Practical Phytochemistry

Laboratory manual

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Course contents:

Wk No.	Topic	Reference in the textbook
1	- Laboratory safety and basic laboratory operation	Laboratory manual p.3
2	**Extraction methods** - Explain all the techniques and apparatuses that will be used to extract the active constituents of the plants - Microscopical identification for calcium oxalate	Laboratory manual p.15
3	**Cardio-active glycosides** - Explain the concept of cardio-active glycosides - List the part and scientific plant name - Learn extraction method - Discuss tests used for identification - Analyze and evaluate the result of work - Perform good laboratory report writing	Laboratory manual p.21
4	**Anthraquinone glycosides** - To study the microscopic characters of Senna - Review the meaning of anthraquinone glycosides - List the part and scientific plant name - List the solvents used in extraction - Discuss tests used for identification - Analyze and evaluate the result of work - Perform good laboratory report writing	Laboratory manual p.29
5	**Saponin glycosides** - Review the meaning of saponin glycosides - List the part and scientific plant name - List the solvents used in extraction - Discuss tests used for identification - Analyze and evaluate the result of work - Perform good laboratory report writing	Laboratory manual p.37
6	**Extraction of tannins** - Review the meaning of tannins and its classification - List the part and scientific plant name - List the solvents used in extraction - Discuss tests used for identification - Analyze and evaluate the result of work - Perform good laboratory report writing	Laboratory manual p.43
7	**Identification of flavonoids** - Review the meaning of flavonoid glycoside and its classification - List the part and scientific plant name - List the solvents used in extraction - Discuss tests used for identification	Laboratory manual p.48

	- Analyze and evaluate the result of work - Perform good laboratory report writing	
8	**Extraction of Piperine** - Review the meaning of alkaloids glycoside and its classification - Identification of piperine from black pepper - Isolation and identification tests of caffeine from coffee and tea - List the solvents used in extraction - Analyze and evaluate the result of work - Perform good laboratory report writing	Laboratory manual p.54
9	**Extraction of Caffeine** - Review the meaning of alkaloids glycoside and its classification - Identification of caffeine from tea and coffee - Analyze and evaluate the result of work - Perform good laboratory report writing	Laboratory manual p.60
10	**Volatile oils** - Review the meaning of volatile oils and its classification - List the part and scientific plant name - List the solvents used in extraction - Discuss tests used for identification - Analyze and evaluate the result of work - Perform good laboratory report writing	Laboratory manual p.65
11	**Isolation of citric acid as calcium citrate** - Review the meaning of citric acid. - List the part and scientific plant name - List the solvents used in extraction - Discuss tests used for identification - Analyze and evaluate the result of work - Perform good laboratory report writing	Laboratory manual p.68
12	**Isolation of pectin** - Review the meaning of polysaccharides. - List the part and scientific plant name - List the solvents used in extraction - Discuss tests used for identification - Analyze and evaluate the result of work - Perform good laboratory report writing	Laboratory manual p.72

Lab 1 Laboratory safety and basic laboratory operation

Part (A) Safety

1-General Instructions

1. Use small quantities as much possible.
2. Clean the test tubes as soon as you finish the test. It is easier to clean a wet tube than a dry tube.
3. Never taste the chemicals used since some substances are poisonous. Also never touch the chemicals with your hand. Always use a spatula.
4. Smoking is not allowed in the laboratory.
5. When smelling substances do not hold your face directly over the container.
6. Working with toxic, lachrymator or irritating chemical must be conducted in fume hoods. In some cases a trap must be used to prevent hazardous gases from escaping into the laboratory atmosphere.
7. Do not point your test tubes at your neighbor or yourself or yourself when heating substances.
8. Most organic solvents are flammable, so never heat flammable substances with direct flame. A hot water bath is used instead.
9. Experiments should never be left unattended.
10. Know the location of fire extinguishers and how to use them.

