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REVIEW ARTICLE

RJCES

Phytoconstituents and Their Mode of Extractions: An Overview

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ABSTRACT

Phytoconstituents are chemical compounds that occur naturally in plants. Some are responsible for colour and other organoleptic properties. The term is generally refer to biologically significant chemicals, but not established as essential nutrients. Some Phytoconstituents with physiological properties may be elements rather than complex organic molecules. An assortment of different Phytoconstituents is scheduled in this review.

A wide range of technologies is available for the extraction of active components and essential oils from medicinal and aromatic plants. The choice depends on the economic feasibility and suitability of the process to the particular situation. The various processes of production of medicinal plant extracts and essential oils are reviewed in this paper.

KEW WORDS Phytoconstituents, extraction, phenolics, alkaloids, Saponins, glycosides, terpenes, tannins, anthraquinones, steroids, essential oils, extraction techniques, medicinal plants.

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INTRODUCTION

Medicinal plants are the richest bio-resource of drugs for traditional systems of medicine, modern medicines, nutraceuticals, food supplements, folk medicines, pharmaceutical intermediates and chemical entities for synthetic drugs. Aromatic plants are a source of fragrances, flavors, cosmeceuticals, health beverages and chemical terpenes. Medicinal and aromatic plants (MAPs) are traded as such in bulk from many developing countries for further value addition in developed countries. The first step in the value addition of MAP bio-resources is the production of extracts using a variety of methods from simple traditional technologies to advanced extraction techniques.

Extraction is the term used for the separation of medicinally significant portions of plant from the inactive components by using selective solvents in different standard extraction procedures. The solvents are selected carefully on the basis of the nature of phyto-components present in the plant.

Types of extraction procedures play a decisive role for the qualitative and quantitative composition of the extracts. The standardized extracts, thus obtained are further processed for inclusion in other dosage forms containing several groups of plant's metabolites.

These extracts are also utilized for isolation and characterization of therapeutically active chemical constituents used in modern medicines.

Therefore, the extraction plays a vital role in order to isolate the chemical constituents in its original form.

TYPES OF PHYTOCONSTITUENTS

PHENOLICS

Phenolics are plant metabolites widely spread throughout the plant kingdom. Recent interest in phenolic stems from their potential protective role, through ingestion of fruits and vegetables, against oxidative damage diseases (coronary heart disease, stroke, and cancers).

Phenolic compounds are essential for the growth and reproduction of plants, and are produced as a response for defending injured plants against pathogens. Phenolic acid compounds are universally distributed in plants. They have been the subject of a great number of chemical, biological, agricultural, and medical studies. They form a varied group that includes the widely distributed hydroxybenzoic and hydroxycinnamic acids. Plant phenolic compounds are diverse in structure but are characterized by hydroxylated aromatic rings (e.g. flavan-3-ols). They are categorized as secondary metabolites. Many plant phenolic compounds are polymerised into larger molecules such as the proanthocyanidins and lignins. Furthermore, phenolic acids may occur in food plants as esters or glycosides conjugated with other natural compounds such as flavonoids, alcohols, hydroxyfatty acids, sterols, and glucosides [1].

ALKALOIDS

Alkaloids are traditionally defined as basic, nitrogen-containing organic constituents that occur mainly in plants. The nitrogen in the alkaloid molecule is derived from amino acid metabolism. Since the amino acid skeleton is often largely retained in the alkaloid structure, alkaloids originating from the same amino acid show similar structural features.

Alkaloids often have pronounced bioactivities and are therefore thought to play an important role in the interaction of plants with their environment. Alkaloids and extracts of alkaloid-containing plants have been used throughout human history as remedies, poisons and psychoactive drugs [2]

Many alkaloids, though poisons, have physiological effects that render them valuable as medicines. For example, curarine, found in the deadly extract curare, is a powerful muscle relaxant; atropine is used to dilate the pupils of the eyes; and physostigmine is a specific for certain muscular diseases. Narcotic alkaloids used in medicine include morphine and codeine for the relief of pain and cocaine as a local anesthetic. Other common alkaloids include quinine, caffeine, nicotine, strychnine, serotonin, and LSD. Aconitine is the alkaloid of aconite. Cinchonine and quinine are derived from cinchona, coniine is found in poison hemlock, and reserpine is an extract of rauwolfia roots. Emetine is an alkaloid of ipecac [3]. SAPONINS

Saponins are phytochemicals found in most vegetables, beans and herbs. The best known sources of saponins are peas, soybeans, and some herbs with names indicating foaming properties such as soapwort, saoproot, soapbark and soapberry. Commercial saponins are extracted mainly from Yucca schidigera and Quillaja saponaria.

