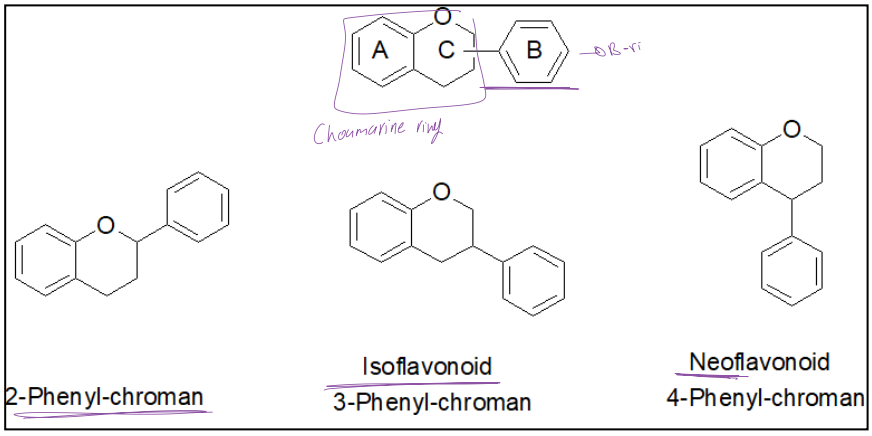
Experiment (8)

Extraction and Identification of Flavonoids

Flavonoids are an important class of natural products; particularly, they belong to a class of plant secondary metabolites having a polyphenolic structure. They constitute one of the most characteristic classes of compounds in higher plants. Many flavonoids are easily recognized as flower pigments in most angiosperm families. However, their occurrence is not restricted to flowers but are found in all parts of plants.

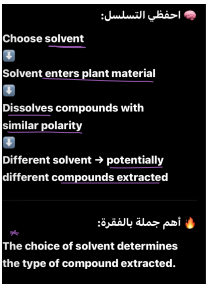
Flavonoids are based on a C15 skeleton with a Chromane ring bearing a second aromatic B ring in position 2, 3 and 4. Flavonoids can be subdivided into different subgroups depending on the carbon of the C ring on which the B ring is attached and the degree of unsaturation and oxidation of the C ring. Flavonoids in which the B ring is linked in position 3 of the C ring are called isoflavones. Those in which the B ring is linked in position 4 are called neoflavonoids, while those in which the B ring is linked in position 2 can be further subdivided into several subgroups on the basis of the structural features of the C ring (e.g.: flavones, flavonols, flavanones, etc.).



In plants, flavonoids have long been known to be synthesized in particular sites and are responsible for the color (yellow or red/blue pigmentation) and aroma of flowers, and in fruits to attract pollinators. Flavonoids protect plants from different biotic and abiotic stresses and act as unique UV filters. Also, function as signal molecules, detoxifying agents and antimicrobial defensive compounds.

Flavonoids are associated with a broad spectrum of health-promoting effects and are an indispensable component in a variety of pharmaceutical, medicinal and cosmetic applications. This is because of their anti-oxidative, anti- inflammatory, anti-mutagenic and anti-carcinogenic properties coupled with their capacity to modulate key cellular enzyme functions. They are also known to be potent inhibitors for several enzymes, such as xanthine oxidase (XO), cyclo- oxygenase (COX), lipoxygenase and phosphoinositide 3-kinase.

Examples of plants with a high flavonoid content include parsley, onions, berries, green tea, and all citrus fruits.



I. Extraction

Extraction: is the separation of medicinally active portions of plant using selective solvents through standard procedures. During extraction, solvents diffuse into the solid plant material and solubilize compounds with similar polarity. The choice of the solvents will determine the type of compound extracted from the samples.

➢ *Common extraction procedures*

✓ Maceration:

In maceration, whole or coarsely powdered plant-drug is kept in contact with the solvent in a stoppered container for a defined period (minimum 3 day) with frequent agitation until soluble matter is dissolved. This method is best suitable for use in case of the thermolabile drugs.

