

**Pharmacognosy**  
**3rd Class, 1st Semester**

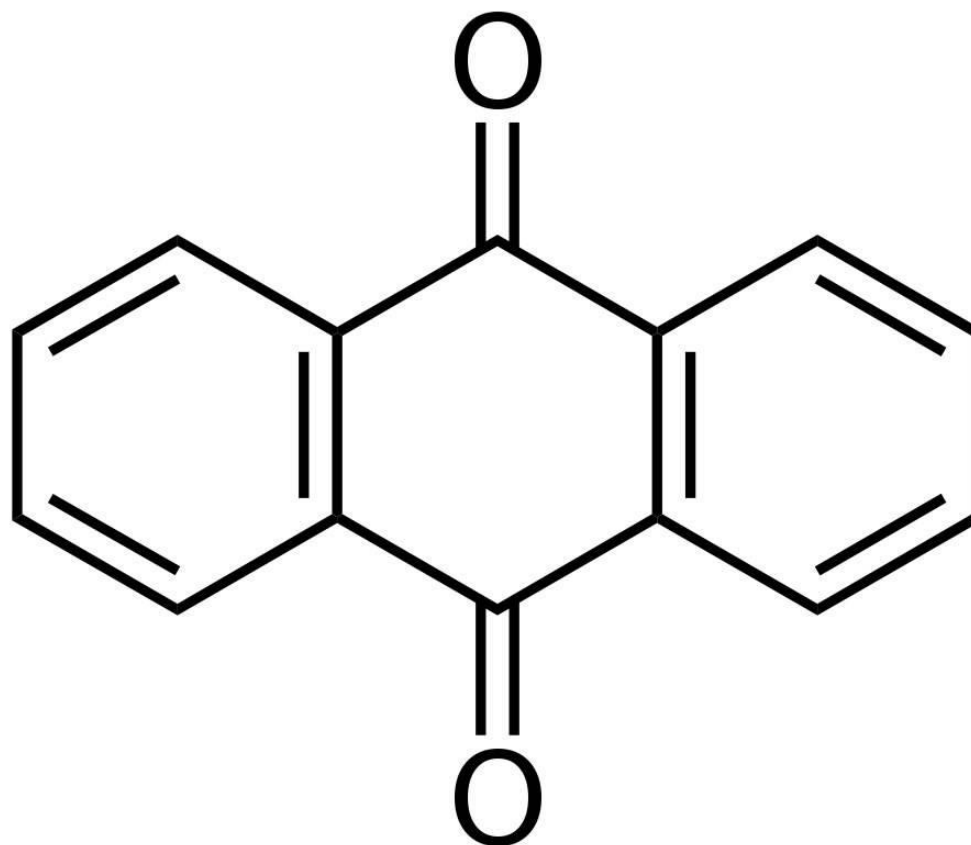
# **ANTHRAQUINONE GLYCOSIDES**

**Lab.2**

# Introduction

- ❖ Anthraquinone and related glycosides are organic compounds found in some plants and have a stimulant cathartics effect by exert their action by increasing the tone of the smooth muscle in the wall of the colon and stimulate the secretion of the water and electrolytes into the large intestine.
- ❖ After the oral administration, the Anthraquinone glycosides are hydrolyzed in the colon by the action of enzymes of the micro flora, to the pharmacologically active free aglycone which usually produce their effect in 8-12 hour after administration, these agents are indicated for constipation in patient who do not respond to milder drugs and for bowel evacuation before investigation procedures or surgery.

