Date
7/10/2020

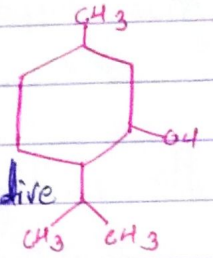
Day - Wednesday

UNIT - 3rd

Isolation, Identification and analysis of Phytoconstituents

[1] Terpenoids

[A] MENTHOL



- Menthol is a monocyclic monoterpenoid derivative of the drug.
- Menthol contains one alcoholic group.
- The menthol is obtained from the leaves of the mentha Pipperencia of family Labitace.
- In the from leaves the pipperminit oil obtained and from this pipperment oil about 50% L menthol is isolated.
- It is soluble in alcohal, ether, chloroform and cyclo-hexane.

Uses Menthol is used as additives in food material, caugh, syrup and other beakery products.

- Used in oral hyeginic product.
- Tobacco, Cigrate.
- Flavour/ coating.

Isolation- **Step 1-** First of all the pipperment oil is obtained from the leaves of mentha pipperasicia by the dillation method.

Step-2 Now remove the moisture from this pipperment oil by using distillation.

Step-3 Now this pipperment oil in plastic packet and tight and store in -60°C for 7 days.

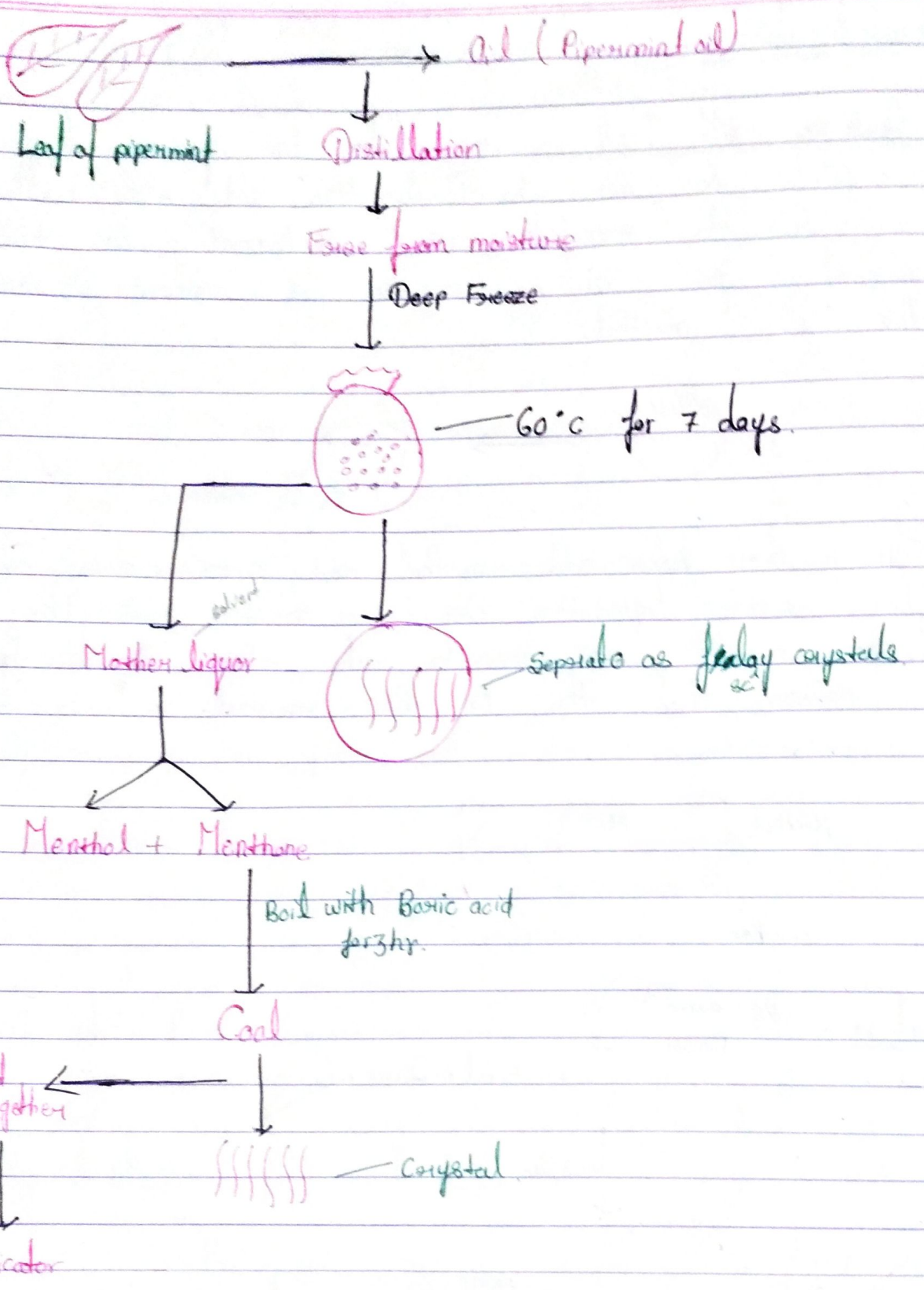
Step-4 Now the menthol is obtained in the form of white flaky crystals and the mother liquor is the separate.

Step-5 In the remaining mother liquor is menthol and menthone is present.

Step-6 Now at the Boric acid in this mixture and boil for the 3 hour, that why the menthene removes and menthal is again obtained.

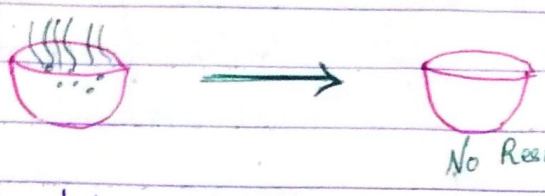
Step-7 Now cool the same ~~temperature~~ procedure and obtained the menthol.

Step-8- At those menthol flash and send into the dessicator for removing the moisture.

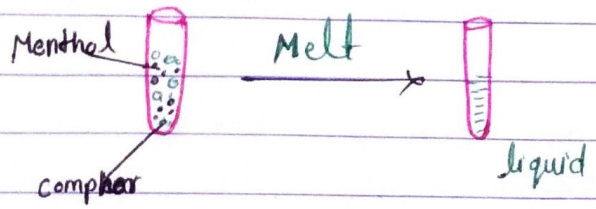

Oil (Peppermint oil)
Leaf of peppermint
Distillation
Free from moisture
Deep Freeze
60°C for 7 days.
Mother liquor
Separate as flaky crystals
Menthol + Menthone
Boil with Boric acid for 3hr.
Cool
Mixed together
Crystal
Dessicator

Identification -

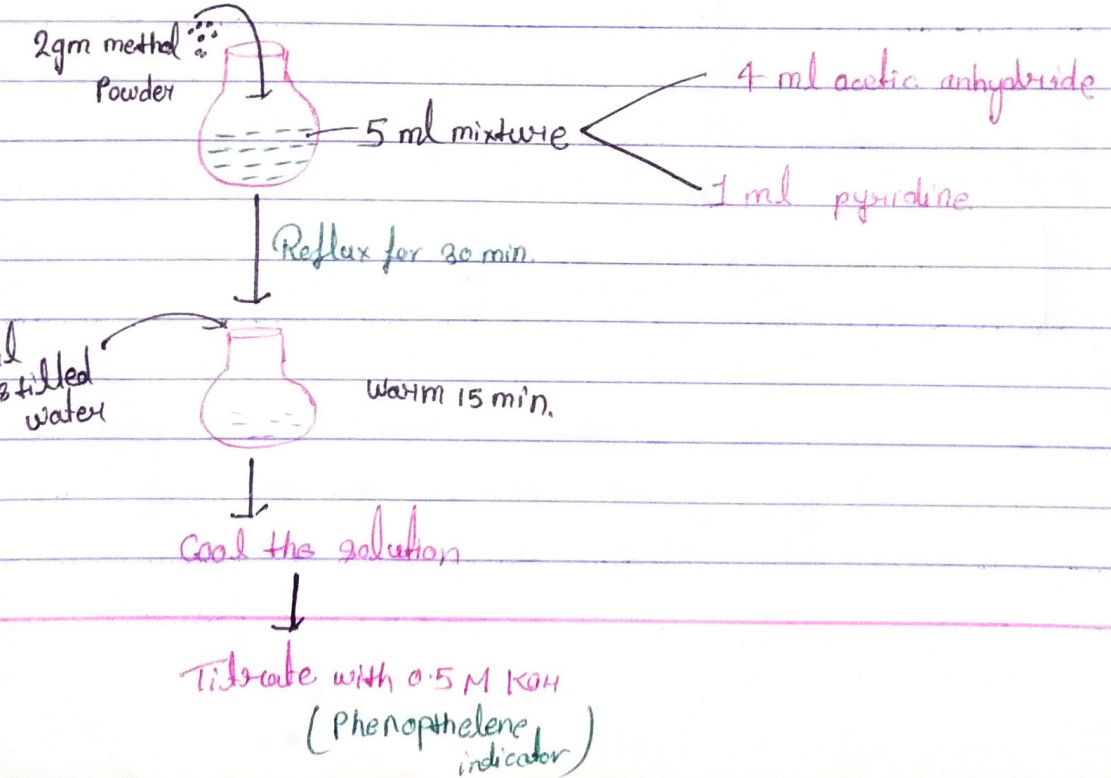
[1] Active 1st Test - Take few grams of menthol crystals add into the Porselin disc and gives heat after heating the all menthols is sirwapted in the form of gas and no residue is remain, it means this is menthol.



