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PHYTOCHEMICAL INVESTIGATION OF HERBAL PRODUCT (PART 1)

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The phytochemistry investigation of plant may involve the following steps :

1 -authentication of the plant

2 - extraction of the plant material

3-separation and isolation of the constituents of interest

4 -characterization of the isolated compounds

5 - investigation of the biosynthetic pathways to particular compounds

6 - quantitative evaluation

7- Parallel to this may be the pharmacological assessment of the separated components, which may, in some investigations, precede the characterization

Authentication of the plant

- Authentication of the plant : all plant material used should be properly authenticated by specialists . the National Herbarium in Iraq is the main institute for authentication of plants.

Extraction

as the term is used pharmaceutically, involves the separation of medicinally active portions of plant or animal tissues from the inactive or inert components by using selective solvents in standard extraction procedures.

The products so obtained from plants are relatively

impure liquids, semisolids or powders intended only for oral or external use.

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 such as ajmalicine, hyoscine and vincristine, which are modern drugs

The choice of extraction procedure depends on the nature of the plant material and the components to be isolated .

- Dried materials are usually powdered before extraction.
- whereas fresh plants ex: can be homogenized or macerated with a solvent such as alcohol. The latter is also particularly useful for stabilizing fresh leaves by dropping them into the boiling solvent.
- The methods of extraction are different , they are either cold or hot extraction depending on whether the components to be isolated are heat stable or not.

- **Choice of the exracting solvents**:
- Alchohol is a general solvent for many plant constituents except most of the fixed oils. Water – immiscible solvents are widely used ex:-light petroleum which is used for extraction of essential & fixed oils, steroids.
- ether & chloroform which might be used for extraction of alkaloids & quinones

- the extraction of organic bases ex: alkaloids usually requires basification of the plant material if a water immiscible solvent is to be used , while for aromatic acids & phenols acidification may be required.

