

Quality control & extraction techniques

Pharmacognosy and Phytochemistry

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Quality Control:

➤ The extract should always be prepared from a plant material of well known quality according to a pharmacopoeia or any other reliable monograph.

➤ **Weight ratio** between the crude drug and the extract should be stated.

➤ **Example:** 1:8 ratio of senna leaves ; 1 g dry extract corresponds to 8 g crude drug.

➤ Control of dry extract should include:

IDENTITY: determined by:

- Organoleptic qualities (color, smell, taste ; SENSES).
- Thin-layer chromatography and high performance liquid chromatography. Compare these of the tested drug to those of a **standard**.

MOISTURE: 5% determined by:

1. **Loss on drying:** at 105 °C suitable for large number of samples.

2. **Karl Fischer titration:**

- Used for **expensive** drugs and samples containing **small** quantities of moisture.
- **Composition of reagent:** a solution of iodine, sulphur dioxide and pyridine in **dry** methanol.
- **Basis:** the reagent is titrated with sample containing water which causes the loss of the **dark brown color**. At the end point, when no more water exists, the color persists.
- Water is equivalent to the reduced iodine (in terms of moles).

SOLVENT RESIDUE:

- For ethanol max. 0.5%. For noxious solvents such as methanol and methylethyl ketone: 0.05 % and 0.002% are the limits.
- **Gas-liquid chromatography, GLC:** the technique preferred.

HEAVY METALS:

➤ Some heavy metals, such as copper, zinc and molybdenum appear as micro components in diet. However, these metals **shouldn't exceed the stated limits.**

➤ Also, metals such as lead, mercury and cadmium **should not exceed the allowed limits.**

Atomic absorption spectrometry

➤ **Atomic adsorption spectrophotometry:** is the technique of determination. The sample is **1. combusted** or **2. digested** by a mixture of conc. Nitric, sulphuric and hydrochloric acids, and then **adsorption of metals** is measure at certain wavelength, e.g. Cd at 228.8 nm, Cu 328.8 nm.

➤ For example, accepted limits: Cd < 0.02 ppm (parts per million), Pb < 0.05 ppm and Hg 0.0005 ppm.

CONTENT OF PHARMACOLOGICALLY ACTIVE COMPUND(S):

➤ To give a reliable dose, these compounds should be determined.

➤ HPLC methods: a complex mixture of substances, GLC: v. oilš.

MICROBIAL CONTAMINATION:

- The pharmacopoeia states the accepted levels and limits of bacteria and fungi existing in samples (per unit weight or volume).
- Absence of bacteria such as. *Salmonella*, *Escherichia coli*, *Enterobacteria*.

TESTS FOR AFLATOXINS, PESTICIDES AND RADIOACTIVE CONTAMINATION:

NOTE: the quality control of *fluid* and *soft* extracts should comprise the same parameters mentioned in the preceding pages except **moisture** for which determination of *dry matter* should be constituted.

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EXTRACTION OF PLANT MATERIAL:

- The choice of the extraction procedure depends on the nature of the plant material and the components to be isolated.
- Dried materials are usually powdered before extraction (decrease in size to increase the surface area available for extraction → Increase in the efficiency of extraction).
- Fresh plants (leaves ... etc) can be macerated with a solvent such as alcohol.
- **Alcohol**: is a **general** solvent for many plant constituents (except most fixed oils).
- **Water-immiscible solvents**: are widely used --- e.g. light petroleum for essential and fixed oils, steroids.
- **Ether and chloroform**: for alkaloids and quinines.
- **Basification** of the plant material during extraction of organic **bases** (e.g. alkaloids) if **immiscible solvent** is to be used.

- Likewise, in case of aromatic acids, acidification may be required (to have them in uncharged form where they become miscible in the organic water-immiscible solvents).

A. CONVENTIONAL EXTRACTION METHODS:

- Most of these techniques are based on the **extractive power** of different solvents with application of **heat** or/and **mixing** (shaking, stirring... etc).

