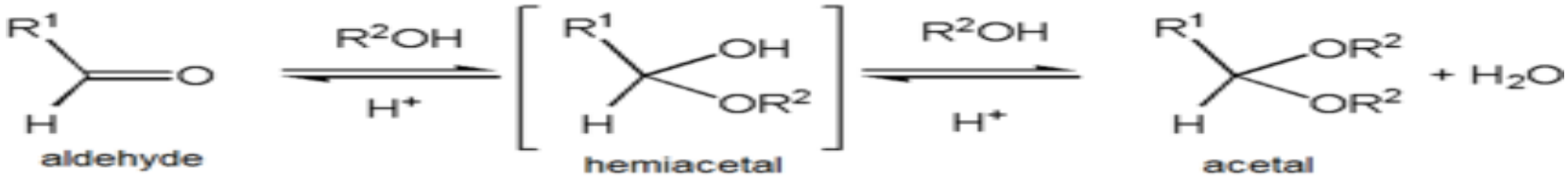
GLYCOSIDES
Cardio active glycosides

Pharmacognosy
3rd Class, 1st
Semester

Lab.1

Introduction

- Glycosides are compounds that yield on hydrolysis , one or more sugar .
- The sugar part is known as **Glycone** and the non-sugar part is the **Aglycone** or called **Genin**.
- Chemically the glycosides are **Acetals** which is a molecule with two single-bonded oxygen atoms attached to the same carbon atom.



- Two forms of glycosides are present the **α-form** and the **β- form**, but the β-form is the one that occur in plants .

- Glycosides are soluble in water and alcohols.
- Inside the body the glycoside will be cleaved to glycone and aglycone parts.
- Glycone part (Sugar): water soluble, insoluble in the organic solvents.
- Increase number of sugars increase water solubility.
- Aglycone part: water insoluble, soluble in the organic solvents.

- Glycosides hydrolyzed by using mineral acids and temperature or by using enzymes such as:

- Emolsin enzyme which is present in Bitter almond seeds.

- Myrosin or Myrosinase enzyme which is present in black mustard seeds.

Generally in the extraction of the glycosides we need the following points

- A polar solvent, which is mostly alcohol, but not water, since water may induce fermentation , in addition water needs high temperatures due to its high boiling point.

- Neutralization of the extract with base, since the presence of acid lead to hydrolysis of the glycoside.

CARDIO ACTIVE GLYCOSIDES

- They are named as cardio active glycosides due to their action on the heart muscle.
- The aglycone part here is a steroid. Which is chemically cyclopenta phenanthrene .
- The steroidal aglycones are two types:

1) Cardenolide (α-β unsaturated-5 member lactones' ring).
2) Bufadienolide(doubly unsaturated 6-member lactones' ring)

