International Journal of Chemical and Analytical Science
ISSN: 0976-1206
Review Article
www.ijcas.info

Application of systematic technologies for the extraction of novel phytoconstituents from pharmacologically important plants

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Medicinal and aromatic plants that constitute one of the major areas in the floral biodiversity also harbor reservoirs of important bioactive compounds. Withdrawal of these bioactive constituents in the form of extraction has always been a challenging job for the researchers in the past as well as in the present. An expansive array of technologies is presented for the extraction of active components and essential oils from medicinal and aromatic plants. However, the application of the discussed extraction procedures depends entirely on the cost- effective viability and appropriateness of the procedure applicable to the particular situation. Keeping this in mind an attempt has been made to give an outline of certain extractants, solvents used in the respective extraction processes with their advantages and disadvantages and their applications in this present review.

Key words – Medicinal and Aromatic Plants (Maps), Maceration, Distillation, Percolation, Essential Oils

INTRODUCTION

Our continent, Asia is one of the largest mega-biodiversity hot spots of our planet. It has an abundance of floral diversity aging higher than that of mankind, mainly concentrated in tropical and subtropical regions. In this diversified floral assemblage both medicinal and aromatic plant species occupies an imperative position; that is well documented through a long-standing practice of traditional medicine, and the potential for social and economic development of medicinal and aromatic plants (MAPs). According to major geographical distributions; South East Asia, China and East Asia, Indian Subcontinent, South West Asia have 50,000, 45,000, 25,000, 23,000 medicinal and aromatic plants, among which 40,000, 18,650, 12,000 and 7,100 are endemic species respectively. Suitable advanced technologies should be used for the industrial exploitation of such a valuable bio-resource, which in the long run will contribute widely for the socio-economic growth of human race.

Medicinal plant extracts:

In Pharmaceutical Chemistry the term “EXTRACTION” is commonly used, having the actual meaning - to separate out bioactive portions from plant tissues with the help of selective solvents following the standardized extraction procedures. The purposes of standardized extraction procedures for crude drugs are to attain the therapeutically desired portion and to eliminate the inert material by treatment with a selective solvent known as *menstruum*. The extract thus obtained can be readily used as active agents in the form of fluid extracts, or it may be further processed to be commercialized in the form of tablets or capsules. Again the crude extract can be further fractionated or partially purified by suitable analytics to isolate the potent chemical entities leading to drug manufacture. Thus, standardization of extraction procedures contributes significantly to the final quality of the herbal drug (Handa, S. S. and Kaul, M. K., 1996).

Criteria for the selection of medicinal plants:

Four basic methods are available for selecting plants for a screening programme to seek medicinal properties, which includes;

(1) Random collection of plants followed by mass screening;
(2) Selection based on ethnomedical uses;
(3) Follow-up of existing literature leads; and
(4) Chemotaxonomic approaches (Suffness and Douros, 1979).

The same advantages and disadvantages of the various approaches to the selection of plants for screening to discover anticancer activity can be associated with approaches to discover antiviral activity in plants (WHO Report, 1989). All factors considered above approach (2) and (3) would seem to be the most cost-effective for finding plants with medicinal properties. Comparison of the different approaches showed the selection method based on folklore to give a five-times higher percentage of active leads, although, in some cases, the same active compounds were isolated from botanically non related active plants. On the other hand, the screening of extracts from random collected plants afforded more novel substances with antiviral properties. Since the screening capacities of researchers are rather limited, so selection of plants based on a combined analysis of ethnomedicinal, phytochemical, taxonomical and toxicological data are rather preferable.

Methods of extraction of medicinal plants:

In this context the traditional methods of extraction from medicinal plants are summarized. Previous researchers (Seader, J. D. and Henley, E. J., 1996; Handa, S. S., 1999; Handa, S. S., Rakesh, D. D. and Vasisht, K., 2006) have extensively standardized these protocols for industrial scale applications.

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Received 12-07-2011; **Accepted** 02-08-2011

1. Maceration

The effective or entire portion of the plant is powdered and then it's placed in a stoppered container with the solvent and allowed to react at room temperature for a period of at least 72hours with recurrent stir until the entire soluble matter gets dissolved. The mixture is then strained, the marc (the damp solid material) is pressed, and the pooled liquids are clarified by filtration or decantation after settling.

2. Infusion

This process is carried out by macerating the crude active material of the plant for a short period of time with cold or boiling water, for the formation of dilute solutions of the readily soluble constituents of the crude herbal material.

