Al-Nahrain University
College of pharmacy

Practical pharmacognosy
3rd stage 1st term

Extraction and Identification of cardiac glycosides

Pharmacist Ayah AlQrimli
Lab Lecture 2

Isolation and identification of cardioactive glycosides

Extraction

- Aim: isolation of the cardioactive glycosides
- Procedure :
- Method of extraction: maceration (soaking)
- Plant used :**Nerium oleander**
- Part used: **dry leaves**



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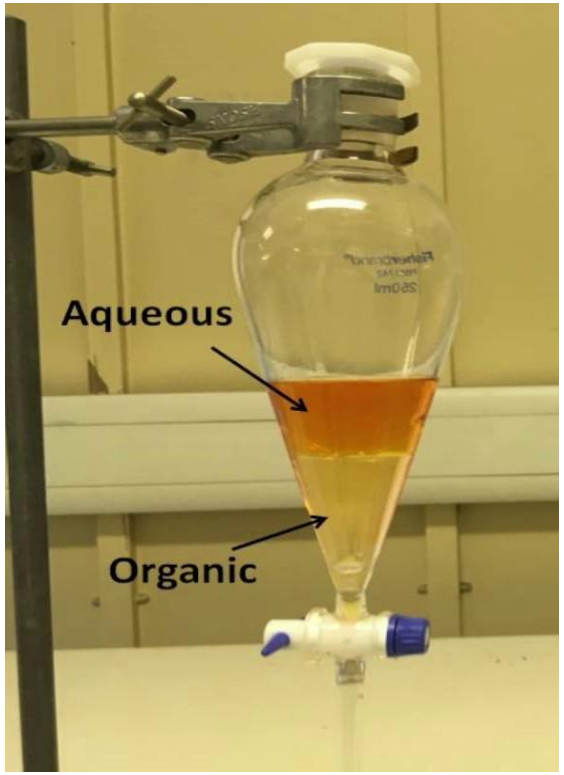
Maceration

Procedure

- Maceration 10gm of the powdered leaf in 100ml of 70% ethanol for 24hrs (prepared previously).
- Take 10ml of the alcoholic extract in a conical flask.
- Add 5ml lead sub acetate solution (to precipitate unwanted material).
- Centrifuge (2 min).
- Decant and take supernatant (upper layer).
- Divide supernatant into two divisions.

Fraction A



Take one division and put in the separatory funnel

↓

Add 10ml of

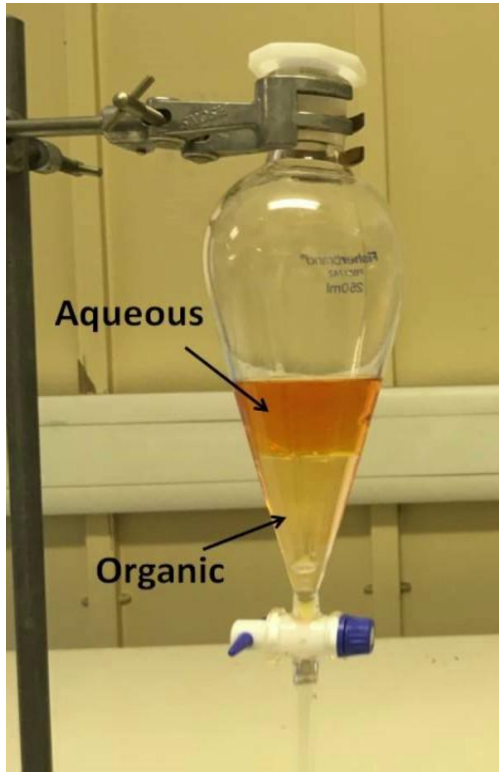
chloroform : ethanol (3:1 v/v)

↓

Shake and stand

Take the organic lower layer and put it in the conical flask (lower layer)

