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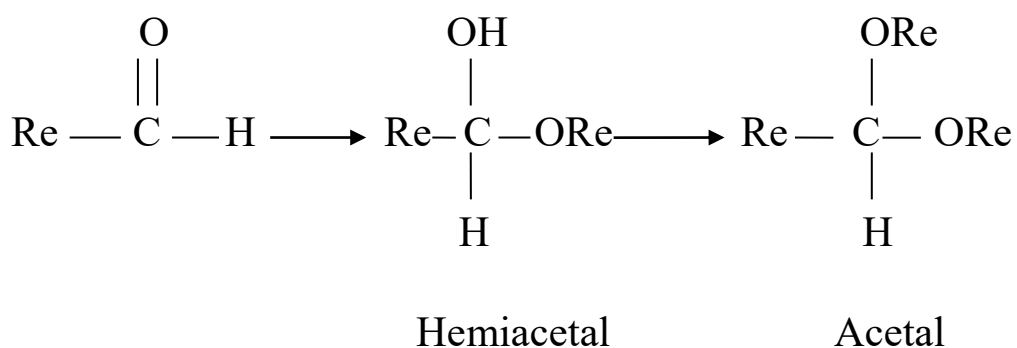
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Glycosides

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**.

In general there are four *basic classes* of glycosides: *C- glycosides*, in which the sugar is attached to the aglycone through C-C bond, and the *O- glycosides* in which the sugar is connected to the aglycone through oxygen –carbon bond, S-glycosides and N-glycosides.

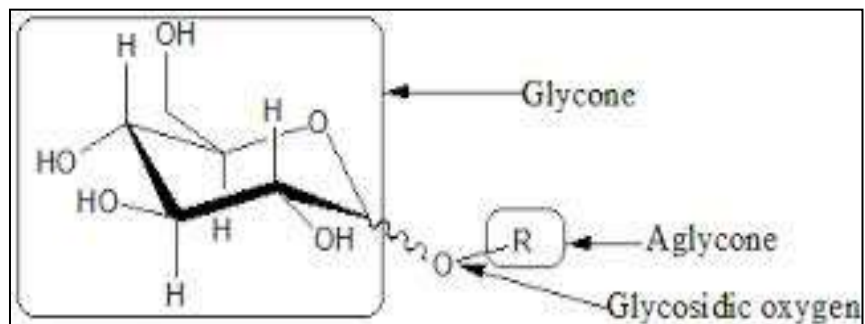
Chemically the glycosides are acetals in which the hydroxyl group (OH) of the glycone is condensed with the hydroxyl group of aglycone. More simply the glycosides may be considered as sugar ether. Two forms of glycosides are present, the α -form and the β -form, but the β -form is the one that occur 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, but in different cells

from those that contain the glycosides, 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.



(Fig.1) General Structure of Glycosides

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

1. A polar 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.

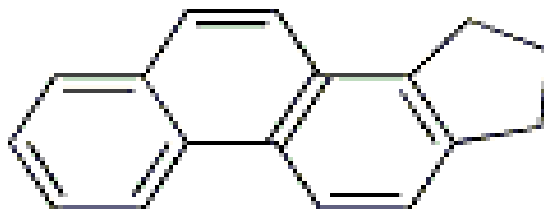
The glycosides are classified according to the chemical structure of the aglycone to:

- Cardioactive glycosides.
- Anthraquinone glycosides.
- Cyanophore glycosides.
- Isothiocyanate glycosides.
- Flavonoid glycosides.
- Alcohol glycosides.
- Aldehyde glycosides.
- Saponin glycosides.
- Lactone glycosides.
- Phenol glycosides.
- Miscellaneous glycosides.

Exp. No.1

Lab.1 Cardio-active Glycosides

They are named so, due to their action on the heart muscle. The aglycone part here is steroid, which is chemically cyclopenta phenanthrene.



Cyclopenta phenanthrene nucleus

The steroidal aglycones are of two types:

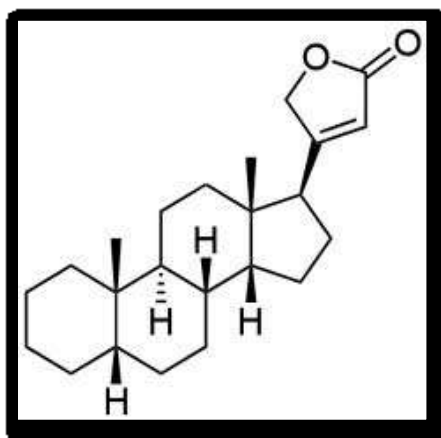
- 1) Cardenolides (α - β unsaturated 5 – member lactone ring).
- 2) Bufadienolides (doubly unsaturated 6-member lactone ring).