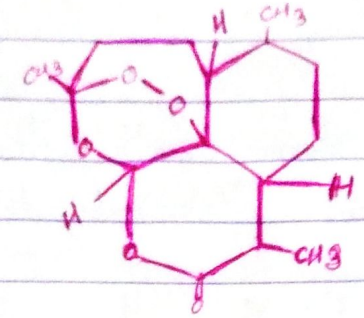
[2] In a test tube add menthol and some camphor crystal after some liquidation this is convert into the eutectic mixture and converts into the liquid form, it proves that this is the menthol.



Estimation -



[B] Artemisinin



- It is also known as molecule of century, it is effective as malaria treatment.
- It is discoverd by the Chinese scientist Mr To You you in the 1972 and it is obtained from the dried leaves of the Artemesia annua.
- It is used as antimalerial and antiparasitic drug.
- The main drawbacks of this drug, it has low bioavailibility and poor pharmacokinetics property.
- WHO is not recommend for the use of artemisinin for long duration because it can creat the resistant in our body.

Isolation -

Step-1- Take the green leaves of artemesia annua, and dried below the 60°C for few days in sunlight.

Step-2 Now ~~rise~~ crushed the dried leaves and convert into the dry powdered form.

Step-3 After the masceration process with the using menthol mathenal extract, the mathenal extract of artemesia is obtained.

Now partition coefficient of this extract with the several number of times untill the clear extract is obtained.

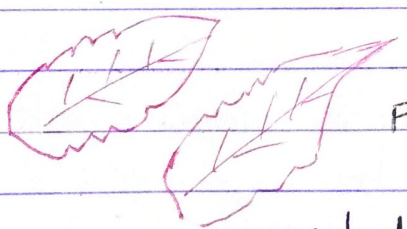
Now add the water in this extract and convert this extract into hydroalcohalic extract.

Step-4 Now partition coefficient of this extract with the hexane and repeat several no. of times untill the clear extract is obtained.

Step-5 Now add the water in this extract and convert this extract into hydroalcohalic extract.

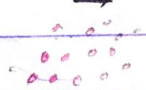
Step-6 Now again this hydrochloride extract is partition coefficient is partition with the acetic acid untill the concentrated solution is obtained.

Step-7 Now when the concentrate extract is obtained then put under the vaccum and deep freeze and the crystal of artemisia is obtained.



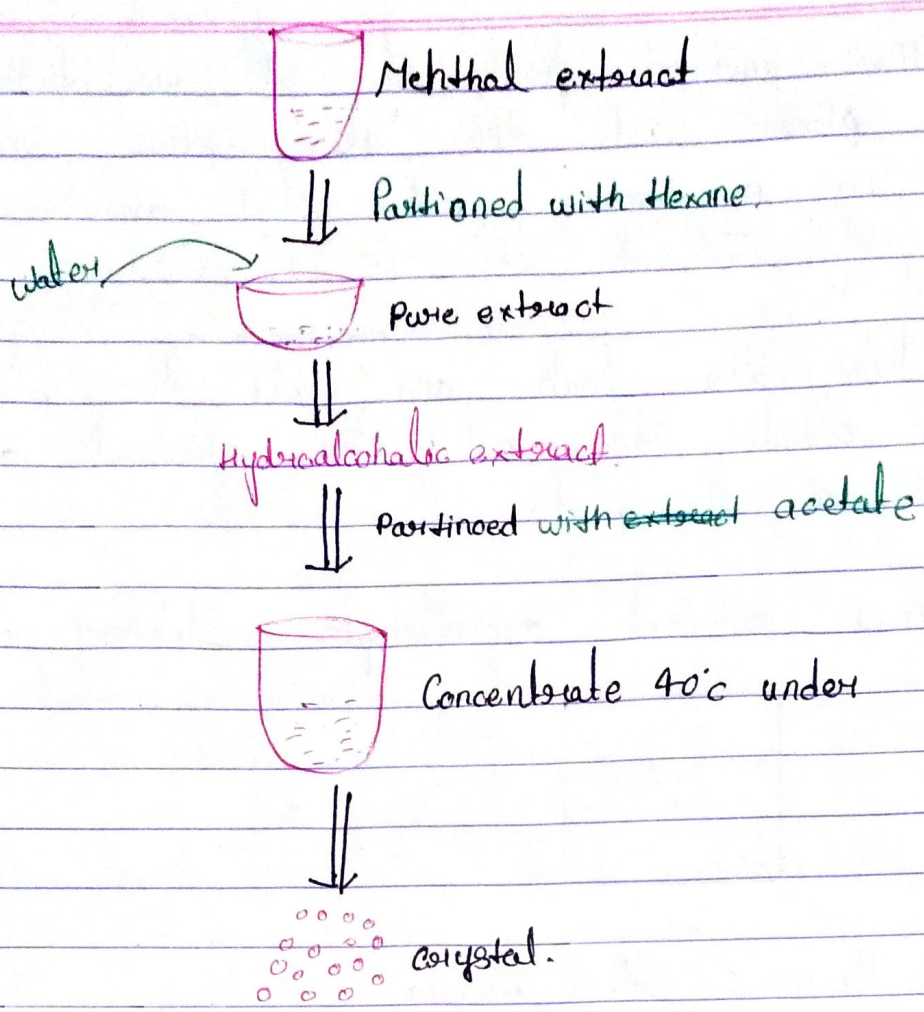
Fresh leaves

⇓ dried at 60°C



Dried powder

⇓ Masceration

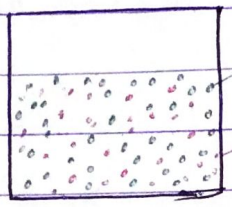


Eastimation/ Analysis - The eastimation of artemisia canbe perform by two method -

[A] HPLC method.
[B] HPTLC Method.

- First of all we make the two both solution. test solution and standard solution.
- The test solution is made by the adding the powder artemisia leaves into the hexane solution and the standard solution is prepared by the adding standard drug into the n- hexane solution.

- In the HPLC method first of all we take the stationary phase and the glass phase and the mobile phase in n-Hexane and ethyl acetate in the ratio of 75 to 25 (75:25).
- Now when take the tank and maintain them add the plate inside the chamber and put the standard and colour sample on the plate.
- And the reagent is used anisaldehyde sulphuric acid reagent



Mobile phase = n Hexane : Ethyl Acetate
75 : 25

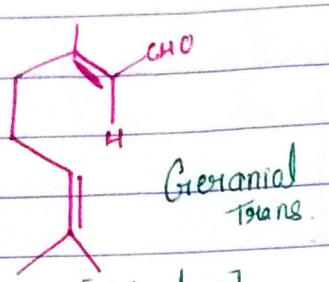
Reagent = Amisaldehyde Sulphuric acid Reagent.

- 110° , 10 - 15 min.

Rf Value = 0.28. standard

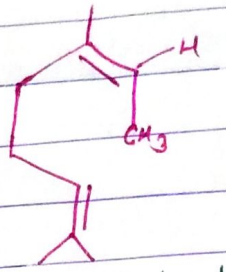
RF value should be 0.28 and the concentration of drug is calculated by the AUC (area under curve method)

[3] CITRAL



Geranial
Trans.

[Citral-A]



Neral Cis.

[Citral-B]

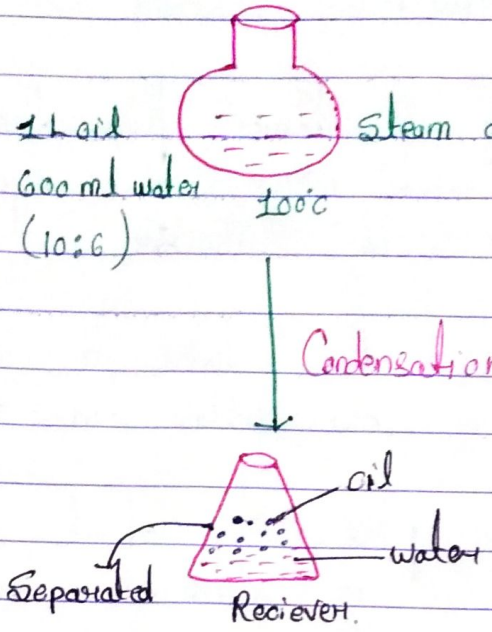
- The citral drug is obtained from the leaves of the plant Lemon gross oil which is cymbopogan citratus and the family is Labeabe
- The citral is the have a Lemony sweet odour and they are yellow in colour.
- In the lemon gross oil about 75-80% citral is present, citral is aldehyde in nature and it is exit into two form. it is terpenoid. it is into two form -

(A) Citral A
(B) Citral B.

- Citral A is Geranial or trans form and citral-B is Neral or Cis form.