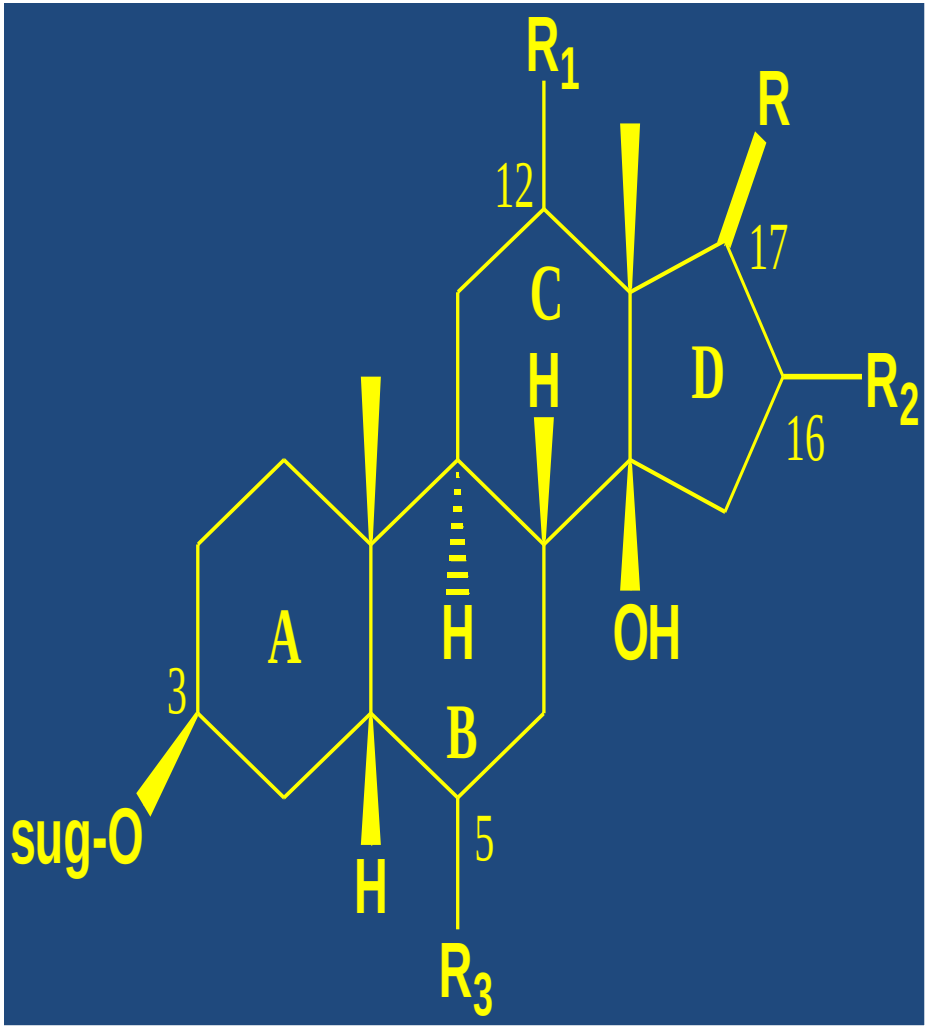
- The more prevalent in nature is the cardenolide type.

For maximum activity of the cardio active glycosides the following points are important :

- 3b-OH group involved in glycosidic linkage.
- 14*b*-OH group at C-14.
- A/B ring junction *cis*
- B/C ring junction *trans*
- C/D ring junction *cis*

Cis means on the same side.

Transe means on the other side or across .



- **The presence of lactone ring at 17-β:**

According to the type of lactone ring Cardiac Glycosides are classified into:

1) Cardinolides:

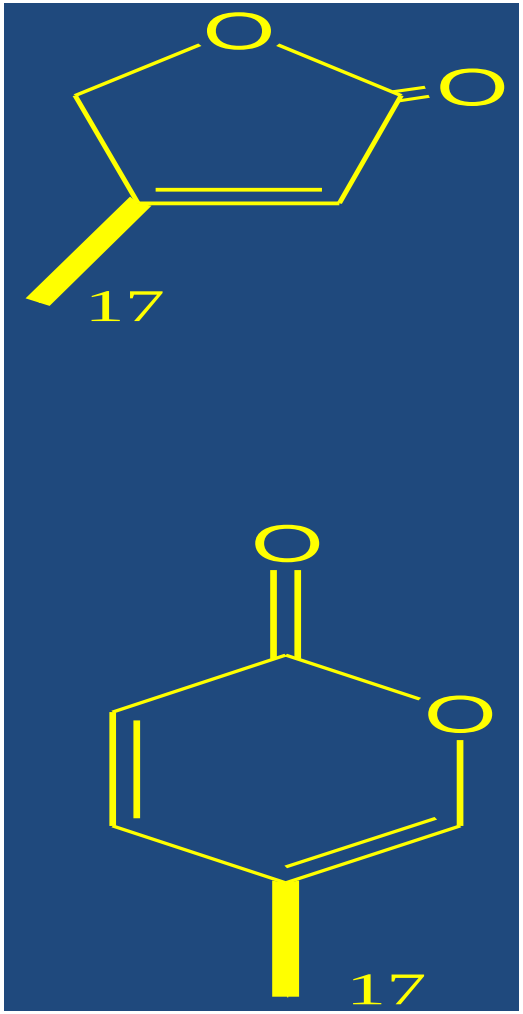
Containing 5-membered unsaturated lactone ring

e.g. *Digitalis* .