2-Laboratory Instructions

1. Throw all solids to be discarded into a waste-paper basket. Never throw matches, filter paper, broken glass, or any insoluble chemicals into the sink. Organic liquids should not be poured into the sink either they are collected in special residues bottle.
2. Read the label twice before using a reagent bottle.
3. The reagent bottles on the side shelves should not be carried to your bench. Pour the solution you require into a beaker and at your bench measure the amount you need. This prevents crowding at the side benches.
4. Never return chemicals to the stock bottles.
5. Do not lay the stopper of a bottle down, as impurities will be picked up and contaminate the stock solution. Also, close reagent immediately after use.
6. At the end of each laboratory period, leave your glassware clean and the top of your bench clean and dry.
7. Study the experiment and read the directions carefully before coming to the laboratory. Know what you are going to do and why, and to be ready to answer questions on the experiment.

3-Reporting results

Students are required to record all data and observations collected during the experiment on the report sheets provided for that purpose.

4-Hazard symbol and designations

The symbols illustrated and described below (pictograms) are intended as optical aids on the type of danger involved with the products. E.g. highly "flammable" is represented by a flame symbol.

- **Explosive:**

Danger: this symbol warms that certain substances are liable to explode.
E.g.: Ammonium dichromate.
Caution: Avoid impact, knocks, friction, sparks fire and heat.

- **Oxidizing (fire-promoting):**

Danger: Such substances can cause flammable substances to catch fire or promote already burning substances hence impede fire fighting.
E.g: Potassium permanganate, sodium peroxide.
Caution: Avoid all contact with flammable substances.

- **Highly Flammable:**

1- Spontaneously flammable substances. E.g.: Aluminum alkyls, Phosphorous.
Caution: Avoid contact with air.
2- Gases, liquefied gases ignitable in air. E.g: Butane, propane.
Caution: Avoid formation of f1ammable gas-air mixtures and keep away from sources of ignition.
3- Substances sensitive to moisture that form flammable gas-air mixtures and keep away from source of ignition.
Caution: avoid contact with moisture or water.
Liquids: with flash points below 21°C but still not "extremely flammable".
Danger class Al (see flammable liquids and danger classes). E.g: Acetone.
Caution: Keep away from naked names, sources of heat and sparks.

- **Very toxic:**

Danger: Inhalation, swallowing or absorption through the skin can cause severe illness and in certain cases death.
E.g.: Thallium and associated compounds, Aniline
Caution: Avoid all contact with the body; do not inhale; if feeling unwell consults a doctor immediately.

- **Harmful:**

Danger: these substances can cause some discomfort if absorbed in to the body.
E.g: Pyridine, oxalic acid.
Caution: Avoids contact with the body; do not inhale; if feeling unwell consults a doctor.

- **Corrosive:**

Danger: Living tissue, but also laboratory equipment, can be damaged by contact with these chemicals.

E.g: Bromine, sulfuric acid, nitric acid.
Caution: Do not inhale vapors and avoid all contact with skill, eyes and clothing.

- **Irritant:**

Danger: Substances with this symbol irritate skin, eyes and respiratory organs.

E.g.: Benzyl chloride, 2-amino ethanol.

Caution: Do not inhale vapors and avoid contact with eyes and skin.

5-First aids

- **Burns:**

In case of burns, use picric acid solution, tannic acid ointment or acriflavine ointment.

- **Acids:**

Wash the skin quickly with water than with sodium bicarbonate solution and again with water. Vaseline or acriflavine ointment should be added.

- **Alkalis:**

Wash the skin with water then with 1% acetic acid. Apply acriflavine ointment.

- **Organic materials:**

Wash the skin with alcohol, then soap and water.

- **Skin cut:**

Wash the skin with alcohol or Dettol®. Add sulfanilamide powder and bandage. If the cut is deep, a suture is necessary.

