grinded, and powdered plant material is placed into a clean container. Water is then poured and stirred. Heat is then applied throughout the process to hasten the extraction. The process is lasted for a short duration usually about 15min. The ratio of solvent to crude drug is usually 4:1 or 16:1. It is used for extraction of water soluble and heat stable plant material.

Percolation:

The apparatus used in this process is called percolator. It is a narrow-cone-shaped glass vessel with opening at both ends. A dried, grinded, and finely powdered plant material is moistened with the solvent of extraction in a clean container. More quantity of solvent is added, and the mixture is kept for a period of 4h. Subsequently, the content is then transferred into percolator with the lower end closed and allow to stand for a period of 24h. The solvent of extraction is then poured from the top until the drug material is completely saturated. The lower part of the percolator is then opened, and the liquid allowed to drip slowly. Some quantity of solvent was added continuously, and the extraction taken place by gravitational force, pushing the solvent through the drug material downward. The addition of solvent stopped when the volume of solvent added reached 75% of the intended quantity of the entire preparations. The extract is separated by filtration followed by decantation. The marc is then expressed and final amount of solvent added to get required volume.

Soxhlet extraction (Hot Continuous Extraction):

This process is otherwise known as continuous hot extraction. The apparatus is called Soxhlet extractor made up of glass. It consists of a round bottom flask, extraction chamber, siphon tube, and condenser at the top. A dried, grinded, and finely powdered plant material is placed inside porous bag (thimble) made up of a clean cloth or strong filter paper and tightly closed. The extraction solvent is poured into the bottom flask, followed by the

thimble into the extraction chamber. The solvent is then heated from the bottom flask, evaporates, and passes through the condenser where it condenses and flow down to the extraction chamber and extracts the drug by coming in contact. Consequently, when the level of solvent in the extraction chamber reaches the top of the siphon, the solvent and the extracted plant material flow back to the flask. The entire process continues repeatedly until the drug is completely extracted, a point when a solvent flowing from extraction chamber does not leave any residue behind. This method is suitable for plant material that is partially soluble in the chosen solvent and for plant materials with insoluble impurities. However, it is not a suitable method for thermolabile plant materials. Advantages. Large amount of drug can be extracted with smaller amount of solvent. It is also applicable to plant materials that are heat stable. No filtration is required, and high amount of heat could be applied. Disadvantages. Regular shaking is not possible, and the method is not suitable for thermolabile materials.

Microwave-assisted extraction:

This is one of the advanced extraction procedures in preparation of medicinal plants. The technique uses mechanism of dipole rotation and ionic transfer by displacement of charged ions present in the solvent and drug material. This method is suitable for extraction of flavonoids. It involves the application of electromagnetic radiation in frequencies between 300 MHz and 300 GHz and wavelength between 1cm and 1 m. The microwaves applied at frequency of 2450 Hz yielded energy between 600 and 700 W. The technique uses microwave radiation to bombard an object, which can absorb electromagnetic energy and convert it into heat. Subsequently, the heat produced facilitates movement of solvent into the drug matrix When polar solvent is used, dipole rotation and migration of ions occur, increase solvent penetration, and assist extraction process. However, when nonpolar solvent is used, the microwave radiation released will produce only small heat; hence, this method does not favor use of nonpolar solvents. Advantages. Microwave-assisted extraction has special advantages such as minimizing solvent and time of extraction as well as increase in the outcome. Disadvantages. This method is suitable only for phenolic compounds and flavonoids. Compounds such as tannins and anthocyanins may be degraded because of high temperature involved.

Ultrasound-assisted extraction:

This process involves application of sound energy at a very high frequency greater than 20KHz to disrupt plant cell all and increase the drug surface area for solvent penetration. Consequently, secondary metabolites will be released. In this method, plant material should dry first, grinded into fine power, and sieved properly. The prepared sample is then mixed with and appropriate solvent of extraction and packed into the ultrasonic extractor. The high sound energy applies hasten the extraction process by reducing the heat requirements. Advantages. Ultrasound-assisted extraction is applicable to small sample; it reduces the time of extraction and amount of solvent used, and maximizes the yield. Disadvantages. This method is difficult to be reproduced; also, high amount of energy applied may degrade the phytochemical by producing free radical.

Pulsed electric field (PEF) extraction

Pulsed electric field extraction significantly increases the extraction yield and decreased the extraction time because it can increase mass transfer during extraction by destroying membrane structures. The effectiveness of PEF treatment depends on several parameters including field strength, specific energy input, pulse number and treatment temperature. PEF extraction is a non-thermal method and minimizes the degradation of the

thermolabile compounds.

Method	**Solvent**	**Temperature**	**Pressure**	**Time**	Volume of organic solvent consumed	Polarity of natural products extracted
Maceration	Water, aqueous and non-aqueous solvents	Room temperature	Atmospheric	Long	Large	Dependent on extracting solvent
Percolation	Water, aqueous and non-aqueous solvents	Room temperature, occasionally under heat	Atmospheric	Long	Large	Dependent on extracting solvent
Decoction	Water	Under heat	Atmospheric	Moderate	None	Polar compounds
Reflux extraction	Aqueous and non-aqueous solvents	Under heat	Atmospheric	Moderate	Moderate	Dependent on extracting solvent
Soxhlet extraction	Organic solvents	Under heat	Atmospheric	Long	Moderate	Dependent on extracting solvent
Pressurized liquid extraction	Water, aqueous and non-aqueous solvents	Under heat	High	Short	Small	Dependent on extracting solvent
Supercritical fluid extraction	Supercritical fluid (usually S-CO_2), sometimes with modifier	Near room temperature	High	Short	None or small	Nonpolar to moderate polar compounds
Ultrasound assisted extraction	Water, aqueous and non-aqueous solvents	Room temperature, or under heat	Atmospheric	Short	Moderate	Dependent on extracting solvent
Microwave assisted extraction	Water, aqueous and non-aqueous solvents	Room temperature	Atmospheric	Short	None or moderate	Dependent on extracting solvent
Pulsed electric field extraction	Water, aqueous and non-aqueous solvents	Room temperature, or under heat	Atmospheric	Short	Moderate	Dependent on extracting solvent
Enzyme assisted extraction	Water, aqueous and non-aqueous solvents	Room temperature, or heated after enzyme treatment	Atmospheric	Moderate	Moderate	Dependent on extracting solvent
Hydro distillation and steam	Water	Under heat	Atmospheric	Long	None	Essential oil (usually non-polar)

distillation						

brief summary of various extraction methods for natural products:

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شناسنامه سند: CoreLab.109

نام سند پروتکل استاندارد استخراج عصاره گیاهان دارویی

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نام کامل فایل پروتکل استاندارد استخراج عصاره گیاهان دارویی

شرح سند روش انجام استاندارد استخراج عصاره گیاهان دارویی را شرح می‌دهد.

تهیه کننده شهربانو نادری