Saponins are glucosides with foaming characteristics. They consist of a polycyclic aglycones attached to one or more sugar side chains. The foaming ability of saponins is caused by the combination of a hydrophobic (fat-soluble) sapogenin and a hydrophilic (water-soluble) sugar part. Saponins have a bitter taste. Some saponins are toxic and are known as sapotoxin.

Saponins have many health benefits. Studies have illustrated the beneficial effects on blood cholesterol levels, cancer, bone health and stimulation of the immune system.

Saponins from Yucca and Quillaja are used in some beverages, such as beer, to produce stable foam. The detergent properties of saponins have led to their use in shampoos, facial cleansers and cosmetic creams [4].

GLYCOSIDES

Glycosides are compounds containing a carbohydrate and a non carbohydrate residue in the same molecule. The carbohydrate residue is attached by an acetal linkage at carbon atom 1 to a non carbohydrate residue or aglycone. The non sugar component is known as the aglycone. The sugar component is called the glycone [5].

S.No.	Types of glycoside	Example		
1	Anthraquinones	Aloe, Rhubarb, Cascara, Senna, Frangula		
2	Cardiac	Digitalis, Squill, Strophanthus, Quabain, Theveti		
3	Saponin	Classifying in two groups: i. Tetracyclic Triterpenoids Saponins Dioscorea bark- Diosgenin Solanum, Berries- Solasodine, Asparagus roots- Sarsapogenin ii. Pentacyclic Triterpenoid Saponins Ginseng- Gingenoside, Licorice- Glycyrrhizin, Senega- Senegin- II, Quillaja-Quillaia, Sarsaparilla- Sarsapogenin		
4	Coumarin and furocoumarin	Visnaga, Ammi, Psoralea		
5	Cyanophore	Wild cherry, Bitter almond		
6	Isothiocyanate	Black Mustard		
7	Flavonoids	 i. Flavone glycosides: Parsley- Api Buchu- Diosmin ii. Flavonol glycosides: Buck wheat- Rutin, Ring (Crategus oxycantha) – Querceti iii. FlacanOne glycosides: Lemon, Sweet orange-Hesperidin, Bitter orange iv. Chalone glycosides: Safflor red-Carthamin, v. Isoflavonoid glycoside Sharapunkha-Tephrosin, Gilas-Prunetrin vi. Anthocyanidin glycosides: Pelargonium flower- Plargonidin, Petunia flower- Petunidin 		
8	Phenol	Arbutin, Slicin		
9	Aldehyde	Vanilla		
10	Bitter	Gentian, Chirata, Picrorrhiza, Quassia		

Classification of glycosides:

TERPENES

The term 'terpene' was given to the compounds isolated from terpentine, a volatile liquid isolated from pine trees. The simpler mono and sesquiterpene is chief constituent of the essential oils obtained from sap and tissues of certain plant and trees. The di and tri terpenoids are not steam volatile. They are obtained from plant and tree gums and resins. Tertraterpenoids form a separate group of compounds called 'Carotenoids'.

Terpenes are the hydrocarbons of plant origin of the general formula $(C_5H_8)_n$ as well as their oxygenated, hydrogenated and dehydrogenated derivatives. Thermal decomposition of terpenes gives isoprene as one of the product. The terpene molecules are constructed of two or more isoprene units joined in a 'head to tail' fashion [6, 7]

Classification of Terpenes

Most natural terpenes hydrocarbon have the general formula $(C_5H_8)_n$. They can be classified on the basis of value of n or number of carbon atoms present in the structure.

