Al-Nahrain University
College of pharmacy

Practical pharmacognosy
3rd stage 1st term

Flavonoid Glycosides

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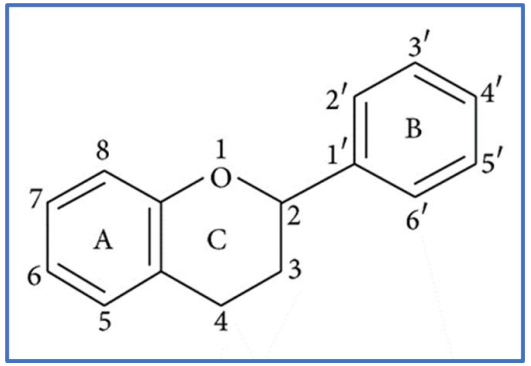
Flavonoids

- Flavonoids from the Latin word flavus meaning yellow, their color most chemical reactions.
- They are natural polyphenols of plant origin that are among the most important compounds in human diet due to their widespread distribution in foods and beverages.
- They can occur both the free form (aglycones) and as glycosides.
- Flavonoids differ in their substituents in the number and position and in the instauration of the middle ring.
- All flavonoids share a basic C6-C3-C6 benzo pyran backbone.



Chemical property

- The glycosides are generally soluble in water and alcohol, but insoluble in organic solvents; the aglycons are only sparingly soluble in water but soluble in ether and other organic solvents.
- **Flavonoids (due to acidic nature) dissolve in base giving yellow-colored solutions which by addition of acid becomes colorless.**
- **Flavonoids tend to form light yellow precipitate with lead acetate.**

Isolation and Identification of flavonoids

- **Aim :** Detection and isolation of flavonoid glycosides from apple peel extract.
- **Extraction method**: maceration.



Apple peel in-vitro biological activities

- Invitro Anticancer activity on **prostate and breast cancer** cell lines.
- **Anti- hypertensive.**
- In treatment of **gastric ulcer caused by H. Pylori.**
- Very **potent antioxidant** since it is flavonoid rich.

Experimental stages

Isolation and identification of flavonoids (Quercitrin/Rutin /Quercetin): From apple peel extract.

- **Extraction** :

  Maceration using 70% hydroalcoholic solvent for 24hrs.

- **Isolation**:

  liquid-liquid partition (*solvent extraction*) using **chloroform.**

- **Identification**:

➢ ***NaOH test***: (yellow sodium phenoxide salt) >> **HCl**: liberates phenolic free acid.

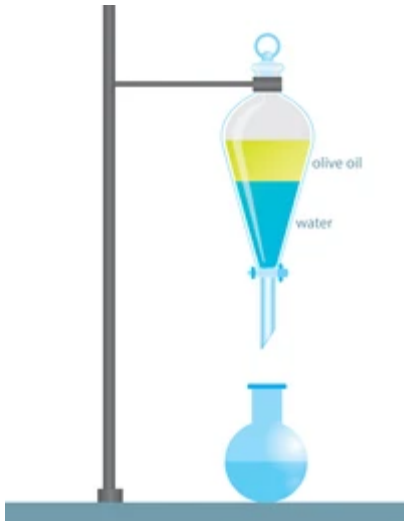
➢ ***Lead acetate test*****:** which forms complex and cause the precipitation of flavonoids.

➢ ***TLC*** : thin layer chromatography : separation of components on silica plate with Rutin and Quercitrin standards.

Procedure:

- From the apple peel alcoholic extract concentrate take **10ml.**
- Divide into two parts:
- **3ml** (**fraction A**)
- **7ml** (add **1ml** 5% **HCl**) >>> boil for **5 min.**

- Partition with chloroform (**7ml**) (*liquid-liquid extraction*).
- **Lower part (organic) fraction B**