Isolation-

Distillation → Lemon gross oil



Step-1 First of all cut the fresh green leaves of lemon gross oil and cut into the pieces and wash and dry.

Step 2- Then after washing by the steam distillation method the lemon gross oil is obtained.

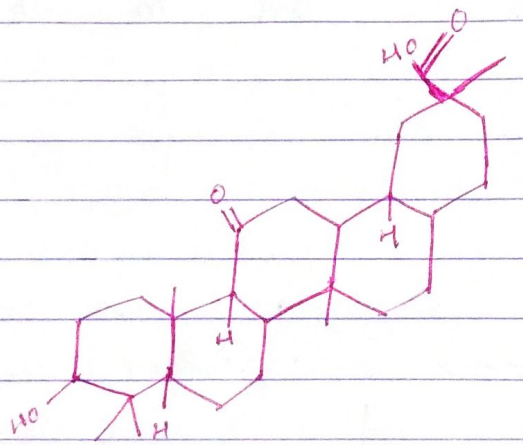
Step 3- Now the lemon gross oil is mixed 1 ltr of lemon gross oil and 600 ml of water in round bottom flask and add reflux condenser and boil at 100°c for steam distillation.

Step 4 - After steam distillation in the reciever we can receive the cold mixture of condensate.

Step 5 - In this condensate the upper layer is oil and the lower layer is water and separate the oil layer and the oil layer is called citral.

GLYCOSIDES

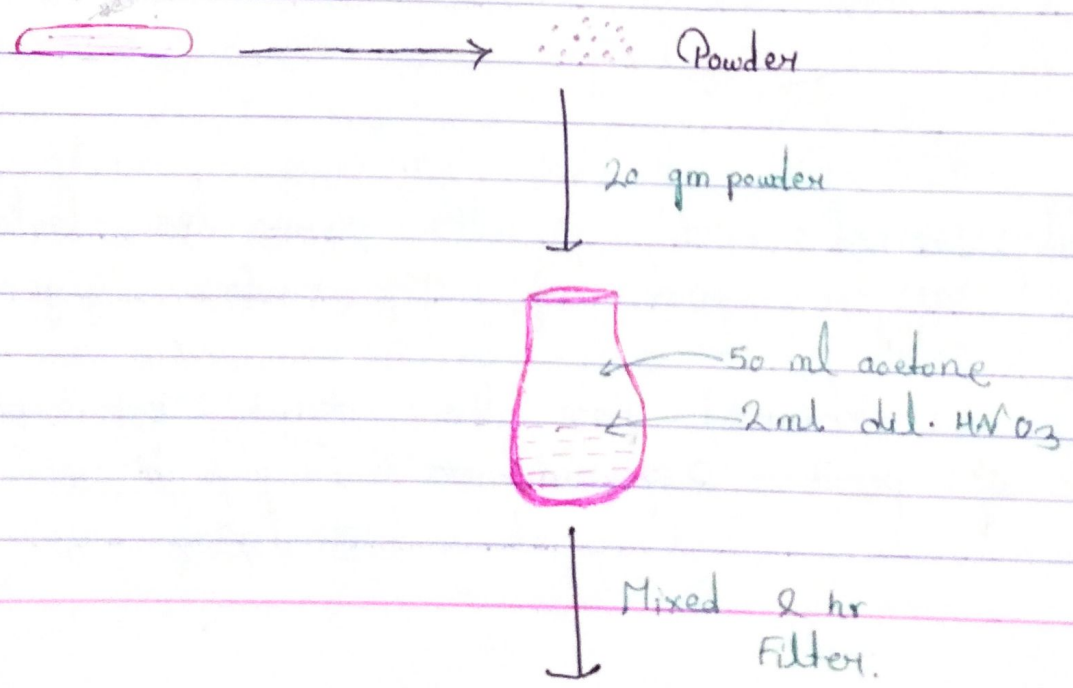
[11] GLYCYRRHETINIC ACID

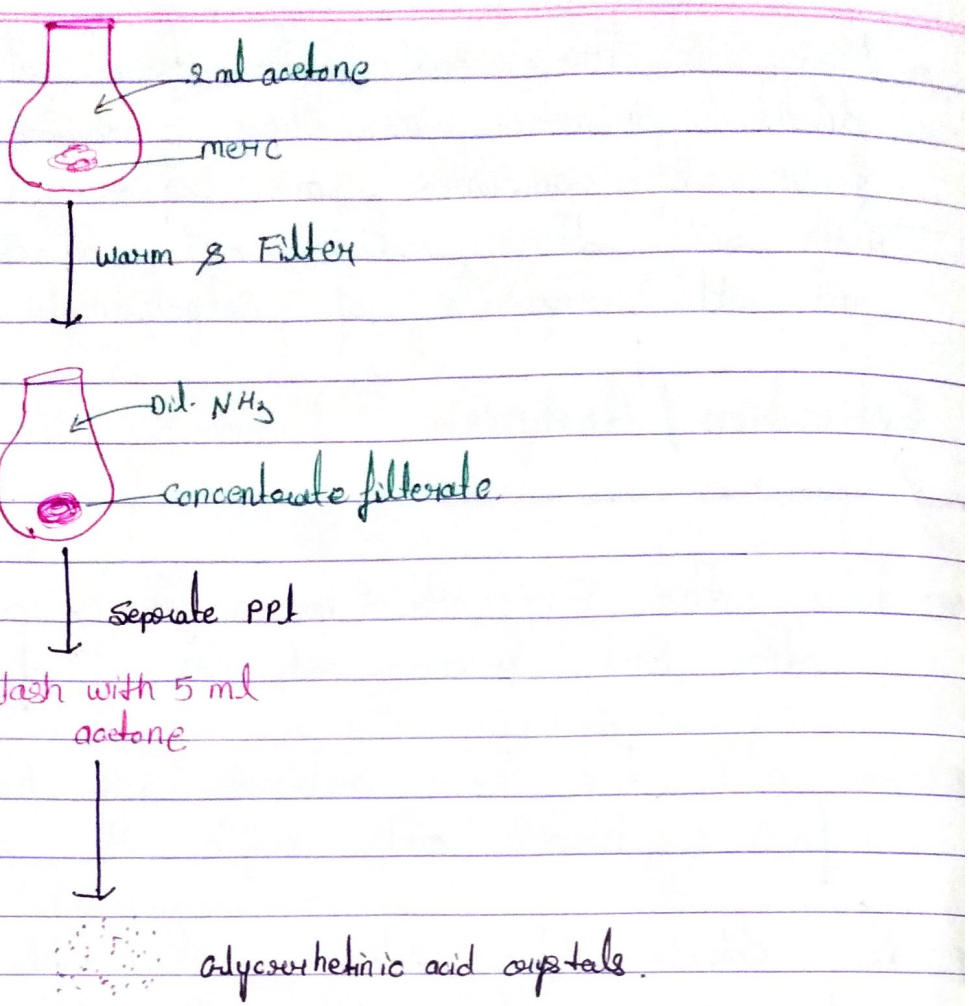


- Glycyrrhetic acid is the pentacyclic triterpenoid aglycon part of glycyrrhetinic glycoside.
- It is obtained from the dried root, pealed root of plant ~~Glycyrrhetinic~~ Glycyrrhizin Glabra Leguminasae.

- In the glycyrrhizin globra the two main active constituent are Glycerrhizin and Glycyrrhizinic acid.
- The Glycerrhizin is the available in two form of salt. it is the available in the Glycerrhizin potassium salt and Glycerrhizin Ca^{++} Salt.
- When the Glycyrrhizinic acid is undergoes the hydrolysis then the ~~Glycyrrhizinic~~ glycosidic linkage acid is breakdown and both the glycon and aglycon part are separated and after the hydrolysis aglycone part Glycyrrhetinic acid is obtained.
- It is used in flavouring agent, it is bitter in taste and it is used in the peptic ulcer and expectorent.

Extraction / Isolation -





- First of all we take the Glycyrrhizin glabra dried peal root and convert into the powder form.
- In the stop cork conical flask 20 gm of powder and 50 ml of acetone and 2 ml of dilute HNO_3 and shake this for 2 hour.
- After shaking 2 hours filter the solution and in the separate flask and add to 20 ml acetone.
- Now again warm the solution though so the water is removes and filter the concentrate form.

- Now in the concentrate form of more, add dilute ammonia so they convert into the salt form of ammonia and after the separate and wash with the acetone and after drying we can get the crystals of Glycyrrhetinic acid.

Estimation / Analysis -

[A] Take the RBF (Round bottom flask). add 25 of glycyrrhetinic acid powder and 250 ml of water.

[B] Now add the Reflex condenser and heat the solution for 6 hour and reflux the material.

[C] Now obtained the extract and add continuously ammonia dropwise and maintain the pH form 6.5 to 7.0.