Fraction B



Take the other division of the extract in the conical flask

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Add 3ml of **4N HCL**

Boiling in water bath for 5 min

↓

Cool and transfer to a separatory funnel

↓

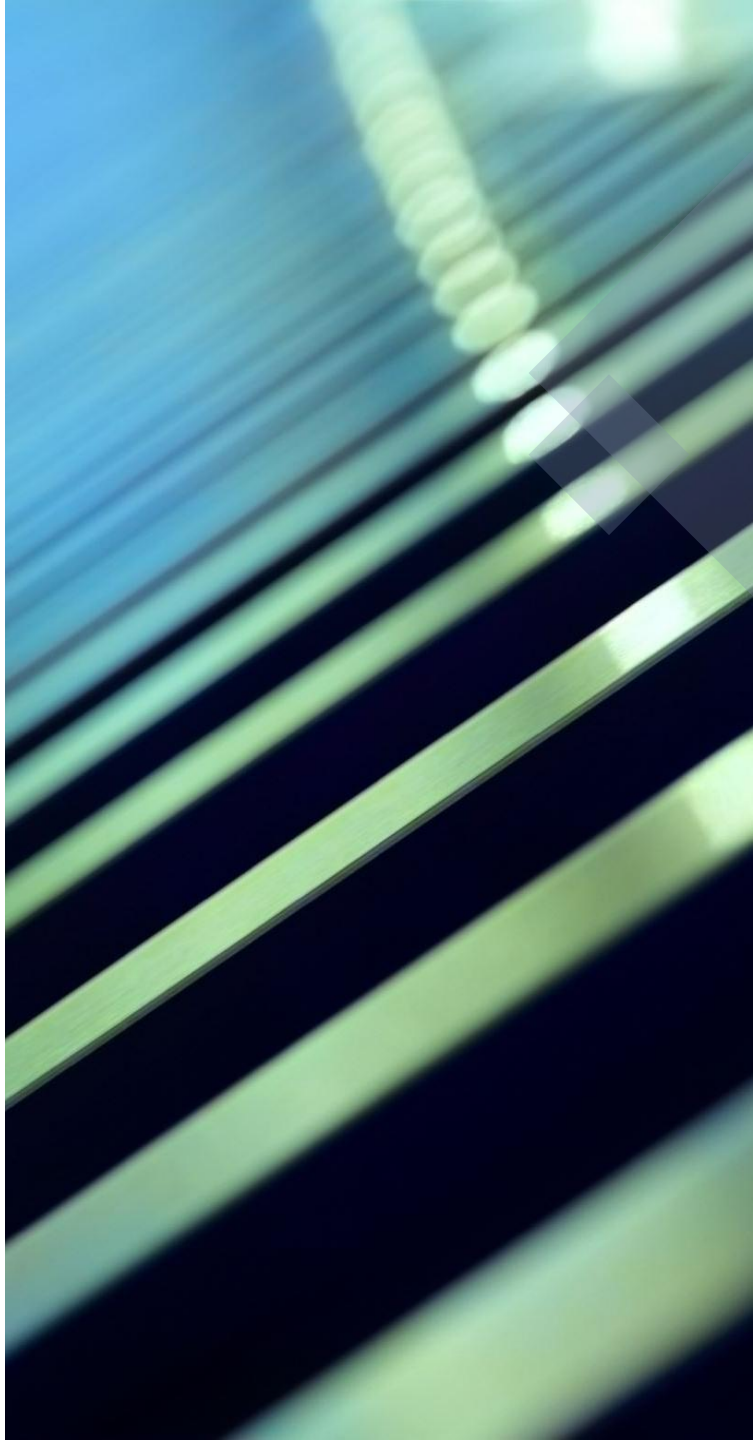
Add 10 ml of **chloroform**

Take lower layer (chloroform layer)

Results:

Fraction A: contain the **whole glycosides**

Fraction B: contain the **aglycon (genin) part only**

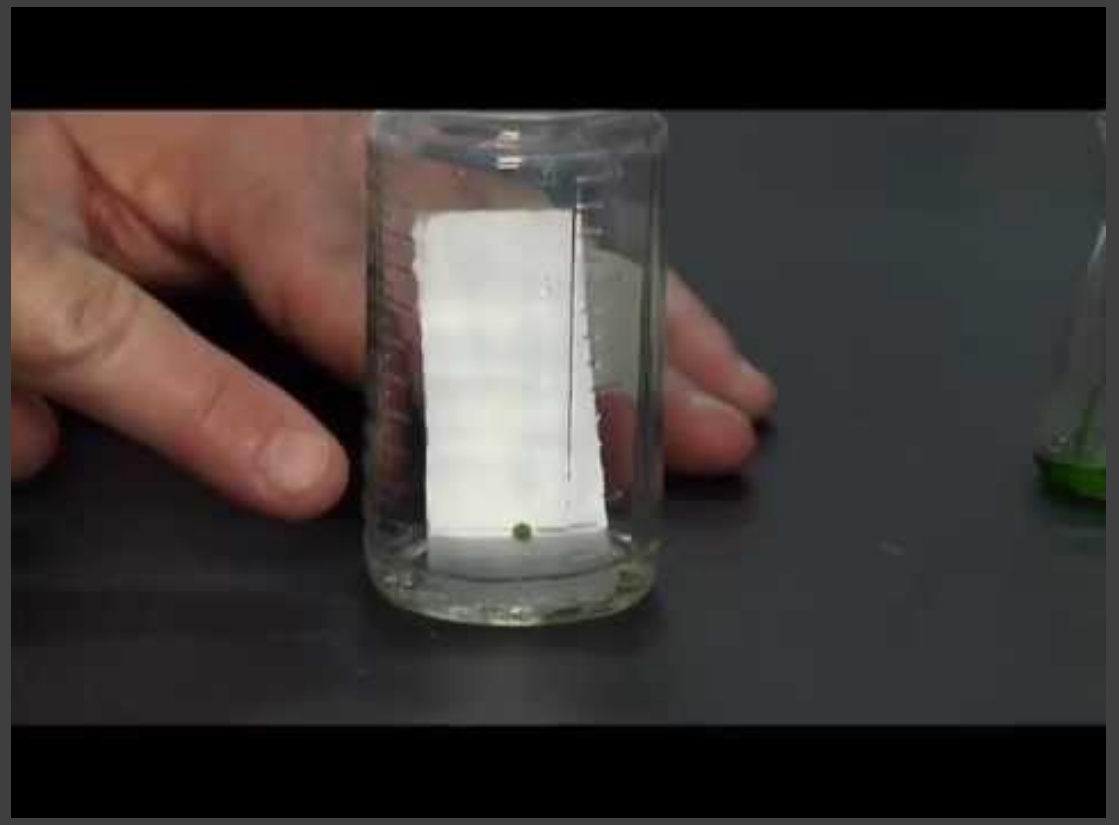


How to detect the C.A glycoside in a fraction A and B?

Chemical tests



Thin layer Chromatography (TLC)



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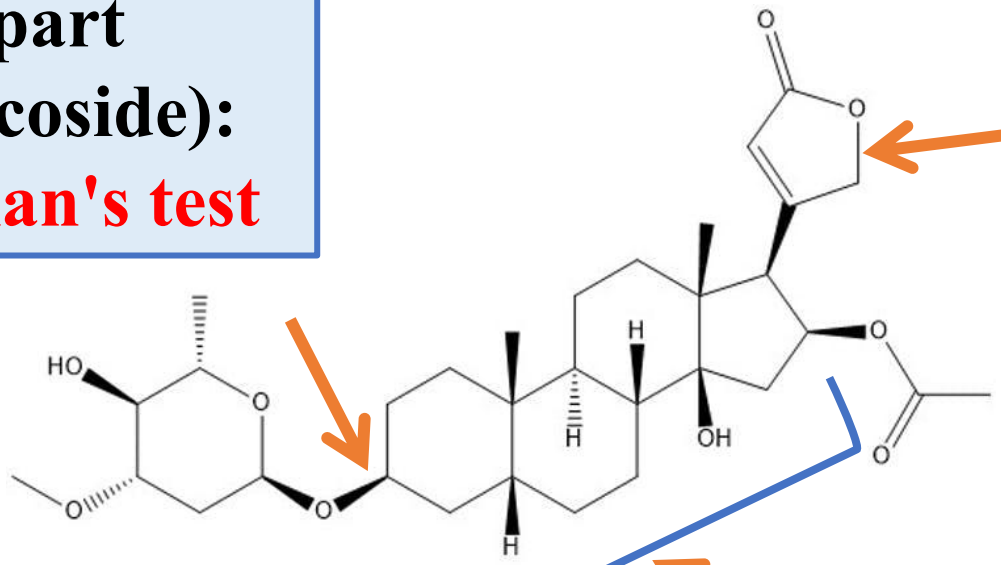
Active sites in cardiac glycosides

