

2. Results:

Fraction A: Contain the whole glycosides.

Fraction B: Contain the aglycone (dianthrone).

Fraction C: Contain the aglycone part (monoanthrone).

Lab. 4

Chemical tests for anthraquinone glycosides

The chemical tests for aloin detection

For extraction of aloin, boil **1 gm** of the crude drugs (**Aloe**) given with **100 ml** of water, add a little of **kieselghur**, filter and use the solution for the following tests:

➤ Schonteten's Reaction (Borax test):

Procedure:

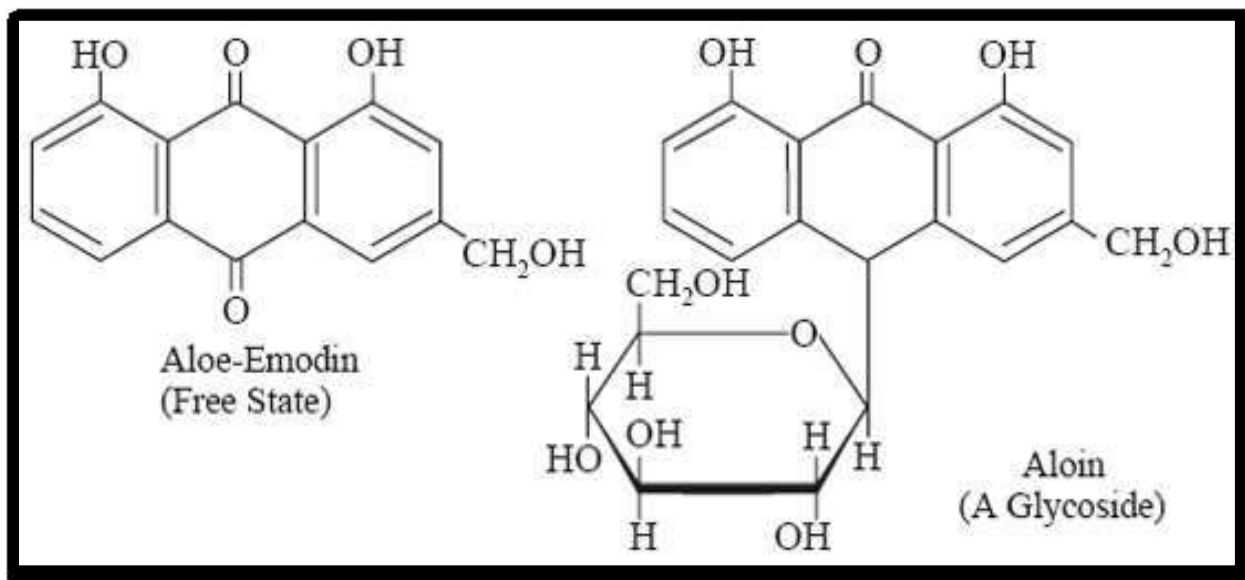
To **2 ml** of the Aloe extract, add **0.1 gm** of **Borax** and heat until dissolved. Pour a few drops of the liquid into test tube nearly full of water.

Results:

A **green fluorescence** is produced.

Discussion:

This **green fluorescence** is due to **aloe-emodin anthranols** liberated from **barbaloin** by hydrolysis with Borax giving this reaction.



➤ **Bromine Test for Aloin:**

Procedure:

Take **2 ml** of the Aloe extract, add an equal volume or an excess of freshly prepared solution of **bromine**. Record the color.

Result:

Borntrager's test

Anthraquinone derivatives are generally detected by **Borntrager's test**

Aim: Identity test for aglycone part of anthraquinone glycosides.

Procedure:

To **5 ml** of the Senna extract (**fraction A**), add **5 ml dilute HCl**, then place the mixture in a separatory funnel and partitioning with **5 ml of benzene** for **1 min**.

Take the upper benzene layer (**free aglycone**) and shake it with **dilute ammonia (10%)**. Check the intensity of the color.

Results:

Pink or **red** color will be produced which is very clear with monoanthrones than dianthrones.

Discussion:

The benzene extracts the **aglycone**, and with ammonia, forms anthraquinone salts, which have **pink** or **red** color.

The identification of anthraquinone glycosides by chromatography:

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

The stationary phase = Silica gel G.

The mobile phase = **n-propanol: Ethyl acetate: Water (60:30:30)**.

The standard compound = Sennoside if available

The spray reagent = Alcoholic KOH 5%w/v.

Mechanism of separation = Adsorption.

Developing = Ascending.

NOTE/ for the best result spray first with 25%w/v nitric acid then heat in the oven after that spray with KOH reagent. This step is done to intensify the color of the spot.

- Prepare **100ml** of mobile phase, and place it in the glass tank.
- Cover the tank with glass lid and allow standing for **45 minutes** before use.
- Apply the sample spots (fraction A, fraction B & fraction C), and the standard spot on the silica gel plates, on the base line.
- Put the silica gel plate in the glass tank and allow the mobile phase to rise to about two-third of the plate.
- Remove the plate from the tank, and allow drying at room temperature, spray first with **25% nitric acid** solution and heat for **10 minutes** at **110 °C**.
- Allow to cool, and then spray with **5% w/v** alcoholic **KOH** solution. Detect the spot formed and calculate the R_f values.

Study problems:

Q1. What are the main differences between three fractions (A, B and C) of anthraquinone glycosides? Explain with structures?

Q2. Give the reasons for:

- a) The use of Borax in Shontetens reaction?
- b) The use of 10% dilute ammonia in Borntrager's test?

Q3. How can you identify an extract containing anthraquinone glycosides?