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EXTRACTION OF MEDICINAL PLANTS

Conventional Methods

- Extraction as the name implies, involves the separation of medicinally active component through the use of **selective** solvents and standard extraction procedures.
- The total extractives obtained from plants are named (**Galenicals**) named after Galen a Greek physician, 2nd century A.D. and the solvent is called **menstruum**.

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What solvent to choose?

- Should be **selective** if the known compound to be extracted is **known**. If **not**, especially when dealing with plant materials with a lot of compounds, it is recommended to choose a solvent **without selectivity**.
- Has a **high capacity** in terms of coefficient of saturation.
- **Doesn't react** with the extracted compounds.
- **Low cost**.
- **Harmless** to man, animal and environment.
- **Volatile**.

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Cold methods

- ❖ **1. Maceration:** We place the plant powder together with the solvent, then we filter.
 - The solvent used varies and the time also varies.
- ❖ **2. Percolation:** Plant material in a column and the solvent is poured to soak the material (**POWDER FORM**).
 - The solvent percolates from a down opening taking with it the extracted material (extractives or derivatives).
 - A lot of liquid is usually used and this is not preferable.
- ❖ **3. Liquid-Liquid extraction:** by using a separatory funnel.
 - ❖ Extract the content from one liquid into the other.
 - ❖ The liquids must be immiscible.

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Cold methods

- **4. Expression method:** used for citrus fruits mainly: extracting essential oils from citrus peels.
- {Expression, also referred to as "cold pressed", is a method where oil is obtained by using high mechanical pressure to literally squeeze the oil from the plant material, e.g. olive oil extraction under high pressure}.
- **5. Enfleurage method:** التشريب for extraction of v. oil from flowers for perfumery.
 - {Enfleurage is a process that uses odorless fats that are solid at room temperature to capture the fragrant compounds exuded (discharged) by plants. The process can be "cold" enfleurage or "hot" enfleurage}.

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Hot extraction methods

- **1. Infusion:** Powder material is suspended in **hot water**.
- Used for the extraction of **v. oil** active constituents.
- {**Infusion** is the process of extracting chemical compounds or flavors from plant material in a solvent such as water oil or alcohol by allowing the material to remain **suspended** in the solvent over time}.
- **2. Decoction:** الإستخلاص بالإغلاء
- **Boil** together the powder and the liquid.
- This is not preferable when dealing with **v. oil**.
- However, it is usually used when there is a need to **soften** hard material by boiling (like fibers).

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Hot extraction methods

- **3. Digestion method:** by application of **gentle heat** to the plant material in order to increase the extractive power of the solvent.
- **4. Continuous hot extraction:**
 - We combine between 2-3 methods in order to extract the component.
 - The powder of the plant is mixed with the liquid for extraction and then there will be a **close circle** for the solvent and this is done by:
 1. Distillation apparatus.
 2. Soxhlet apparatus.
- **5. Clevenger:**
 - ❖ It is a hydrodistillation method extraction of **v. oils**.
 - ❖ The material to be extracted is immersed in water, which is then boiled.

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A. CONVENTIONAL EXTRACTION METHODS:

- Most of these techniques are based on the **extractive power** of different solvents with application of **heat** or/and **mixing** (shaking, stirring... etc).

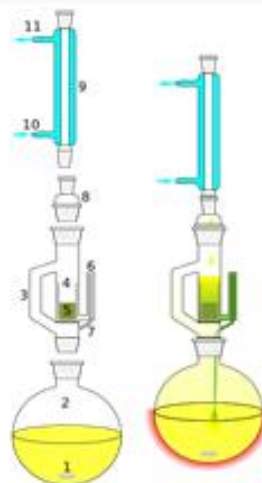
1. Soxhlet extraction:

- By German Van Soxhlet (1879) for extracting lipids.
- Now, for various bioactive compounds from diverse natural sources.
- Used as a model of **comparison** with the **new** techniques.
- **BASIS:** The plant powder is placed in a cellulose thimble in an extraction chamber, which is placed on top of a collecting flask beneath a reflux condenser. A suitable solvent is added to the flask, and the set up is heated under reflux (heat source). When a certain level of condensed solvent has accumulated in the thimble, it is siphoned into the flask beneath and takes the solute with it.