Factor affecting extraction

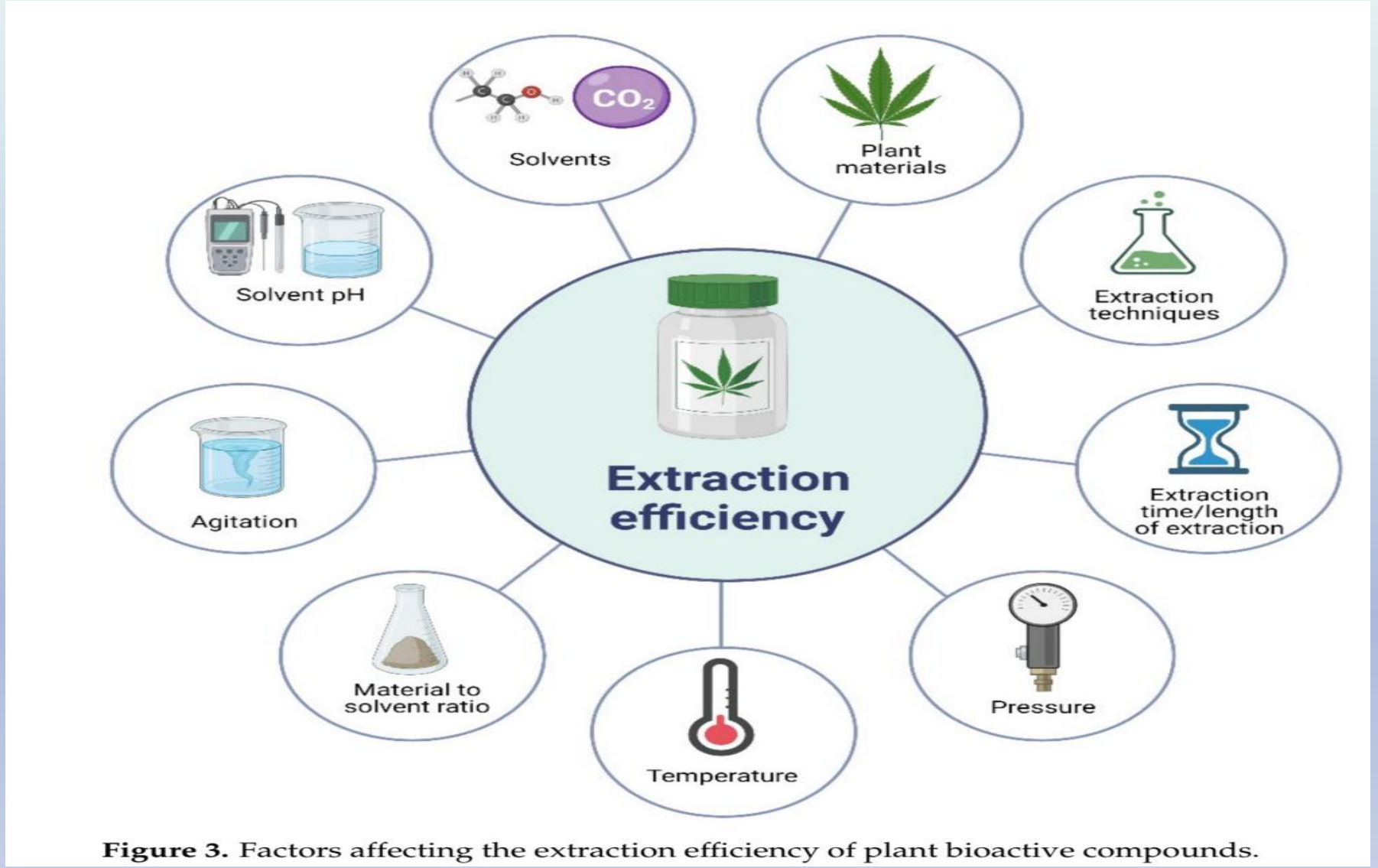
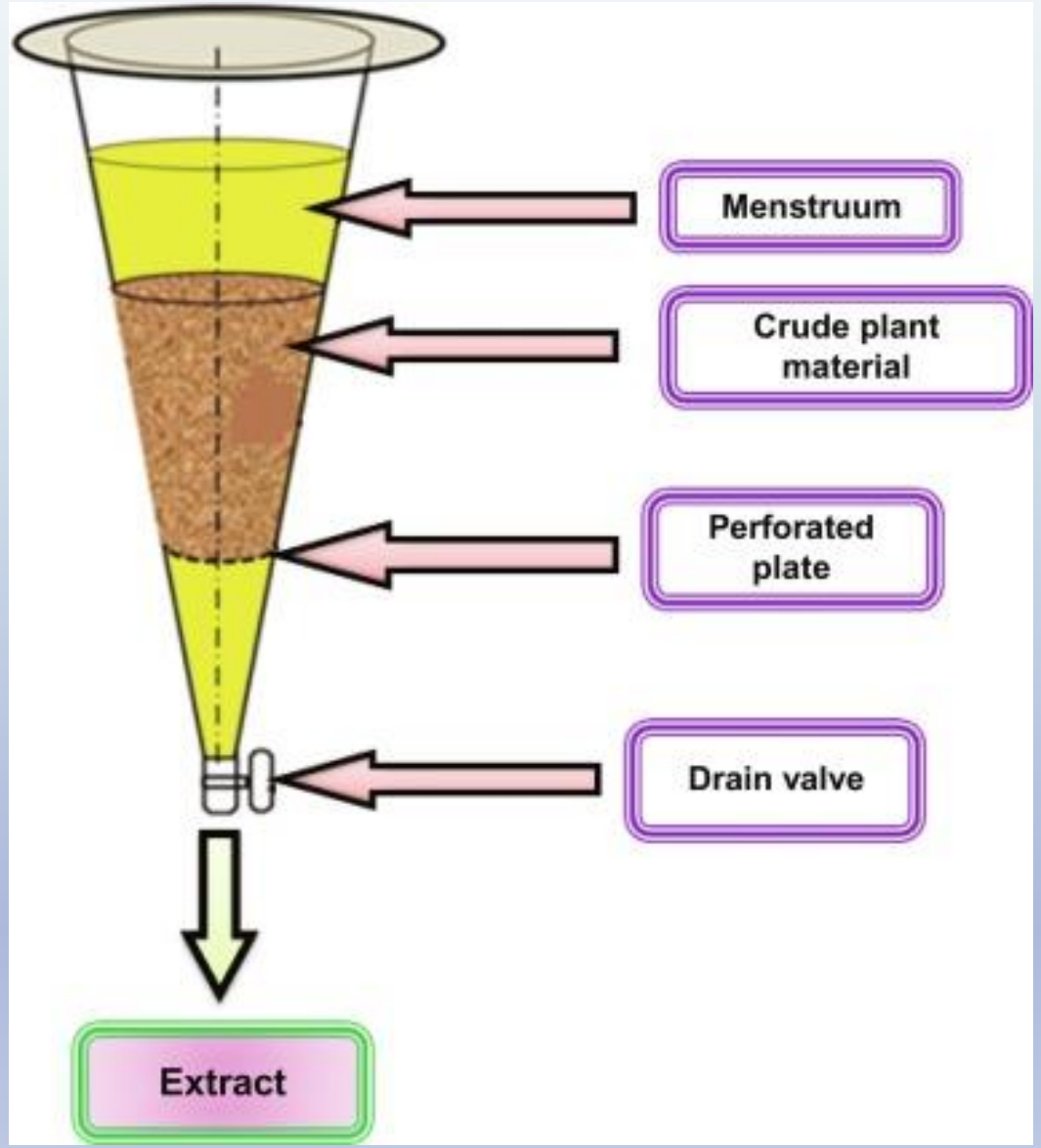