The more prevalent in nature is cardenolides type.

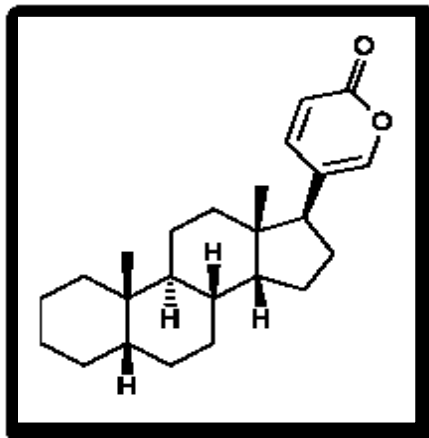
For maximum activity of cardioactive glycosides the following points are important:

- 1) 17 - β –lactone ring (cardenolide or bufadienolide).
- 2) 3 - β – OH (involve in glycosidic linkage).
- 3) 14 - β -OH.
- 4) CATSC (C= *cis* between two rings (A&B). A= *Anti* one ring (5&9). T=*Trans* between two rings (B&C). S= *Syn* in one ring (8&18). C= *cis* between two rings (C&D).

As represented bellow:



Cardenolide



Bufadienolide

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 *purpurea* 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 cardioactive glycosides

Aim: To isolate the cardio active glycosides.

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 **20 ml** of alc. Extract in conical flask

↓
Add

10 ml of **lead sub acetate** solution

(Mixing & standing for **5 min_s**.)

↓
Centrifuge

(5 min_s.)

Decant and take the supernatant (upper layer)

↓
Add

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

↓
Centrifuge

(5 min_s.)

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

Fraction A

Take one division and put in the separatory funnel



Add

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



(Shake& stand)

Take 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, decants and we 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 min_s)

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.

Cardio-active glycosides

Lab II Chemical tests for identification of cardio-active glycosides

Color reactions of cardio-active glycosides for cardinolides

1. Reaction due to (--CH₂--)group of the lactone ring

➤ Baljet's Test

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.

➤ Raymond 's Reaction:

Equipment and Reagents:

Test tube, 10% sodium hydroxide solution, 1% m-dinitrobenzene.

Procedure:

To 1ml of fraction A add **1-2 drops** of **10% sodium hydroxide** and few drops of an alcoholic solution of **1% m-dinitrobenzene**.

Result: Pink color.

➤ Kedde's Reaction :

Equipment and Reagents:

Test tube, 1% 3, 5-dinitrobenzoic acid, 0.5 N aqueous methanolic KOH (50 %).

Procedure:

To a solution of glycoside add a solution of **1% 3,5-dinitrobenzoic acid** in 0.5N aqueous **methanolic KOH (50%)**. Report the color.

Result: **Pink** color.

➤ **Legal's Reaction:**

Few mls of the glycoside or the purified extract of the crude drug is dissolved in pyridine. When sodium hydroxide and sodium nitroprusside are added alternatively, a transient **blood-red** color develops. This is a test for the unsaturated lactone ring of the genin.

2. Reaction due to the steroidal nucleus:

➤ **Lieberman's Sterol Reaction:**

Equipment and Reagents:

Test tube, porcelain dish, anhydrous acetic acid. Conc. H_2SO_4 .

Procedure:

Take **1ml** of fraction A in a test tube then add **5ml** of **anhydrous acetic acid** and shake well. Take **4 drops** of the above mixture and place in a porcelain dish, then add **one drop** of **conc. H_2SO_4** .

Result:

A change of color from **rose**, through **red**, **violet** and **blue** to **green**. The colors are slightly different from compound to compound.

Discussion:

This reaction is due to the steroidal part of the molecule and it is characteristic of the **aglycone** of the scillarenin type (unsaturated steroidal part).

3. Reaction due to presence of 2-deoxy sugar

➤ **Keller- Killian's Test**

Equipment & Reagents:

Test tube, glacial acetic acid, 0.1% of ferric chloride solution, Conc. H_2SO_4 .

Procedure:

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. **H₂SO₄** and add to the above mixture in drops so as to make two layers.

Results:

Two layers are formed; the upper one has **light bright green** color. The lower layer has transparent clear color (H₂SO₄ layer). The junction appears as a **reddish – brown** ring.

Identification of cardioactive glycosides by chromatography:

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

The stationary phase = Silica gel G.

The mobile phase = **Chloroform: Ethanol: Water (7:3:1)**

Or **Ethyl acetate: Methanol: Water (75:10:5).**

Or **Ethyl acetate: Methanol: Water (81:11:8)**

The standard compound = **Oleandrin**.

