ISOLATION OF ESCIN

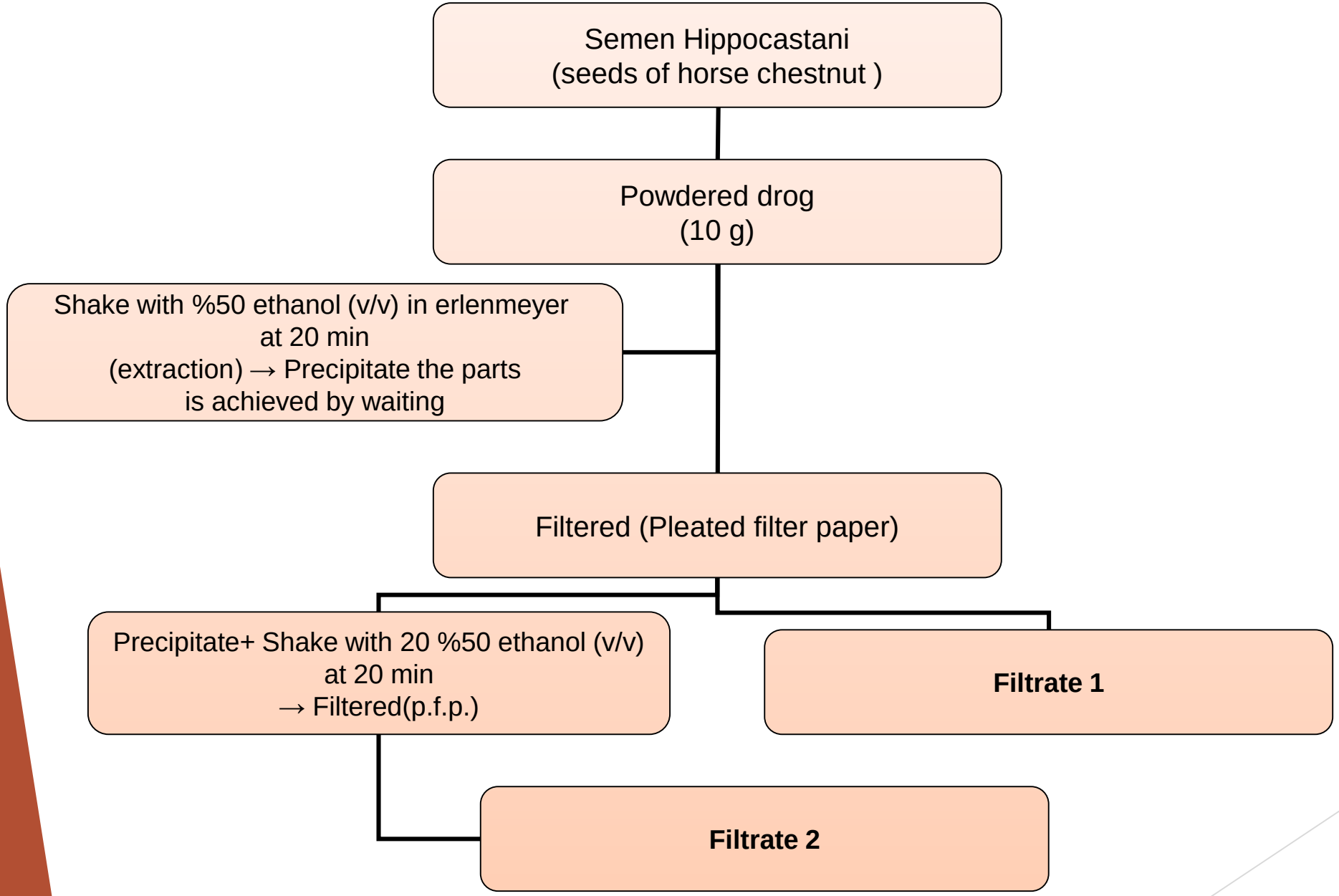
ESCIN

Escin is a pentacyclic triterpene, existing in α and ß forms, and is isolated from the seeds of the horse chestnut (*Aesculus hippocastanum*) (Hippocastanaceae).

There are 30 kinds of saponosides in the complex of Escin which resemble each other.

Essin is found in plant like K^+ salt.