✓ Infusion:

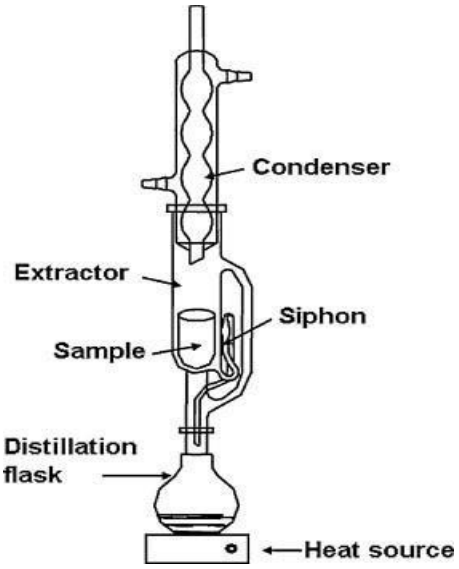
It is a dilute solution of the readily soluble components of the crude drugs. Fresh infusions are prepared by macerating the solids for a short period of timewith either cold or boiling water.

✓ **Decoction:**

This method is used for the extraction of the water soluble and heat stable constituents from crude drug by boiling it in water for 15 minutes, cooling, straining and passing sufficient cold water through the drug to produce the required volume.

➢ *Soxhlet extraction (hot continuous extraction):*

Soxhlet extraction is only required where the desired compound has a limited solubility in a solvent, and the impurity is insoluble in that solvent. If the desired compound has a high solubility in a solvent then a simple filtration can be used to separate the compoundfrom the insoluble substance. The advantage of this system is that instead of many portions of warm solvent being passed through the sample, just one batch of solvent is recycled. This method cannot be used for thermolabile compounds as prolonged heating may lead to degradation of compounds.



✓ The basic parameters influencing the quality of an extract are:

1. Plant part used as starting material
2. Solvent used for extraction
3. Extraction procedure

✓ Variation in extraction methods usually depends upon:

1. Length of the extraction period.
2. Solvent used.
3. pH of the solvent.
4. Temperature.
5. Particle size of the plant tissues.
6. The solvent-to-sample ratio.

⭐ مقارنة سريعة مع الطرق اللي قبل:

Method	الفكرة الأساسية	الحرارة؟	Thermolabile؟
Maceration	نقع طويل ≥ 3 أيام	❌	✅ مناسبة
Infusion	نقع قصير بالماء	غالبًا ماء ساخن/ حسب الطريقة	حسب المادة
Decoction	غلي 15 دقيقة	🔥	❌
Soxhlet	استخلاص ساخن مستمر + إعادة تدوير المذيب	🔥🔥	❌

أهم كلمة في Soxhlet: recycling of one batch of solvent.

Practical work:

1. TLC:

- Plants to be used:
  - Arnica flowers
  - Grapefruit peels
  - Orange peels
  - Calendula flowers
- References: Rutin, Hesperidin, Quercetin
- Solvent system:
  Ethylacetate: Formic acid: Glacial acetic acid: Water (100:11:11:24)
- Detection:
  1- UV 365
  2- 2- Spraying agent: Ferric chloride solution.

- Procedure:
  1. Extract 1g of the plant with 10 ml of ethanol by heating on a water bath for 5 minutes, then filter.
  2. Prepare your TLC plate as instructed and develop in the solvent system.
  3. Spray your TLC with the spraying reagent.
  4. Analyze your results and calculate the R_f values.

$$Rf = \frac{\text{Distance traveled by compound}}{\text{Distance traveled by solvent front}}$$

2. Chemical tests:

- Shinoda's test: to 1ml of the alcoholic solution of plant extract, add Mg metal and then add HCL drop wise, detect the color.
- To 1ml of alcoholic solution of hesperidin, add few drops of $AlCl_3$ solution and detect the difference in the intensity of the yellow color.
- Repeat the tests for Rutin solution.