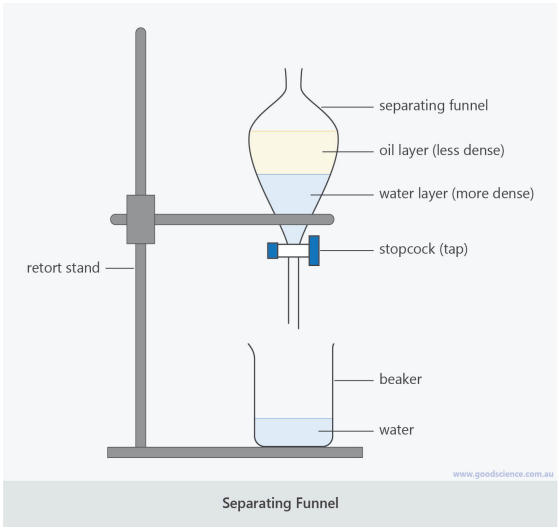
- **Eyes:**

If any chemical comes to the eye, wash with water or boric acid solution. Medical aid should be asked immediately.

Part (B) basic laboratory operation

1. Separatory funnel

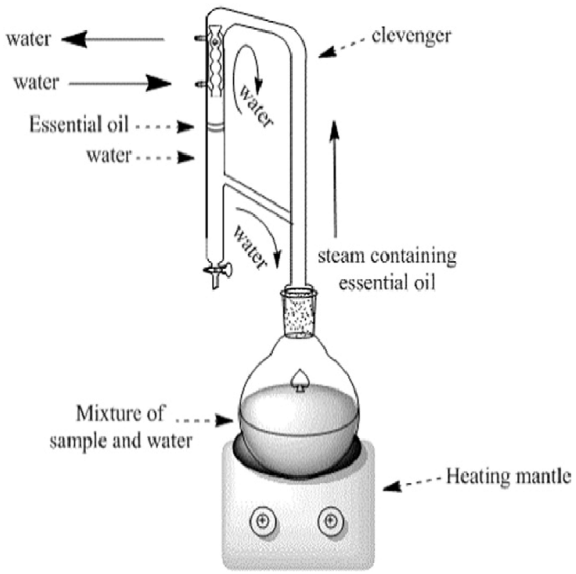
Used in liquid-liquid extractions to separate (partition) the components of a mixture into two immiscible solvent phases of different densities



Separatory funnel

2. Clevenger apparatus

Used for extraction of essential oils from a mixture of plant and water

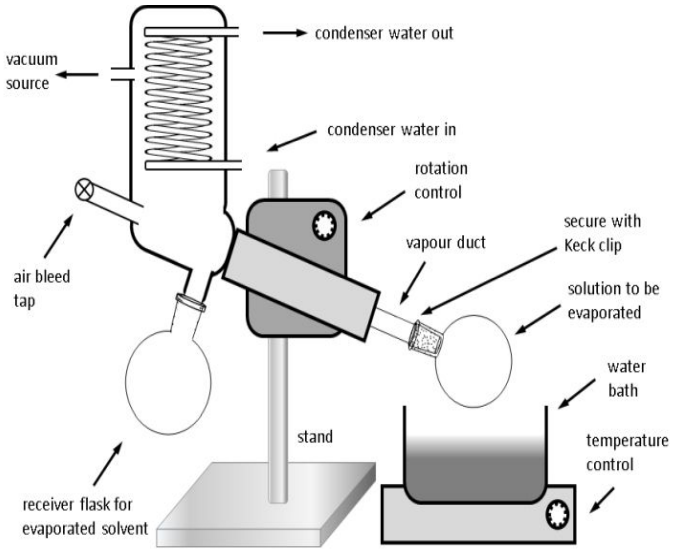


Clevenger apparatus

3. Rotary evaporator

Used in chemical laboratories for the efficient and gentle removal of solvents from samples by evaporation.

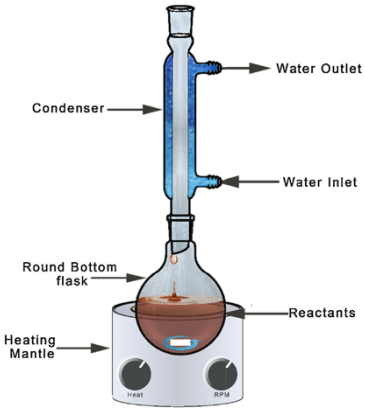
The purpose of distillation is to separate a given mixture into its components based on their respective volatilities, through the process of evaporation and condensation (liquid-gas-liquid).
1. to concentrate non-volatile components in a mixture (for example, concentrating the purest and freshest flavors from a blood orange by removing the water);
2. to extract the volatile aroma and flavor molecules from mixtures gently and at low temperatures (for example, extracting the desired flavors from a blend of alcohol, herbs, and fruit without heating the mixture up).