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A figure and a schematic representation of a Soxhlet extractor:

1: Stirrer bar 2: Still pot (the still pot should not be overfilled and the volume of solvent in the still pot should be 3 to 4 times the volume of the Soxhlet chamber) 3: Distillation path 4: Thimble 5: Solid 6: Siphon top 7: Siphon exit 8: Expansion adapter 9: Condenser 10: Cooling water in 11: Cooling water out.



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Advantage

- The main **advantage** of Soxhlet extraction is that it is a **continuous** process, that, **one batch** is enough for the process, which means saving in solvent use (**economical** process somehow).

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INFUSION

Infusion:

- {The process of extracting chemical compounds or flavors from plant material in a solvent such as water, oil or alcohol, by allowing the material to remain suspended in the solvent over time}
- The drug is usually **coarsely** powdered (50 gm). **Very fine powder is avoided.**
- Moisten the drug in a suitable vessel, provided with a cover, with 50 ml of cold water.
- Allow to stand for 15 minutes.
- Then add 900 ml of **boiling** water, cover the vessel tightly.
- Allow it to stand for 30 minutes.
- Then, strain the mixture, pass enough water to make the infusion measure 1000 ml
- Some drugs are supplied in accurately weighed muslin bags for preparing specific amounts of infusion.



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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}.
- Is that form of maceration which consists of the application of **gentle heat** to the substance which is being treated.
- It is used in those cases where a moderately elevated temperature is unobjectionable.
- Heat increases solvent power for the extraction.
- If the solvent is readily volatilized at the temperature employed, it is necessary to attach a **reflux condenser** to the vessel in which the digestion is being conducted so that the solvent may be **recovered** and **returned**.

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DIGESTION



Digestion

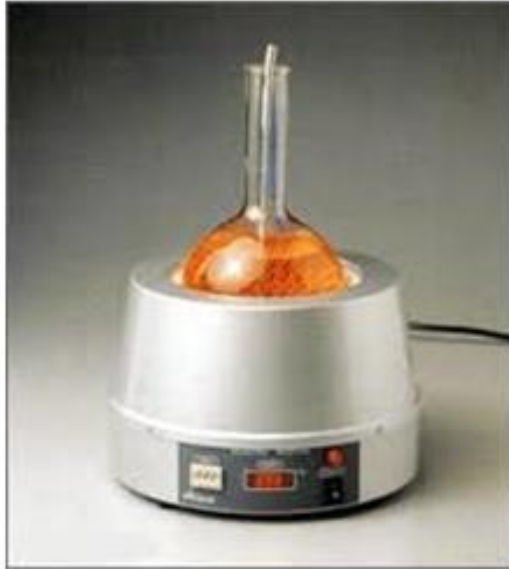


- { • In digestion process, the drug is extracted by heating at a particular pressure. This will increase the penetration power of the menstruum, so that there is complete extraction of the drug. Precautions should be taken so that the increased temperature may not harm the active constituents of the drug.
- The apparatus known as “Digester” is used for extraction of the drug by this method. It is a vessel made up of metal. The whole of the drug along with the menstruum is placed in the body of the digester. Place the cover over it and bolt it with the help of nuts. The drug is treated with menstruum for a definite period under specified conditions of temperature and pressure. }