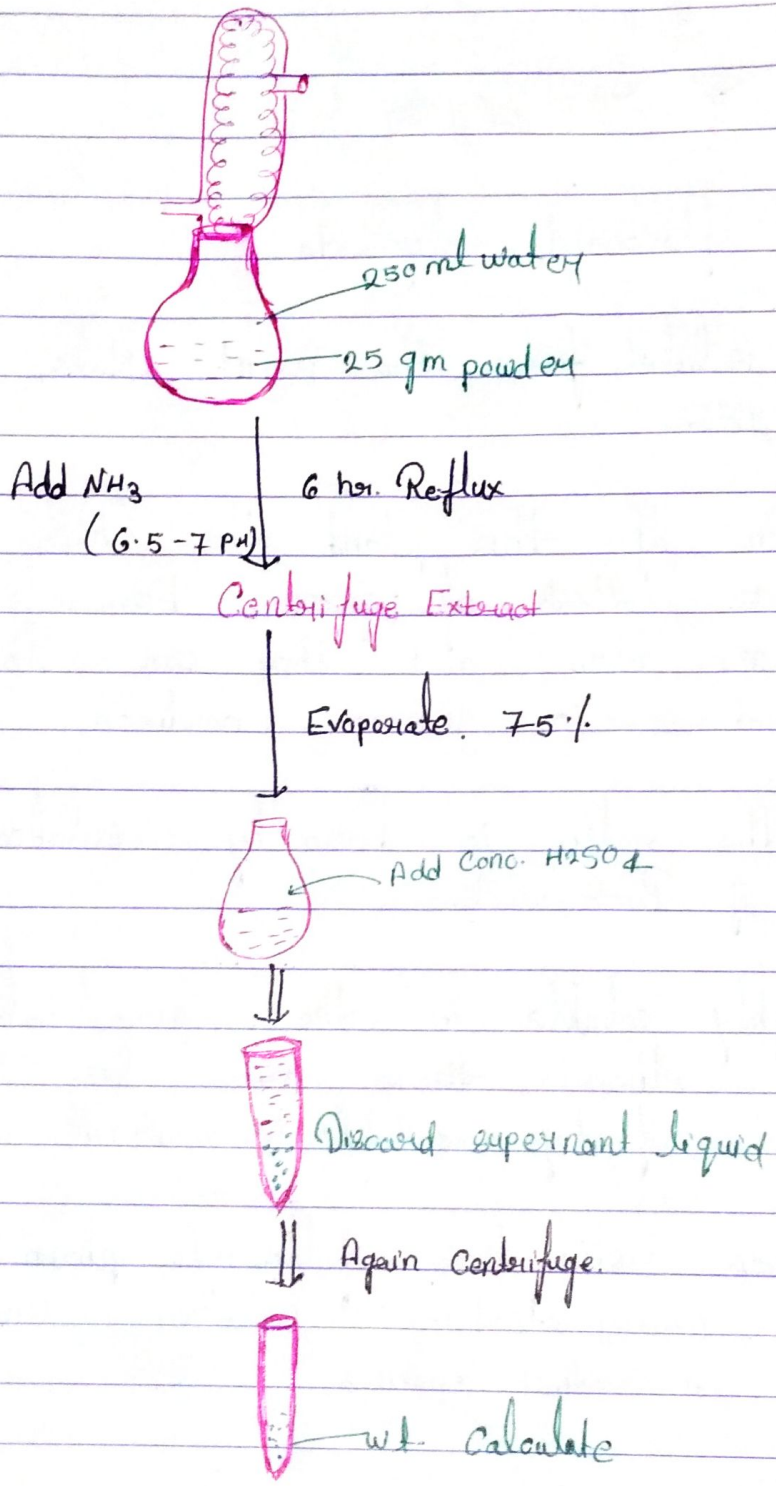
[D] Now centrifuge the extract, by heating evaporate the 75% of solvent.

[E] Now again taken this extract in the round bottom flask and add concentrated H_2SO_4.

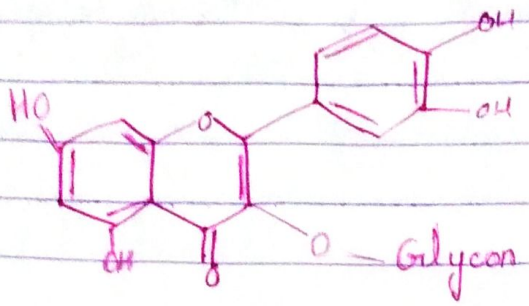
[F] Now again send for the centrifugation and discard the supernatent liquid during centrifugation add $NaCO_3$ to reduce the pH by 4.

[G] Now again send the extract in the next container and maintain the pH again 6 by adding ammonia and after centrifugation the material is obtained.

[4] Now dry this material and calculate the weight from the intial and final weight, we can calculate the percentage of the material.



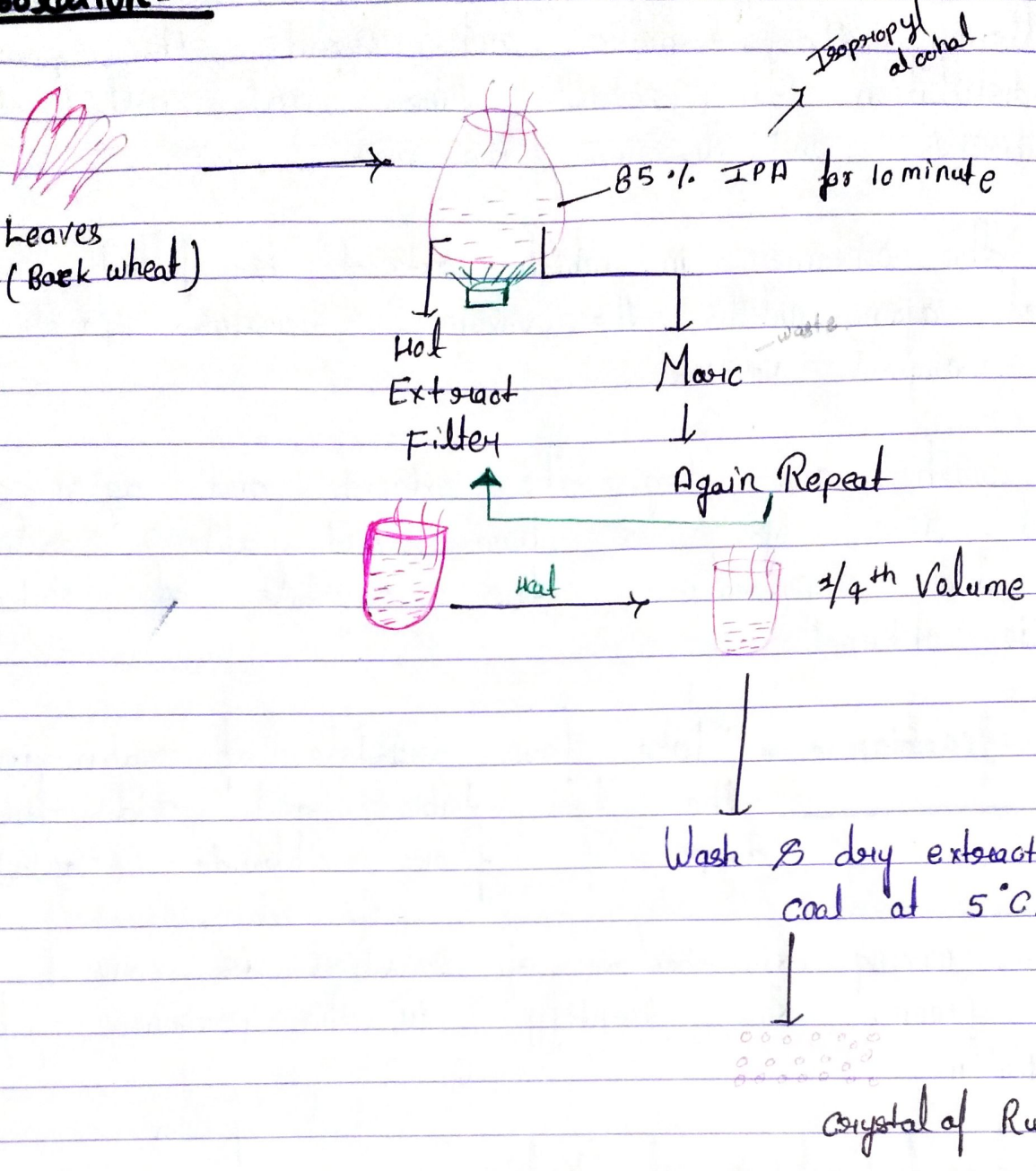
21 RUTIN



- Rutin is the flavonoid Glycoside.
- It is first isolated from the plant Ruta Graveolens is 1860.
- The common name of this plant the Rue and it is also called as Herb of grase because its leaves colour are blue and they can tolerate the high temperature in summer condition.
- In India the rutin is basically isolated from the leaves of Buck weat.
- Rutin are highly soluble in the organic solvents like menthol and ethenol. they are less soluble in cold water but freely soluble in warm water.
- As per evidence rutin have following pharmacological action like neuroprotective. sedative. Analgesic and Anti-diuretic in the nature.

IPA- Iso propyl alcohal.

Isolation-

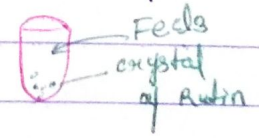


- First of all select the green leaves of Back wheat or Ruta graveolens add the leaves add into the container.
- Add 85 % IPA in the container till the all leaves are somemerc.