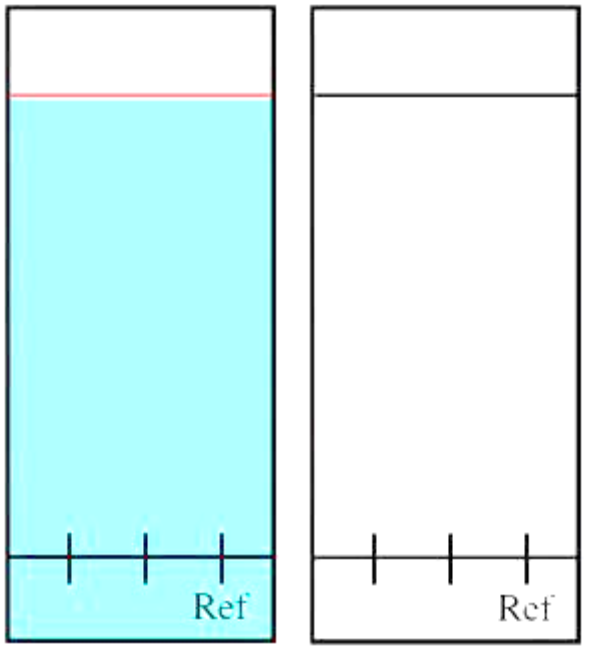


THIN LAYER CHROMATOGRAPHY

- **Apply 4 spots on your TLC plate**:
- **Fraction A** : whole glycosides (flavonoid glycoside).
- **Fraction B** : contains aglycons (flavonoid).
- **Standard compound**: **Quercitrin and Rutin.**
- **Mobile phase :**

chloroform: acetic acid: methanol :water (60:32:16:4)

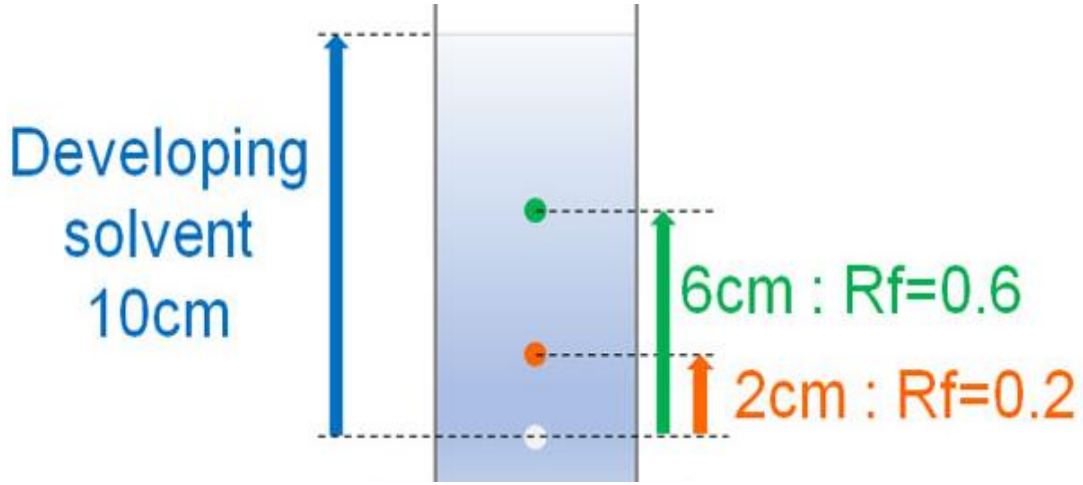
- **Development.**
- **Detect spots under UV-254 nm.**



Thin layer chromatography

- **Calculate the Rf value for all three spots**.
- Rf = distance travelled by compound / distance travelled by solvent.
- Detect the present of flavonoids in fraction A and B.

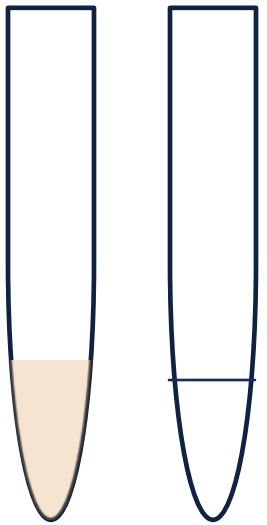
$$Rf = \frac{\text{distance moved by the compound}}{\text{distance moved by the solvent}}$$



CHEMICAL IDENTIFICATION

Base NaOH reaction:

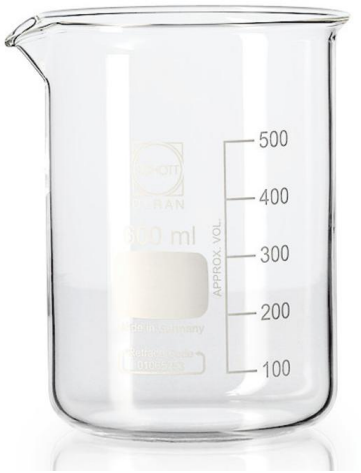
- On **2ml** of the organic layer fraction B:
- **Add 5 drops of 5% aqueous NaOH.**
- **Add 5 drops of HCl.**
- **A positive results for flavonoid presence:**
- Base gives a deep **bright yellow color.**
- Acid turns the solution **colorless.**



CHEMICAL IDENTIFICATION

Lead acetate test

- Lead acetate causes precipitation of flavonoids.
- To **2ml** of fraction B:
- Add 3 drops of **lead acetate solution.**
- **Yellow precipitate will appear.**



REPORT

Please include:

- Name of experiment:
- Aim of experiment:
- Procedure:
- Results:
- Discussion;
- Name of the group members.