Rotary evaporator

4. Reflux

Is a distillation technique involving the condensation of vapours and the return of this condensate to the system from which it originated.
It is used in chemistry to supply energy to reactions over a long period

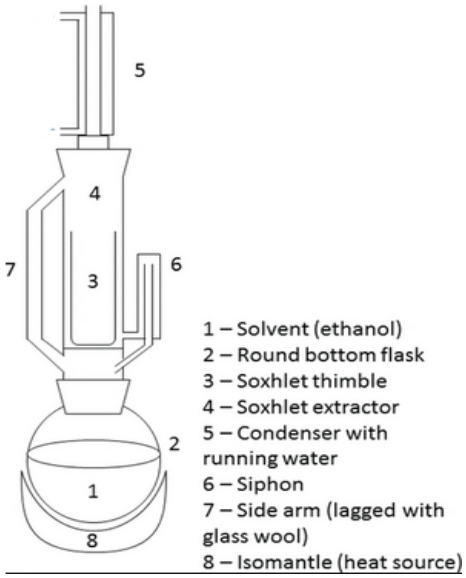


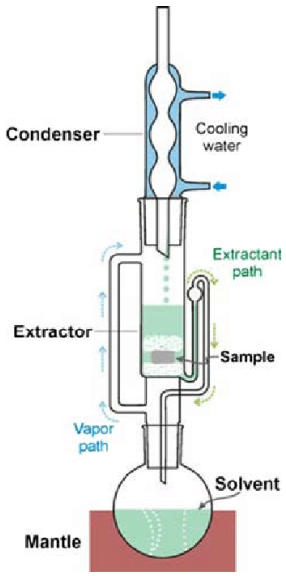
Reflux apparatus

5. Soxhlet extraction

When a compound of low solubility needs to be extracted from a solid mixture a Soxhlet extraction can be carried out. The technique places a specialised piece of glassware in-between a flask and a condenser. The refluxing solvent repeatedly washes the solid extracting the desired compound into the flask.

A Soxhlet Extractor has three main sections: A percolator (boiler and reflux) which circulates the solvent, a thimble (usually made of thick filter paper) which retains the solid to be laved, and a siphon mechanism, which periodically empties the thimble.





Sohxlet apparatus

6. Thin layer chromatography (TLC)

Is a method for identifying substances and testing the purity of compounds.

TLC is a useful technique because it is relatively quick and requires small quantities of material.

Separations in TLC involve distributing a mixture of two or more substances between a stationary phase and a mobile phase.

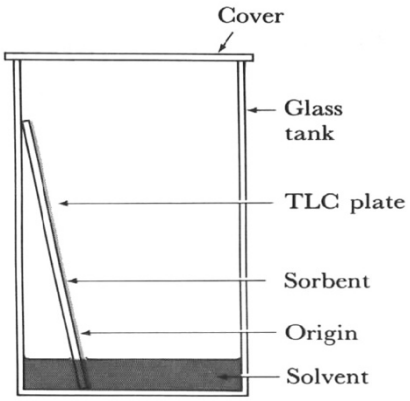
The stationary phase:

Is a thin layer of adsorbent (usually silica gel or alumina) coated on a plate.

The mobile phase:

Is a developing liquid which travels up the stationary phase, carrying the samples with it.

Components of the samples will separate on the stationary phase according to how much they adsorb on the stationary phase versus how much they dissolve in the mobile phase.



Thin layer chromatography (TLC)

7. Microscopical examination

Preparation of drugs for microscopical examination and general use of reagents The following aims should be kept in mind for the microscopical examination of advanced crude drugs:

1. The determination of the size, shape and relative positions of the different cell and tissues.
2. The determination of chemical nature of the cell walls.
3. The determination of the form and chemical nature of cell content.