The spray reagent = Lieberman's reagent **or** sulphuric acid reagent (5% ethanolic H₂SO₄)

Mechanism of separation = Adsorption.

Developing = Ascending.

Other mobile phases:

Butanone: Xylene: Formamide (50:5:4),

Chloroform: tetrahydrofuran: Formamide (50:50:6).

Procedure:

1. Prepare **100ml** of mobile phase, and place it in the glass tank.
2. Cover the tank with glass lid and allow standing for **45 minutes** before use.

3. Apply the sample spots (fraction A & fraction B), and the standard spot on the silica gel plates, on the base line.
4. Put the silica gel plate in the glass tank and allow the mobile phase to rise to about two-third the plate.
5. Remove the plate from the tank, and allow drying, and then detecting the spots by the use of the spray reagent and heat the plates at **105 -110 °C** for **5-10 min** in the oven.
6. Note the spots, and calculate the R_f value for each spot.

Study problems:

Q1. What is the meaning of CATSC, explain with structure?

Q2. Give the reasons for:

- a) The addition of lead sub acetate, sodium phosphate and anhydrous sodium sulphate to the extract?
- b) The use of chloroform: ethanol in partitioning of fraction A?
- c) The use of HCl in the extraction procedure of cardio-active glycosides (Fraction B)?
- d) The use of chloroform alone in partitioning of fraction B?
- e) The use of picric acid in the performance of Baljet's test?
- f) The use of glacial acetic acid in Keller Killian test procedure?
- g) The addition of conc.H₂SO₄ in Keller-Kellian test& Lieberman's sterol reactions?

Q3. How can you identify an extract containing cardio-active glycosides?

Exp. No. 2

[Lab.3] Anthraquinone Glycosides

Anthraquinone and related glycosides, are stimulant cathartics, exert their action by increasing the tone of the smooth muscle in the wall of colon and stimulate the secretion of 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 **aglycones** which usually produce their effect in **8 -12 hrs**. After administration, these agents are indicated for constipation in patient who do not respond to milder drugs and for bowel evacuation before investigational procedure or surgery.

Stimulant laxative are habit forming so the long-term use may result in laxative dependence and loss of normal bowel function. The glycosides of anthranols and anthrones elicit a more drastic reaction than do corresponding anthraquinone glycosides and cause discomforting and gripping action. The drugs mostly used are **cascara, frangula, hypericum** and **Senna**.

Aloe and **Rhubarb** are not recommended due to their irritating actions which increase the chance for gripping effect. The anthraquinone hydrolyzed to give **aglycone** which are **di, tri, or tetra –hydroxyanthraquinone** .Also there are **antherone, dianthrones** and **oxanthrones**.

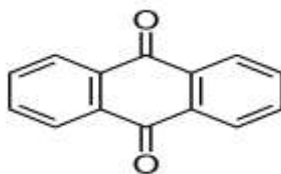


Fig (1): Chemical structure of anthraquinone

Isolation and identification of the anthraquinone glycosides:

1. Extraction:

Aim: To isolate the anthraquinone glycosides.

Reagents:

- ❖ Conc. HCl acid.
- ❖ Chloroform.
- ❖ 60% w/v ferric chloride solution.

Procedure:

Method of extraction: Decoction.

Plant used: Senna Cassia acutifolia, Cassia angustifolia

Family Leguminosae.

Part used: Dry leaves.



Senna

Extraction procedure

Place **0.5 gm** of powdered dry leaves of Senna in **50 ml** of **water**

↓ Boiling (**15 min_s**)

Cool & filter



Place the filtrate (20 ml) in separatory funnel and extract by shaking with

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



Upper layer

(**Aqueous layer**)