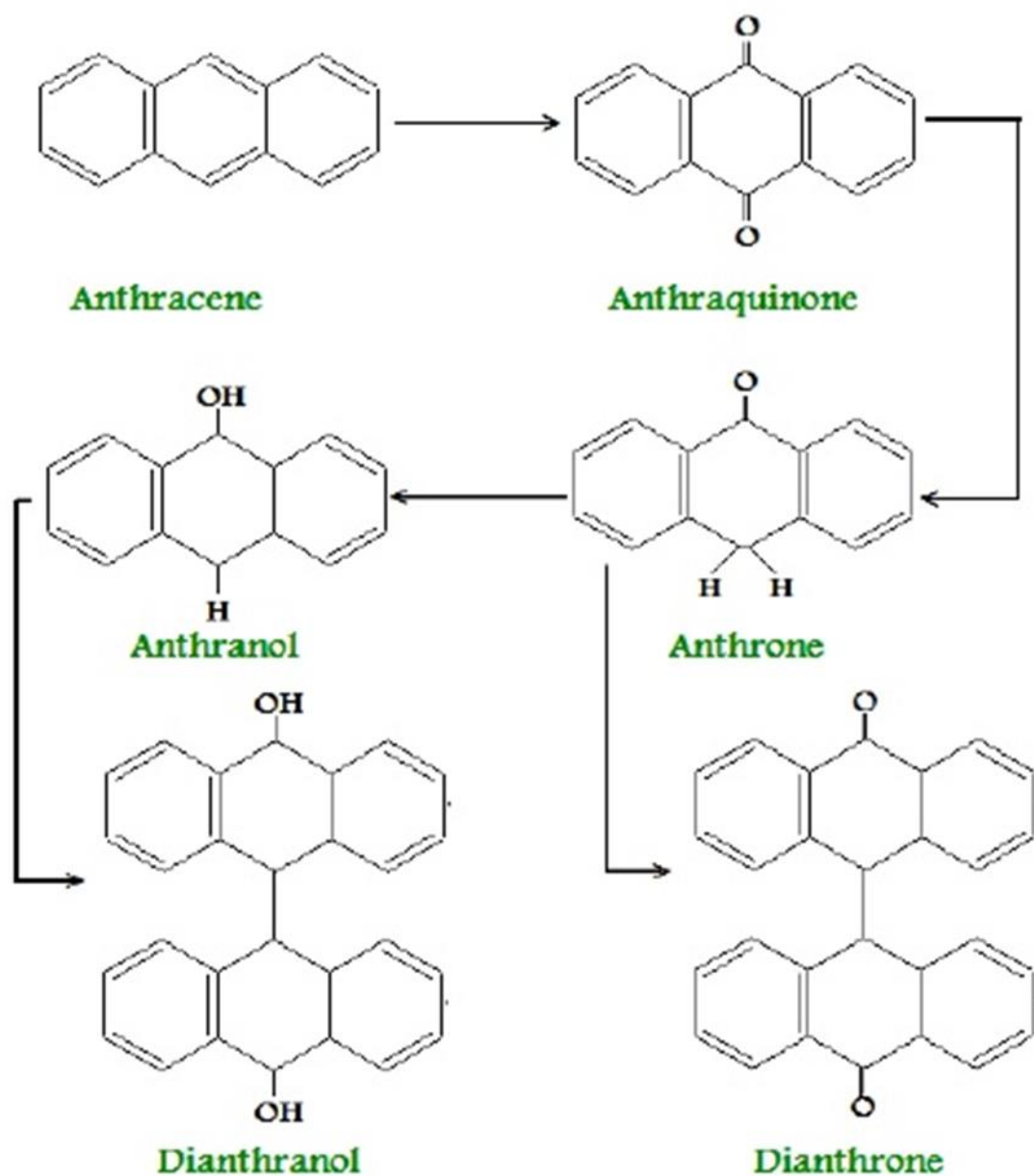
- ❖ Stimulant laxative are habit –forming so the long term use may result in laxative dependence and loss of the normal bowel function and become lazy so for this reason its better just to eat foods with lots of fibber and drink water, fresh juices that will cleanse system.
- ❖ The glycoside of anthranols and anthrones which is reduced anthraquinone derivatives elicit a more drastic reaction than do other anthraquinone, and cause discomfort and gripping action.
- ❖ The drugs mostly used are, cascara, frangula, casanthranol and senna. The aloe and rhubarb are not recommended due to their irritating action, which increase the chance for gripping effect.
- ❖ The Anthraquinone hydrolyzed to give aglycone, which are Di, Tri or Tetra- hydroxyanthraquinone.
- ❖ Also there are anthrone, dianthrone and oxanthrone.



