Practical pharmacognosy

Third year

1st/term

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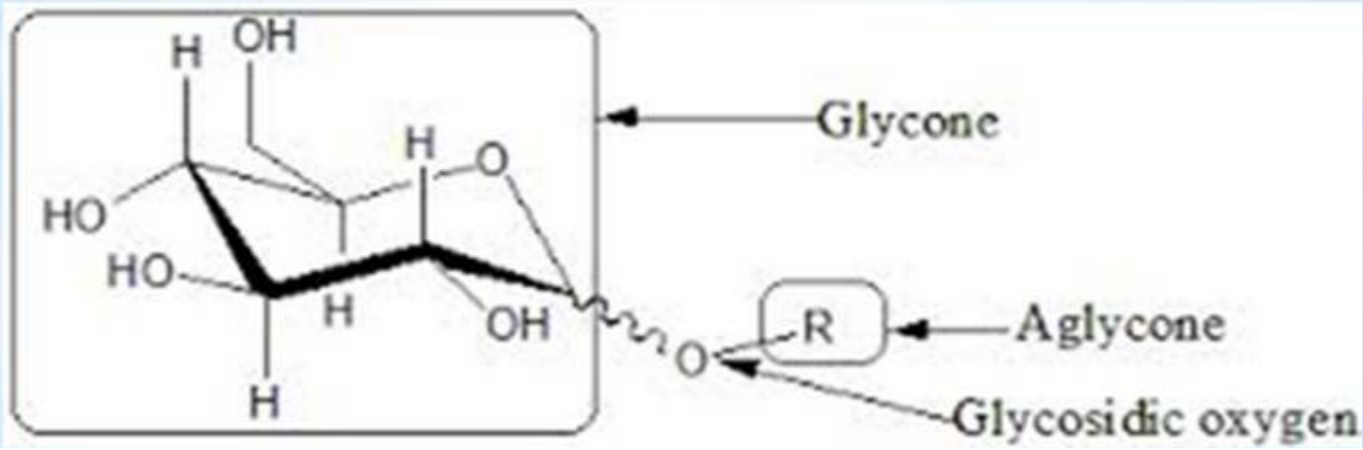
Lab.1

Glycosides

- are compounds that yield on hydrolysis, one or more **sugar part** and another **non-sugar** part.
- The sugar part is known as **glycone** , and the non-sugar part is the **aglycone.**

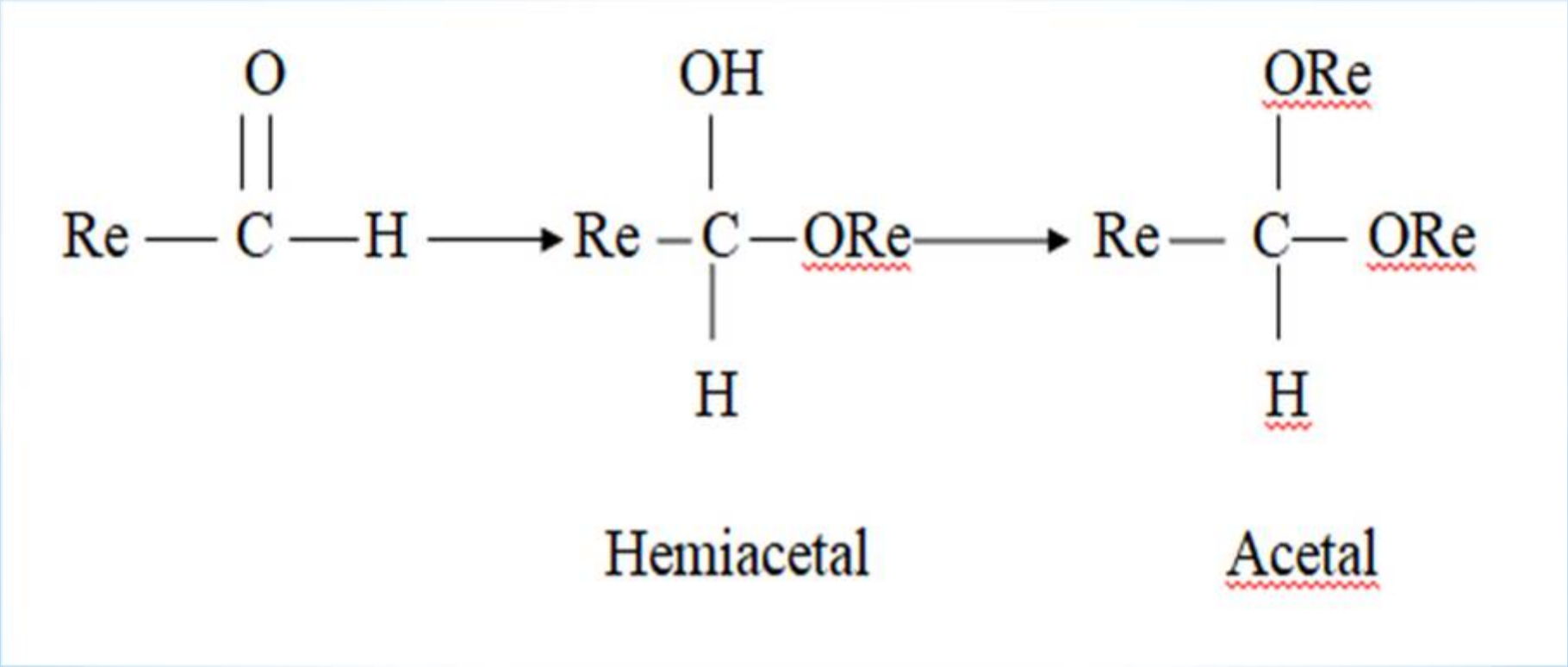
There are two basic classes :