3. Digestion

This is a form of maceration technique to increase the solvent efficiency of the menstruum, in which moderate heat is used during the extraction process of thermostable herbal components.

4. Decoction

This process is one of the most suitable methods for the preparation of Ayurvedic extracts called "kawath" that involves boiling of the crude herbal extract in a specific volume of water for a definite time, which is allowed to cool and then filtered. The starting ratio of crude extract to water is fixed, e.g. 1:4 or 1:16; the volume is then brought down to one-fourth its original volume by boiling during the extraction process. Then the concentrated extract is filtered and used as such or processed further.

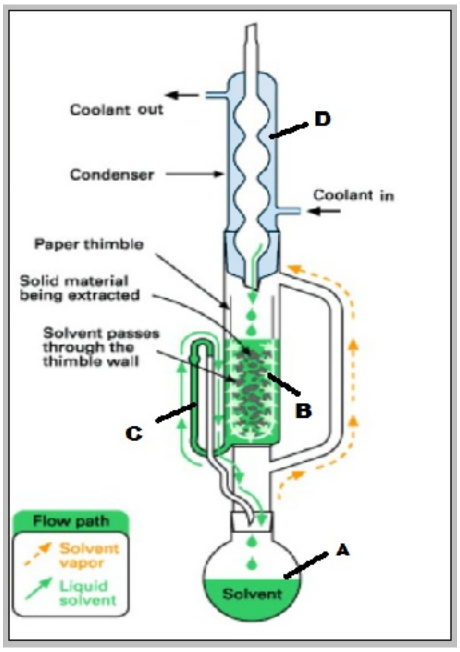
5. Percolation

This process is practiced most often to extract out the active key ingredients in the preparation of tinctures and fluid extracts. For this process a narrow, cone-shaped vessel open at both ends, namely percolator is commonly used. The solid ingredients are moistened with an appropriate amount of the specified menstruum and allowed to rest 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 is then 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.

6. Hot Continuous Extraction

Soxhlet apparatus shown in (Fig. 1) is commonly used for this method, the finely ground coarse plant material is placed in a porous bag or "thimble" made of strong filter paper, which is placed in chamber B of the Soxhlet apparatus. The extracting solvent in flask A is heated, with the help of heating mantle and its vapors condense in condenser D. The condensed extractant drips into the thimble containing the coarse plant material, and extracts it by contact. When the level of liquid in chamber B rises to the top of siphon tube C, the liquid contents of chamber B siphon into flask A. This process is continuous and is carried out until a drop of solvent from the siphon tube does not leave residue when evaporated. The advantage of this method, compared to previously described methods, is that large amounts of drug can be extracted with a much smaller quantity of solvent. This method of extraction is highly economic in terms of time, energy and consequently from monetary point of view.

Figure 1: Soxhlet apparatus



7. Counter-current Extraction

This process is highly proficient, requiring less time and posing no risk from high temperature when compared to continuous hot extraction. In this process, wet crude material is pulverized with the help of toothed disc disintegrators to produce fine slurry, where the material to be extracted is moved unidirectional within a cylindrical extractor where it comes in contact with the extraction solvent. The more the starting material moves, the more concentrated the extract becomes. For the completion of the extraction process both the flow rates of the solvent and material should be optimized. Finally, sufficiently concentrated extract comes out at one end of the extractor while the marc falls out from the other end.

This extraction process has significant advantages:

i) A unit quantity of the plant material can be extracted with much smaller volume of solvent as compared to other methods like maceration, decoction, and percolation.

ii) CCE is commonly done at room temperature, which spares the thermolabile constituents from exposure to heat which is employed in most other techniques.

iii) As the pulverization of the drug is done under wet conditions, the heat generated during comminution is neutralized by water. This again spares the thermolabile constituents from exposure to heat.

8. Ultrasound Extraction

The procedure increases the permeability of cell walls and produces cavitation, as it involves the usage of ultrasound frequencies ranging from 20 kHz to 2000 kHz. Although the process is useful in some cases (Taylor, L. T, 1996).

This extraction process has significant disadvantages:

i) Its large-scale application is limited due to the higher costs.

ii) Deleterious effect of ultrasound energy (more than 20 kHz) on the active constituents of medicinal plants through formation of free radicals leading to consequent undesirable changes in the drug molecules.

9. Phytonics Process

The products mostly extracted by this process are high quality natural fragrant essential oils, flavors and biological extracts which can be directly used without further physical or chemical treatment. The Phytonics process involves the use of a novel non-toxic solvent based on hydrofluorocarbon -134a, having a boiling point of - 25° C and a vapor pressure of 5.6 bar at ambient temperature and technology to optimize its remarkable properties in the extraction of plant materials.