**General Anthraquinone Structure**

## Chemistry:

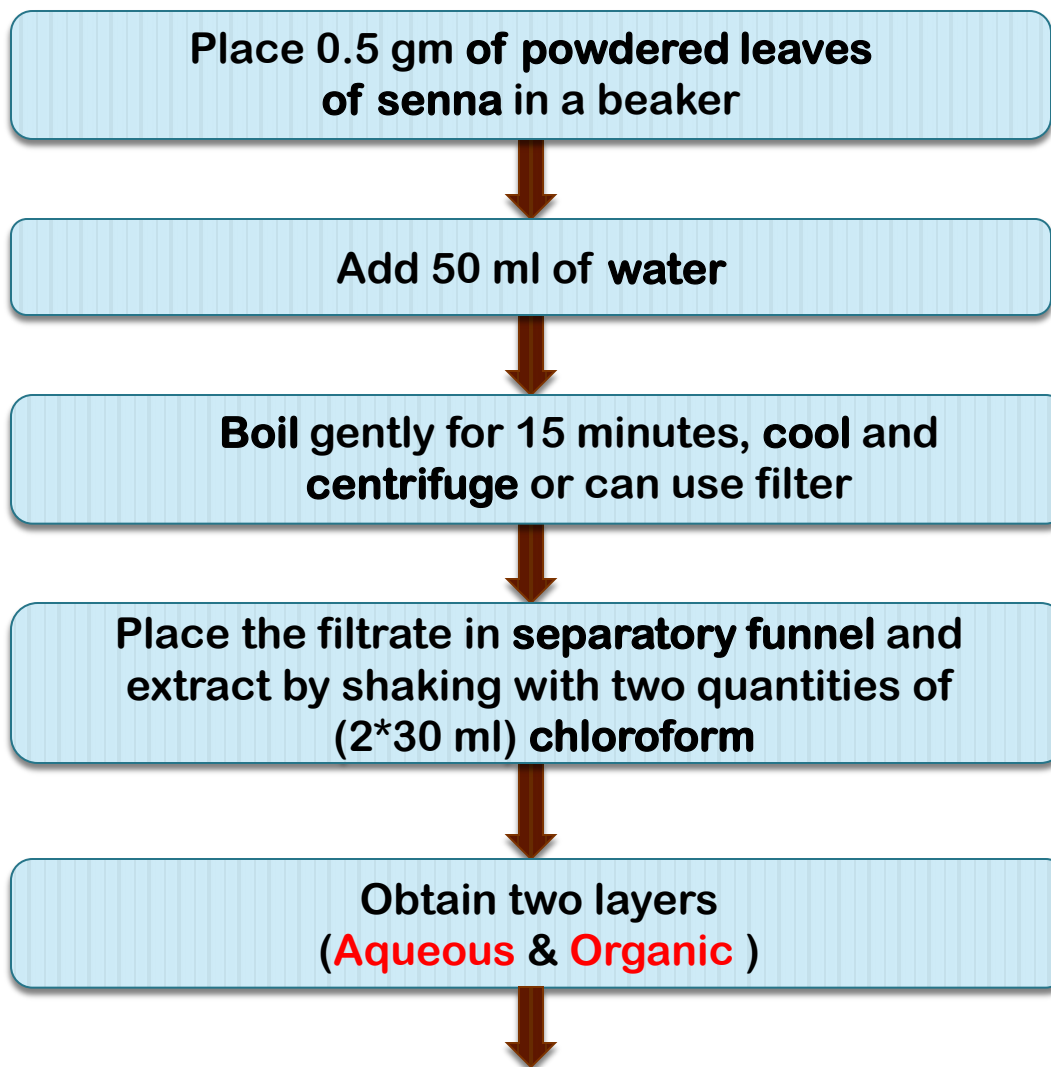
*Chemically these are related to anthracene.*



# Senna Plant



# Extraction OF Anthraquinone glycosides (Senna)



**Aqueous Layer**

**Fraction B  
Organic Layer**

Combine the chloroform and then concentrate to about 1ml

Placed in small flask and evaporate carefully almost to dryness on a rotary evaporator

**Fraction A**

- 1) The other part is place in a 250 ml round bottom flask
- 2) Adding to it 3.5 of ferric chloride solution (60%w/v).
- 3) Reflux for 20 minutes
- 4) Add 2ml of concentrated HCL acid , continue heating for further 20 minutes , shaking the flask occasionally to dissolve as possible of the precipitate, and allow cooling.
- 5) Place the hydrolysate in a separatory funnel and extract with two quantities of (2\*30ml) chloroform