S.No	No. of C atoms	Value of n	Class
1	10	2	Monoterpenoids (C ₁₀ H ₁₆)
2	15	3	Sesquiterpenoinds (C ₁₅ H ₂₄)
3	20	4	Diterpenoids (C ₂₀ H ₃₂)
4	25	5	Sesterpenoids (C ₂₅ H ₄₀)
5	30	6	Troterpenoids (C ₃₀ H ₄₈)
6	40	8	Tetraterpenoids (C ₄₀ H ₆₄)
7	>40	>8	Polyterpenoids (C ₅ H ₈)n

Each class can be further subdivided into subclasses according to the number of rings present in the structure.

i) Acyclic Terpenoids: They contain open structure.

ii) Monocyclic Terpenoids: They contain one ring in the structure.

iii) Bicyclic Terpenoids: They contain two rings in the structure.

iv) Tricyclic Terpenoids: They contain three rings in the structure.

v) Tetracyclic Terpenoids: They contain four rings in the structure.

TANNINS

Tannin is an astringent, plant polyphenolic compound that binds to and precipitates proteins and various other organic compounds including amino acids and alkaloids.

The term tannin refers to the use of wood tannins from oak in tanning animal hides into leather; hence the words "tan" and "tanning" for the treatment of leather. However, the term "tannin" by extension is widely applied to any large polyphenolic compound containing sufficient hydroxyls and other suitable groups to form strong complexes with various macromolecules.

The tannin compounds are widely distributed in many species of plants, where they play a role in protection from predation, and perhaps also as pesticides, and in plant growth regulation The astringency from the tannins is what causes the dry and puckery feeling in the mouth following the consumption of unripened fruit or red wine. Likewise, the destruction or modification of tannins with time plays an important role in the ripening of fruit and the aging of wine.

Tannins have molecular weights ranging from 500 to over 3,000 and up to 20,000 [8].

Classification of Tannins

Based on specific structural characteristics and chemical properties:

1. Gallotannins

Gallotannins are the simplest hydrolysable tannins, containing a polyphenolic and a polyol residue. Although a great variety of polyol residues are possible, most of the gallotannins isolated from plants contain a polyol residue derived from glucose.

2. Ellagitannins

With more than 500 natural products characterized so far, the Ellagitannins form by far the largest group of known tannins. Ellagitannins are formed from the gallotannins by the oxidative coupling of at least two galloyl units

3. Complex tannins

The structures of the complex tannins are built up from a gallotannin unit or an ellagitannin unit, and a catechin unit. One example from this substance class is acutissimin A, having a flavogallonyl unit

(nonahydroxytriphenoyl unit) bound glucosidically to C-1, and linked via three further hydrolysable ester bridges to the glucose derived polyol.

4. Condensed tannins

Condensed tannins are oligomeric and polymeric proanthocyanidins consisting of coupled flavan-3-ol (catechin) units (oligomeric or polymeric proanthocyanidins = condensed proanthocyanidins = condensed tannins). Biosynthetically the condensed tannins are formed by the successive condensation of the single building blocks, with a degree of polymerization between two and greater than fifty blocks being reached. The coupling pattern of the catechin units in condensed tannins can vary considerably. ANTHRAOUINONES

Anthraquinones are a class of aromatic compounds with a 9,10-dioxoanthracene core. So far, 79 naturally occurring anthraquinones have been identified which include emodin, physcion, cascarin, catenarin, and rhein. A large body of literature has demonstrated that the naturally occurring anthraquinones possess a broad spectrum of bioactivities, such as cathartic, anticancer, anti-inflammatory, antimicrobial, diuretic, vasorelaxing, and phytoestrogen activities, suggesting their possible clinical application in many diseases. Despite the advances that have been made in understanding the chemistry and biology of the anthraquinones in recent years, research into their mechanisms of action and therapeutic potential in autoimmune disorders is still at an early stage [9]

STEROIDS

Plant steroids are types of natural organic compounds found in plants. Many types of plant steroids exist and play important roles in the biological processes of plants, such as growth and development, cell division, and resistance to damage from environmental stresses like cold weather. Some plant steroids are also useful for their effects when consumed by human beings because their presence decreases the amount of cholesterol in the bloodstream. Plant steroids should not be confused with anabolic steroids used to increase muscle mass, which are a synthetic substance that imitates the effects of human androgenizing hormones such as testosterone.