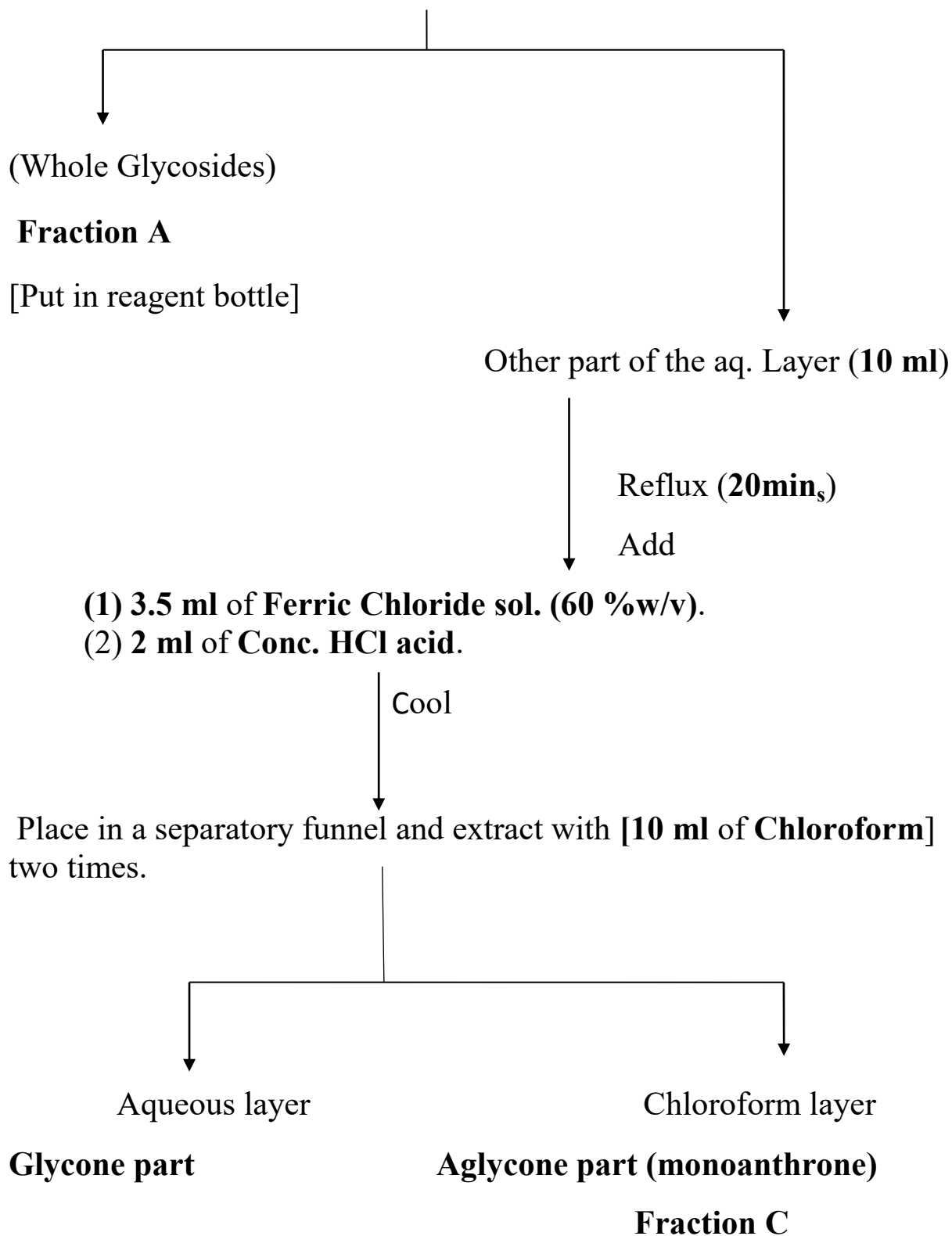
Combine lower layer

(**Chloroform layer**)

[Free aglycone (dianthrone)]

Fraction B

The upper aqueous layer will be divided into two parts



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:

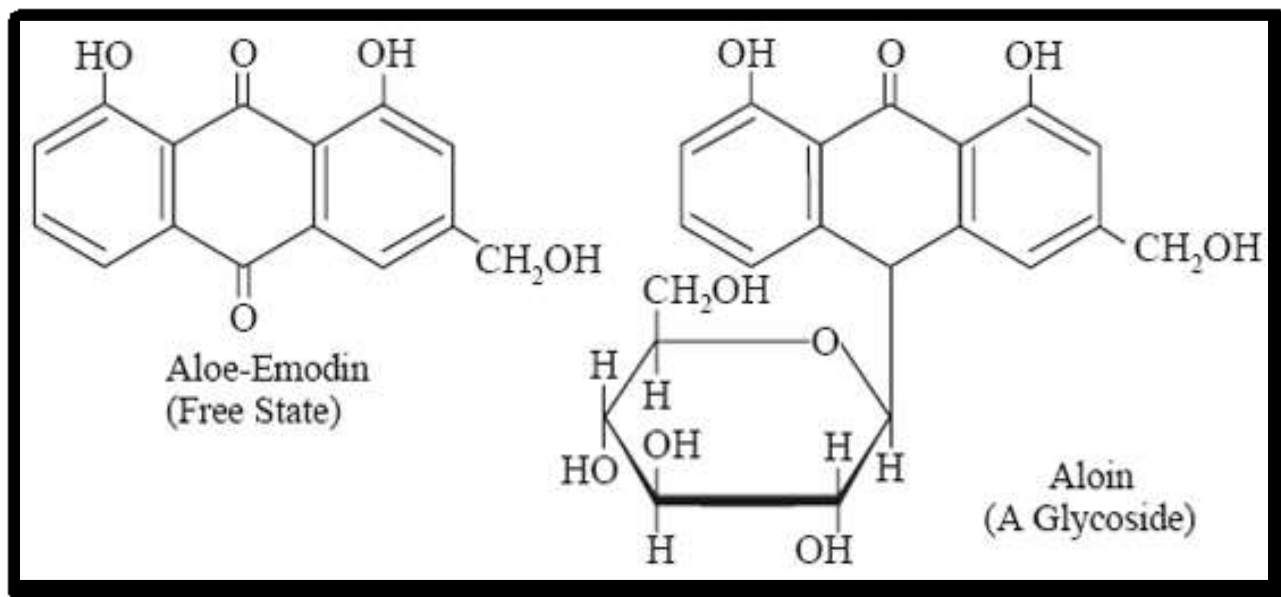
To **2 ml** of the Aloe extract, add **0.1 gm** of **Borax** and heat until dissolved. Pour a few drops of the liquid into test tube nearly full of water.