2) Bufadienolides:

Containing 6-membered unsaturated lactone ring

e.g. *Squill*



Plants containing cardio active glycosides

1) **Digitalis (digitalis or fox glove)** ***Digitalis purpurea*** **.**

- The family **Scrophulariaceae**.
- The name digitalis is from the Latin digitus which means finger refers to finger- shaped.
- While purpurea refers to the purple color of the their flower.
- This plant contains a number of glycosides as digitoxin , gitoxin and gitaloxin.

2) ***Digitalis lanata*** of the same family, from which the digoxin is obtained.

3) ***Nerium oleander*** of the family Apocyanaceae. Here the glycoside is oleanderin .

Aim: To isolate the cardioactive glycosides.

Equipment and Reagents

- Large beaker & two medium size beakers.
- Two conical flasks.
- Centrifuge & Centrifuge tubes.
- Separatory funnel.
- Water bath.

- 70% ethanol.
- Lead sub acetate.
- 10% sodium phosphate solution.
- Chloroform: Ethanol (3:1 v/v).
- Anhydrous sodium sulphate.
- 4N HCl acid.
- Chloroform.

Procedure

Maceration ***10 gm*** of the powdered leaf in ***100 ml*** of *70%**ethanol*** for **24 hrs. (**Prepared previously)

Take ***10 ml*** of alc. Extract in conical flask

↓ Add

10 ml of ***lead sub acetate*** solution
(Mixing& standing for **5 mins.)**

↓ Centrifuge
(5 mins.)

Decant and take the supernatant (upper layer)

↓ Add

10 ml of *10%**sodium phosphate*** solution

↓ Centrifuge
(*5 mins.*)

Take supernatant and divide in to ***two*** divisions

Fraction A

Take one division and put in a separatory funnel

↓ Add

[***10 ml*** of ***Chloroform: Ethanol*** *(3:1 v/v)*] two times

↓ (Shake& stand)

Combine the organic lower layer and put it in the conical flask

↓ Add

Small quantity of ***Anhydrous sod. Sulphate*** & allow standing for few minutes until get a clear solution, decant the Chloroform-ethanol extract and reduce the volume on water bath to get the whole glycoside.

Fraction B

Place the other division of the extract in the conical flask

↓ *Add*

3 ml of ***4N HCl***

↓ *Boiling in water bath*

(***15 mins***)

Cool &transfer to a separatory funnel

↓ *Add*

[***10 ml*** of ***Chloroform***] tow times

Combine the chloroform extracts (lower layers)

↓ *Add*

Small quantity of ***Anhydrous sod. Sulphate*** & allow standing for few minutes until get a clear solution then decant the chloroform layer and concentrated on water bath to about 1ml. and we get the aglycone part.

Additions in the procedure

- **Lead sub acetate** is added to precipitate tannins and other unwanted material.
- **10% sodium phosphate** solution is added to take the excess of lead sub acetate.
- Use of **chloroform-ethanol** in partition is due to the fact that the chloroform will take the genin part while the ethanol will take the glycoside there will be no loss in the glycoside.
- **Anhydrous sodium sulphate** is added in during mixture since the anhydrous form will act as an adsorbent.
- **4N HCL** is used to hydrolyze the glycoside to glycone and aglycone parts.
- Use of **chloroform** alone is to extract the genin part So fraction A, will contain the whole glycoside, while fraction B will contain only the genin part.

The Chemical Tests

1. Baljet's Test:

Aim: The identification of the cardio active glycosides.

