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PHYTOCHEMICALS

Phytochemicals are secondary metabolites which have different health benefits and with respect to plants, they possess color, aroma and flavor. There are different extraction methods of phytochemicals which have been used from the past and which are novel. Those novel techniques are very efficient and they will enable to extract large yields from small amount of plant material. Further, there are some techniques which can be used for both qualitative and quantitative measurements. Gas chromatography, liquid chromatography, high performance liquid chromatography and high performance thin layer chromatography are some advanced techniques which can be used for quantitative analysis of phytochemicals. The aim of this study is to elaborate different extraction methods and different qualitative and quantitative techniques for screening phytochemicals from plant materials.

EXTRACTION-Is the separation of medicinally active portions of plant (and animal) tissues using selective solvents through standard procedures. The products so obtained from plants are relatively complex mixtures of metabolites, in liquid or semisolid state or in dry powder form (after removing the solvent), & are intended for oral or external use.

Medicinal plants-The Medicinal plants constitute an effective source of both traditional and modern medicines, herbal medicine has been shown to have genuine utility and about 80% of rural population depends on it as primary health care. [WHO, (2005)]

Medicinal plants are the richest bio-resource-drugs of traditional systems of medicine,modern medicines,food supplements,folk medicines,pharmaceutical intermediates,chemical entities for synthetic drugs.

Classes of preparations *viz*.,

- Decoctions,
- Infusions,
- Fluid extracts,
- Tinctures,
- Pilular (semisolid) extracts,
- Powdered extracts.

The general techniques of medicinal plant extraction

- ✓ 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,
- ✓ Phytonic extraction (with hydrofluorocarbon solvents).

Extraction techniques For aromatic plants

- Hydrodistillation techniques (water distillation, steam distillation, water and steam distillation),
- Hydrolytic maceration followed by distillation,expression and enfleurage (cold fat extraction)
- Headspace trapping,
- Solid phase micro-extraction,

- Protoplast extraction,
- Microdistillation,
- Thermomicrodistillation,
- Molecular distillation.

Properties of a good solvent in plant extractions

- 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 extract so obtained is separated out from the marc (exhausted plant material) by allowing it to trickle into a holding tank through the built- in 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.

Steps Involved in the Extraction of Medicinal Plants

Size reduction

Extraction

Filtration

Concentration

Drying

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.

Extraction

Medicinal plants

- Cold aqueous percolation
- Hot aqueous extraction (decoction)
- Solvent extraction (cold / hot)

Aromatic plants

- Essential oils
- Concretes
- Bsolutes
- Pomades
- Esinoids

Selection of plant

- 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 were 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 72h.

Filtration

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- The marc is retained at the false bottom, and the extract is received in the holding tank.
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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).

Variation in extraction methods

- Length of the extraction period,
- Solvent used,
- pH of the solvent,
- Temperature,
- Particle size of the plant tissues,
- The solvent-to-sample ratio.

Parameters for Selecting an Appropriate Extraction Method

- Authentication of plant material by botanist.
- Use the right plant part + the age of plant + the time, season & place of collection.
- The nature of its chemical constituents.

- Grinding methods & powdering techniques.
- Nature of constituents (polar/nonpolar).
- The quality of water / menstruum.
- The design & material of fabrication of the extractor.
- Analytical parameters of the final extract,(TLC/HPLC).

The general techniques of medicinal plant extraction

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- ✓ 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,
- ✓ phytonic extraction (with hydrofluorocarbon solvents).

Extraction methods

Maceration

The whole powdered material is allowed to contact with the solvent which is in a stoppered container for a particular time period with frequent agitation . At the end of the process the solvent is drained off and the remaining miscella is removed from the plant material through pressing or centrifuging. Maceration is not an advanced technique since active ingredients cannot be totally extracted

Percolation

A percolator which has a narrow cone shaped vessel open at both ends is used for this technique.The plant material is moistened with the solvent and allowed to place in a percolation chamber. Then the plant material is rinsed with the solvent for several times until the active ingredient is extracted. The solvent can be used until its point of saturation.

Soxhlet extraction

This method is widely used when the desired compound has a limited solubility in the particular solvent and impurities are less soluble in the solvent. The finely ground sample is placed ina porous bag or "thimble" which made out of filter paper or cellulose. The solvent which the desired compounds are going to extracted is kept in the round bottom flask.

Supercritical fluid extraction

Supercritical gases such as carbon dioxide, nitrogen, methane, ethane, ethylene, nitrous oxide, sulfur dioxide, propane, propylene, ammonia and sulfur hexafluoride are used to extract active ingredients. The plant material is kept in a vessel which filled with a gas under controlled conditions such as temperature and pressure. The active ingredients which dissolved in the gas separate when both temperature and pressure are lower . The important factor of this technique is the mass transfer of the solute in the supercritical solvent. Generally, temperature and pressure has the biggest influence. However the effect of the pressure is more direct. As the pressure increases, higher densities are achieved by the supercritical fluid. Thus the density of the medium increases and the solubility of the solute will be increased. In order to get higher yields the process has to be optimized. Using response surface methodology the optimum parameters can be found.

Microwave assisted extraction

In this method microwave energy facilitate the separation of active ingredients from the plant material into the solvent. Microwaves possess electric and magnetic fields which are perpendicular to each other. The electric filed generates heat via dipolar rotation and ionic conduction. As high as the dielectric constant of the solvent, the resulting heating is fast. Unlike the classical methods, microwave assisted extraction heats the whole sample simultaneously. During the extraction, heat disrupts weak hydrogen bonds due to dipole rotation of molecules and the migration of dissolved ions increases the penetration of solvent in to the sample or matrix

Ultrasound assisted extraction

This is an advanced technique which has the capability of extracting large amount of bioactive compounds within shorter extraction time. The main advantage of this technique is the increasing the penetration of solvent into the matrix due to disruption of cell walls produced by acoustical cavitations. And also this achieves at low temperatures and hence this is more suitable for extraction of thermally unstable compounds

Maceration

- The whole / coarsely powdered crude drug is placed in a stoppered container with the solvent.
- Allow to stand @ room temperature for a period of at least 3 days with frequent agitation until the soluble matter gets dissolved.
- The mixture then is strained, the marc (the damp solid material) is pressed,
- The combined liquids are clarified by filtration or decantation after standing.
- This method is best suitable for use in case of the thermolabile drugs.