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 intensity 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 **110⁰ 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?

Exp. No. 3

[Lab.5] Saponin Glycosides

Saponins are high-molecular-weight glycosides, consisting of a sugar moiety linked to a triterpene or steroid aglycone. This group of glycoside is widely distributed in higher plants. Saponin glycosides form colloidal solution in water that foam upon shaking, this is due to **a decrease in the surface tension action done by saponin glycosides**, as a result of the hydrophobic/hydrophilic characteristics of the saponin, and due to this property the saponins are used in the manufacturing of beer, and soap.

Saponins have a bitter, acrid taste, and drugs containing them are usually sternutatory and otherwise irritating the mucus membrane.

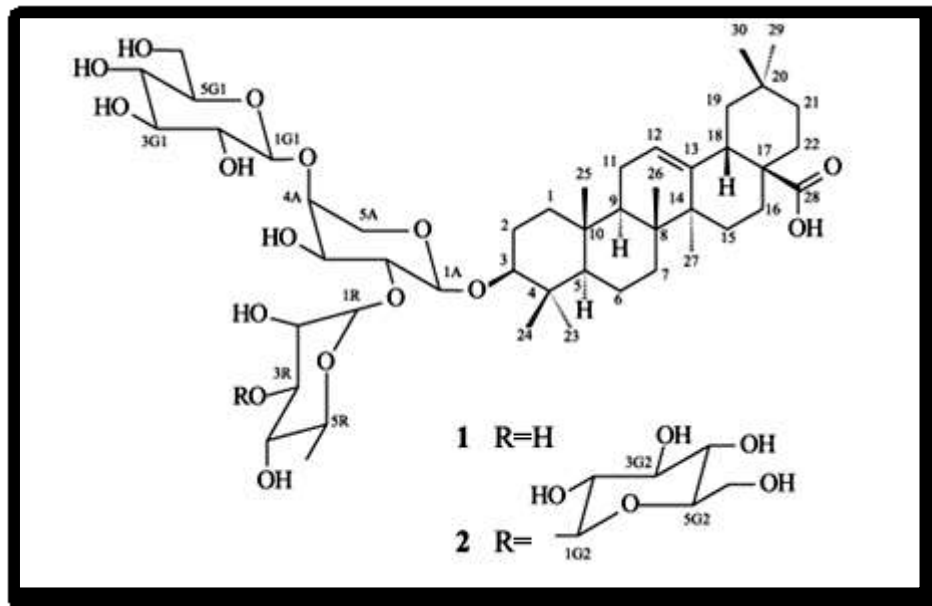
They destroy red blood corpuscles by hemolysis and are toxic especially to cold blooded animals therefore many saponins are used as fish poisons. The more poisonous saponin is often called **sapotoxin**, many are toxic to insects and mollusks, and some are used to control schistosomiasis snails.

Saponin upon hydrolysis yield an aglycone known as **sapogenin**, which are crystallized upon acetylation, therefore this process is used for purification.