Figure 3. Factors affecting the extraction efficiency of plant bioactive compounds.

- **Cold extraction:**
- **1.Percolation:** it is usually one of the most widespread methods employed for plant extraction since it does not require much manipulation or time .
- The equipment used is a conical glass container with a tap at the base of the apparatus used to set the rate of the solvent elution. Hot or cold solvent may be used. In the former case , a metalic percolator is required .


Menstruum
Crude plant material
Perforated plate
Drain valve
Extract

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.

- Very fine powders , resins , & powder that swell or give a viscous eluent cannot be extracted by this method since perculation would be disrupted. The sample should be coarsely fragmented , &particles that pass through a 3-mm sieve would be adequate. Particles of too large a size may produce a high-elution rate precluding the necessary equilibrium for the dissolution of the metabolites , & the menstruum (solvent) would percolate unsaturated .

- **2.Maceration :**
- In this method the plant is introduced into a suitable container & a sufficient quantity of the required solvent is added & the container is tightly closed & left in away from heat & light for 24 hours, after which the solvent can be replaced by a new quantity (after filtering the first quantity) for another 24 hours & so on until there is exhaustion of the active constituents. Sometimes one time of maceration is enough & this might be left for more than 24 hours.

- The efficiency of this method may be increased by occasionally shaking the container or by using a mechanical or magnetic stirrer to allow homogenization of the final solution & saturation of the solvent. It is a discontinuous method & the solvent should be renewed until the plant material is exhausted.
- This requires occasional filtration steps that may produce loss of solvent, metabolites &/or plant material. Such problems may be avoided in part by suspending the ground material in a tied bag in the upper part of the solvent.

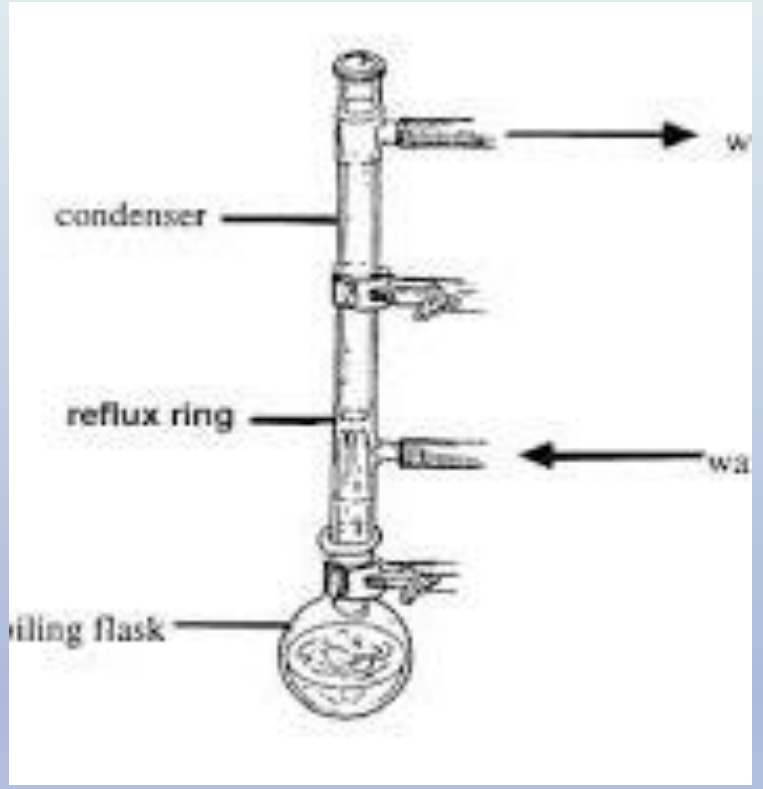
- **Hot extraction** (for heat stable material):
- There are different methods for hot extraction:
- **<u>Non continuous hot extraction:</u>**
- 1.**Infusion:** where by the plant is introduced in a container & a hot solvent is poured on it & the container is covered & left for a certain time then strained.
- 2. **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.
- 3.**Decoction**: In this method the plant is boiled with the solvent principally water for certain time taking in consideration the quantity of the solvent so that to avoid dryness and burning of the plant material.