Equipment & 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:

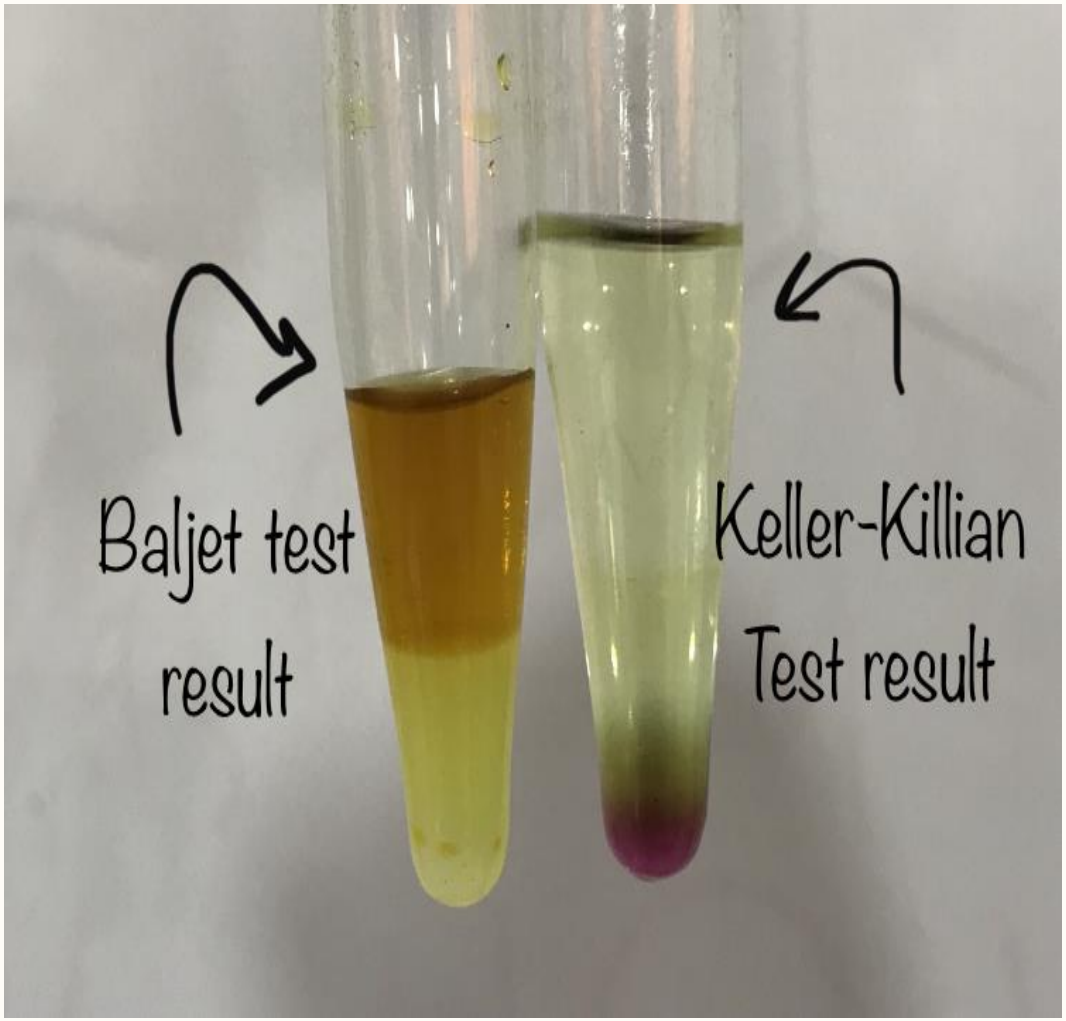
Turbid , yellow to orange in color.

2. Keller- Killian's Test

Aim: The identification of the cardio active glycosides.

Equipment & Reagents:	***Procedure:***
• Test tube. • Glacial acetic acid • 0.1 % of ferric chloride solution. • Conc. H_2SO_4.	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***.*** H_2SO_4 and add to the above mixture in drops to make two layers.

Results: Two layers are formed; the upper one has ***light bright green*** color. The lower layer has transparent clear color (H_2SO_4 layer).The junction appears as a ***reddish –brown*** ring.



The chemical tests results


THANK
YOU