Sugar part
(whole glycoside):
Keller Killian's test



Lactone ring :
Legal's test
Baljets test

Sterol aglycon:
Lieberman's Burchard test

Identification by chemical tests

GENERAL

Keller Killian's test: A/B

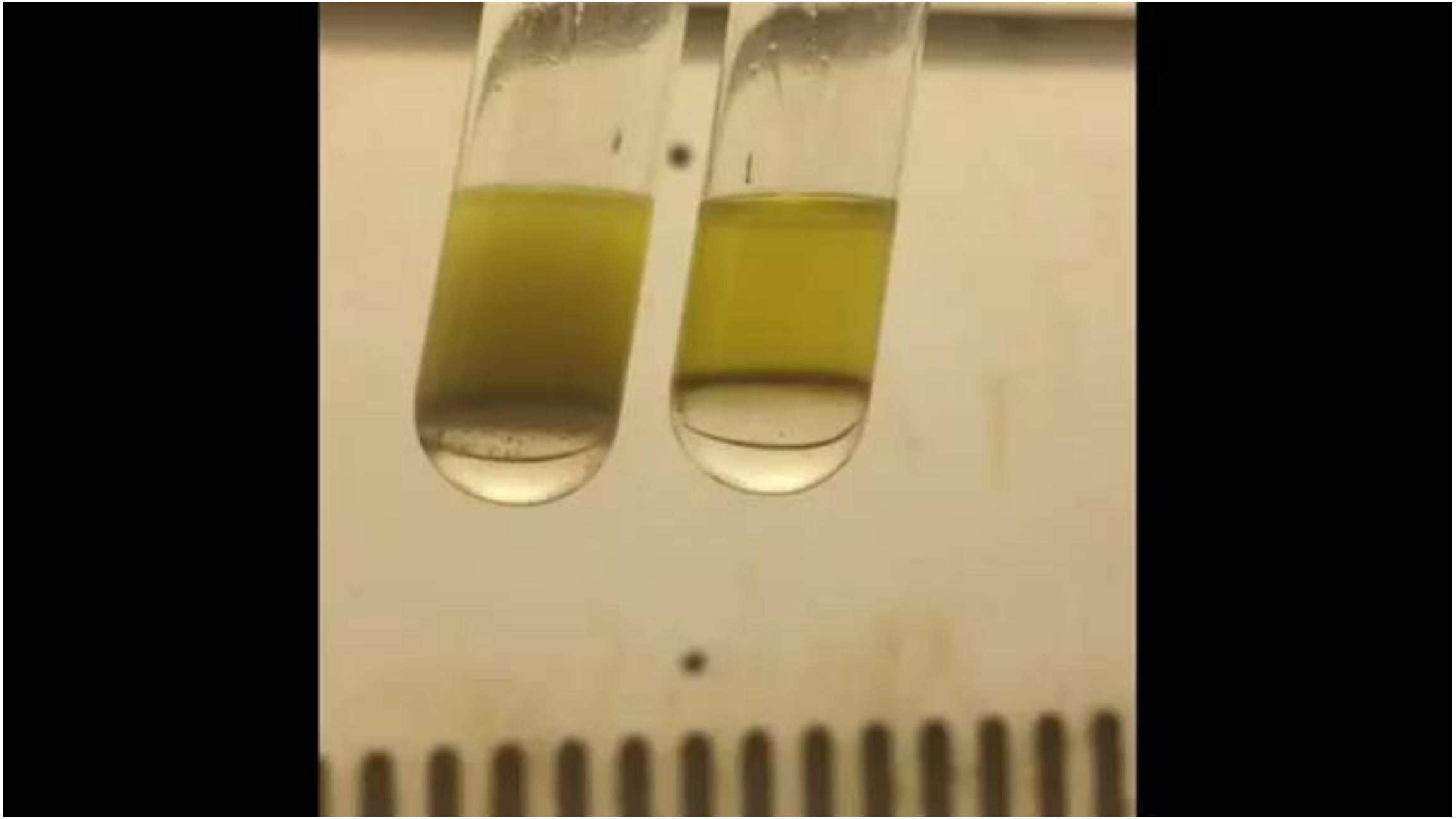
-Take 1ml of fraction A /B and 2ml of glacial acetic acid and 1 drop of 0.1% of ferric chloride solution

-add 1 to 2 drops of concentrated H_2SO_4 carefully to make two layers

- **Results :**

Two layers are formed the upper light green , lower layer is transparent color while the junction appears reddish-brown ring (charred sugar).