- Now remove the filterate in the separate container and collect the marc and repeat this procedure of distillation for several times and collect the filterate and discard the marc.
- Now the container in which extract is filled is heated again until the volume remains 1/4 th of their orginal volume.
- Now wash and dry the extract and again cool at 5°c for 1 hour and after cooling at low temperature the crystals of Rutin is obtained.

Identification -

- Take few crystals of rutin in the test tube and add few drops of feroic chloride ($FeCl_3$).
- After mixing the colour of solution is convert into the green, this identify in the presence of Rutin.

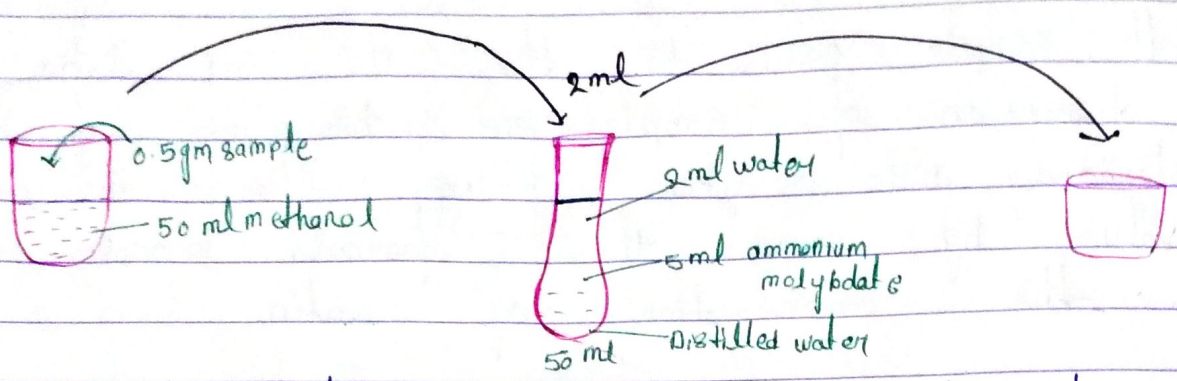


Estimation / Analysis of Rutin -

The estimation of rutin canbe perform by UV spectroscopy -

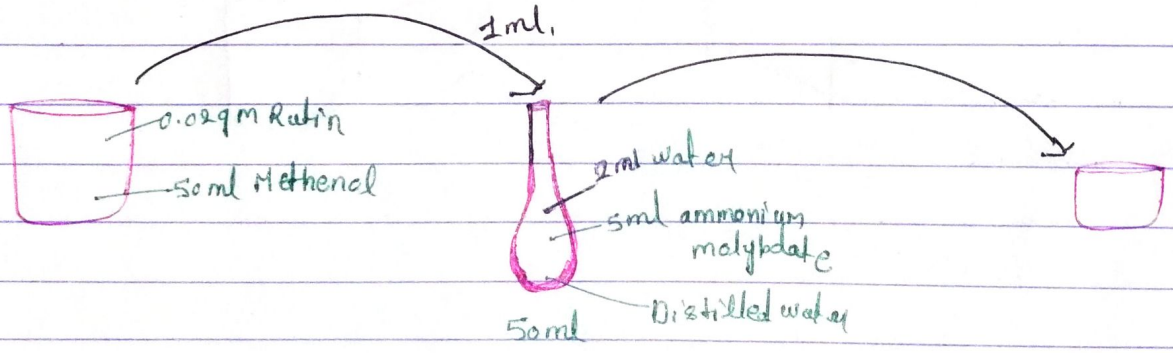
[A] Sample Solution
[B] Standard solution.

Preparation of Sample Solution-



- Take 0.5 gm of sample powder and add into the 50 ml of HPLC methenal.
- After mixing 2 ml of filtrate add into the 50 ml volumetric flask, add 2 ml of water, add 5 ml of ammonium molybdate and makeup the volume with the distilled water.

Preparation of Standerd Solution-



- Take 0.02 gm of standard rutin Powder and add into the acetyl acetate methenal, after take 1 ml of filterate into 50 ml of volumetric flask add 2 ml of distilled water, add 5 ml

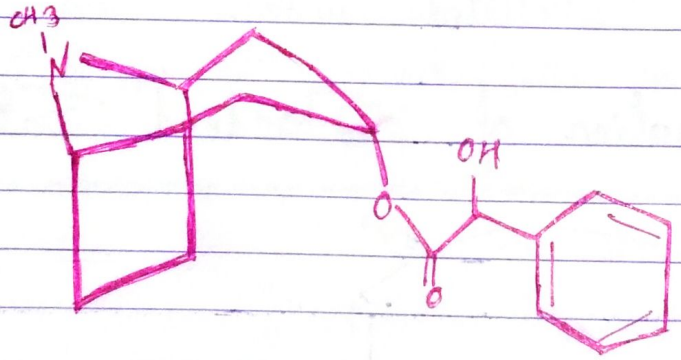
ammonia molybdate and make-up the volume with distilled water.

- Both sample send to the UV and take the absorption of sample and absorption of standard, and after getting the absorption value by using the formula and calculate the concentration of rutin.

V.V. most

ALKALOIDS

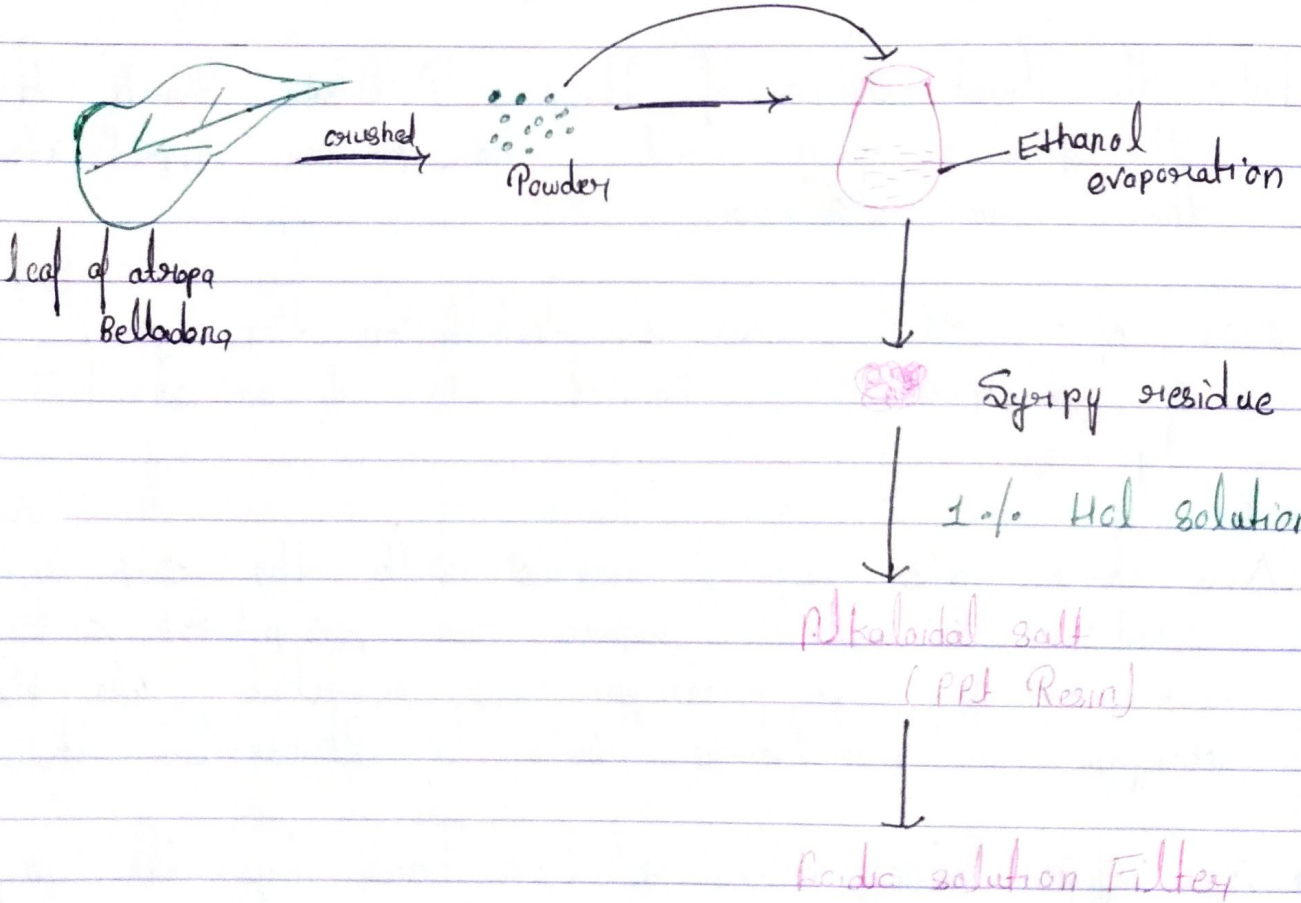
[1] ATROPINE

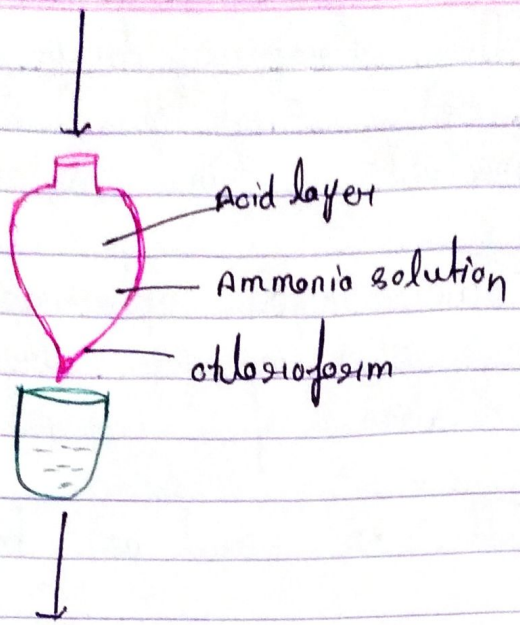


- Atropine is a kind of tropane alkaloids class.
- It is obtained from the dried leaves of the plant Atropa Belladona belonging to family Solanace.