Menstruum: solvent

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DIGESTION



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Distillation

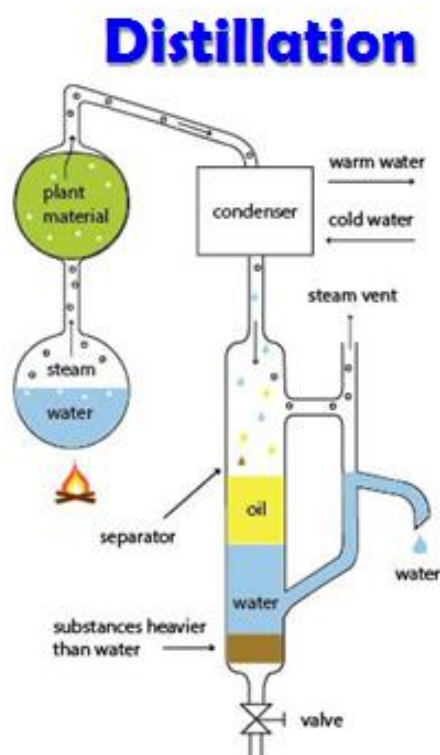
- **Hydrodistillation:** is a traditional method for extraction of bioactive compounds and essential oils from plants.
- *Organic solvents are not involved* and it can be performed before dehydration of plant materials.
- **There are 3 types of Hydrodistillation:**
 1. Water distillation
 2. Water and steam distillation
 3. Direct steam distillation.
- In hydrodistillation:
 - ❖ **First**, the plant materials are packed in a still compartment;
 - ❖ **Second**, water is added in sufficient amount and then brought to **boil**.

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Distillation

- ❖ Alternatively (or), **direct steam** is injected into the plant sample. Hot water and steam act as the main influential factors to **free** bioactive compounds of plant tissue.
- ❖ Indirect cooling by water **condenses** the vapor mixture of water and oil.
- ❖ Condensed mixture flows from condenser to a separator, where oil and bioactive compounds separate **automatically** from the water.

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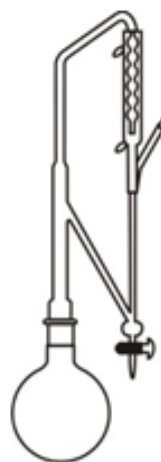


Distillation of essential oils

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Clavenger apparatus for extraction of v. oils

- The amount of v. oil in v. oil-containing drugs may be determined by **distillation**.
- In this method the drug is **distilled with water in the proper Clevenger apparatus**.
- This is a **continuous distillation** apparatus in which the separated v. oil is caught in a trap and determined by volume.



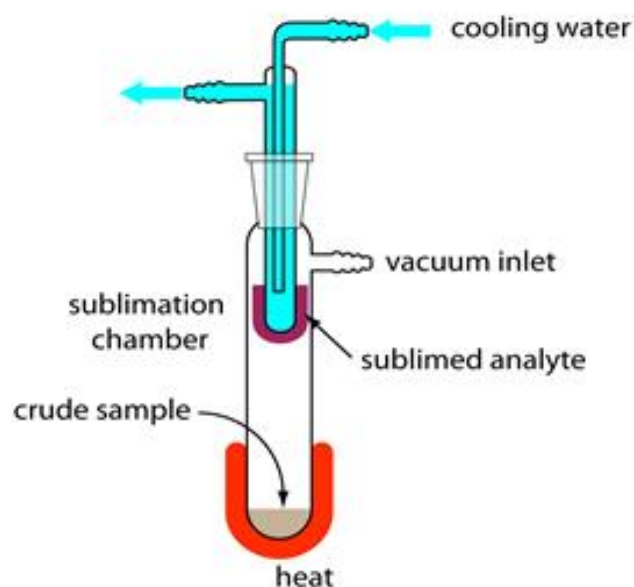
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sublimation purification

- *Sublimation is a technique used by chemists to purify compounds. A solid is typically placed in a sublimation apparatus and heated under vacuum. Under this reduced pressure, the solid volatilizes and condenses as a purified compound on a cooled surface (cold finger), leaving a non-volatile residue of impurities behind. Once heating ceases and the vacuum is removed, the purified compound may be collected from the cooling surface. For even higher purification efficiencies a temperature gradient is applied, which also allows for the separation of different fractions*

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SUBLIMATION APPARATUS



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DECOCTION

- The plant material is placed together with the solvent in a container.
- The container is placed on a source of heat, either direct flame or on water bath **until boiling**.
- This method is used for hard parts of plant like root or bark which don't contain heat-labile active constituents.
- The solvent for extraction used depends on the chemical nature of active constituents.
- The extract separated from the marc by filtration

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Cold methods

1. Maceration:

- This is a simple widely used procedure that involves leaving the *pulverized* plant to **soak** in a suitable solvent in a closed container.

