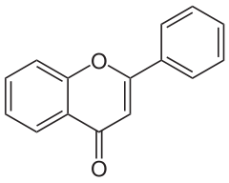
# Exp.03: flavonoids extraction

**Objectives:**

- Extraction flavonoids from orange

**Introduction:**

Flavonoids are a class of plant secondary metabolites Consisting of 2 benzene rings linked via a heterocyclic pyrone or pyran ring they are benzo-γ-pyrone derivatives so not open by alkalis.



**The basic flavonoid skeleton can have a large number of substitutions on it:**

-Hydroxyl groups

-Sugars-e.g. glucose, galactose, rhamnose.

Sugars and hydroxyl groups: increase the water solubility of flavonoids

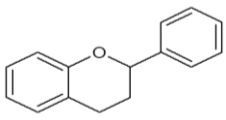
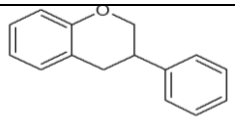
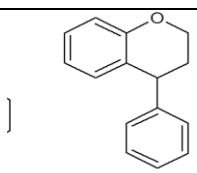
-Methyl and isopentyl groups: make flavonoids lipophilc

-If no sugar: -AGLYCONE

-With sugar: -GLYCOSIDE

**Classification of flavonoids:**

According to the position of the linkage of the aromatic ring to the benzopyrano (chromano) moiety, it divided into three classes:

| | |
|---|---|
| 1- The flavonoids (2-phenylbenzopyrans) |  |
| 2- isoflavonoids (3-benzopyrans) |  |
| 3- the neoflavonoids (4-benzopyrans) |  |

| | |
|---|---|
| 4-Minor Flavonoids | |
| 5-Anthocyanins | |
| 6-Bioflavonoids | |

All share a common chalcone precursor, and therefore are biogenetically and structurally related.

**Uses:**

**- For humane:**

Seem to have major health benefits for humans such as Antioxidant and other medicinal use

**- For plants:**

Protection from animal Breading photosynthesis

**Tests of flavonoids:**

The extracts were dissolved in ethanol, filtered and subjected to following test.

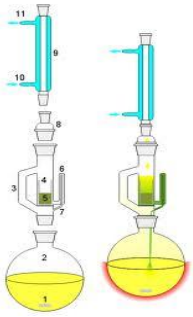
**(a) Shinoda test:**

To the test solution, few drops of concentrated hydrochloric acid (HCL) were added. Then the magnesium turnings were put into the solution andobserved for appearance of pink red color.

**(b) Zinc Hydrochloride reduction test**

To the test solution add a mixture of Zinc dust and conc. Hydrochloric acid. It gives red color after few minutes

**Experiment Procedure:**

<table>
<tr><th>Step</th><th colspan="2">Procedure</th></tr>
<tr><td rowspan="4">1</td><td colspan="2">- 10 g of orange in round- bottom flask</td></tr>
<tr><td colspan="2">- The flavonoids extraction from orangewill performed using soxhlet extractor (Fig. 1)</td></tr>
<tr><td colspan="2">- Add alcohol (Ethanol) in spherical flask.</td></tr>
<tr><td colspan="2">- Heat for 1 h</td></tr>
<tr><td colspan="2">  <br>Fig. 1</td></tr>
<tr><td>2</td><td colspan="2">Concentrated the solution.</td></tr>
<tr><td>3</td><td colspan="2">TLC using two system<br>- First system: Chloroform-methanol (95:5 v/v).<br>- Second system: Ethyl acetate- methanol-water (60:5:4 v/v/v).</td></tr>
<tr><td>4</td><td colspan="2">Notes the color of spot under UV light(brown).</td></tr>
<tr><td>5</td><td colspan="2">Use $AlCl_3$as a reagent and notes the change in spot color under UV light (yellow).</td></tr>
<tr><td>6</td><td colspan="2">Calculate $R_f$ for flavonoid spot in two systems.</td></tr>
<tr><td>7</td><td colspan="2">Performed:<br>(a) Shinoda test:<br>To the test Solution, add few fragments of Magnesium and concentrated Hydrochloric acid drop wise crimson red.<br>(b) Zinc Hydrochloride reduction test:<br>To the test solution add a mixture of Zinc dust and conc. Hydrochloric acid. It gives red color after few minutes</td></tr>
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