Experimental Procedure:



Filtrate 1 + Filtrate 2 = A ml

- *The total volume of the filtrates is measured. In this way the drog is extracted 2 times.*
- *Drog is a seed, so it carries a huge of amount fatty oil. Fatty oil can pass into ethanol if high concentrated alcohol is extracted.*
- *This situation is avoided by using 50% ethanol.*
- *So, 50% ethanol is a selective solvent.*

Filtrate 1 + Filtrate 2 = A ml

The alcohol level of A ml of extract is increased to 65 % (v/v). The reason for the raising of the alcohol level is the need for a solvent that dissolves the escin complex during further processing. Precipitate of escin complex is inhibited, when passing through ion-exchange resin.

To do this, firstly calculated is raise to alcohol level to 65 % for 100 ml of 50% ethanol:

$V_1 \times C_1 + V_2 \times C_2 = V_s \times C_s$

$100 \times 50 + V \times 96 = (100 + V) \times 65$

$V = 48,4$ ml

100 ml, %50 ethanol → 48,4 ml EtOH R (if you add, it will be %65)

A ml, %50 ethanol → X ml EtOH R (needed)

The volume of the 96% ethanol to be added to the extract = X = 0,484 A ml

The calculated amount of 96% ethanol(X ml) is added to total filtrate (A ml).

- **We will use a glass column to apply the extract to the cation exchange resin.**
- **Filling column :**
- **Cotton (not too tight)**
- **Filter paper (same diameter as the column)**
- **10 g resin+ EtOH (up to 2/3 of the column, slowly, filled with resin to prevent air from entering)**
- **Add the EtOH until the resin passes 1-2 cm → To prevent the resin from drying out**
- **The extract is applied.**
- **Tap dropwise into a beaker the following is taken.**

The resulting extract is concentrated to until 1/4 of its volume in the water bath.

When the crystallization starts, the beaker is taken at a cold place and the process is expected to be completed.

The crystals are filtered by filtration through filter paper is weighed.

The filter paper is dried and the % efficiency is calculated.

10 g Drog → A g Escin

100 g Drog → X g Escin

% X Escin

Ion Exchange Resins

- It is the substances used to purify the active substances obtained from natural sources.

2 types:

1- Cation exchangers : (+) to change the charged ions

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2- Anion exchangers: (-) to change the charged ions.

- Escin is in the form of K^+ salt in the plant, the cation exchange resin is used in the experiment.

K^+-escin+ H^+-Resin $\rightarrow$ H^+-escin + K^+-Resin

(complex)

The escin complex is obtained in pure form.

Resin Regeneration

- Once the resins are used, they lose their ion-exchange properties because they lose all of their ions.
- Resins must be regenerated to be reusable.

Resin Regeneration

- Containing %1 H_2SO_4 % 65 EtOH is added to the column. Wait 5-10 min.
- Flows slowly from the tap.
- H^+ ions are reloaded into the resin.
- Wash with only 65 % EtOH until the wash water is neutral.

- $2\ \text{Resin-}K^+ + H_2SO_4 \rightarrow 2\ \text{Resin-}H^+ + K_2SO_4$

Foaming Test

This determination is made to examine the amount of saponin present in the drog.

Foaming Index: 1It is the dilution degree of a 10 ml saponin solution which forms a permanent foam at a height of 1 cm after being shaken for 15 seconds in a 6 mm diameter tube for 15 minutes (Inverse of concentration).

Experimental Procedure

Decoction of 0.1 % is prepared from Radix Saponariae albae.

Add cold water to the erlenmayer and boil it for 30 minutes and filtered from cotton.

Check the acidity of the solution with Turnusol paper (blue→red = acid) If acidic character is indicated, it is neutralized with 1 % Na_2CO_3. (Saponins is hydrolyzed in an acid medium that is required for neutralization foaming.)

When the solution is cooled, it is transferred to a volumetric flask and completed to 100 ml.

Experimental Procedure

- Thereafter the test tube 10 from the decoction is taken as follows:

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

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The tubes were shaken vigorously vertically for 15 sec and after waiting 15 min, the degree of the dilution is calculated on the tube 1 cm.

Calculation

- Example: 1 cm foam in 7.tube;

7 ml decoction+ 3 ml water

100 ml decoction 0,1 g drog

7 ml decoction x

x = 0,007 g drog

C = 0,007 g / 10 ml

FI = 10/0,007 FI = 1428