- *"There must be no barriers to freedom of inquiry ... There is no place for dogma in science. The scientist is free, and must be free to ask any question, to doubt any assertion, to seek for any evidence, to correct any errors. ... We know that the only way to avoid error is to detect it and that the only way to detect it is to be free to inquire. And we know that as long as men are free to ask what they must, free to say what they think, free to think what they will, freedom can never be lost, and science can never regress."*

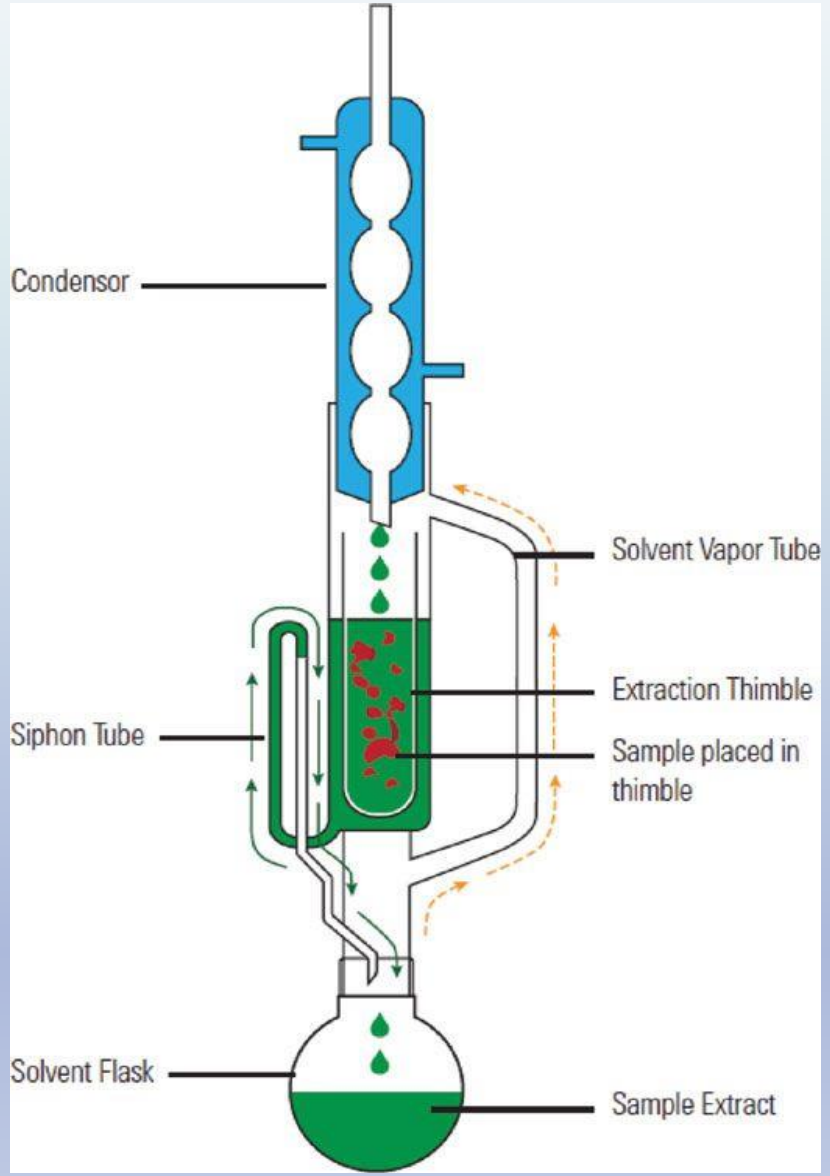
J. Robert Oppenheimer

- **<u>Continuous hot extraction</u>:**

1.**Reflux extraction**: Here the plant is boiled with the solvent in a round flask on which a condenser is placed to insure a complete extraction with out reduction in the quantity of the solvent.