- The atropine drugs are very soluble in ethanol and slightly soluble in chloroform and it is insoluble in water.
- The various pharmacological activities like in the opthalmology mydriasis is present for the dilation of pupil.
- It is used as bradycardiya.
- It is used as a anticholinergic drug so it is used as a antidote of acetylcholine poisoning.

Isolation/ Extraction -





Solvent removed

↓

React with oxalic acid

↓

Atropine oxalate crystal

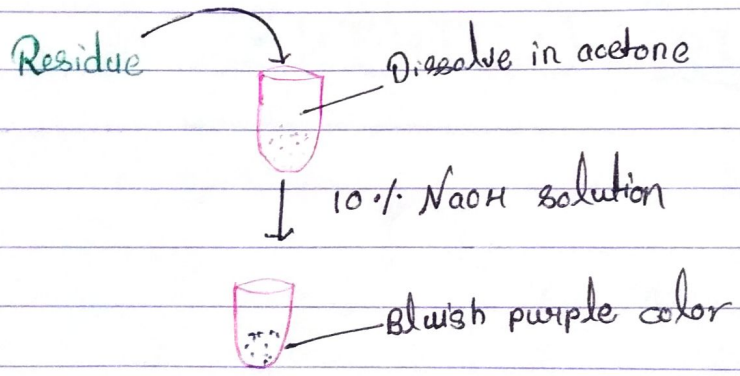
- Take the dried leaves of Atropa Belladona, crush into the powder form and the powder dipped into the ethanol solution.
- Now, after the evaporation distillation the syrupy residue of atropine is obtained, in which resins are present.
- Now take the syrupy residue with the 1% HCl solution, so the resins are precipitated and remove and the atropine is converted into the atropine hydrochloride salt or alkaloidal salt.
- Now filter the acidic solution and by the using of separating funnel, separate into the ammonia

solution and they chloroform solution and the then chloroform solution and the solvent residue is obtained.

- This solvent residue is again evaporated and the solid material is obtained.
- This solid material is react with oxalic acid so the salty crystal of atropine oxalate is obtained.

Identification Test

Ratherasinkam's Test



The atropine can be identify the Ratherasinkam's test, in this test residue is dissolve in acetone in the test tube and after then add few drops of 10% NaOH solution then the solution is convert into the bluish purple color, it means atropine is present.

Eastimation / Analysis -

The atropine canbe estimated by the using thin layer chromatography method. [TLC].

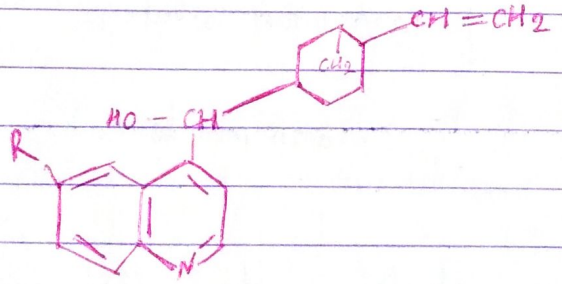
In which the mobile phase and stationary phase are used by calculating the Rf value and concalate the concentration of atropine.

Stationary Phase - Silica Gel G

Mobile Phase - Acetone : Water : Ammonia solution
90 : 7 : 3

V.V.most

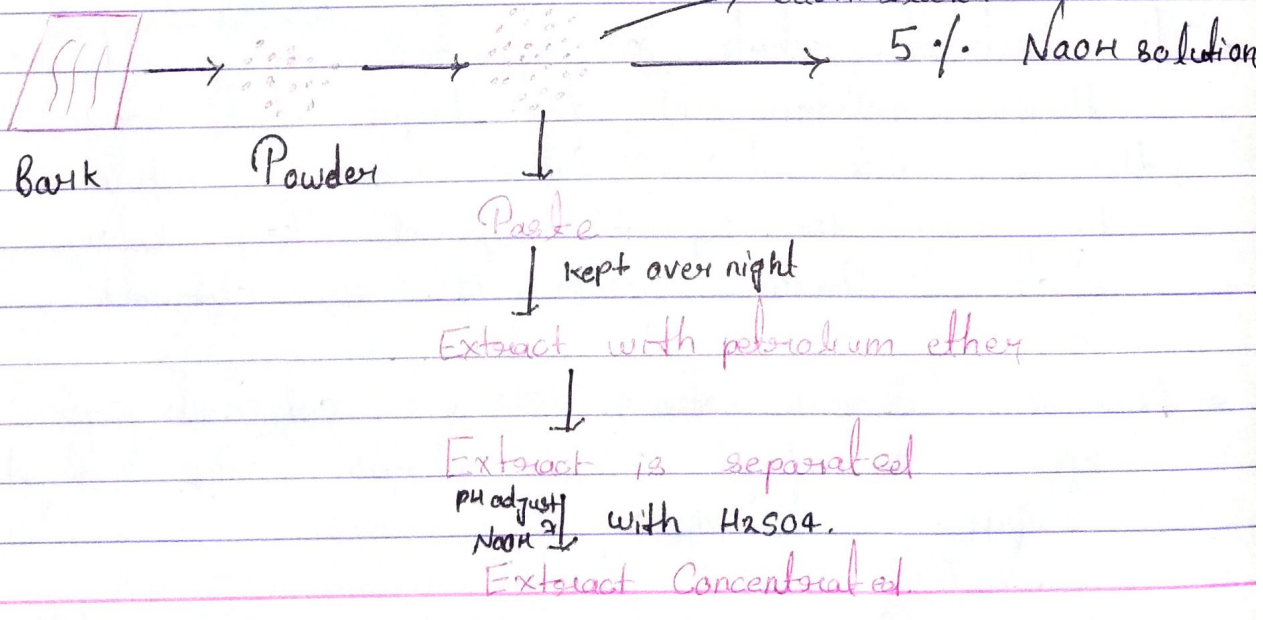
[2] QUININE



- The drug quinine is belongs to the class quinoline alkaloids.
- It is obtained from the dried bark of the plant Cincona officanalis belonging to family Rubiaceae.

- In the bark of the Cincona officanalis about 25 types of different - different alkaloids are present in which four are most important - Quinine, Quinidine, Cinchonine, and Cinchonidine.
- The quinine and quinidine molecule, cinchonine and cinchonidine molecule are stereo isomer to each other.
- Quinine is soluble in the benzene, chloroform, propyl alcohal and Glycerol alcohal and it is highly soluble in the peteralium ether.
- It is used as the antiparasitic and antimalerial drug it is effective against all species of plasmodium like plasmodium falsiperum, plasmodium ovale, Plasmodium qulex and plasmodium malate.

Isolation-



↓

Cool and Centrifuge

↓

Crystals of quinine SO_4

↓ warm with dil. H_2SO_4, dil NH_3

Filtered and separated

↓

Dried weight.

- Take the dried bark and crush into the powder form.
- In the dried powder of quinine add calcium hydroxide powder and with the help of 5% NaOH solution make into the paste.
- Kept the paste for overnight to dry.

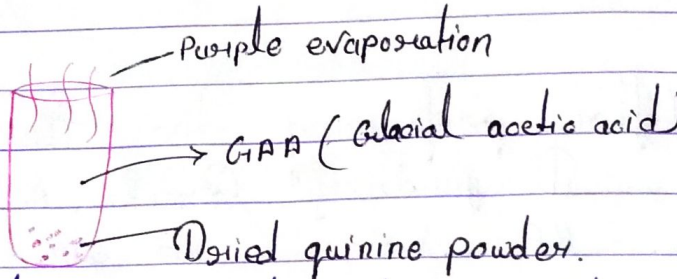
Now the paste is extracted with petrolium ether with the help of separating funnel.

- The extract which is separated, now add dilute H_2SO_4 solution and few drops of NaOH to adjust the pH, so the solution becomes alkaline and by using the separating funnel keep extract untill the alkaline extract is not obtained.
- Now the alkaline extract is cool and centrifuge and after centrifugation we obtain the crystals of quinine sulphate.