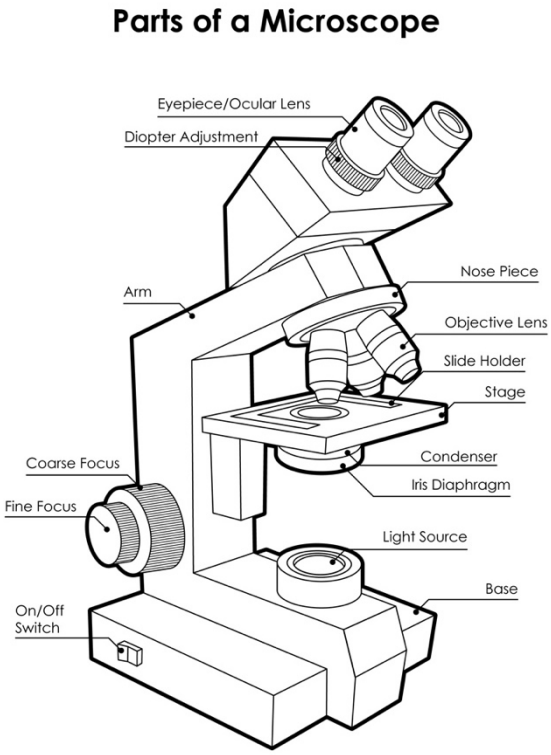
Disintegration serves for the isolation of the specific tissues and bleaching and defatting techniques for observing deeply colored materials and fatty seeds respectively. Almost certainly, clearing reagents will be required together with the range of suitable stains for cell wall and cell contents.

Reagent used as mounting reagent:
Chloral hydrate reagent: dissolve 80 gm of chloral hydrate in 20 ml water, a valuable and widely used reagent, heating at water bath and maceration of the plant powder well give a better result since defatting and clearing action of the reagent well be better, this reagent well dissolve many cell content as fat, resin starch and calcium oxalate.

Phloroglucinol/ HCl reagent: a 1% solution in 90% ethanol with conc. HCl as a test for lignin. Lignified structured well be colored pink/red.
Hydrochloric acid is a powerful clearing agent and it will dissolve many cells content as ca oxalate and starch.

Diluted ethanol reagent: different strength are used for preserving material and for hardening, alcohol acts as a clearing reagent by dissolving oils, resins, chlorophyll, etc., while it does not dissolve gum and mucilage (therefore it is useful for plant powder containing them).

Iodine reagent: this gives blue color with starch and hemicelluloses.
Smith's reagent: is suitable mounting reagent for starch, it is composed of equal amount of water, glycerin and 50% acetic acid.



Videos: basic laboratory operation

A. Reflux:

1. **https://youtu.be/_SzYqPdUkFQ**

2. **https://youtu.be/5Cnyf1k4pUA**

B. Rotary evaporator:

1. **https://youtu.be/FkBhsZy39Ck**

2. **https://youtu.be/jdMUJkKpx_0**

3. **https://youtu.be/zObkDGldsus**

C. Soxhlet extraction:

1. **https://youtu.be/tUDdkU2Of4s**

2. **https://youtu.be/mLq35x0g46g**

D. Clevenger apparatus:

1. **https://youtu.be/nZFjmbRrkek**

E. Solvent Extraction:

1. **https://youtu.be/C11H_1Bxzs0**

F. Thin-Layer Chromatography (TLC):

1. **https://youtu.be/qdmKGskCyh8**

2. **https://youtu.be/w0FyQ_tO15U**

G. Extraction:

1. **https://youtu.be/j2F5SvBsOdE**

H. Microscope Parts, Function, and Care

1. **https://youtu.be/pujis3QsA_I**

Report Sheet **Date:**

Title:		
Student name:		**Section:**
Objective:		
Apparatus name	**Drawing**	**Uses**

Report Sheet **Date:**

Title:		
Student name:		**Section:**
Objective:		
Apparatus name	**Drawing**	**Uses**

Report Sheet **Date:**

Title:		
Student name:		**Section:**
Objective:		
Apparatus name	**Drawing**	**Uses**

Lab 2 Extraction Methods and Microscopical identification for calcium oxalate

A. Extraction methods

Extraction involves the separation of the medicinally active constituents of plants or animal tissues from the active or inert component by using solvent (s) and by using one of the standard extraction procedures.