All steroids have a characteristic chemical structure based around carbon atoms linked by single or double bonds and arranged into four interconnected rings. Additional groups of atoms called functional groups are bonded to the carbon atoms in the rings at various points, which vary from one steroid to another. Different steroids have different properties that vary according to the number of double bonds in the carbon rings and the composition of the attached functional groups.

The most biologically prominent plant steroid is brassinolide ($C_{28}H_{48}O_6$), which is important to the development of plant cells and promoting the plant's growth. It is part of a larger class of plant steroids called brassinosteroids. Brassinolide is synthesized from campesterol ($C_{28}H_{48}O$), another plant steroid that is part of a group of similar steroid compounds called phytosterols. Other examples of phytosterols, also commonly called plant sterois, include beta-sitosterol($C_{29}H_{50}O$) and brassicasterol ($C_{28}H_{46}O$) [10].

ESSENTIAL OIL

An essential oil is a concentrated hydrophobic liquid containing volatile aroma compounds from plants. Essential oils are also known as volatile oils, ethereal oils, aetherolea, or simply as the oil of the plant from which they were extracted, such as oil of clove. An oil is "essential" in the sense that it contains the "essence of" the plant's fragrance—the characteristic fragrance of the plant from which it is derived. The term essential used here does not mean indispensable as with the terms essential amino acid or essential fatty acid which are so called since they are nutritionally required by a given living organism.

Essential oils are generally extracted by distillation, often by using steam. Other processes include expression, solvent extraction, absolute oil extraction, resin tapping, and cold pressing. They are used in perfumes, cosmetics, soaps and other products, for flavoring food and drink, and for adding scents to incense and household cleaning products [11]

VARIOUS EXTRACTION TECHNIQUES FOR MEDICINALLY SIGNIFICANT PLANTS

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 may be ready for use as a medicinal agent in the form of tinctures and fluid extracts, it may be further processed to be incorporated in any dosage form such as tablets or capsules, or it may be fractionated to isolate individual chemical entities. Thus, standardization of extraction procedures contributes significantly to the final quality of the herbal drug.

METHODS OF EXTRACTION OF MEDICINAL PLANTS

MACERATION

The word 'maceration' means 'softening'. This is the simplest method of crude drug extraction and is official in Indian Pharmacopoeia. In this process, the material to be extracted is placed in a closed vessel and suitable solvent (menstruum) is added and left for 7 days with occasional shaking. The liquid is then strained off and the solid residue (Marc) is pressed to remove the solution as much as possible. The strained and expressed liquids are mixed and clarified by filtration.

In case of vegetable and animal tissues, sufficiently long time is allowed for the menstruum to diffuse through the cell wall to solubilise the constituents present within the cells and for the resulting solution to diffuse out.

Occasional shaking brings about rapid equilibrium between intra and extra cellular fluids thereby bringing fresh menstruum to the particle surface for further extraction.

A closed vessel is used to prevent the loss of menstruum by evaporation and avoids batch to batch variations. The process takes several days for complete extraction [12]

Types of maceration

1. Modified maceration:

It is essentially used for extracting unorganized drugs. Ex: gums, resins etc. This process is quick because the soluble constituents are directly exposed to menstruum due to lack of cellular structure. The process includes, Comminute the drug with menstruum, place in closed vessel for 2-7 days, agitate the mixture occasionally, strain and filter the strained liquid, wash the Marc with fresh menstruum. The filtrate should be collected in a dry receiver.

2. Multiple maceration:

Multiple maceration is aimed at achieving maximum extraction by using portions of total volume of menstruum for successive maceration. The volume of menstruum for each maceration can be calculated as follows, For double maceration, volume of menstruum (total volume - vol.retained drug)/2 + for 1st maceration = vol. retained by drug. For triple maceration, volume of menstruum for 1st maceration = (total volume - vol. retained by drug)/3 + vol. retained by drug.