The acid with ferric chloride hydrolyzes the glycoside into glycon and aglycon while sulphuric acid will burn the sugar



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Identification by chemical tests

GENERAL A/B

Baljets test

-Take 1ml of fraction A and 2ml of picric acid and 1 drop of NaOH

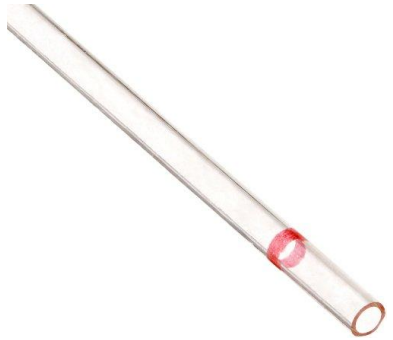
- **Results :**

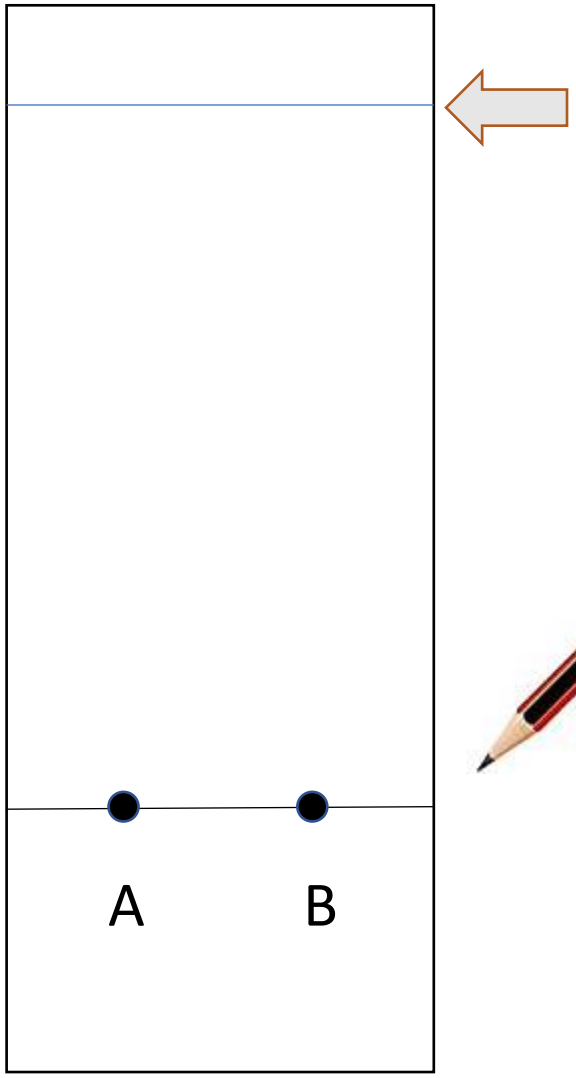
Turbid yellow color

The use of picric acid is to hydrolyze the glyoseide to aglycon and aglycone parts,
The use of sodium hydroxide is to neutralize the acidity in the solution.

The color is product due to the aglycone portion (genin).

TLC





Front line (3/4 the plate)



Base line



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- **Mobile phase**

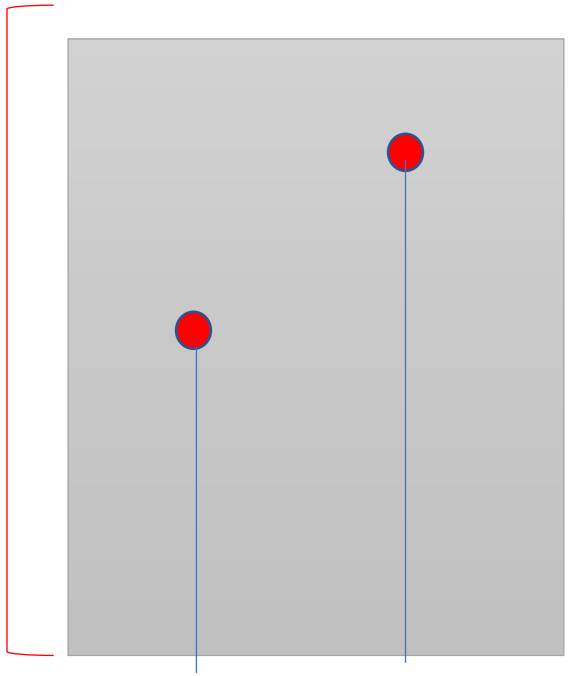
  Ethyl acetate :methanol :water (81:11:8)
- **Spotting**

