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.

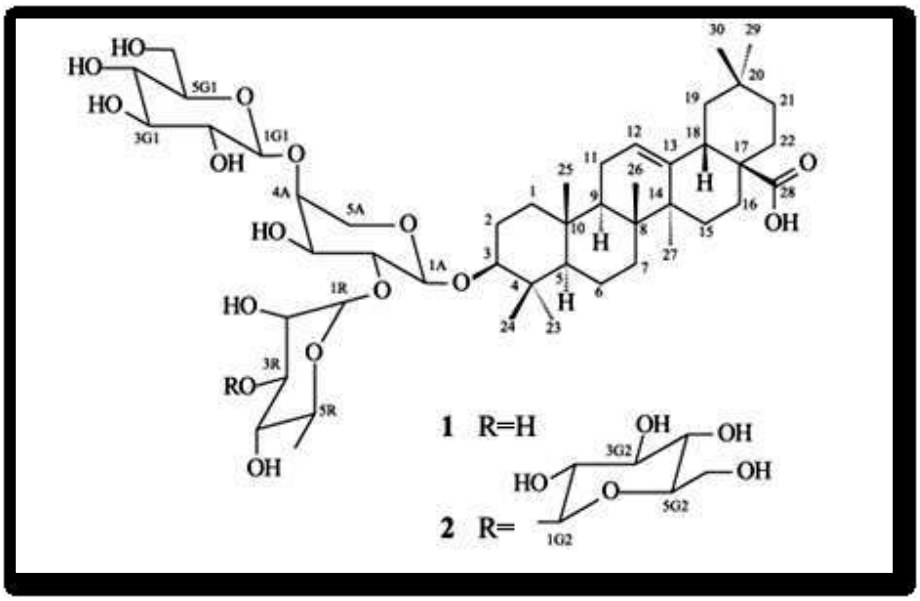
Saponin upon hydrolysis yield an aglycone known as **sapogenin**, which are crystallized upon acetylation, therefore this process is used for purification.

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

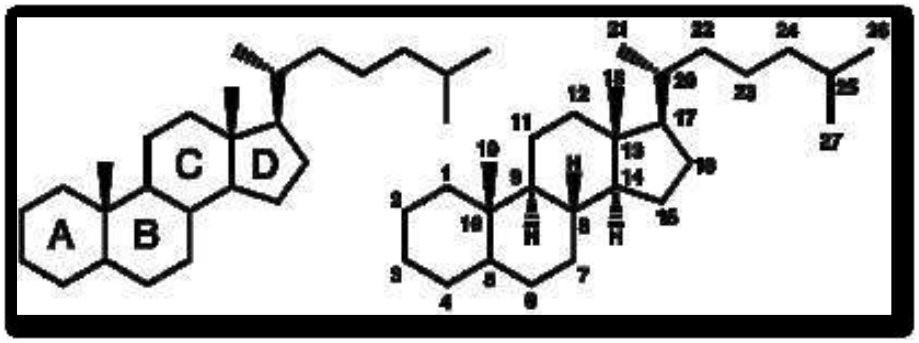
1. Pentacyclic triterpenoid saponins (acidic, and the C-atom is C_{30})

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 H_2SO_4acid** and boil gently for **3-5 min_s** .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_s** 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?