PHARMACOGNOSY-I PRACTICE

Extraction of Saponins

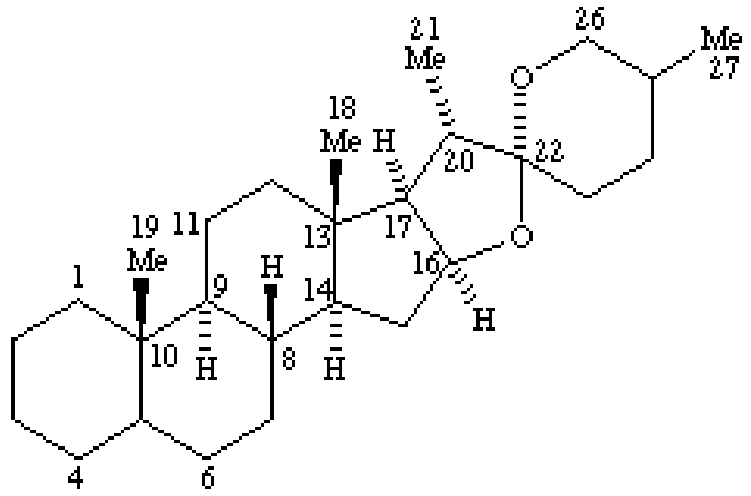
&

Identification of Steroidal and Triterpenoid Saponins

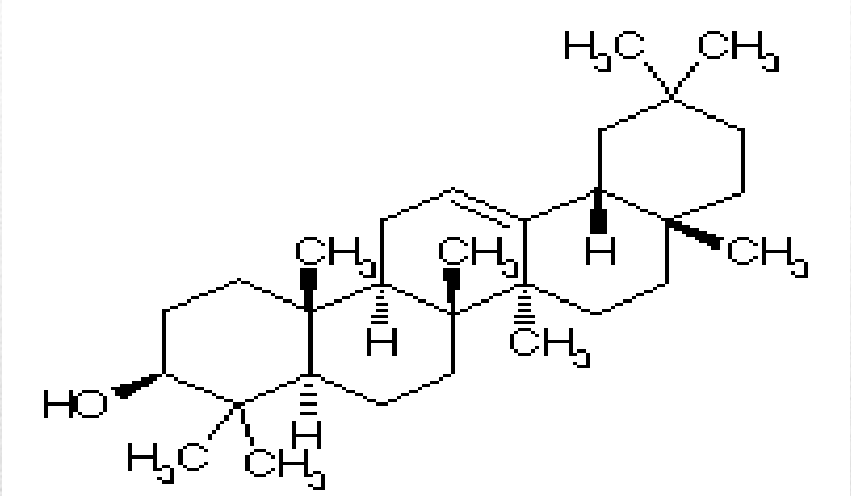
SAPONİNS

- **Saponins are glycosides of triterpenes, steroids or steroid alkaloids which have a very wide distribution in natural sources. Many saponins have detergent property and give stable foam in water based on their surface activity. They also show haemolytic activity. They are highly toxic when injected to the blood.**
- **Saponins are amorphous, odorless compounds with bitter taste.**
- **Saponin glycosides are hydrolysed by acids to give an aglycone (sapogenin) and various sugars and related uronic acids.**

Saponins are classified as steroidal and triterpenoid saponins according to the structure of the aglycone (sapogenin).



Steroidal skeleton (27C)



Triterpenoid skeleton (30C)

STEROIDAL AND TRITERPENOID AGLYCONES

- ***"Saponin"*** **is the commercial name of saponins which is extracted from Radix Saponaria albae.**
- **Saponin is an emulsifying agent and used in pharmaceutical products and textile industry.**

Radix Saponariae albae

SAPONIN

EXTRACTION OF SAPONINS

Take 10,???? g dried and powdered sample (R. Saponariae albae)