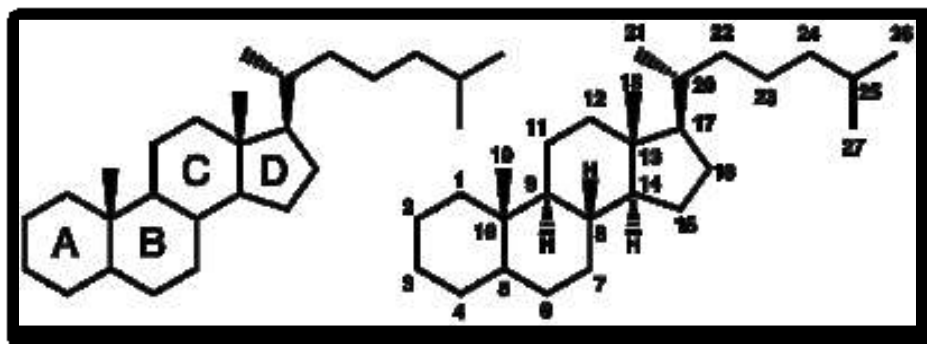
According to the structure of the aglycone, two kinds of saponin are recognized:

1. Pentacyclic triterpenoid saponins (acidic, and the C-atom is C_{30})
2. Steroidal saponins (neutral, C- atom is C_{27}).

Both of these have glycosidic linkage at C-3.



Pentacyclic triterpenoid saponin



Steroidal saponin

Extraction & Identification of the Saponin Glycosides:

Procedure:

Method of extraction: Decoction.

Plant used: Saponaria officinalis family: Caryophyllaceae.

Part used: Dry root.



Saponaria officinalis

Add **0.1 gm** of saponaria root in coarse powder to **20 ml** distilled water in a beaker and boil gently for **2-3 minutes**. Filter hot and allow cooling:

- Dilute **5ml** of the filtrate with water and shake vigorously.
- To the remaining of the filtrate add **5ml** of **dilute H₂SO₄ acid** and boil gently for **3-5 min**, .The aglycones are obtained by acid hydrolysis and are insoluble in water but are soluble in **90% alcohol**.
- Make the filtrate obtained from (b) alkaline with **NaOH**, (litmus paper) and then carry **Fehling's test** or **Benedict's test** (**5 ml** filtrate + **2 ml** of Benedict's reagent heat for **10 min**, on boiling water bath).

Specific reaction:

1. The Hemolytic Test

Aim: Identity test (**specific**) for saponin glycosides

Reagents:

- ❖ 10% solution of blood in normal saline.
- ❖ Normal saline.

Procedure:

Take two test tubes and place in each one **5ml** of a **10% solution of blood** in normal saline. To one of them, add **5ml** of **normal saline** solution and to the other one add **5ml** of the extract of Saponaria root .Shake both tubes gently and notice the result.

Results:

The test tube containing 5 ml of the extract of Saponaria will cause **blood hemolysis**.

2. Foam Index (according to Kofler)

Foam index is a value, which is used to express the quantity of the saponin glycosides in the crude drugs.

The method is based upon the property of saponin to form foam when shaken with water. The foam index signifies the dilution of the substance or drug to be tested which gives a layer of foam **1cm** high if the aqueous solution is shaken for **15 seconds**, and then allow standing for **15 minutes** before reading is made.

Foam Index

Aim: Identity test (**specific**) for saponin glycosides

Equipments & Reagents:

- ❖ 10 Test tubes having the same diameter.
- ❖ Graduated pipette.
- ❖ 0.1% decoction from the powdered drug.
- ❖ 1% solution of sodium carbonate.

Procedure:

1. Prepare **0.1%** decoction from the powdered drug, neutralized it by adding solution of **1% sodium carbonate** drop wise (litmus paper) and filter.

2. In to 10 test tubes having the same diameter, **1 to 10 ml** of this decoction is added respectively using a graduated pipette, complete the volume to **10 ml** with **distilled water**.
3. Shake the content of each test tube thoroughly for **15 seconds** and allow to stand for **15 minutes**.
4. After this time, the reading is made in the test tube containing the most dilute solution with a ring of foam **1 cm** height.

For example: When this is occur in the test tube number 8, which contains 8ml of the decoction and 2 ml of water, then 8 ml of 0.1% decoction corresponds to 0.008 gm of the drug and the dilution is calculated from the following calculation:

Gm	ml	
0.1	100	
X	8	X= 0.008 gm. of saponin in 8ml of decoction.

Gm	ml	
0.008	10	
1	X	X= 10/0.008
		X= 1250

That means the ring of foam 1cm high is formed by a solution diluted 1:1250. The foam index is therefore 1250.

Note/ The addition of sodium carbonate is to convert the acidic saponins that may be present in the decoction, to salts, which are soluble in water.

Study problems:

Q1. How many kinds of sapogenin are found in the medicinal plants? Explain with structures?

Q2. Give the reasons for:

- a) The addition of H_2SO_4 and boiling during the extraction of Saponaria root?
- b) Alkalinization with NaOH in Fehling's or Benedict's tests?

