Thin Layer Chromatography Characterization of Flavonoids

Introduction:

- Flavonoid is a class of plant secondary metabolites based around phenylbenzopyrone structure.
- Flavonoids are most commonly known for their antioxidant activity.
- Flavonoids are also commonly referred to as bioflavonoids because all Flavonoids are biological in origin.
- They have been referred to as nature's biological response modifiers because of strong experimental evidence of their ability to modify the body's reaction to allergens, viruses and carcinogens.
- They show anti-allergic, anti-inflammatory, anti-microbial and anti-cancer activity. In addition, flavonoids act as powerful antioxidants, protecting against oxidative and free radical damage.
- The beneficial effects of fruits, vegetables and tea have been attributed to flavonoid compounds rather than to known nutrients and vitamins.

- They are widely distributed in plants producing yellow or red/blue pigmentation in flowers and protection from attack by microbes and insects.

Important Dietary Sources:

- All citrus fruits, berries, onions, green tea and dark chocolate are good sources of flavonoids.

- The citrus bioflavonoids include hesperidine, quercetin, rutin and tangeritin.

Classification:

- Over 5000 naturally occurring flavonoids have been characterized from various plants.

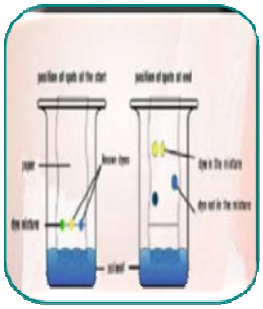
- They have been classified according to their chemical structure and are usually divided into 6 subgroups:

- Flavones
- Flavonols
- Flavanones
- Flavan-3-ols
- Isoflavones
- Anthocyanidins

Rutin (Ruta herb):

- It is a citrus flavonoid glycoside (a sugar of quercetin) found in some plants.
- It combines with cations, supplying nutrients from the soil to the cells in plants.
- In humans, it attaches to the iron ion Fe^{2+}, preventing it from binding to hydrogen peroxide and creating a highly reactive free radical that may damage cells.
- It is an antioxidant and therefore plays an important role in inhibiting some cancer.

Principle of thin layer chromatography:



- TLC is a simple, quick, and inexpensive procedure that indicates how many components are in a mixture.

- A TLC plate is a sheet of glass, metal, or plastic which is coated with a thin layer of a solid adsorbent (usually silica or alumina). A small amount of the mixture to be analyzed is spotted near the bottom of this plate. The TLC plate is then placed in a shallow pool of a solvent in a developing chamber

so that only the very bottom of the plate is in the liquid. This liquid, or the eluent, is the mobile phase, and it slowly rises up the TLC plate by capillary action.

Aim of the experiment:

-In this experiment you will identify flavonoids by TLC in different herbs.

Material:

- Ruta herb, chamomile flowers, caraway.

- Adsorbent: silica gel G.

- Solvent: ethyl acetate: formic acid: water (8:1:1).

Procedure:

1- Preparation of extract:

-Boil 3g of powdered drug + 30ml of methanol for 2min, cool and filter. Use the filtrate for chromatography applying.

2- Reference substance:

- Dissolve 2.5mg of rutin in 10ml of methanol.

3- Development:

- Develop the spotted plate at room temperature to a distance of 12cm.

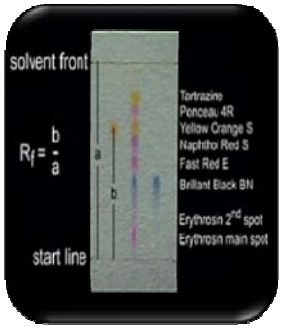
- Mark the front and allow the solvent to evaporate off at room temperature.

4- Detection:

- Spray the plate with the reagent consisting of (15ml 3% boric acid solution and 5ml 10% oxalic acid) heat the plate and examine in UV light.

5- Evaluation of the chromatogram:

- Flavonoids treated with boric and oxalic acids give compounds which after heating fluorescence yellowish green.(see the figure)



6- Results:

- Determine R_f values of reference flavonoids and flavonoids contained in the tested extracts.

References:

- www.wikipedia.org

- El-Olemy, M. Al-Muhtadi, F. Afifi, A. Experimental Phytochemistry. A Laboratory Manual. Riyadh: College of Pharmacy, King Saud University; 1994.

Results Sheet