**Fraction C**  
Concentrate the bulked chloroform extracts to about 3ml



## Results:

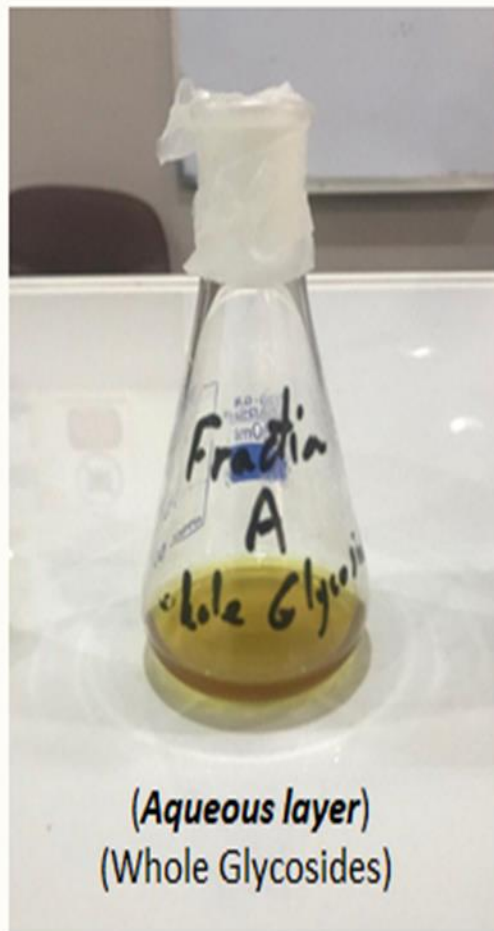
**Fraction A** : Contain the whole glycosides.

**Fraction B** : Contain the aglycone (dianthrone) .

**Fraction C** : Contain the aglycone part (monoanthrone).



# Procedure



*(Aqueous layer)*  
*(Whole Glycosides)*

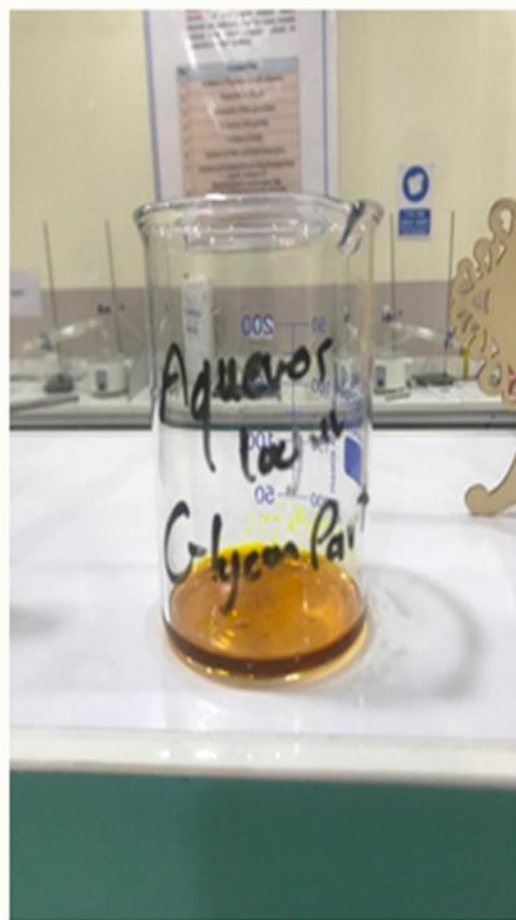
Fraction A



*(Chloroform layer)*  
*(Free aglycone) (Dianthrone)*

Fraction B

# Procedure



Aqueous layer  
(Glycone part)



Chloroform layer  
**Aglycone part (Monoanthrone)**  
Fraction C

# Discussion

- ❖ The chloroform layer will contain the free aglycone (fraction B) , while the aqueous layer will contain the glycoside as a whole , since sugar is water soluble (fraction A) .
- ❖ The use of ferric chloride and HCL and reflux is to break the C-C bond in the dimer, which is very strong bond so need strong conditions.

# The chemical test for anthraquinones

## A. GENERAL TESTS:

### 1. Borax Test:

**Aim:** To identify the anthraquinone glycosides .

**Equipments and reagents:-**

- Test tube.
- Small beaker.
- Dilute acid.
- Water bath.
- Borax.

**Procedure:** Add 0.5 gm of borax to 5ml of the extract and heat until dissolved . Pour a few drops of the liquid into a test tube nearly full of water. A green fluorescence is produced.

## 2. Bromine Test for Aloin:

**Aim:** Identification of the anthraquinone glycosides in general.

### Equipments & Reagents:

- ✓ Test tube.
- ✓ Bromine solution.

### Procedure:

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

## B. Specific Test:

### Borntrager's test :

**Aim:** Identity test for aglycone part of anthraquinone glycosides.

### Equipments & Reagents:

-Separatory funnel    -Test tube    - Dilute HCl    -Benzene    - Dilute ammonia (10%).