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

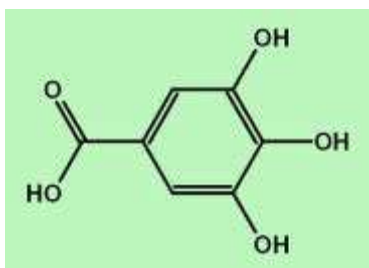
Exp. No. 4

[Lab.6] Tannins

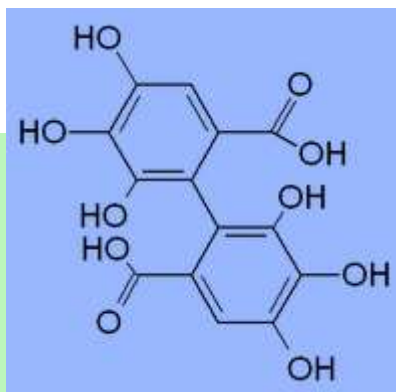
Tannins compromise a large group of complex substances that are widely distributed in the plant kingdom. Chemically tannins are complex substances; they usually occur as mixtures of poly hydroxyphenols that are difficult to separate because they do not crystallize. Tannins are divided according to the identity of the phenolic nuclei involved, and on the way they are joined into two classes:

1 .Hydrolysable tannins:

This class consists of **gallic acid** and related polyhydroxy compounds (**hexahydroxydiphenic acid**) and their derivatives esterified with glucose. They are termed hydrolysable tannins due to ease of esters to hydrolyze to phenolic acids and sugar. They were formerly known as pyrogallol tannins.



Gallic acid



Hexahydroxydiphenic acid

2. Nonhydrolysable tannins or condensed tannins:

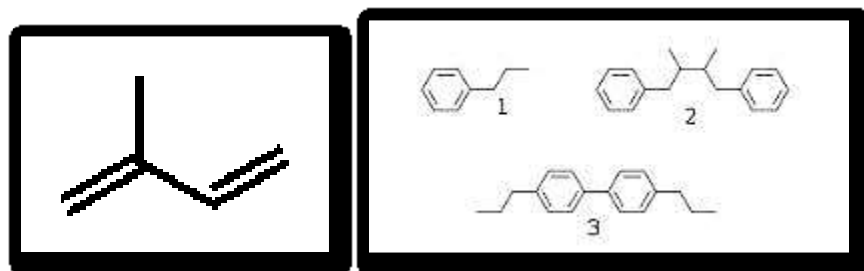
This class contains only phenolic **nuclei** but frequently linked to carbohydrates or proteins. When treated with hydrolytic agents, these tannins tend to polymerize, yield insoluble usually **red**-colored products known as **phlobaphenes**.

[Lab.7] Volatile Oils

They are odorous principles found in various plant parts. Because they evaporate when exposed to the air at room temperatures, they are called **volatile oils**; they are also called essential or etherical oils.

Volatile oils are colorless as a rule, particularly when they are fresh, but on long standing they may oxidize and resinify, thus darkening in color, to prevent this darkening, they should be stored in a cool, dry place in tightly stoppered, preferably full, amber glass containers.

As a rule, volatile oils are immiscible with water, but they are sufficiently soluble to impart their odor to water. They are soluble in ether, alcohol and most organic solvents. Many volatile oils consist largely of **terpenes** (terpenes are natural products whose structures may be divided into isoprene units).



Isoprene unit

Phenylpropanoids

Another major group of volatile oil constituents are the **phenylpropanoids**. (These compounds contain the C_6 phenyl ring with an attached C_3 propane side chain).

Generally volatile oils and volatile oil-containing drugs are divided into the following classes:

- | | |
|------------------|--------------------|
| 1. Hydrocarbons. | 5. Phenols |
| 2. Alcohols. | 6. Phenolic ethers |
| 3. Aldehydes. | 7. Oxide |
| 4. Ketones. | 8. Esters |

Essential oils are derived from various sections of plants:

- **Leaves**- Rosemary, Basil, Eucalyptus.
- **Flowers**- Rose, Lavender, Clove.
- **Seeds**- Fennel, Anise, cumin.
- **Bark**- Cinnamon.
- **Rhizome**- Ginger.

Pharmacological Uses of Volatile Oils:

- Carminative as for **Rosemary oil**
- Antitussive as for **Eucalyptus**.
- Antiseptic as **Clove oil**.
- Aromatherapy, alternative medicine as **Lavender Oil**.

Isolation and identification of the volatile oils:

Aim: extraction of the volatile oil by water distillation method, determination of the percentage yield and identification of the major chemical constituents of isolated oil.

Procedure:

Method of extraction: hydrodistillation by Clevenger apparatus

Plant used: Clove *Syzygium aromaticum*

Family: Myrtaceae,

Part used: dried flower buds

Cloves are the flower buds of *Syzygium aromaticum* (clove tree). Major constituents of clove oil are Eugenol (nearly 85 %) and Eugenol acetate (10 %).