Advantages of the process:

i) Unlike other processes that employ high temperatures, the phytonics process is cool and gentle and its products are never damaged by exposure to temperatures in excess of ambient.

ii) No vacuum stripping is needed which, in other processes, leads to the loss of precious volatiles.

iii) The process is carried out entirely at neutral pH and, in the absence of oxygen; the products never suffer acid hydrolysis damage or oxidation.

iv) The technique is highly selective, offering a choice of operating conditions and hence a choice of end products.

v) It is less threatening to the environment.

vi) It requires a minimum amount of electrical energy.

vii) It releases no harmful emissions into the atmosphere and the resultant waste products are innocuous and pose no effluent disposal problems.

viii) The solvents used in the technique are not flammable, toxic or ozone depleting like chloroflurocarbons.

ix) The solvents are completely recycled within the system.

Disadvantages of the process:

i) By most standards this is a poor solvent.

ii) The solvent does not mix with mineral oils or triglycerides

iii) The solvent does not dissolve plant wastes.

Applications:

The phytonics process can be used for extraction of the production of antibiotics, in the herbal drug industry, in the food, essential oil and flavor industries, and in the production of other pharmacologically active products. In particular, it is used in the production of top quality pharmaceutical - grade extracts, pharmacologically active intermediates, antibiotic extracts and phytopharmaceuticals. The technique is also used in refining crude products obtained from other extraction processes. It provides extraction without waxes or other contaminants. It helps remove many biocides from contaminated biomass.

Parameters for selecting an appropriate extraction method:

1. The plant species should be aptly authenticated by botanist / taxonomist before performing extraction. Any foreign matter should be completely eliminated.
2. The right plant part and, for quality control purposes, the age of plant and the time, season and place of collection should be recorded.
3. Conditions used for drying the plant material largely depend on the nature of its chemical constituents. Hot or cold blowing air flow for drying is generally preferred.
4. Grinding methods should be specified and techniques that generate heat should be avoided as much as possible.
5. Powdered plant material should be passed through suitable sieves to get the required particles of uniform size.
6. Nature of constituents.
   - If the non-polar constituent's contain the therapeutic values, a non-polar solvent may be used.
   - If the constituents are heat sensitive, extraction methods like cold maceration, percolation and CCE are preferred. For heat stable constituents, Soxhlet extraction and decoction are useful.
   - Appropriate safety measures should be taken while dealing with those organic solvent degrading constituents e.g. flavonoids and phenyl propanoids.
   - In case of hot extraction, higher than required temperature should be avoided. Some glycosides are likely to be degraded by the continuous exposure to higher temperature.
   - Standardization of extraction time is important, as inadequate time leads to incomplete extraction and if the extraction time exceeds, superfluous constituents may also be extracted.
   - The number of extractions required for complete extraction is as important as the duration of each extraction.
7. The water or menstruum quality parameters used should be precise.
8. Concentration and drying procedures should make sure that the safety and constancy of the active constituents. Drying under reduced pressure (e.g.; rotavapor) is widely used. Lyophilization, although pricey, is increasingly employed in modern days.
9. The extractor design as well as its material of production is to be taken into consideration.
10. Analytical parameters of the final extract, such as TLC and HPLC fingerprints, should be documented to monitor the quality of different batches of the extracts.

Steps involved in the extraction of medicinal plants:

In order to extract medicinal ingredients from plant material, the following sequential steps are involved:

1. Size reduction
2. Extraction
3. Filtration
4. Concentration
5. Drying

1. Size Reduction

The dried plant material is further reduced to a size between 30 and 40 mesh, (can be altered as per requirement) by placing it inside a hammer mill or a disc pulverizer with built-in sieves. The size of the particle is restricted by varying the speed of the rotor clearance between the hammers and the lining of the grinder and also by varying the opening of the discharge of the mill.

Objective of the process

i. The plant material is powdering in order to rip open its organ, tissue and cell structures so that its medicinal ingredients are exposed to the extraction solvent.

ii. Furthermore, size reduction maximizes the surface area, which in turn enhances the mass transfer of active components from plant material to the solvent.