The products that obtained from plants are relatively impure liquids, semisolid or powders, intended only for oral or external use. These total extractive products are called Galenical, Which came from the name Galen, the 2nd century Greek physician.

Methods of extraction can be divided into:

1- **Cold Methods.**
2- **Hot methods.**

1. Cold extraction methods:

Is the process whereby a substance is extracted from a mixture via cold solvent. The procedure carried out at room temperature (15-25 °C).

A. Maceration:

This simple widely used procedure involves leaving the pulverized plant to soak in a suitable solvent in a closed container .simple maceration is performed at room temperature by mixing the ground drug with the solvent (drug solvent ratio : 1:5 or 1:10) and leaving the mixture for several days with occasional shaking or stirring. The main disadvantage of maceration is that the process can be quite time-consuming, taking from a few hours up to several weeks.

B. Percolation:

Percolation (from Lat. percōlāre, to filter) concerns the movement and filtering of fluids through porous materials. The powdered plant material is soaked initially in a solvent. In a percolator, additional solvent is then poured on top of the plant material and allowed to percolate slowly (drop wise) out of the bottom of the percolator. Additional filtration of the extract is not required because there is a filter at the outlet of the percolator.

2. Hot Extraction Methods:

A. Infusion:

Infusion is the process of extracting chemical compounds or flavors from plant material in a solvent such as water, oil or alcohol by allowing the material to remain suspended in the solvent over time. In this procedure we have special container called 'Infusion pot' which contain sieves and cover with heavy lid. After the addition of the solvent ,boiling water, left for a while for the extraction of active constituent during that time the volatile oil evaporated with steam and condenses on the lid, after that we take the solvent which contain the active constituent.

B. Decoction:

The term dates back to 1350–1400 ,from present participle stem of Latin decoquere (meaning to boil down), de "from"+ coquere "to cook". Decoction is a method of extraction by boiling, of dissolved chemicals, from hard plant material, which may include stems, roots, bark and rhizomes on a source of heat or direct flame then agitating until the active constituents will be dissolved in the solvent. Here the solvent used depend on the active constituent and source of heat e.g.

chloroform and ether can't be used because we used direct source of heat. In addition to that the active constituent should be heat stable.

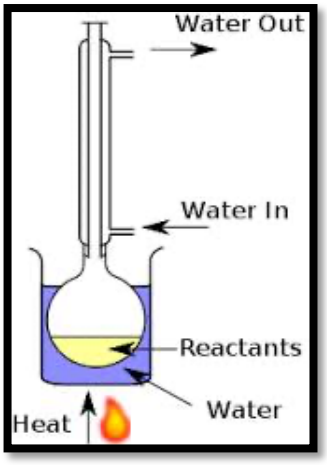
C. **Digestion:**

In this method the plant material is placed together with the solvent and application of gentle heat, so that the solvent will increase its power for extraction and this method is used in cases were moderately elevated temperature is required. e.g. Tea is the brew made from the leaves of the Camellia sinensis plant. It is the beverage most consumed worldwide, after water.

D. Contiuous hot extraction methods:

a) Reflux condenser:

Plant material is immersed in a solvent in a round-bottomed flask, which is connected to a condenser. The solvent is heated until it reaches its boiling point. As the vapor is condensed, the solvent is recycled to the flask.



b)Soxhlet apparatus:

The plant powder is placed in a cellulose thimble in an extraction chamber, which is placed on top of a collecting flask beneath a reflux condenser. A suitable solvent is added to the flask, and the setup is heated under reflux. When a certain level of condensed solvent has accumulated in the thimble, it is siphoned into the flask beneath.

-The main advantage of Soxhlet extraction is that it is a continuous process for the extraction of active constituents decomposed by direct heat.

