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A REVIEW

SUPERCRITICAL FLUID EXTRACTION OF HERBAL DRUGS AND THEIR CHARACTERIZATION

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ABSTRACT:

Among the different extraction methods, supercritical fluid extraction (SFE) has gained wide acceptance in recent years because of increasing stringent environmental regulations. SC-CO_2 refers to supercritical fluid extraction that use carbon dioxide as a solvent. Different parameters as temperature,pressure and solvent mass governing the extraction yield.Supercritical fluid extraction has been considered as a clean and environmental friendly green processing technique and sometimes an alternative to organic solvent-based extraction of natural products. Supercritical fluid extraction has the most applications in food science,natural product,byproduct recovery.pharmaceutical and environmental science.The process of good quality and standardization of herbal medicines were discussed.

Keyword: Supercritical fluid extraction,Extraction,Herbal medicine,Standardization,Application .

1. INTRODUCTION:

Medicinal herbs have a long history of use in improving human health and curing diseases because they provide unlimited components with complex chemical structure and a wide bioactivities. Herb is a plant or a part of a plant, commonly leaves, roots, seeds and flowers.Herb extraction is the most important step. Several extraction methods were used and their extraction efficiencies were compared. Supercritical fluid extraction have been used widely as alternative technique to conventional liquid extraction due to its several distinct properties [1]. It produced excellent mass transfer properties, ease solubility control due to temperature and pressure.

This extraction method also is an energy efficient economically viable and environmental friendly process. carbon dioxide is the most widely used supercritical fluid for extraction of natural products for foods and medicines because it is non flammable, inexpensive,odorless, non-toxic,chemically insert,tasteless[2,3],available at high purity levels,environmental friendly solvent.Further it is easily removed from the extract ,since it is a gas in the ambient condition.

Due to its relatively low critical temperature (31.1^{o}c), thermal sample decomposition is reduced [4]. CO_2 is a non polar solvent, so extraction of polar compounds is difficult but this problem can be easily solved by adding small amounts of organic modifier such as methanol.

2. DEFINITION OF SUPERCRITICAL FLUID

A fluid is said to be supercritical, when its pressure and temperature exceed their respective critical values (Tc-critical temperature and Pc-critical pressure)[12].

The critical point for a fluid is defined as the maximum temperature at which a gaseous substance can be liquefied.In the phase diagram (fig 1), the critical point located at the right upper end and the phase area beyond of this point is the SCF region. A substance below the critical temperature is either a vapor or a liquid while one above is either gaseous or supercritical depending on the pressure.SCF has a unique thermo-physical property. As the pressure, is increased, the density of the gas increase without significant increased in viscosity while the ability of the fluid to dissolve compounds also increases.Hence by changing the pressure we can extract desired ingredient from the plant material [5, 6].

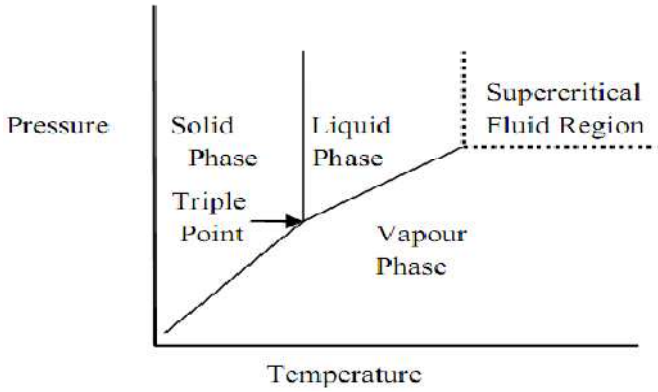

Figure (1): Typical diagram of supercritical region

3. PROPERTIES OF SUPERCRITICAL FLUIDS (SCF)

i. SCF have lower viscosity and higher diffusion rate than liquids make the mass transfer during extraction rapid.

ii. The solvent power of a SCF can be controlled by changing the pressure and the temperature.

iii. Many SCF have a low critical temperature and this enables extractions to be carried out at a low temperature and may avoid decomposition of labile compounds.

iv. Concentration of the extract by distillation of solvent is also eliminated since many SCF are gases at ambient temperature and are lost by vaporization.

v. SFE can also be coupled to chromatographic (HPLC, GC) and spectroscopic techniques to provide specific identification.