  2 spots A/B
- **Detection**
- **Liberman spray reagent**

Or Spray reagent with 5% ethanolic H2SO4

- **Heat on heater**

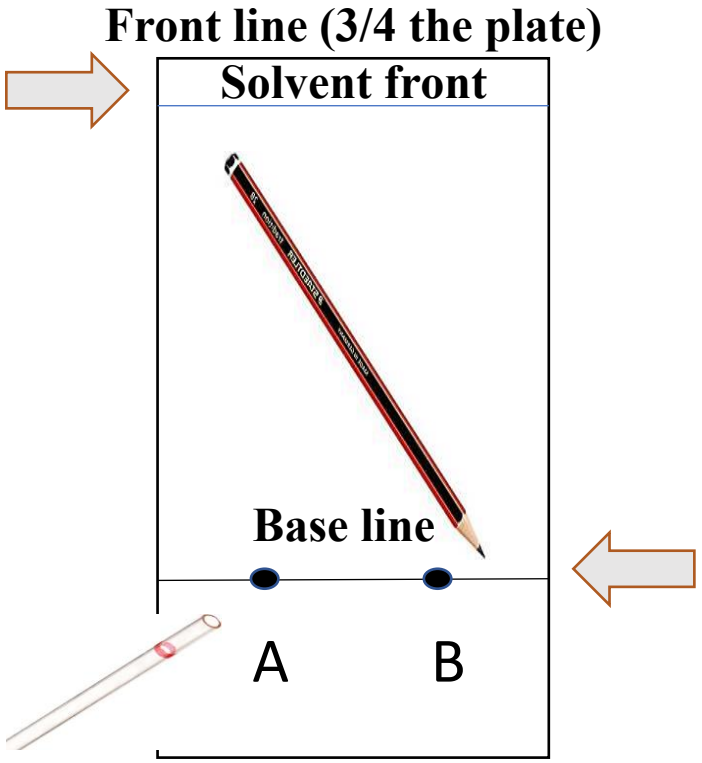
  UV light 360nm>> light blue



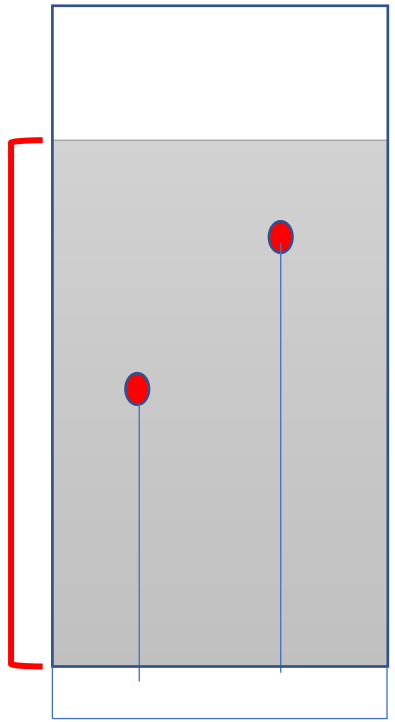
TLC: thin layer chromatography



- **Stationary phase:** Silica
- **Mobile phase:** Ethyl acetate :methanol :water (81:11:8)
- **Mechanism:** adsorption
- **Activation :** put the oven for 5 min.
- **Spotting:** apply 2 spots A and B
- **Detection** :
- Spray reagent with diluted **H2SO4 solution.**
- **Heat on heater**
- Visualize the spot by **UV light 360nm** >> **light blue fluorescence.**



- **Calculate the Rf value for A and B spots**
- *Rf = Distance travelled by the compund / distance travelled by the solvent*



Report for each student

NAME :

AIM :

PROCEDURE :

MAIN STEPS.

RESULTS :

FOR EACH FRACTION AND EACH TEST

TLC RESULTS

DISCUSSION