- **2.Soxhlet extraction**: In this method a special apparatus is used called the soxhlet in which the powdered plant is placed in thimble (which is made from cellulose) & the thimble is introduced in the apparatus after plugging it i.e the thimble with cotton wool & the apparatus is placed on a round flask containing the solvent & a cooling condenser is placed on the top of the flask


Condensor
Solvent Vapor Tube
Extraction Thimble
Siphon Tube
Sample placed in thimble
Solvent Flask
Sample Extract

- The solvent is boiled & gets up to the condenser through a side tube , after condensation the solvent will get down & drop on the top of the thimble & extract the plant material inside it. After the trough of the apparatus is filled with the solvent , the solvent will return to the flask by syphoning & so on , the process is repeated until complete extraction.

- The main advantage of extraction using a Soxhlet apparatus is that it is an automatic , continuous method that does not require further manipulation other than concentration of the extractive & saves solvent by recycling it over the sample. Moreover , this method is not time-consuming , since for a standard-sized sample (500 g), the extraction time is less than 24 h. An obvious disadvantage is that the extractives are heated during the period of extraction at the boiling point of the solvent employed & thermally labile compounds such as carotinoids may hydrolyze , decompose , or produce artifacts.

Ultrasound Extraction (Sonication)

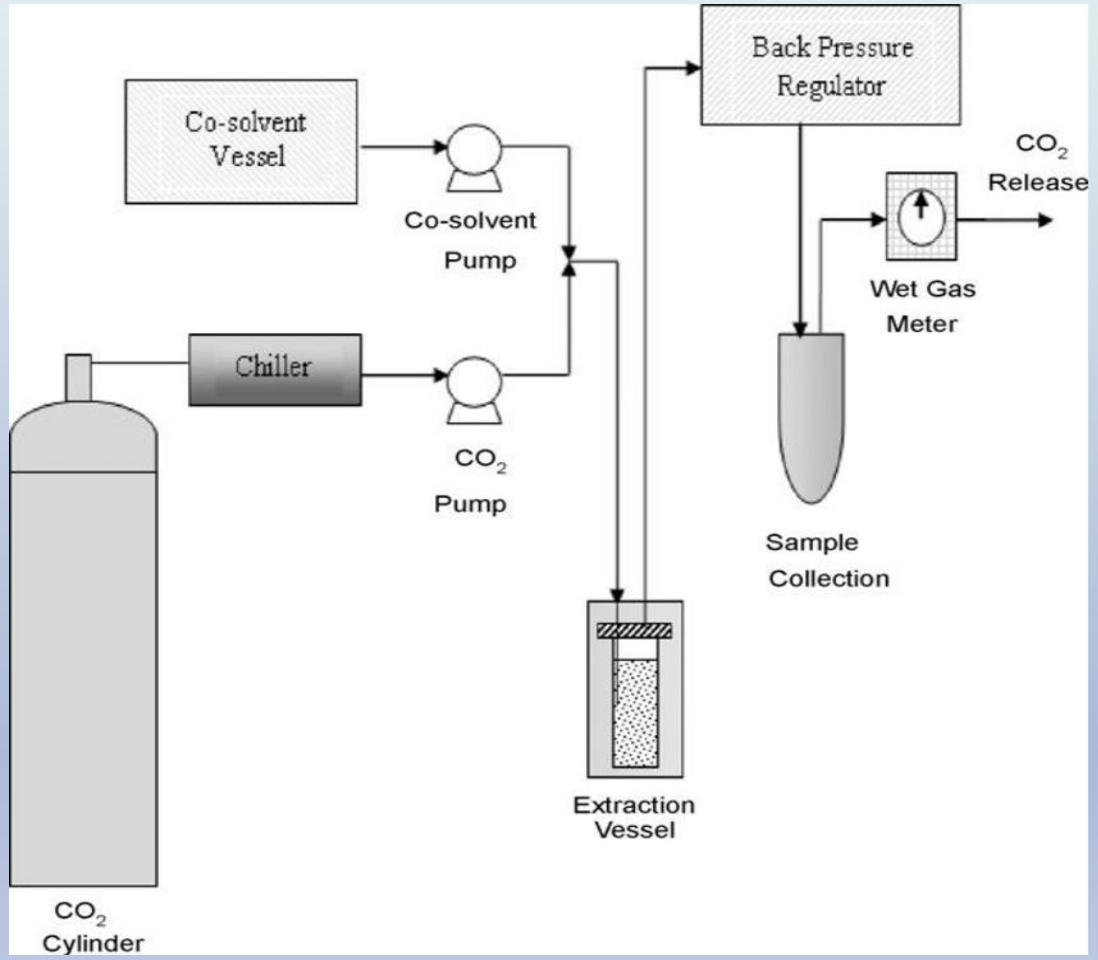
The procedure involves the use of ultrasound with frequencies ranging from 20 kHz to 2000 kHz; this increases the permeability of cell walls and produces cavitation. Although the process is useful in some cases, like extraction of rauwolfia root, its large-scale application is limited due to the higher 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.