Efficiency of extraction increases as the number of maceration's increases.

3. Vacuum extraction:

This process employs a specially designed maceration vessel with arrangement for connecting it to vacuum line. This process increases the permeability of the cell walls considerably and facilitates extraction in a much shorter time.

INFUSION

An infusion is a very simple chemical process used with plants that are volatile and dissolve readily, or release their active ingredients easily, in water, oil or alcohol. They are typically dried herbs, flowers or berries. The liquid is typically boiled (or brought to another appropriate temperature) and then poured over the herb, which is then allowed to steep in the liquid for a period of time. The liquid may then be strained or the herbs otherwise removed from the liquid.

The amount of time the herbs are left in the liquid depends on the purpose for which the infusion is being prepared. Usually steeping for not more than 15 to 30 minutes, or until the mix cools, will create a beverage with optimal flavor.

Quantities of the herb and liquid used will vary according to the herb or how strong the infusion is required to be. A common proportion used is 28 g (one ounce) of herb to 0.5 L (one pint) of liquid [13].

There have been several accessories and techniques for removing the steeped or left over products that were used to infuse liquids such as water, oil, or alcohol.

DIGESTION

Digestion is a form of maceration with slight warming during the extraction process, provided that the temperature does not alter the active ingredients of plant material and so there is greater efficiency in the use of menstruum.

The most used temperatures are between 35° and 40°C., although can rise to no higher than 50°C. This process is used with the tougher plant parts or those that contain poorly soluble substances.

The parts of the plant were introduced to extract in a container with the liquid pre-heated to the indicated temperatures, maintained for a period that may vary between half an hour and 24 hours, shaking the container regularly [14]

DECOCTION

The **decoction** is used for active ingredients that doesn't modify with temperature.

In this process the plant is boiled in water for 15 to 60 minutes (depending on the plant or the active ingredient to extract), it's cooled, strained and added enough cold water through the plant to obtain the desired volume.

Depending on the consistency of the parts to extract, decoction times will be more or less long; generally roots, leaves, flowers and leafy stems are boiled in water for about 15 minutes, while the branches and other hard parts can require up to an hour, during this time the evaporated water must be replaced [15]. Once the decoction is done it is necessary to filter the liquid through a cloth. Doses are similar to the infusion, a plant part per ten of water, except for the plants with high mucilage content in this case will be 1/20 to prevent the solution takes much viscosity. The decoctions are prepared for using in the moment and should not be stored for more than 24 hours.

PERCOLATION

Percolation is an extraction process that involves the slow descent of a solvent through a powdered substance until it absorbs certain constituents and drips out through the filtered bottom of the container. The main advantages of percolation are a more complete extraction of constituents, shorter processing time, and increased flexibility in processing. The disadvantages include the need for more equipment, additional complexity in processing, and the incompatibility of percolation with certain herbs [16] Percolation depends upon the basic principle of diffusion for extraction: diffusion flows from a gradient of greater concentration to a lesser concentration. Percolation, however, has the advantage of a continuous gravity driven flow of fresh, unsaturated solvent that works its way down through the marc. Since the soluble constituents are constantly wicked out of the marc by a less concentrated solvent the process can continue until the constituents are completely exhausted. This process does not require any agitation and

SOXHLET EXTRACTION

can usually be completed in two days.

When active constituents of the drug are not freely soluble in the solvent or difficult to be displaced from the cells of the drug, then it becomes necessary to extract the crude drug by the action of hot menstruum for a considerable length of time. The fixed oils from seeds and alkaloids from the drug are extracted by continuous hot percolation process using benzene, chloroform, petroleum ether etc.

The apparatus used for continuous hot percolation process is soxhlet apparatus which consist of three parts:

- 1. Flask containing boiling solvent.
- 2. Soxhlet Extractor in which the drug to be extracted is packed. It has a side tube which carries the vapours of the solvent from the flask to the condenser and a siphon tube which siphons over the extract from soxhlet extractor to the flask.
- 3. A condenser in which the vapours of the solvent are condensed again into solvent.