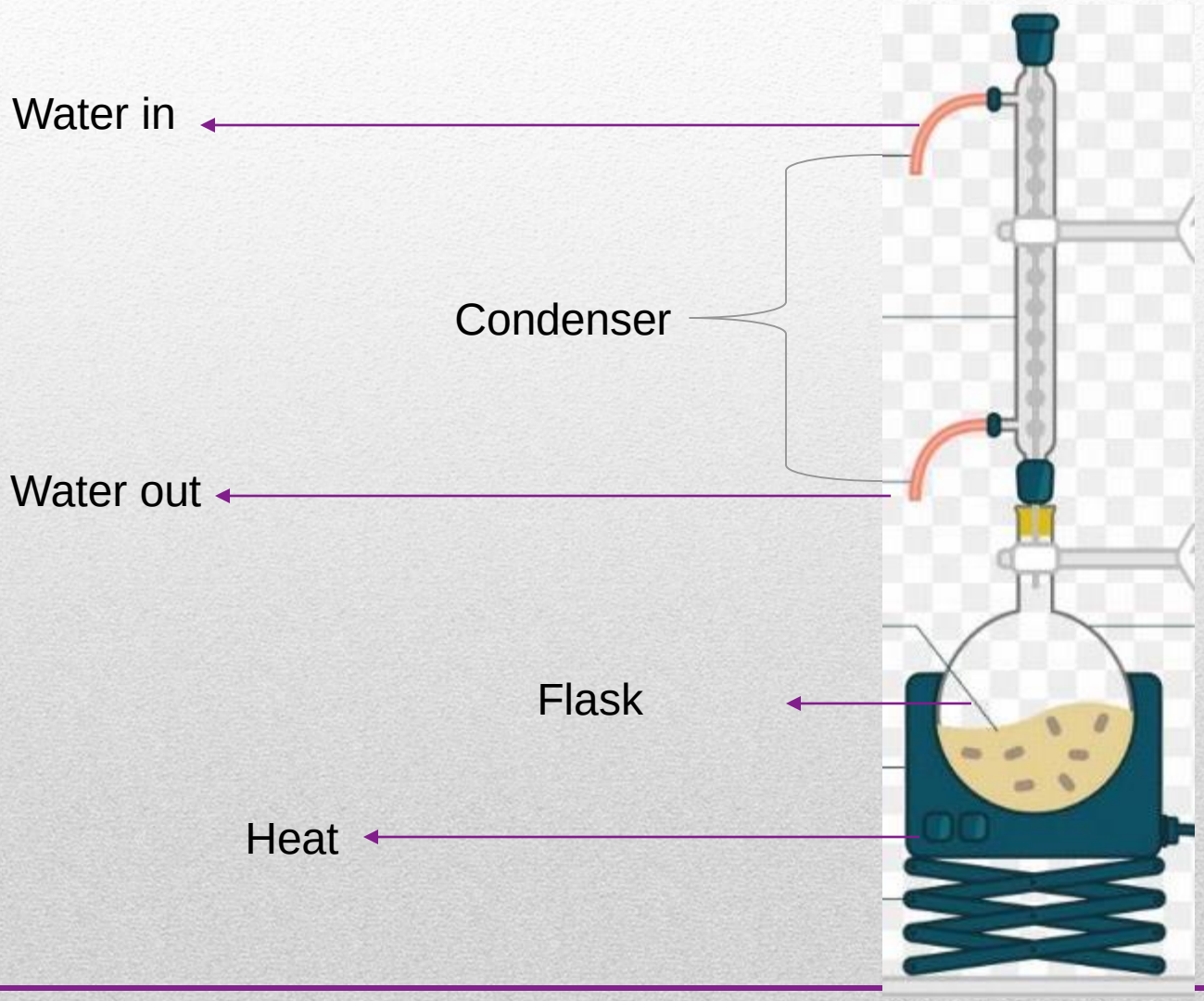
Petroleum ether is used for removing resine and lipophilic substances

Extract with petroleum ether (150 ml) under reflux for 15 min (+ boiling stone)

And then decant the filtrate (petroleum ether). Evaporate the sediment in a boiling water bath for a few minutes to dryness

Dilute the residue to 150 mL with EtOH. And then extract with EtOH under reflux for 30 min.

