

Practical Pharmacognosy

3rd. Stage

1st semester

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Lab.2



The Chemical Tests

1. Baljet's Test:

Aim: The identification of the cardio active glycosides in general.

Equipments & Reagents:

- Test tube.
- Picric Acid.
- Sodium hydroxide solution.

Procedure :

Take 1ml of fraction A, add 2 drops of Picric acid then make it alkaline with Sod. Hydroxide solution. (litmus paper).

Results:

Turbid , **yellow to orange** in color.

2. Keller- Killian's Test

Aim: The identification of the cardio active glycosides in general.

Equipments & Reagents:

- Test tube.
- Glacial acetic acid
- 0.1 % of ferric chloride solution.
- Conc. H₂SO₄.

Procedure:

Take 1ml of fraction A, and 2ml of glacial acetic acid, add 1 drop of 0.1 % of ferric chloride solution. Take 1ml of conc. H₂SO₄ and add to the above mixture in drops so as to make two layers.

Results:

Two layers are formed; the upper one has **light bright green color**. The lower layer has transparent clear color (H₂SO₄ layer). The junction appears as **a reddish –brown ring**.

**Keller_ellian's
test
result**



**Baljet's test
result**

**Identification of cardioactive
glycoside**

Other Chemical Tests for the Identification of Sterol Glycosides:

1. Raymond 's Reaction:

Aim: To identify the sterol nucleus.

Equipments and Reagents:

- Test tube.
- 10% sodium hydroxide solution.
- 1% m-dinitrobenzene.

Procedure:

To 1ml of fraction A add 1-2 drops of 10% sodium hydroxide and few drops of an alcoholic solution of 1% m-dinitrobenzene.

Result:

Pink colour appears.

2.Kedde's Reaction :

Aim : To identify the sterol nucleus.

Equipments and Reagents:

- Test tube.
- 1% 3,5-dinitrobenzoic acid.
- 0.5 N aqueous methanolic KOH (50 %).

Procedure:

To a solution of glycoside add a solution of 1% 3, 5-dinitrobenzoic acid in 0.5N aqueous methanolic KOH (50%).Report the colour.

3.Lieberman's Sterol Reaction:

Aim: To identify the sterol nucleus.

Equipments and Reagents:

- Test tube.
- Porcelain dish.
- Anhydrous acetic acid.
- Conc.H₂SO₄.

Procedure:

Take 1ml of fraction A in a test tube then add 5ml of anhydrous acetic acid and shake well. Take 4 drops of the above mixture and place in a porcelain dish, and then add one drop of conc.H₂SO₄.

Result:

A change of color from **rose**, through **red**, **violet** and **blue** to **green**. The colors are slightly different from compound to compound.



The Identification of Cardio active Glycosides By Chromatography:

By the use of thin layer chromatography (T.L.C)

- The stationary phase = Silica gel G.
- The mobile phase = Chloroform: Ethanol: Water (7:3:1)

Or Ethyl acetate: Methanol: Water (75:10:5).

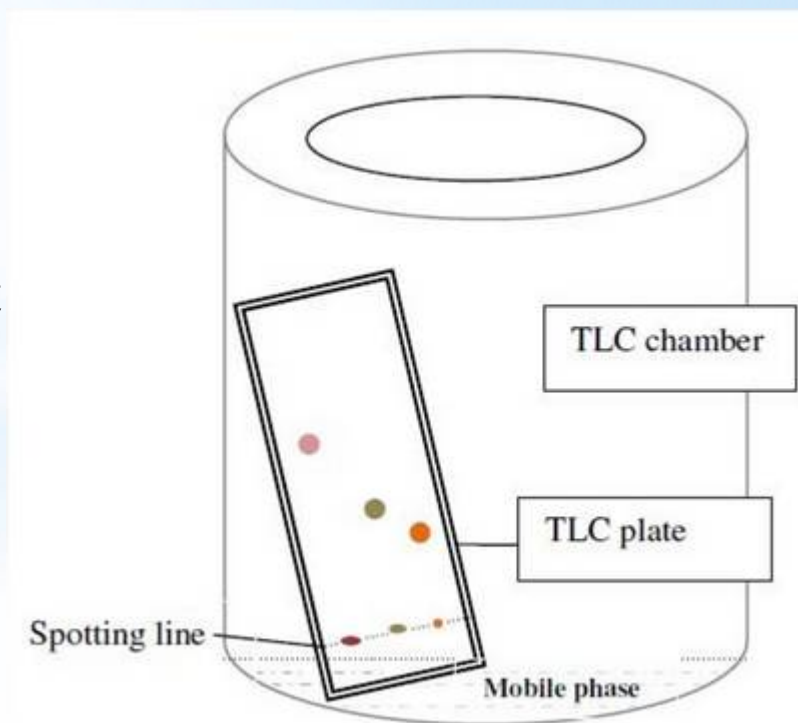
- The standard compound = Oleandrin.
- The spray reagent = Lieberman's reagent.
- Mechanism of separation = Adsorption.
- Developing = Ascending.
- Other mobile phases :

Butanone: Xylene: Formamide (50:5:4)

Chloroform: tetrahydrofuran: Formamide (50:50:6).

Procedure:

- 1) Prepare 100ml of mobile phase, and place it in the glass tank.
- 2) Cover the tank with glass lid and allow standing for 45 minutes before use.
- 3) Apply the sample spots (fraction A & fraction B), and the standard spot on the silica gel plates, on the base line.
- 4) Put the silica gel plate in the glass tank and allow the mobile phase to rise to about two-third the plate.
- 5) Remove the plate from the tank, and allow drying, and then detecting the spots by the use of the spray reagent and heat the plates at 105 -110 °C for 5-10 mins in the oven.
- 6) Note the spots, and calculate the R_f value for each spot.



Thank you