❖ **Simple maceration:**

- **Simple maceration** is performed at room temperature by mixing the ground drug with the solvent (drug solvent ratio: 1:5 or 1:10) and leaving the mixture for several days with *occasional* shaking or stirring. The extract is then **repeated** from the plant particles by straining (squeezing).
- The process is repeated for **once** or **twice** with **fresh** solvent.
- Finally the last residue of extract is pressed out of the plant particles using a mechanical press or a centrifuge.

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- **Kinetic maceration:** differs from simple by *continuous* stirring.
- **DISADVANTAGE:** Time-consuming; it takes from few hours to several weeks.

2. Percolation:

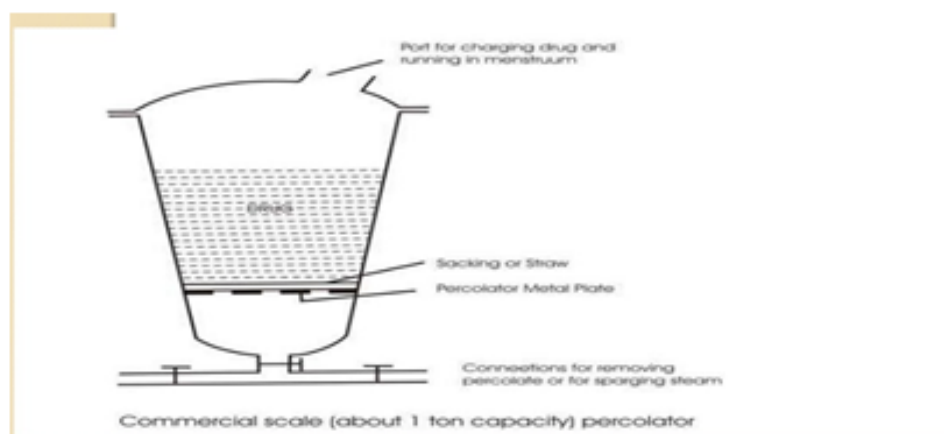
- the **powdered** plant material is **soaked** initially in a solvent in a percolator.
- Additional solvent is then poured on top of the plant material and allowed to percolate slowly (**dropwise**) out of the bottom of the percolator.
- Additional filtration of the extract is **not** required because there is a filter at the outlet of the percolator.

Advantages:

1. Percolation is adequate for both initial and large-scale extraction.
2. It is a simple and popular technique.

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Percolator:



The main **disadvantages** are:

1. Fine powders and materials such as resins and plants that swell excessively (e.g., those containing mucilage's) can **clog** the percolator.
2. If the material is not **distributed homogeneously** in the container, the solvent may not reach all areas and the extraction will be incomplete.

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3. Hydrodistillation: is a traditional method for extraction of bioactive compounds and essential oils from plants. **Organic solvents are not involved** and it can be performed before dehydration of plant materials.

• There are 3 types of Hydrodistillation:

1. Water distillation
2. Water and steam distillation
3. Direct steam distillation.

• In hydrodistillation:

- ❖ **First**, the plant materials are packed in a still compartment;
- ❖ **Second**, water is added in sufficient amount and then brought to **boil**.
- ❖ Alternatively (or), **direct steam** is injected into the plant sample. Hot water and steam act as the main influential factors to **free** bioactive compounds of plant tissue.
- ❖ Indirect cooling by water **condenses** the vapor mixture of water and oil.
- ❖ Condensed mixture flows from condenser to a separator, where oil and bioactive compounds separate automatically from the water.