2. Extraction

Extraction of the plant material is carried out in four different ways:

i) Cold aqueous percolation
ii) Hot aqueous extraction (decoction)
iii) Solvent Extraction

i) Cold Aqueous Percolation

The powdered plant material is macerated using water and then poured into a tall column to which cold water is added until the powdered material gets immersed entirely. It is allowed to stand for 24 h so that water-soluble ingredients attain equilibrium in the water. The enriched aqueous extract is then concentrated in multiple - effect evaporators to a particular concentration. Some diluents and excipients are added to this concentrated extract, which is then ready for medicinal use.

ii) Hot Aqueous Extraction (Decoction)

This is done in an open-type cylindrical extractor, made from type 316 stainless steel and has a diameter (D) greater than the height (H), i.e. the H/D ratio is roughly around 0.5. The bottom of the vessel is welded to the dished end and is provided with an inner false bottom with a filter cloth. The outer vessel has a steam jacket and a discharge valve at the bottom. One part of powdered plant material and sixteen parts of demineralized water are fed into the extractor. Heating is done by injecting steam into the jacket. The material is allowed to boil until the volume of water is reduced to one-fourth of its original volume. By this time the medicinal ingredients present in the plant material have been extracted out.

iii) Solvent Extraction

The principle behind solid-liquid extraction is that when a solid material comes in contact with a liquid, namely solvent, the mass transfer of soluble active components gets into the solvent, and this takes place in a concentration gradient. The rate of mass transfer decreases as the concentration of active components in the solvent increases, until equilibrium is reached between the two. Moreover, if the solvent that is in equilibrium with the plant material is replaced with fresh solvent, the concentration gradient is changed. This gives rise to different types of extractions: cold percolation, hot percolation that is already described above and in case of concentration process the enriched extract from percolators or extractors, commonly known as miscella, is fed into a wiped film evaporator where it is concentrated under vacuum to produce a thick concentrated extract.

3. Filtration

The extract obtained is separated out from the marc 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 recovered in the holding tank, from which it is pumped into a sparkler filter to get rid of fine or colloidal particles from the extract.

4. Concentration

A thick concentrated extract is obtained when the enriched extract, known as miscella, is fed into a wiped film evaporator where it is concentrated under vacuum. Thus, the thick concentrated extract is further subjected to vacuum drying to produce a marc which is further pulverized and used directly for the desired crude drug formulation.

5. Spray Drying

The above extract filtrate is subjected to high pressure spray drying with a pump at a controlled feed rate and temperature, to obtain dry powder. The desired particle size of the product is obtained by controlling the inner temperature of the chamber and also 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 directly used.

Aromatic plant extracts:

The volatile isolates that are commercialized from aromatic plants include essential oils that are isolated by distillation whereas concretes, absolutes, pomades and resinoids are obtained by solvent extraction (Handa S. S. and Kaul, M. K., 1997).

1. Concrete

Concrete is a hydrocarbon soluble rich, waxy, semisolid, dark-colored extract obtained from fresh flowers, herbs, and leaves by the use of hydrocarbon class of solvent such as butane, pentane, hexane and petroleum ether.

2. Absolutes

Absolute is an alcohol-soluble volatile concentrated form of Concretes that are used in perfumery. The concrete is mixed with absolute alcohol for the preparation of absolute, with thorough agitation in a vessel, during which the temperature is kept at 40°-60° C. The mixture thus formed is cooled down to -5° to -10° C to precipitate out the wax, which is then removed by passing the solution through a rotary filter. The filtrate from the rotary filter is pumped into a primary evaporator, where it is concentrated to about 10% alcohol content. Finally, the concentrated extract is pumped into an agitating-type evaporator, where the alcohol is carefully removed under high vacuum.

3. Resinoids

Resinoid is an extract that is obtained from dry and powdered resinous materials using a hydrocarbon solvent.

The extraction process is same as that of concrete production.

4. Pomades

Pomades are extracted by a cold fat extraction method known as enfleurage. The fat along with fresh flowers is spread out on glass plates contained in wooden frames that are stacked in piles leaving behind a clear margin near the edges. After the perfume oils have been absorbed from the flowers, the residual flowers are hand removed. The above mentioned steps are repeated until the fat surface is totally enriched with perfume oils.

5. Essential Oils

In present days the cosmetic industries are very much competitive in the production and consumption of essential oils in the form of perfumes. Other industries are not lagging behind as they are under extensive production of detergents, soaps, pharmaceutical products, confectionery, food products, soft drinks, alcoholic beverages and insecticides. The overall yield and quality of essential oil depends gravely on its production technology. The traditional technologies leading to the production of essential oil includes, water and steam distillation, cohobation, maceration and enfleurage are the most traditional and commonly used methods (De Silva, T. K., 1995). In Table 1 and 2 the essential oils are discussed elaborately.