In general terms, supercritical fluids have properties between those of a gas and a liquid.

Table 1**:** Critical properties for some components commonly used as supercritical Fluids[7]

Critical properties of various solvents				
Solvent	**Molecular weight (g/mol)**	**Critical temperature(K)**	**Critical Pressure MPa (atm)**	**Critical density (g/cm^3)**
Carbon dioxide (CO2)	44.01	304.1	7.38	0.469
Water(H2O)(acc.IAPWS)	18.015	647.096	22.064 (217.755)	0.322
Methane (CH4)	16.04	190.4	4.60 (45.4)	0.162
Ethane (C2H6)	30.07	305.3	4.87 (48.1)	0.203
Propane (C3H8)	44.09	369.8	4.25 (41.9)	0.217
Ethylene (C2H4)	28.05	282.4	5.04 (49.7)	0.215
Propylene (C3H6)	42.08	364.9	4.60 (45.4)	0.232
Methanol (CH3OH)	32.04	512.6	8.09 (79.8)	0.272
Ethanol (C2H5OH)	46.07	513.9	6.14 (60.6)	0.276
Acetone (C3H6O)	58.08	508.1	4.70 (46.4)	0.278
Comparison of gases, supercritical fluids and liquids				

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	Density (kg/m3)	Viscosity(μ Pa· s)	Diffusivity (mm^2/s)
Gases	1	10	10-1
Supercritical fluids	100-1000	50-100	0.01-0.1
Liquids	1000	500-1000	0.001

Table 2: Density, diffusivity and viscosity for typical liquids, gases and supercritical fluids [7].

4. SUPERCRITICAL FLUID EXTRACTION (SFE) PROCESS:

The process of SFE system is shown in Fig. (2), and describe as follow. Raw material is charged in the extraction tank which is equipped with temperature controllers and pressure valves at both ends in order to keep desired extraction conditions. The extraction tank is pressurized with the fluid by the pumps that also used for the circulation of the fluid in the system. From the tank the fluid and the solubilized components are transferred to the separator where the solvation power of the fluid is decreased by increasing the temperature or decreasing the pressure of supercritical fluid extractor [8]. The product is then collected via a valve located in the lower part of the separators [8, 9].

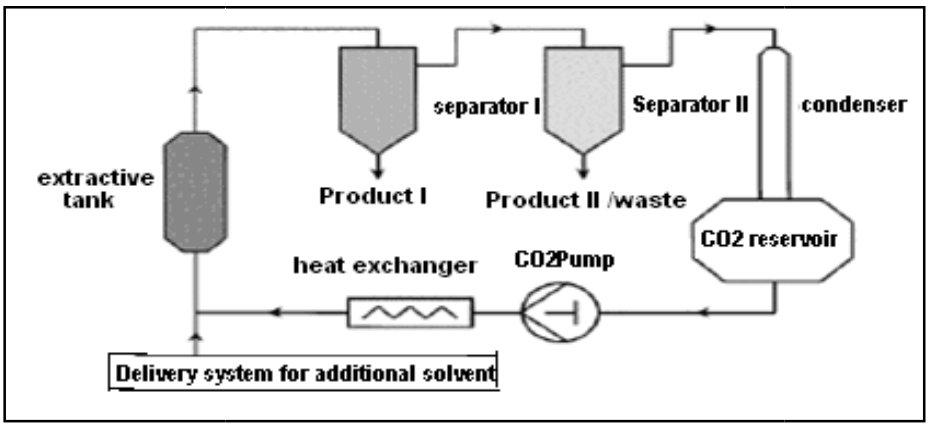

Figure 2: A simplified drawing of a process-scale

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5. OTHER METHODS OF EXTRACTION OF MEDICINAL PLANTS

5.1 MACERATION

In this process, the whole or coarsely powdered crude drug is placed in a stoppered container with the solvent and allowed to stand at room temperature for a period of at least 3 days with frequent agitation until the soluble matter has dissolved. The mixture then is strained, the marc (the damp solid material) is pressed, and the combined liquids are clarified by filtration or decantation after standing [10].