### Procedure:

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

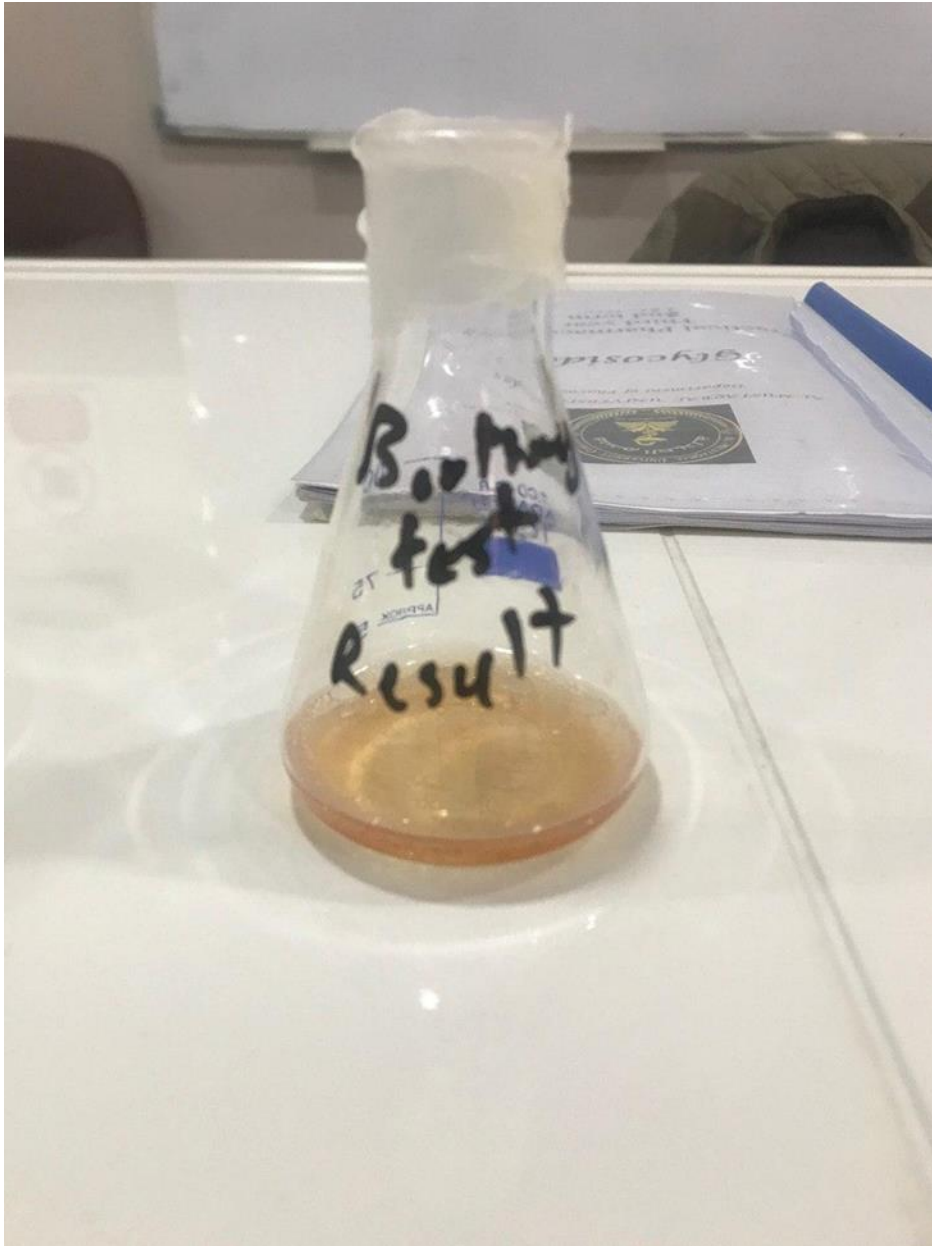
Take the upper benzene layer (*free aglycone*) and shake it with **dilute ammonia (10%)**. Check the intensity of the color.

### Results:

Pink 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 color.



**Borntrager's test**



# Identification by chromatography by the use of T.L.C technique

- ❖ The stationary phase = silica gel G
- ❖ The mobile phase = n-propanol : ethyl acetate: water (40: 40:30)
- ❖ Spray reagent =alcoholic KOH (5% w/v)
- ❖ (For better 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 spots.)

# Procedure

Prepare the solvent system and place it in the glass tank, leave it for 45 minutes before use.

Apply the sample fraction A, B, C, each sample with standard on the separated plate.

Place the three plates in the tank and allow the mobile phase to rise to about two-thirds the plates then after 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 spots formed and calculate the R<sub>f</sub> value.



THANK YOU

---