Extraction Under Reflux



And then, filter the extract while hot.
because saponins precipitate while cool.

Concentrate the filtrate until 15-20 ml of the solution is left (under the distillation apparatus)

Subsequently, take 20 ml diethyl ether in a beaker, pour dropwise the concentrated extract into diethyl ether portion to precipitate the saponin

To obtain the sediment, filter the mixture through a tared plain filter paper

EXTRACTION OF SAPONINS

Dry the filter paper in the oven (60˚C). Calculate to total saponin content.

10,.... g sample	A g saponin
100	x

X= content %

TOTAL SAPONIN CONTENT

IDENTIFICATION OF STEROIDAL AND TRITERPENOID SAPONINS

- **Identification of Steroidal Saponins**

  1) Salkowski's Test

  2) Lieberman-Burchard's Test

- **Identification of Triterpenoid Saponins**

  1) Anisaldehyde-Sulphuric acid's Test

IDENTIFICATION OF SAPONINS

SALKOWSKI'S TEST

- **Mix 1 micro-spatula steroidal saponin sample and 1-2 ml chloroform in a test tube and dissolve.**
- **Carefully incline the test tube and pour dropwise concentrated H_2SO_4, using a dropper, along the sides of the tube.**
- **Observe the yellow-red colour at the junction of two liquids. And then shake the test tube, come out the blood red colour.**

LIEBERMAN-BURCHARD'S TEST

- **Mix 1 micro-spatula sample and 1-2 ml chloroform in a test tube and dissolve.**
- **Evaporate the solution in a porcelain dish to dryness.**
- **Dissolve the residue in 1 ml of glacial acetic acid and cautiously transfer into a test tube.**
- **Carefully incline the test tube and pour dropwise concentrated H_2SO_4, using a dropper, along the sides of the tube.**
- **Observe the violet colour at the junction of two liquids. And then the colour becomes blue-green.**

ANISALDEHYDE-SULPHURIC ACID TEST

Take 1 micro-spatula sample in a porcelain capsule. And add 1-2 drops anisaldehyde-sulphuric acid reagent**

Heat it in a boiling water bath

Observe the pink-violet colour

**Preparation: Mix 1ml anisaldehyde,1ml MeOH ve 0.1ml concentrated H_2SO_4

Foaming Test

- This determination is made to determine the amount of saponin present in the drog

- **Foam Index:** The dilution degree (inverse of concentration) of 10 ml saponin solution which forms a permanent foam at 1 cm height of in a tube of 6 mm in diameter after being shaken for 15 seconds and left for 15 min.

Experimental Procedure

- Decoction of 0.1 % is prepared from Radix Saponariae albae.
- Add cold water to the erlenmayer and boil it for 30 minutes , then filter from cotton when it is hot.
- Control the acidity of the solution with Turnusol paper (blue→red = acidic)
- If acidic character is indicated, it is neutralized with 1 % Na_2CO_3. (Neutralization is required since saponins are hydrolyzed in acid medium and don't form foam.)
- When the solution is cooled, transfer to a volumetric flask and complete to 100 ml.

Experimental Procedure

- Thereafter the decoction is taken to 10 test tubes as explained below:

1. tube → 1 ml decoction + 9 ml water
2. tube → 2 ml decoction + 8 ml water
3. tube → 3 ml decoction + 7 ml water .....

The tubes were shaken vigorously vertically for 15 sec and after waiting 15 min, the degree of the dilution of the tube where the foam is 1 cm at height is recorded.

Calculation

- Example: If 1 cm foam is in Tube 7;

7 ml decoction+ 3 ml water

100 ml decoction 0.1 g sample

7 ml decoction x

x = 0.007 g sample

Cons. = 0.007 g / 10 ml

F.I. = 10/0.007 F.I. = 1428