The drug to be extracted is packed in a paper cylinder made from a filter paper and it is placed in the body of soxhlet extractor. The solvent is placed in the flask. When solvent is boiled on heating the flask, it gets converted into vapours. These vapours enter into the condenser through the side tube and get condensed into hot liquid which falls on the column of the drug. When the extractor gets filled with the solvent, the level of siphon tube also raise up to its top. The solvent containing active constituents of the drug in the siphon tube siphon over and run into the flask, thus emptying the body of extractor .This alternation of filling and emptying the body of extractor goes on continuously. The soluble active constituents of the drug remain in the flask while the solvent is repeatedly volatilized. The process of filling and emptying of the extractor is repeated until the drug is exhausted. Normally the process is repeated about 15 times for complete exhaustion of the drug [17].

AQUEOUS ALCOHOLIC EXTRACTION BY FERMENTATION

Some medicinal preparations of Ayurveda (like asava and arista) adopt the technique of fermentation for extracting the active principles. The extraction procedure involves soaking the crude drug, in the form of either a powder or a decoction (kasaya), for a specified period of time, during which it undergoes fermentation and generates alcohol in situ; this facilitates the extraction of the active constituents contained in the plant material. The alcohol thus generated

also serves as a preservative [18].

If the fermentation is to be carried out in an earthen vessel, it should not be new, water should first be boiled in the vessel. In large-scale manufacture, wooden vats, porcelain jars or metal vessels are used in place of earthen vessels. Some examples of such preparations are karpurasava, kanakasava, dasmularista. In Ayurveda, this method is not yet standardized but, with the extraordinarily high degree of

advancement in fermentation technology, it should not be difficult to standardize this technique of extraction for the production of herbal drug extracts.

COUNTER CURRENT EXTRACTION

In counter-current extraction (CCE), wet raw material is pulverized using toothed disc disintegrators to produce fine slurry. In this process, the material to be extracted is moved in one direction (generally in the form of 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 and material and their flow rates are optimized. The process is highly efficient, requiring little time and posing no risk from high temperature. Finally, 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 [18].

This extraction process has significant advantages:

1. 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.

2. CCE is commonly done at room temperature, which spares the thermo labile constituents from exposure to heat which is employed in most other techniques.

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

4. The extraction procedure has been rated to be more efficient and effective than continuous hot extraction.

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 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 [18].

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.

SUPERCRITICAL FLUID EXTRACTION

Supercritical Fluid Extraction (SFE) Systems extract chemical compounds using supercritical carbon dioxide instead of an organic solvent. The supercritical fluid state occurs when a fluid is above its critical temperature (Tc) and critical pressure (Pc), when it is between the typical gas and liquid state. Manipulating the temperature and pressure of the fluid can solubilize the material of interest and selectively extract it. The sample is placed in an extraction vessel and pressurized with CO_2 to dissolve the sample. Transferred to a fraction collector, the contents are depressurized and the CO_2 loses its solvating power causing the desired material to precipitate. The condensed CO_2 can be recycled [19].

SFE applications in the food, pharmaceutical, and fine chemical industries:

- 1. Decaffeinating of coffee and tea
- 2. Extraction of essential oils (vegetable and fish oils)
- 3. Extraction of flavors from natural resources (nutraceuticals)
- 4. Extraction of ingredients from spices and red peppers
- 5. Extraction of fat from food products
- 6. Fractionation of polymeric materials
- 7. Extraction from natural products

CONCLUSION

To achieve a suitable concentration of the Phyto-constituents, contained in the plants, it is necessary to perform several procedures through which active ingredients are extracted with the adequate solvents, selected according to the solubility and stability of the beneficial substances. Different extraction methods allow obtaining products in pharmaceutical forms suitable for oral or external dosage. From these procedures extractive techniques have been developed that allow obtaining phyto-constituents in pure form. Prior to extraction treatment the plant must be cleaned carefully to avoid contamination with other plants or foreign mechanical particles. The aim is the extraction of substances using an appropriate solvent called menstruum solvent.

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