- Now this quinine sulphate is again warm with the solution of diluted H_2SO_4 by adding few drops of ammonia, then filter, separated and dry.
- They we obtained the crystals of quinine.

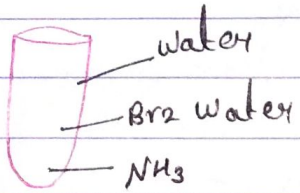
Identification-

[1]



Take a testtube add few gm of dry powder of quinine, and added few drops of GAA and make the solution after heating the vapours of purple colour is produced, this indicates the presence of quinine.

[2] Thalloquinine Test-



Take few gm of quinine powder add distilled water, add Br_2 water and add amonia and after heating it convert into green colour, it indicates the presence of quinine.

Estimation / Analysis - The estimation of quinine is done by the thin layer chromatography method.

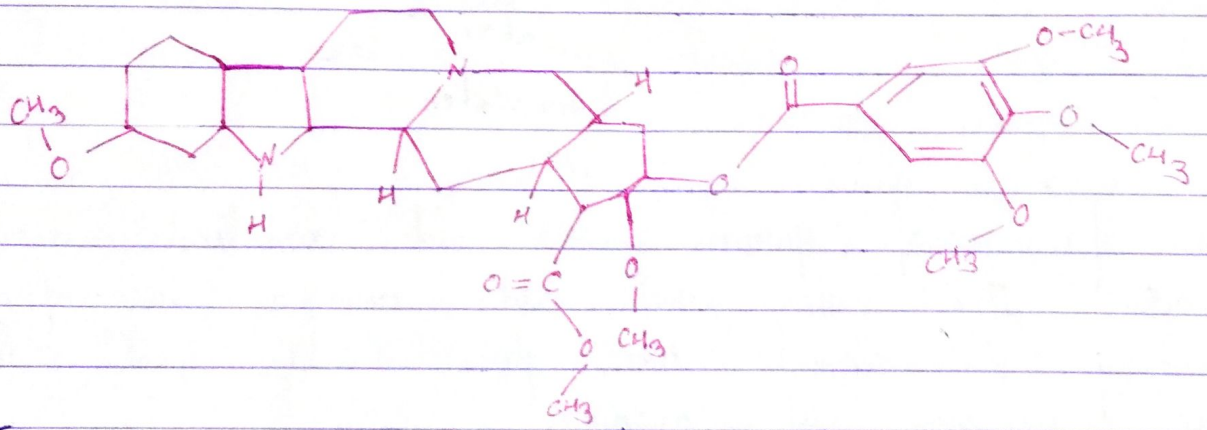
On this technique the Silica Gel G is used and the mobile phase the solution of mixture of chloroform, methanol and ammonia is used in the ratio of 60 : 10 : 1.

Mobile Phase = Chloroform : Methenol : Ammonia
60 : 10 : 1

After performing the TLC test the visualizing agent Dragendraff Regent is used and calculating the RF Value. we can calculate the concentration.

[3] RESERPINE

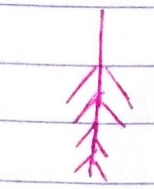
Imp



The reserpine is the alkaloid of Indole class.

- It is obtained from the dried root of Rauwolfia Serpentina belonging to family Apocynaceae.
- It is commonly known as Sarpgandha in India. which is used for the snake bite and fever.
- It is soluble in the chloroform, methylene, chloride, GAA and Benzene.
- It has different pharmacological action along with the snake bite action, such as it is used as antihypertensive drug and thiazide diuretics.

Isolation -



Root → Powder → Powder | Benzene soxhlet

10% Na_2CO_3 Solution

Extract

Ether | Evaporate

Concentrated extract

Dil. HCl | Separating funnel

Acid layer separate

Add NH_3

Further extract with CH_2Cl_2 (chloroform)

Chloroform layer is washed with 10% Na_2CO_3

Residue dissolve, in CH_3OH

cool.

Crystal of reserpine.

- First of all take the dried root of Sarpgandha and convert into powder form.
- This powder is mixed in the 10% Na_2CO_3 solution, after this wet powder are now going for the Soxhlet extraction with using solvent benzene, then we obtained the extract.
- Now add few drops of ether in the extract and evaporate the extract to get the concentrated extract.
- Now in concentrated extrat is adding into separating funnel with the dilute HCl and separating the acid layer is obtained.
- Now this acid layer is evaporated and add few drops of NH_3 till the solution becomes alkaline.
- Now extract is further separated with the chloroform ($CHCl_3$) and the $CHCl_3$ layer is washed again with 10% Na_2CO_3.
- Now the residue is obtained and dissolve in the methenol and send to the refrigerator for deep freezing then after cooling obtained the crystal of reserpine.

Identification and Estimation - The identification and estimation test of reserpine is done by the TLC method

By using this method first of all we prepare the stationary phase in which the TLC plate with silica gel G apply.

S phase - TLC plate with silica Gel G.

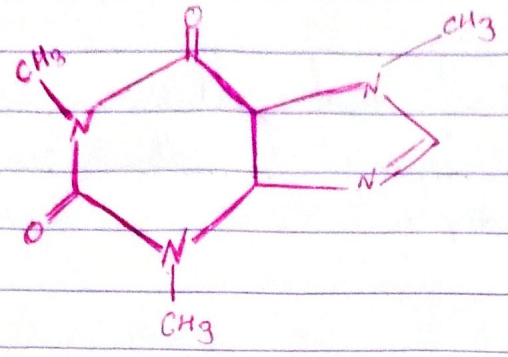
And in the TLC chamber the mobile phase solvent is used chloroform and acetone in the ratio of 7:3 and after development of chamber and running the solvent of over the plate, the visualising agent basically Anisaldehyde sulphuric acid reagent is used.

Mobile Phase - chloroform : Acetone
7 : 3.

Visualising agent - Anisaldehyde sulphuric acid reagent.

It canbe estimated with the help of non aqueous titration

[4] CAFFIENE



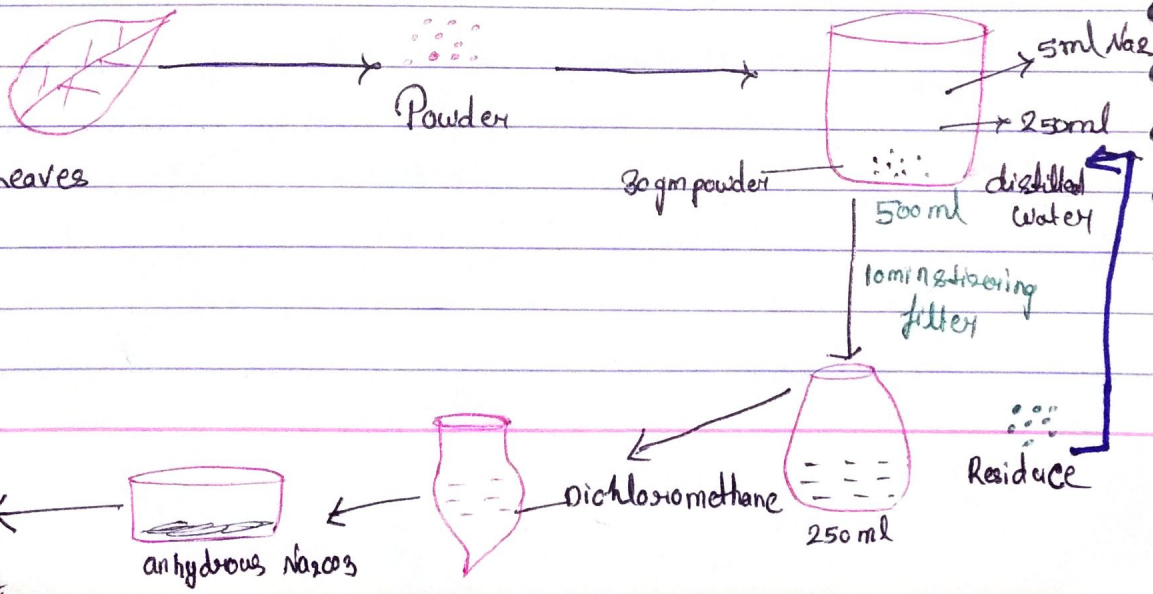
- It is the purine alkaloide drug.
- Caffiene is obtained from the different - different concentration in different different plant.

Example -

Coffee seed	–	Coffea arabica	1-2%
Tea leaves	–	Camellia Sinensis	1-5%
Mate leaf	–	Liex paraguvensis	0.2 - 2%

- It has different pharmacological action in our body, it act as CNS stimulant, it act as the cardiac muscle stimulant and it act as respiratory stimulant.
- It is soluble in pyrrole, petrolium ether and tetrahydro furon.