Infusion

- Fresh infusions are prepared by macerating the crude drug for a short period of time with cold or boiling water.
- These are dilute solutions of the readily soluble constituents of crude drugs.

Digestion

- This is a form of maceration in which gentle heat is used during the process of extraction.
- It is used when moderately elevated temperature is not objectionable.

- The solvent efficiency of the menstruum is thereby increased.

Image=microwave Digestion system

Decoction

In this process, the crude drug is boiled in a specified volume of water (1;4) for a defined time,

- Volume is reduced to 1/4th the original,
- It is then cooled and strained / filtered.
- This procedure is suitable for extracting water- soluble, heat-stable constituents.
- Typically used in preparation of Ayurvedic extracts
  = “quath” / “kawath”

Percolation

- Used most frequently to extract active ingredients in the preparation of tinctures and fluid extracts.
- The solid ingredients are moistened with an appropriate amount of the specified menstruum,
- Allowed to stand for approximately 4 hours in a well closed container, After stand time, the mass is packed & the top of the percolator is closed.

- the mixture is allowed to macerate in the closed percolator for 24h ,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.

        - The mixed liquid is clarified b
          filtration or by standing
          followed by decanting.

Hot Continuous Extraction (Soxhlet)

- The finely ground crude drug is placed in a porous bagor "thimble" made of strong filter paper, which is placed in chamber of the Soxhlet apparatus.

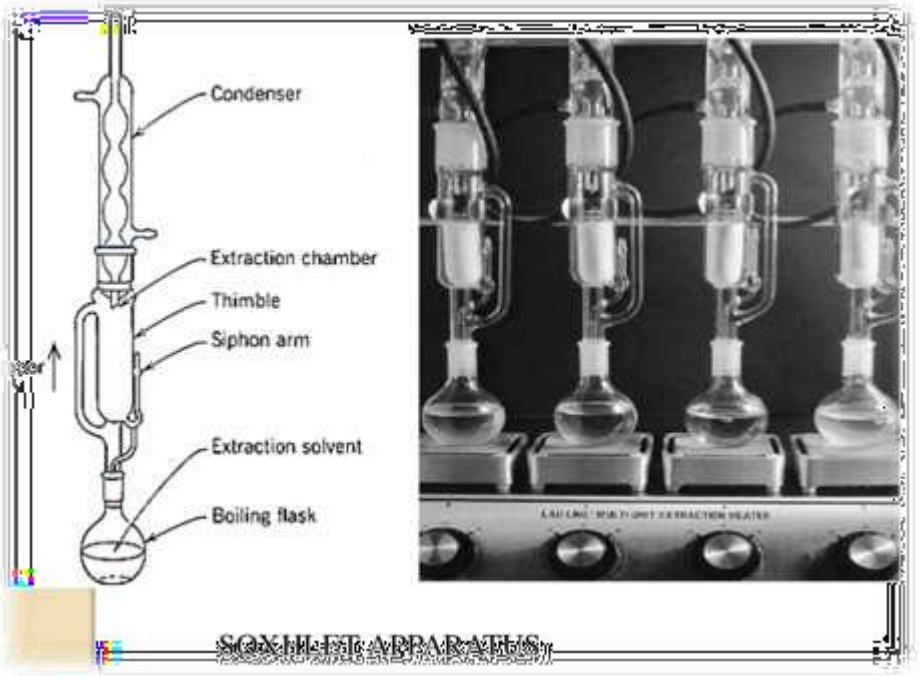
- The extracting solvent in flask is heated, and its vapors condense in condenser.

- The condensed extractant drips into the thimble containing the crude drug & extracts it by contact.

Soxhlet apparatus

- When the level of liquid in chamber rises to the
  top of siphon tube, the liquid contents of chamber siphon into flask

- This process is continuous and is carried out until a drop of solvent from the siphon tube does not leave residue when evaporated.



Counter-current Extraction

- Wet raw material is pulverized using toothed disc disintegrators to produce a fine slurry.
- Material to be extracted is moved in one direction generally in the form of a fine slurry within a cylindrical extractor where it comes in contact with extraction solvent.
- The further the starting material moves, the more concentrated the extract becomes.
- Complete extraction is thus possible when the quantities of solvent & material. Their flow rates should be optimized.
- sufficiently concentrated extract comes out at one end of the extractor while the marc, practically free of visible solvent falls out from the other end

Ultrasound Extraction (Sonication)

- The procedure involves the use of ultrasound with frequencies ranging from 20 kHz to 2000 kHz.
- This increases the permeability of cell walls & produces cavitation.

Eg: extraction of rauwolfia root.

- Deleterious effect: Ultrasound energy (>20 kHz) on the active constituents of medicinal plants through formation of free radicals and consequently undesirable changes in the drug molecules.

Supercritical Fluid Extraction

- Cylindrical extraction vessels are used.
- The collection of the extracted analyte following SFE is another important step: significant analyte loss can occur during this step.
- CO_2 as the extracting fluid.
- Organic solvents are frequently added to the CO_2 extracting fluid to alleviate the polarity limitations
- Thecomponentrecoveryratesgenerallyincreasewith increasing pressure/temperature.

References

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