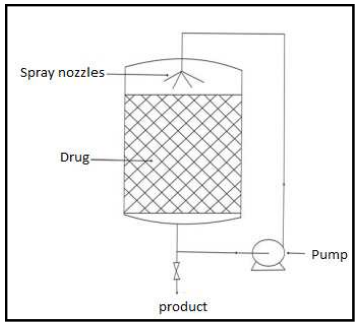

Figure 3: *Circulatory extraction*

5.2 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 [11].

5.3 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 [11].

5.4 DECOCTION

In this process, the crude drug is boiled in a specified volume of water for a defined time; it is then cooled and strained or filtered. This procedure is suitable for extracting water-soluble, heatstable constituents. This process is typically used in preparation of Ayurvedic extracts called

"quath" or "kawath". The starting ratio of crude drug 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 procedure. Then, the concentrated extract is filtered and used as such or processed further [11].

5.5 PERCOLATION

This is the procedure used most frequently to extract active ingredients in the preparation of tinctures and fluid extracts. A percolator (a narrow, cone-shaped vessel open at both ends) is generally used. The solid ingredients are moistened with an appropriate amount of the specified menstruum and allowed to stand 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 then is 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 [12].

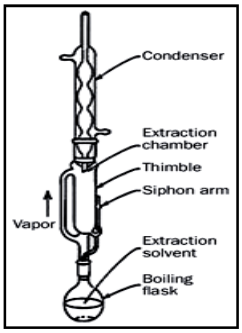

Figure 4: commercial scale (about 1 ton capacity) percolator

5.6 HOT CONTINUOUS EXTRACTION (SOXHLET)

In this method, the finely ground crude drug is placed in a porous bag or "thimble" made of strong filter paper, which is placed in chamber E of the Soxhlet apparatus. The extracting solvent in flask A is heated, and its vapors condense in condenser D. The condensed extractant drips into

the thimble containing the crude drug, and extracts it by contact. When the level of liquid in chamber E rises to the top of siphon tube C, the liquid contents of chamber E 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 effects tremendous economy in terms of time, energy and consequently financial inputs. At small scale, it is employed as a batch process only, but it becomes much more economical and viable when converted into a continuous extraction procedure on medium or large scale [14].

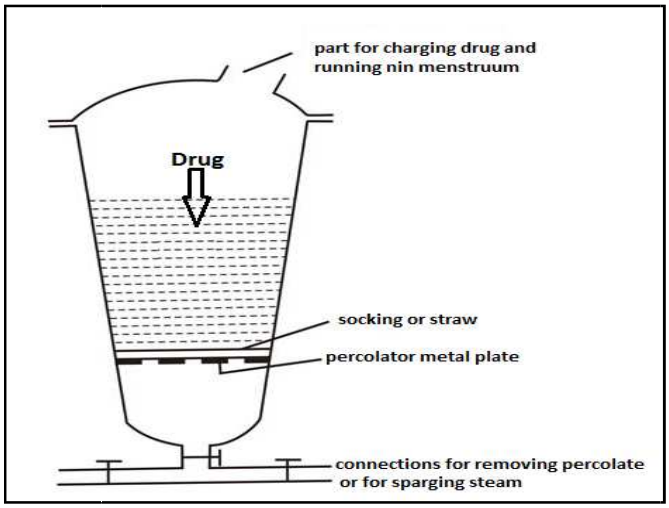

Figure 5: *Soxhlet apparatus for hot extraction*

5.7 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. 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 [12].