- C- glycosides, in which the sugar is attached to the aglycone through **C-C bond**.
- O- glycosides in which the sugar is connected to the aglycone through **oxygen –carbon bond**.



Chemically:

- glycosides are acetals.
- Two forms of glycosides are present, the **α-form** and the **β-form**, but the β-form is the one that occur in plants, even the hydrolytic enzymes act on this type.



- Inside the body the glycosides will be cleaved to glycone and aglycone parts, the **glycone** part confers on the **molecule solubility** properties, thus is important in the **absorption and distribution** in the body, while the **aglycone** part is responsible for the **pharmacological activity.**
- Generally all glycosides are hydrolyzed by boiling with **mineral acids.**
- on the other hand the presence of **specific enzyme** in the plant tissue, are able to hydrolyzed the glycosides, such as the **emulsin** enzyme which is present in the almond kernel, and the **myrosin** enzyme which is found in the black mustard seeds.

The glycosides are classified according to the chemical structure of the aglycone to:

1. Cardioactive glycosides.
2. Anthraquinone glycosides.
3. Saponin glycosides.
4. Cyanophore glycosides.
5. Isothiocyanate glycosides.
6. Flavonoid glycosides.
7. Alcohol glycosides.
8. Aldehyde glycosides.
9. Lactone glycosides.
10. Phenol glycosides.
11. Miscellaneous glycosides.

Generally in the extraction of glycosides we have to consider the following points:

1.Apolar solvent, which is mostly alcohol, but not water, since water may induce fermentation, in addition water need high temperature due to its high boiling point.

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

3.Use of heat is to **inhibit the activity of hydrolytic enzymes** that present in the plant cell.

Cardioactive Glycosides

- They are named due to their action on the heart muscle.
- The aglycone part here is steroid, which is chemically ***cyclopentaphenanthrene*** .

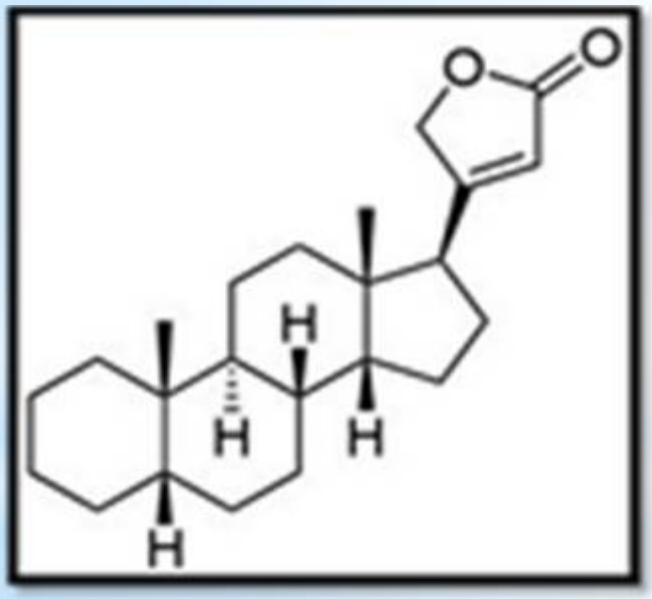
cyclopentaphenanthrene nucleus

The steroidal aglycones are of two types:

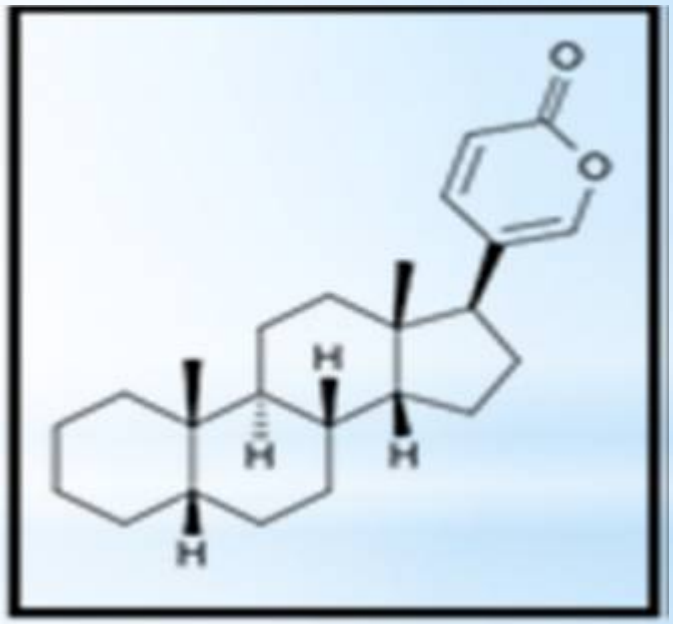
1)**Cardinolides** (α-β unsaturated 5 – member lactone ring).

2)**Bufadienolides** (doubly unsaturated 6-member lactone ring).

The more prevalent in nature is cardinolides type.



Cardenolide



Bufadienolide

For maximum activity of cardioactivce glycosides the following points are important:

1)17 -β –lactone ring (cardinolide or bufadinolide).

2)3 -β - OH.

3)14 -β-OH.

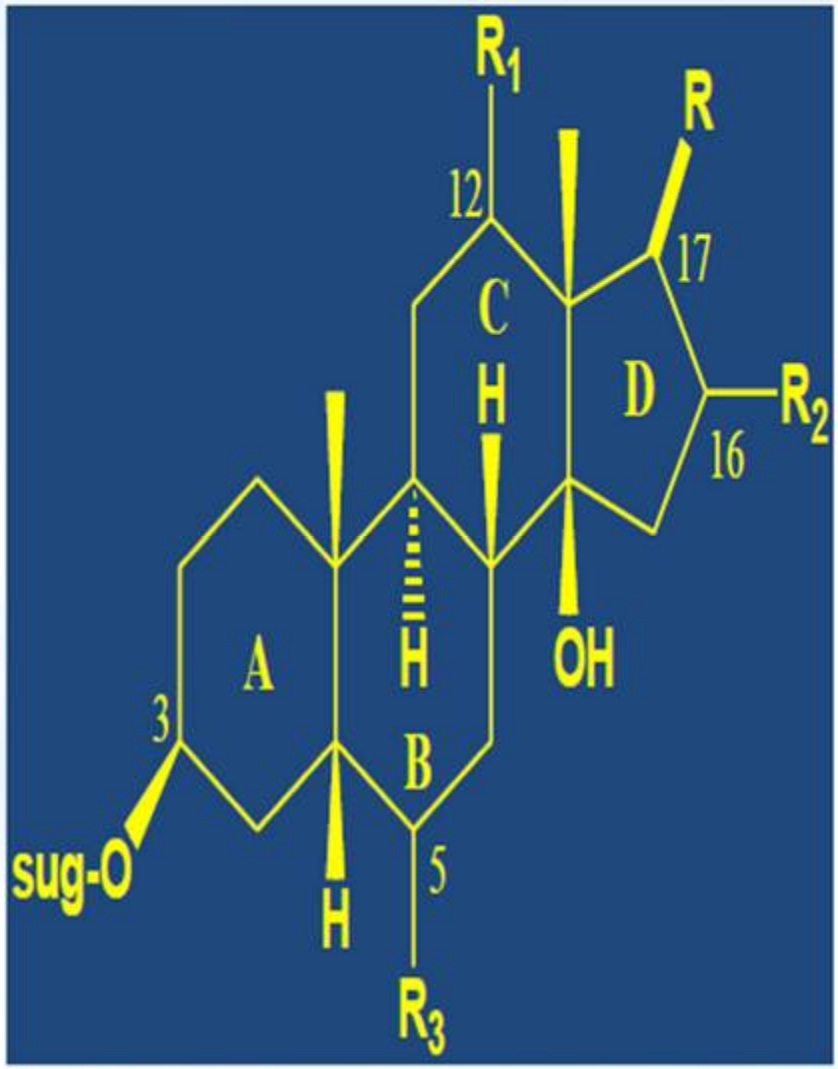
4)CATSC

(C= cis between two rings (A&B).

A= Anti in one ring (5&19).

T=Trans between two rings (B&C).

S= Syn in one ring (8&18).



Plants Containing Cardioactive Glycosides:

1)Digitalis (digitalis or foxglove) Digitalis purpurea of the family Scrophulariaceae.