Supercritical Fluid Extraction

Supercritical fluid extraction (SFE) is an alternative sample preparation method with general goals of reduced use of organic solvents and increased sample throughput.

The factors to consider include temperature, pressure, sample volume, analyte collection, modifier (cosolvent) addition, flow and pressure control, and restrictors. Generally, cylindrical extraction vessels are used for SFE and their performance is good beyond any doubt.

The collection of the extracted analyte following SFE is another important step: significant analyte loss can occur during this step, leading the analyst to believe that the actual efficiency was poor.


Back Pressure Regulator
Co-solvent Vessel
Co-solvent Pump
CO_2 Release
Wet Gas Meter
Chiller
CO_2 Pump
Sample Collection
Extraction Vessel
CO_2 Cylinder

There are many advantages to the use of CO2 as the extracting fluid. In addition to its favorable physical properties, carbon dioxide is safe and abundant.

But while carbon dioxide is the preferred fluid for SFE, it possesses several polarity limitations. Solvent polarity is important when extracting polar solutes and when strong analyte-matrix interactions are present.

Organic solvents are frequently added to the carbon dioxide extracting fluid to alleviate the polarity limitations.

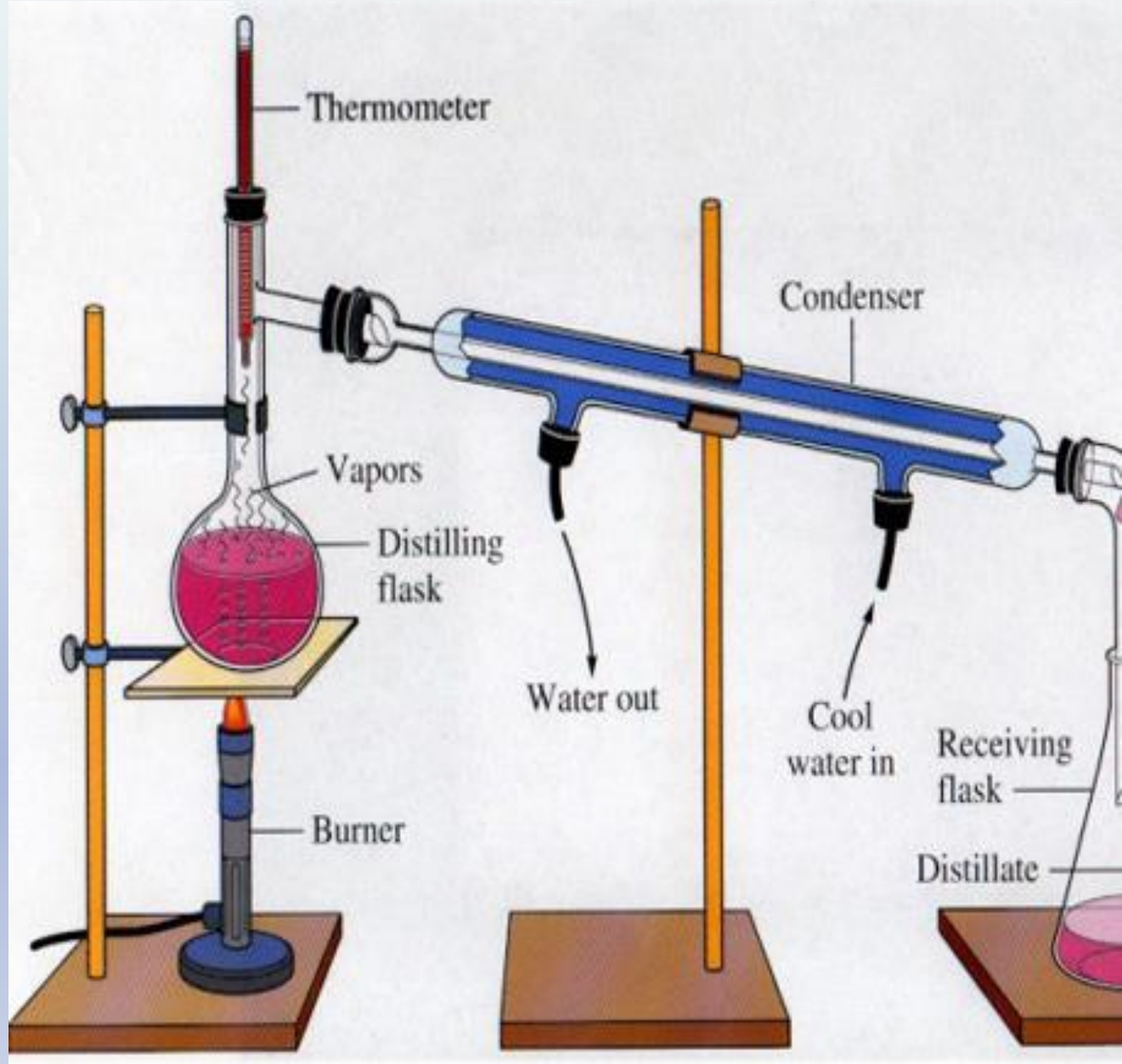
The extraction procedure possesses distinct advantages:

i) The extraction of constituents at low temperature, which strictly avoids damage from heat and some organic solvents.

ii) No solvent residues.

iii) Environmentally friendly extraction procedure.

- **3.Distillation** : which is either :
- -steam distillation
- -fractional distillation which is used for separation of compounds with different boiling point



Parameters for Selecting an Appropriate Extraction Method

i) Authentication of plant material should be done before performing extraction. Any foreign matter should be completely eliminated.

ii) Use the right plant part and, for quality control purposes, record the age of plant and the time, season and place of collection.

iii) 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.

iv) Grinding methods should be specified and techniques that generate heat should be avoided as much as possible.

v) Powdered plant material should be passed through suitable sieves to get the required particles of uniform size.

vi) Nature of constituents:

a) If the therapeutic value lies in non-polar constituents, a non-polar solvent may be used. For example, lupeol is the active constituent of Crataeva nurvala and, for its extraction, hexane is generally used. Likewise, for plants like Bacopa monnieri and Centella asiatica, the active constituents are glycosides and hence a polar solvent like aqueous methanol may be used.

b) If the constituents are thermolabile, extraction methods like cold maceration, percolation are preferred. For thermostable constituents, Soxhlet extraction (if nonaqueous solvents are used) and decoction (if water is the menstruum) are useful.

c) Suitable precautions should be taken when dealing with constituents that degrade while being kept in organic solvents, e.g. flavonoids and phenyl propanoids.

d) In case of hot extraction, higher than required temperature should be avoided. Some glycosides are likely to break upon continuous exposure to higher temperature.

e) Standardization of time of extraction is important, as:

- Insufficient time means incomplete extraction.
- If the extraction time is longer, unwanted constituents may also be extracted. For example, if tea is boiled for too long, tannins are extracted which impart astringency to the final preparation.

f) The number of extractions required for complete extraction is as important as the duration of each extraction.

vii) The quality of water or menstruum used should be specified and controlled.

viii) Concentration and drying procedures should ensure the safety and stability of the active constituents.

Drying under reduced pressure (e.g. using a Rotavapor) is widely used. Lyophilization, although expensive, is increasingly employed.

Separation & isolation of the constituents

Different methods may be used in this matter ex:

- 1.**Sublimation** : which is some times used on the whole drug , as in the isolation of caffeine from tea , or for the purification of materials present in a crude extract.
- **2.Distillation** : fractional distillation has been traditionally used for the separation of the components of volatile mixtures , mainly components of volatile oils.
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- **3.Fractional liberation** : some groups of compounds may be separated by fractional liberation from a mixture ex: when a mixture of alkaloid bases is shaken with NaOH solution the phenolic alkaloids will be separated as salts.

- **4.Fractional crystallization** : the method exploits the differences in solubility of the components of a mixture in a particular solvent .Some times derivatives of the particular components are employed ex: picrates of alkaloids , osazones of sugars.
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The END •