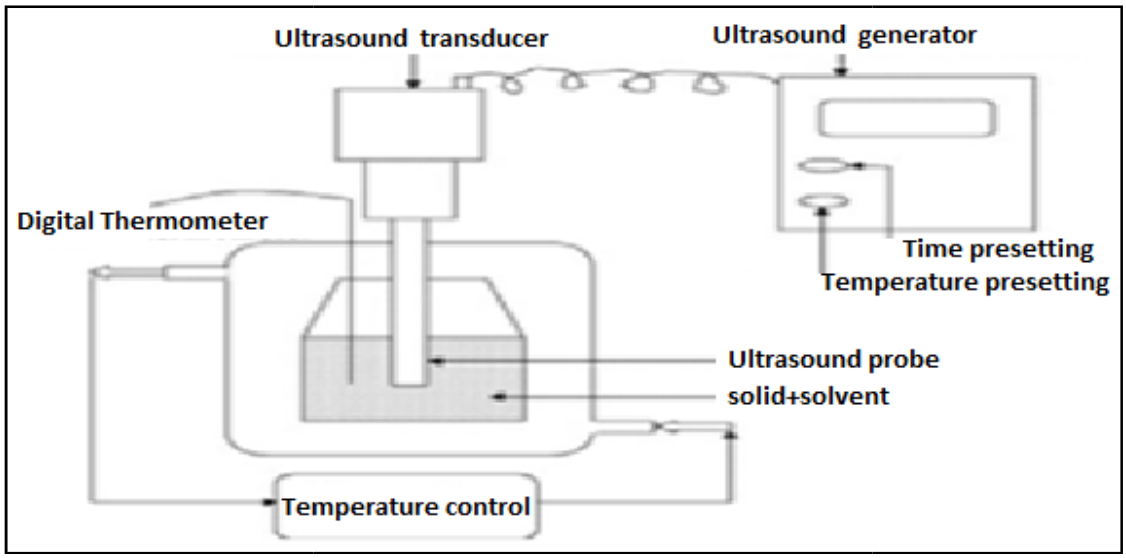

Figure 6: Ultrasound Extraction (Sonication)

5.8 PLANT TISSUE HOMOGENIZATION:

Plant tissue homogenization in solvent has been widely used by researchers. Dried or wet, fresh plant parts are grinded in a blender to fine particles, put in a certain quantity of solvent and shaken vigorously for 5-10 min or left for 24 h after which the extract is filtered. The filtrate then may be dried under reduced pressure and redissolved in the solvent to determine the concentration. Some researchers however centrifuged the filtrate for clarification of the extract [13].

5.9 SERIAL EXHAUSTIVE EXTRACTION:

It is another common method of extraction which involves successive extraction with solvents of increasing polarity from a non polar (hexane) to a more polar solvent (methanol) to ensure that a wide polarity range of compound could be extracted. Some researchers employ soxhlet extraction of dried plant material using organic solvent. This method cannot be used for thermo labile compounds as prolonged heating may lead to degradation of compounds [13].

The following table(3) show Comparison between SCF extraction and other extraction methods:[27].

no	Solvent extraction	SCF extraction
1	Solvent presence is unavoidable. The residual ppm level of the solvent depends on the type of solvent used.	Totally free of solvents and hence very pure.
2	Heavy metal content is also unavoidable and depends on the solvent, the recycle method for the solvent, the source of the raw material, and the material of construction of the contact parts of the machinery.	Totally free of heavy metals since they are not extractable even if they are present in the raw material. No heavy metals are present in CO2 and the equipment.
3	Inorganic salt content cannot be avoided, using the same concept as above.	Totally free of inorganic salts using the same explanation as above.
4	Polar substances get dissolved along with the lipophilic substances from the raw material due to poor selectivity of the solvent.During solvent removal operation,these polar substances form polymers, which lead to dark color of extract and poor flow characteristics.All this renders the extract to look different from the basic components in the raw material and hence it is more of a "pseudo" natural extract.	No such possibility since there is very high selectivity of CO2 and no chance of polar substances forming polymers. In addition the operating temperature is only 40-50 degree Celsius.
5	Both polar as well as non polar colors are extracted.	Only non polar colors get extracted.
6	Solvent removal requires extra unit operations and hence the cost and recovery of useful material is lower.	No extra unit operations needed and yield of useful material is very high.