The name digitalis is from Latin digitus which means finger refers to finger – shaped, while purprea refers to purple color of their flower. This plant contains a number of glycosides as **digitoxin , gitoxin and getaloxine.**

2)Digitalis lanata of the same family,

from which the **digoxin** is obtained.

3)The plant used in our laboratory is **Nerium oleander** of the family Apocyanaceae. The main glycoside of which is **oleandrin**.

Nerium oleander

Isolation and Identification of the Cardioactive Glycosides:

1.Extraction:

Aim: To isolate the cardioactive glycosides.

Equipments:

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

Reagents:

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

Procedure:

Method of extraction: Maceration.

Plant used: Nerium oleander.

Part used: dry leaves.

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:

Fraction A

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:

Fraction B

Results:

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

Fraction B : Contain the **aglycone (genin)** part only.

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:

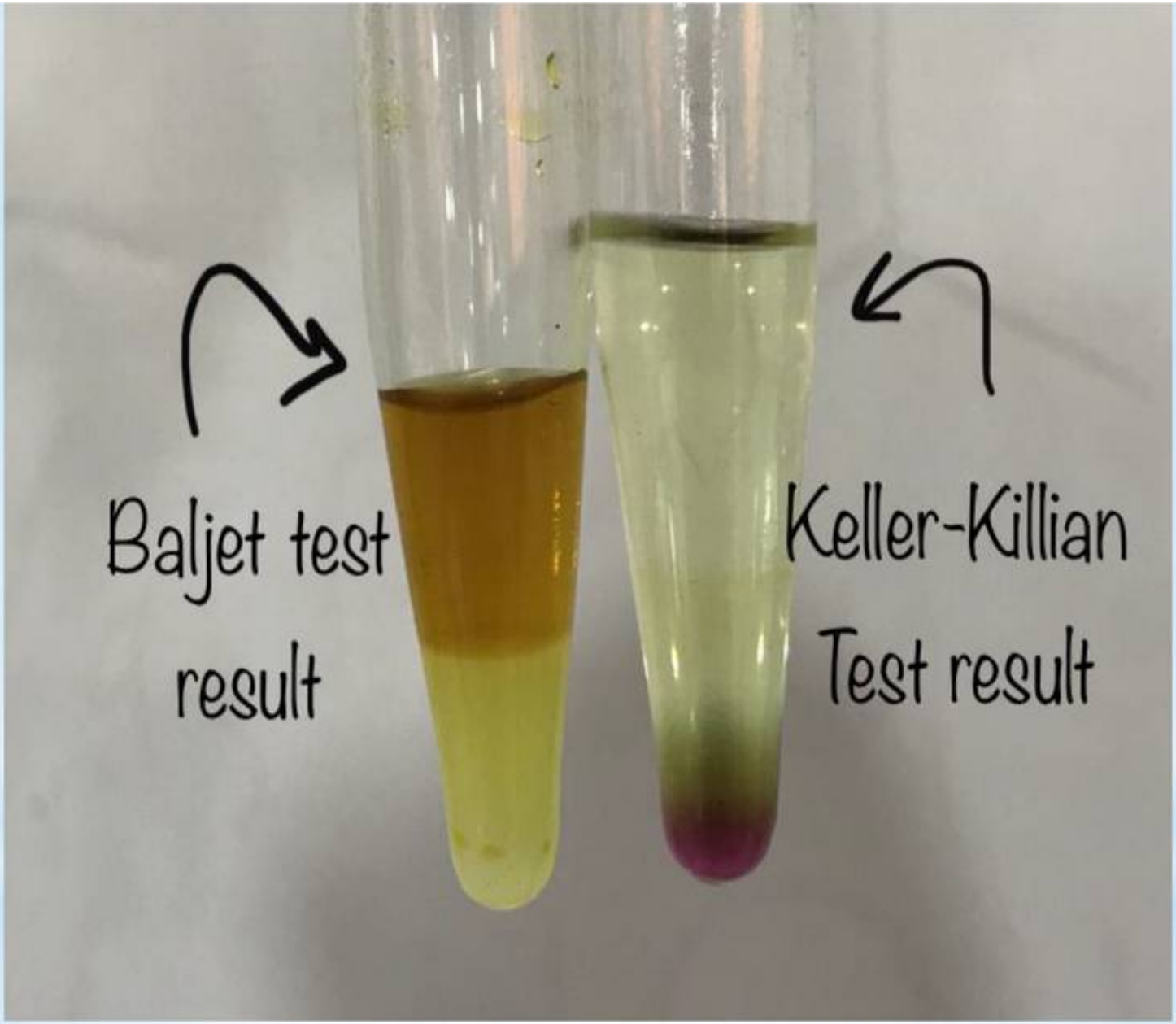
Turbid , yellow to orange in color.

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!