Disadvantages: at high extraction temperatures, some volatile components may be lost; therefore, **it is not convenient for thermo labile compound extraction.**

- The **efficiency** of the conventional methods depends on the **choice of solvents**, which in turn depends on the **polarity** of the solute (drug/s to be separated), and hence increasing the **affinity** between the solute and solvent.
- The following points should be considered when extracting a certain drug:
 1. Affinity between solute (the drug) and solvent.
 2. Use of a co-solvent (a secondary solvent added in small quantities to enhance the primary solvent extracting power).
 3. Environmental safety.
 4. Human toxicity.
 5. Financial feasibility.

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Liquid-liquid extraction

- **Liquid-liquid extraction:**
- Extraction is carried out using a **separatory funnel**.
- Extract the content from one liquid into the other.
- The two liquids must be **immiscible**.



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Expression

- Expression is a method of fragrance extraction where raw materials are **pressed, squeezed or compressed** and the oils are collected.
- In contemporary times (at present), the only fragrant oils obtained using this method are **the peels of fruits in the citrus family**.
- Citrus peel oils are expressed mechanically, or *cold-pressed*.
- Due to the large quantities of oil in citrus peel, citrus-fruit oils are cheaper than most other essential oils.

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Scarification

- Citrus fruits are known to provide volatile oil from their peel.
- The standard procedure for extraction is known as **eculle** by which the peel is twisted clockwise or anticlockwise by the aid of forceps, then placed in the apparatus which consists of bowl of copper lined with projections.
- Upon stirring of apparatus electrically, it will rapture the oil ducts in the peel of citrus.

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Enfleurage method

- ❖ Glass plates in a frame (called a chassis) are covered with highly purified and odorless vegetable or animal **fat** and the petals of the botanical matter that are being extracted are spread across it and pressed in.
- ❖ The flowers are normally freshly picked before being encased in their fatty bed.
- ❖ The petals remain in this greasy compound for a few days or a couple of weeks (depending on the botanic material used) to allow the essence to disperse into the compound, where the then depleted petals are removed and replaced with a fresh harvest of petals.

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Enflourage method

- This process is repeated until the greasy mix is saturated with the essence, and needs to be repeated a couple of times until saturation is achieved.
- When the mix has reached saturation point the flowers are removed and the enfleurage pomade **معجون عطري** (the fat and fragrant oil) then is washed with **alcohol** to separate the extract from the remaining fat, which is then used to make soap.
- As soon as the alcohol evaporates from the mixture, the remaining is the essential oil.
- This is a very labor-intensive **كثيفة العمالة** way of extraction, and needless (unimportant) to say a very costly way to obtain essential oil and is **nowadays only sometimes** used to extract essential oil from tuberose and jasmine.

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Enfleurage method



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B. NON-CONVENTIONAL EXTRACTION TECHNIQUES:

- Conventional extraction techniques have the following drawbacks/ **disadvantages**:
 - 1) Longer time extraction.
 - 2) Requirement of costly and highly pure solvents.
 - 3) Need to evaporate of huge amount of solvent.
 - 4) Low extraction selectivity.
 - 5) Decomposition of thermo-labile subsatnaces owing to the temperatures employed.
- To overcome these limitations of conventional extraction methods, new and promising extraction techniques are introduced. These techniques are referred as nonconventional extraction techniques. They are:

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Non-conventional extraction techniques:

1. **Ultrasound-assisted extraction (UAE):**
2. **Pulse-electrical field extraction (PFE):**
3. **Enzyme-assisted extraction (EAE):**
4. **Microwave-assisted extraction:**
5. **Pressurized liquid extraction (PLE):**
6. **Supercritical fluid extraction.**

Generally, these techniques collectively have the following advantages:

1. Time saving.
2. High capacity and productivity.
3. Use no solvents or just lesser amounts.
4. Selectivity.
5. Some can be automated.
6. Some can accomplish complete extraction.

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7. Suitable for extracting thermo-labile substances.
8. Only small sample size is required.
9. Some can be coupled directly to chromatographic analysis, and thus they are suitable for highly volatile substances.
10. Recycling and reuse of solvent is possible in some methods, a matter that decreases solvent waste.

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