Table 3: Comparison between SCF extraction and other extraction methods

6. QUALITY CONTROL AND STANDARDIZATION OF HERBAL MEDICINES

Several problems not applicable to synthetic drugs often affected the quality of herbal drugs. For instance:

1. The active principles are in most cases unknown.
2. The source and quality of the raw material are variable.
3. Plant materials are chemically and naturally variable.
4. Herbal drugs are usually mixtures of many constituents.
5. Chemo-varieties and chemo cultivars exist.
6. Selective analytical methods or reference compounds may not be available commercially [15]. Generally, all medicines, whether they are synthetic or of plant origin should fulfill the basic requirement of being safe and effective [16-22].According to WHO (World Heath Organization) [23], standardization and quality control of herbals is the process involved in the physicochemical evaluation of crude drug covering aspects. Attention is normally paid to such quality indices such as:

1) Macro and microscopic examination: For identification of right variety and search of adulterants.
2) Foreign organic matter: This involves removal of matter other than source plant to get the drug in pure form.
3) Ash values: Total ash, sulphated ash waters soluble ash and acid insoluble ash etc. are taken to judge the identity and purity of crude drug.
4) Moisture content: It helps to reduce errors in the estimation of the actual weights of drug material.
5) Extractive values: There are indicative weights of the extractable chemical constituent of crude drug under different solvents environment.
6) Qualitative chemical evaluation: It covers identification and characterization of crude drug with respect to photochemical constituent.
7) Chromatographic examination: It includes identification of crude drug based on the use of major chemical constituents as markers.

8) Quantitative chemical evaluation: To estimate the amount of the major classes of constituents.
9) Toxicological studies: This determines the pesticide residues, potentially toxic element to establish the absence or presence of potentially harmful microorganisms.
10) Crude fiber: This helps to determine the woody material component which is a criterion for judging purity.

7. APPLICATIONS OF SFE:

7.1 APPLICATION OF SFE TO ENANTIOMERIC SEPARATION:

The applicability of SFE as an effective and green technique for enantioseparations is known since the late 1990s. In these processes, diastereomeric salts or complexes of the racemic compounds and resolving agents are formed before the extraction step. The selected resolving agent is added in less than stoichiometric ratio to the racemic compound. The unreacted enantiomers are extracted with the supercritical solvent, and are collected as a powder after depressurization of the solution [24].

7.2 METALS RECOVERY USING SUPERCRITICAL FLUIDS:

Removal of heavy metals from solid matrices and liquids remain a big challenge and, although various methods have been described for this purpose, SFE seems to be one of the most promising Complexing agents used in conventional solvent extraction.

7.3 SFE IN FOOD TOXICOLOGY AND ECOTOXICOLOGY:

There are several compounds with serious health implications which determination can be done using SFE, the main areas of application include food toxicology and ecotoxicology.

7.4 SOLVENT REMOVAL AND NEW DRUG DELIVERY FORMULATIONS:

To increase the bioavailability of poorly water soluble drugs, an increasing number of pharmaceutical formulation technologies are being developed. In the case of polar compounds which are not soluble in supercritical fluids (particularly CO_2), SCFs could be used as antisolvent; the solution which consisting of an organic solvent, completely miscible with the SCF, and a solid material dissolved in this solvent is sprayed into a high pressure vessel filled with SCF. The supercritical fluid is used to extract the solvent instead of the

analytic the spectroscopic and chromatographic characterization of triflusal (2 acetoxy-4-(trifluoromethyl) benzoic acid) delivery systems prepared by using supercritical impregnation technologies. Triflusal is an antithrombogenic drug structurally related to acetylsalicylic acid [25].

There are other applications such as natural pesticides, De-nicotinization of tobacco (tar free tobacco), food preservatives, herbal medicines, pesticides (Neem) [26], removal of fat from foods, enrichment of vitamin E from natural sources, removal of alcohol from wine and beer, encapsulation of liquids for engineering solid products, extraction of functional compounds.

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