Table 1. Plant Organs Containing Essential Oils

Active Component	Plant Organs / products	Plant species producing active compounds
Essential Oils	Leaves	Mentha, Ocimum, Lemongrass, Jamrosa, etc.
	Leaves & Stem	Cinnamon, Verbena, Geranium, etc.
	Flower	Rose, Jasmine, Orange, Clove, Lavender, etc.
	Barks	Cinnamon, Canella, Cassia, etc.
	Woods	Cedar, Santal, Pine, etc.
	Roots	Angelica, Vetiver, Saussurea, etc.
	Rhizomes	Ginger, Curcuma, Orris, etc
	Fruits	Orange, lemon, Juniper, etc.
	Seeds	Fennel, Coriander, Dill, Nutmeg, etc.
	Gums / Resins Exudations	Balsam, Benzoin, Storax, etc.

Methods of Producing Essential Oils:

In the following process commonly known as hydrodistillation, the aromatic plant is packed inside a still along with the addition of sufficient quantity of water and boiled with an alternation of live steam into the plant charge in order to free out the essential oils from the oil glands of the plant tissue (Lawrence, B. M., 1995; Verrall, M., 1996; Scheffer, J. J. C., 1997). However, it is possible to obtain better yield and quality of oils by:
(1) Maintaining the temperature as low as possible,
(2) Using as less quantity of water, in the case of steam distillation, and
(3) Last and the most important, thoroughly comminuting the plant material and packing it homogeneously prior to distillation. There are three types of hydrodistillation for isolating essential oils from plant materials; namely - water distillation, water and steam distillation, direct steam distillation.

Modern Methods of Extraction of Essential Oils:

Traditional methods of extraction of essential oils have been extensively discussed and these are the methods most widely used on marketable range. However, with hi-tech encroachment, new techniques have been urbanized which may not inevitably be widely used for commercial fabrication of essential oils but are considered worthy in certain situations, such as the production of pricey essential oils in a natural state without any modification of their thermosensitive mechanism or for the extraction of essential oils for micro-analysis (Werkhoff, P., Brennecke, S. and Bretschneider, W., 1998; Baser, K. H. C., 1999). These techniques are as follows:

- Controlled instantaneous decomposition (CID)
- Headspace trapping techniques
- Membrane extraction
- Microdistillation
- Microwave distillation
- Molecular spinning band distillation
- Phytosol extraction
- Protoplast technique
- Simultaneous distillation extraction (SDE)
- Solid phase micro-extraction (SPME)
- Supercritical fluid extraction (SFE)
- Thermomicrodistillation

The above techniques involves through grounding towards biochemistry, thermodynamics, analytics and molecular biology techniques.

Table 2. Heterogeneous Chemical Groups Present In Essential Oils

Active Component	Function Groups	Chemical groups present in active components
Essential Oils	Acids	Benzoic, Cinnamic, Myristic, Isovaleric, etc.
	Aldehydes	Citral, Citronellal, Benzaldehyde, Vanillin, etc.
	Alcohol	Geraniol, Menthol, Linalool, Terpineol, etc.
	Ester	Benzoates, Acetates, Salicylates, etc.
	Ketones	Camphor, Carvone, Thujone, etc.
	Oxides	Cineol, etc.
	Phenol Ethers	Anethol, Safrol, etc
	Phenol	Eugenol, Thymol, Carvacrol, etc.
	Hydrocarbons	Cymene, Myrcene, Sabinene, Storene, etc.
	Terpenes	Limonene, Pinene, Camphene, Cedrene, etc.

CONCLUSION

In the present review medicinal and aromatic plants (MAPs) are extensively covered up in terms of selection of the correct species, extraction procedures, different positive and negative sides of these procedures, and their scientific applications in various industries. Some of the major constraints in sustainable industrial exploitation of these plants are due to the fact that the developing countries have underdeveloped agricultural practices and post harvesting procedures for MAPs. In addition to the above mentioned reasons; lack of qualified, trained, skilled manpower, socioeconomic instabilities, proper transportation facilities are also the key factors behind poor yield of the economic products obtained from MAPs.

ACKNOWLEDGEMENT

The authors are thankful to authorities of Faculty of Marine Sciences, Annamalai University, for constant encouragement and support; and to INSPIRE Division, Department of Science and Technology, New Delhi for providing financial support during the period of study.

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Source of support: Nil, Conflict of interest: None Declared