(a)

(b)

Fig1: (a) Clove tree and (b) clove buds

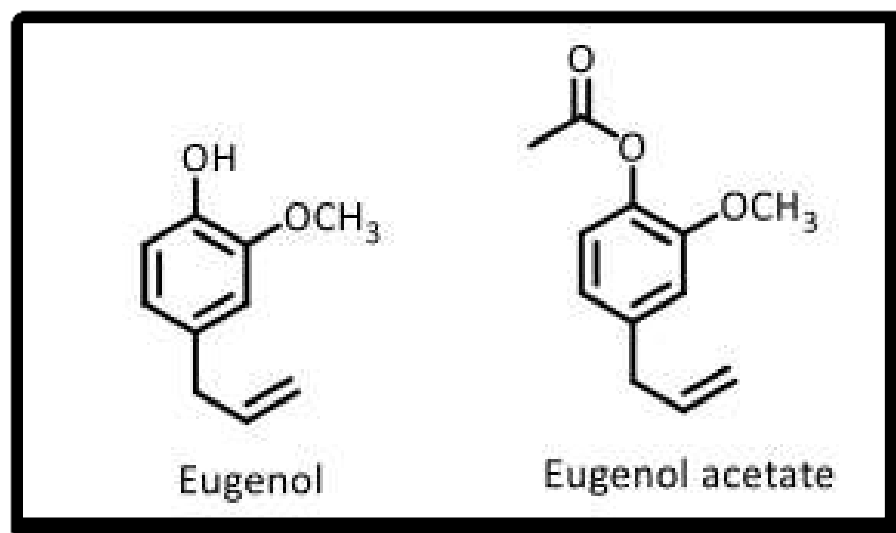


Fig. 2: Chemical structures of eugenol and eugenol acetate



Clevenger apparatus

(Oil heavier than Water)

Clevenger apparatus

(Oil lighter than water)

Fig. 3: Clevenger apparatus

Procedure:

- Weigh out **20 gm** of the plant material (coarse powder) and place into a distilling flask; add few pieces of porous earthenware.
- Add **200 ml** distilled water to the flask and shake well. Add another **200 ml** of water by rinsing the neck of the flask.
- Connect the distilling flask with the still head of the apparatus. By the means of the pipette or washing bottle, fill the receiver with water until over flows.
- Connect the condenser of the apparatus with the cooling water (from the tap).

- Heat the distilling flask until the boiling starts. Record the time of the beginning of distillation, and continue the distillation for one hour.
- Switch off heating. Allow the graduated receiver to cool. Read off the volume of the volatile oil (count all small divisions in the receiver of the layer of oil).
- Calculate the %v/w of the volatile oil content of drug.

$$\% \text{ V/W} = \text{vol. of oil} / \text{wt. of plant} \times 100$$

Identification of clove volatile oils

1. By simple chemical test as reaction of volatile oil with potassium permanganate solution or with bromine solution.
2. By chromatography as gas chromatography GC and TLC

Thin layer chromatography (TLC) for clove oil.

- ❖ The stationary phase = Silica gel G.
- ❖ The mobile phase = **Toluene: ethyl acetate (93:7).**
- ❖ The sample (oil) = clove oil.
- ❖ The standard compound = eugenol
- ❖ The spray reagent = **Vanilline _ Sulphuric acid / Ethanol (10%v/v).**
- ❖ Mechanism of separation = Adsorption.
- ❖ Developing = Ascending.

Procedure:

- 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 spot 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 the plate.
- Remove the plate from the tank, and allow drying, and then detecting the spots by the use of the spray reagent and heat the plates at **120°C** until the spot's color intensity is reached in the oven. Detect the spot and calculate the R_f value.

Study problems:

Q1. How many kinds of Volatile-oil according to the basic nuclei? Explain with structures?

Q2. How can you determine the volatile content of crude drugs?

Q3. How can you identify an extract containing Volatile oils?

Both rhubarb and senna leaves contain [sennosides](#) and anthrone [glycosides](#), which, on their own, have no [purgative](#) activity and are not readily absorbed by the small intestine following [oral administration](#). The real active ingredients are senna aglycones, which are produced through the hydrolysis of [sennoside](#) by enzymes known as β -d-glucosidases, which are secreted by intestinal [Bifidobacteria](#). Together, they can play a purgative role after being absorbed by the small intestine. And among them, rhein anthrone has potent purgative activity³³ (Fig. 5.2). Likewise, compounds like [aloe-emodin](#) glycoside that contain an anthrone skeleton have no purgative effect.