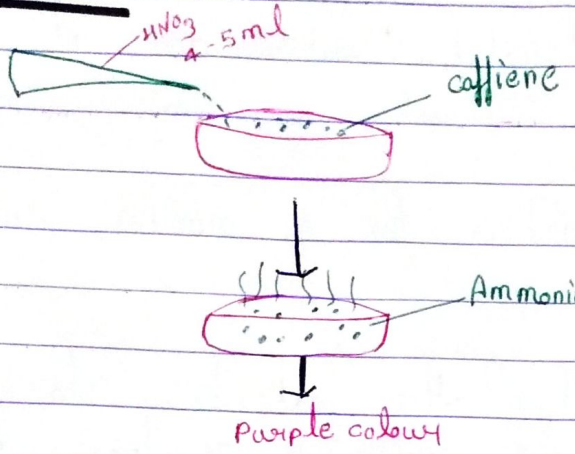
Isolation -



- First of all take the dried leaves of coffiene and crush into the powder form.
- Now take the 30 gm of powder in 500 ml of beaker, add 250 ml distilled water and add 5 ml of Na_2CO_3 solution.
- Now stirr this mixture for 10 minutes and boil for 10 minute.
- After boiling filter this filterate into 250 ml of conical flask and remaining residue. is again go for filteration and coming the extract in 250 ml conical flask.
- Now this 250 ml solution is add into the separating funnel with the help of dichloromethane extract in the separating funnel.
- Now the caffiene material is dissolve into the dichloromethane layer and this dichloromethane layer is powr into the separte container.
- This container contains the Na_2CO_3 crystals which is used as absorbent and the the absorb the moisture in the dichloromethane layer is present.
- Now dichloromethane layer is clear, now powr into the other container, heat evaporate and collect the residue this residue is filter and washed with ethanol and the crystal of coffiene is obtained.

Identification Test -

Murexide Test -



- Take a porslet dish, add the 4 to 5 crystals of caffiene and add 4 to 5 ml of HNO_3 solution.
- After adding HNO_3 solution, and fumes starts appear and the fumes becomes stop then add ammonium hydroxide solution and the mixture is convert into the purple colour, then this purple colour is identified in the presence of Caffiene.

Estimation - Estimation of caffiene is perform by the TLC method.

In this method the stationary phase is silica Gel G, add the mobile phase is uses three mixture of acetone, methenol and water in the ratio of 100: 13: 5: 10 and for visualising agent KI Chamber is used.

The RF value is calculated and with the help of RF value concentration of caffiene is calculated.

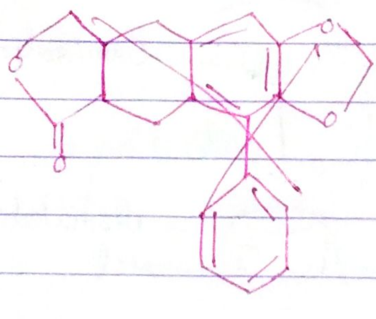
S Phase – Silica Gel G

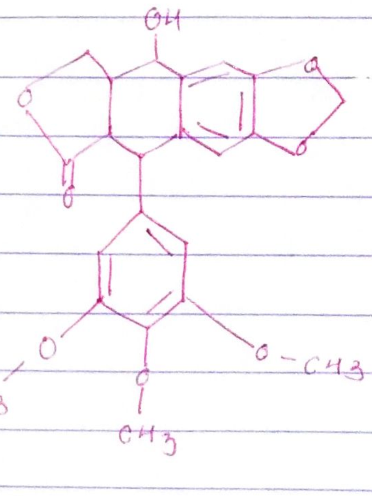
Mobile Phase – Acetone : Methenol : Water
100 : 13.5 : 10.

Visualisingagent – KI chamber

RESINS

PODOPHYLLOTOXIN

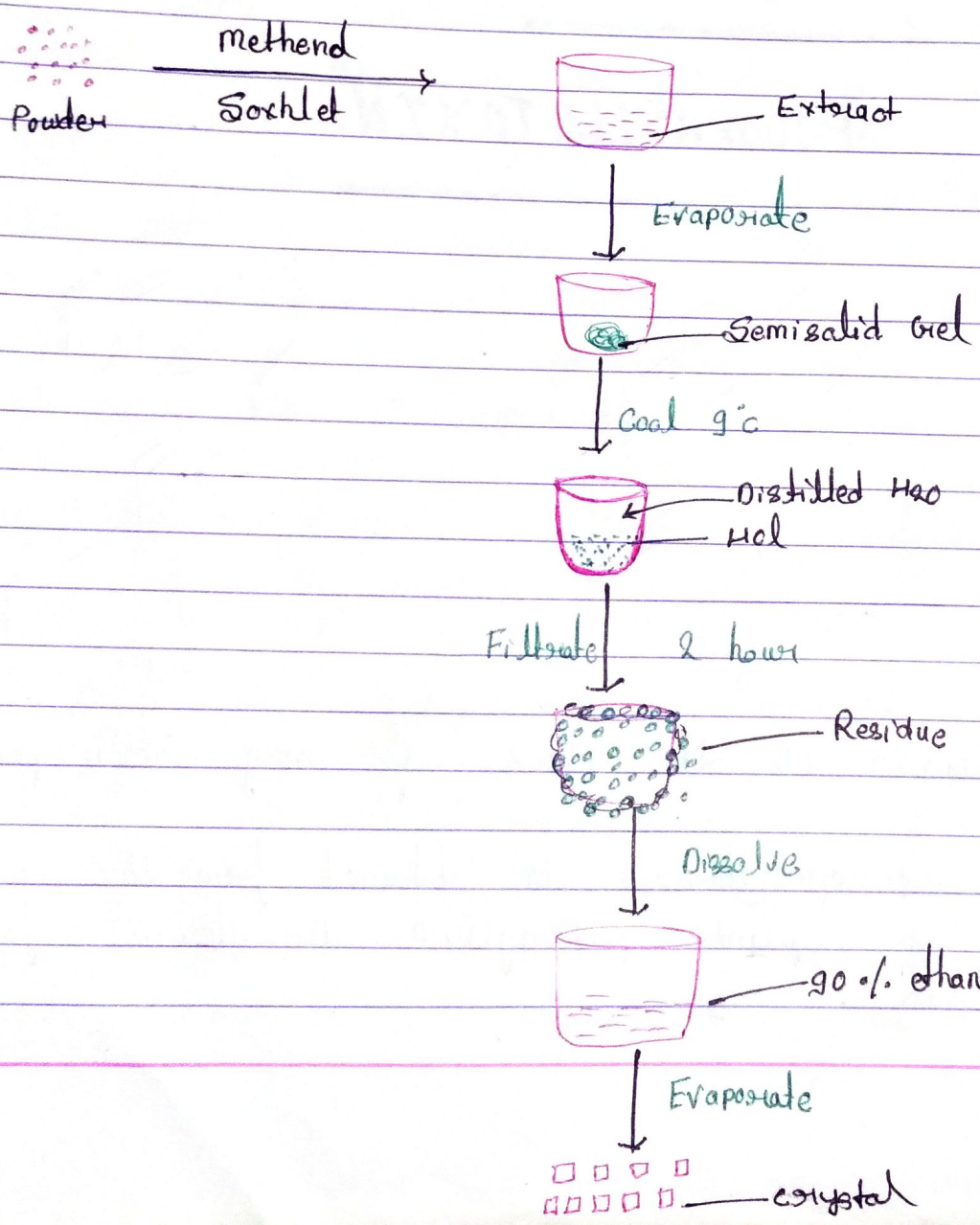




- Podophyllotoxin is the class of drug resin podophylli
- The drug podophyllotoxin is obtained from the root and rhizome of plant podophyllum hexanderum family Berberidaceae.

- Podophyllumtoxin drug is used as anticancer drug which is used in the several types of cancer such as Testicular cancer, Lungs cancer, Leukemia and ovarian cancer.
- It is insaluble in ethyl ether and it is slightly saluble in water.
- It is saluble in acetone and benzene and it is very highly saluble in ethanol and chloroform.

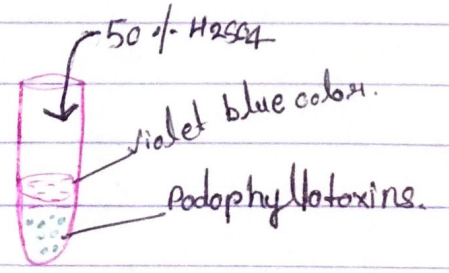
Isolation -



- Take the powder form of podophyllum drug and by using soxhlet apparatus and using methenol as a solvent, extract is obtained.
- Now evaporate this extract and get the residue or semisolid gel residue.
- Now in this semisolid residue add few drops of Hcl and distilled water and allow to cool at 5°c for 2 hours.
- After 2 hour the crystalline residue of podophyllum is obtained, now this residuce is dissolve into the 90% ethanol solution and after evaporating the ethanol we can get the crystals of podophyllotoxin

Identification -

In identification test, take few crystals of podophyllotoxin in a test tube and add 50% H_2SO_4 solution and after dissolution violet blue colour is appear which indicates the presence of podophyllotoxins.



Estimation/Analysis - The estimation or analysis of podophyllotoxins perform by the TLC method.

In this TLC method the stationary phase used, reverse phase 18, 60°F 2545 TLC plate.

Mixture of acetonitrile and water in the ratio of 4:6 and after calculating the RF value is obtained about 0.41 then it is pure as per IP.

Stationary Phase - RP 18 60F° 2545 - TLC plate.

Mobile Phase - Acetonitrile : Water
4 : 6

RF Value = 0.41