C)Clavenger:

In this method we used a special apparatus which is called 'Clavenger', it is used mainly for extraction of volatile compounds, e.g. orange peels has been used for the extraction of orange oil.

Clevenger Apparatus
(Oil heavier than Water)

Clevenger Apparatus.
(Oil lighter than water)

The student will learn in laboratory how to extract Piperine from black pepper Using Soxhlet extractor and rotary evaporator

B. Calcium Oxalate

Calcium oxalate are found in plants as a result of interaction of oxalic acid (a metabolic product) with calcium salt; e.g. Ca-sulphate and the consequent precipitation upon super saturation of the cell sap with this salt. Different types of calcium oxalate crystals with various shapes can be used as a diagnostic element for plant identification. It is usually sufficient to describe the general form and size of the crystals, without reference to a crystallographical class, the most common form encountered are prisms (senna, hyoscyamus, liquorices); rosettes (rhubarb, senna, clove); bundles of acicular crystals (squill); microsphenoidal or sandy crystal (belladonna). When calcium oxalate is present, it is important to record the type of the crystal, shape and distribution. The cells containing calcium oxalate differ from those which don't contain calcium oxalate in size, form or content, and are often called idioplast.

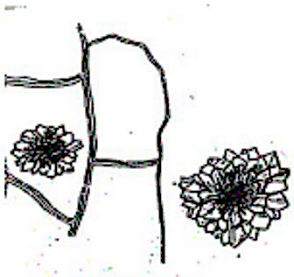
Procedure: Clear different plant powders using chloral hydrate solution and examine the various types of calcium oxalate crystals.

The crystals can be identified as calcium oxalate if they are insoluble in acetic acid and caustic alkali, but soluble in hydrochloric acid and sulfuric acid without effervescent.

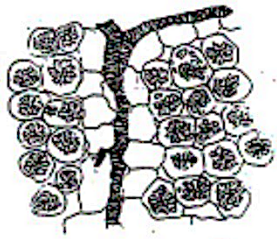
Acicular crystals.


Crystal sheath.


Raphides.


Clusters


Microsphenoidal crystals
(Idioblast)


Crystal layer

Report Sheet **Date:**

Title:		
Student name:		**Section:**
Objective:		
Type of Ca-oxalate	**Drawing**	**Reagent**

Lab 3 Glycosides ISOLATION and identification tests of cardiac glycoside (digoxin)

Objective: To extract and identify Digoxin

Glycosides:

a. Compounds containing a carbohydrate (sugar) and a non-carbohydrate molecule (called the aglycone).
b. Classification according to the sugar unit such as glucose = glucoside, rhamnose = rhamnoside, etc. (a term hence formed by dropping "ose" and adding "oside."
c. Also classification schemes as specific properties:
    1. Simple phenolic compounds existing as Arbutin found in Bearberry leaves.
    2. Cardioactive—steroid-like, act on heart, some poisonous; Digitalis (Digitalis purpurea), Lily of the valley (Convallaria majalis).
    3. Anthraquinone— Senna, Frangula, Cascara, Aloe
    4. Saponin:
        a. common characteristics—bitter taste, hemolytic activity, forms stable foams when shaken in water
        b. Notable actions—hepato-protective, adaptogenic, immuno-modulating, hormonal modulation, anti-microbial, antiinflammatory, expectorant (stimulant to mucosal secretions), diuretic, tonic.
        c. Common to plants such as—Siberian ginseng, gentian, Liquorice, Korean ginseng, American ginseng, Sage, Comfrey, Dandelion, Fenugreek (antiinflammatory, hypotensive, hypoglycemic)
    5. Isothiocyanate—Mustard
    6. Flavonol—Ginkgo, Milk thistle, Hawthorne berry
    7. Alcohol—salicin from Willow (Salix), Poplar (Populus), wintergreens
    8. Aldehyde—Vanilla

Physical Properties of Glycosides:

a. Glycosides are crystalline or amorphous substances.
b. Glycosides are soluble in water or alcohols and insoluble in organic solvents like benzene and ether.
c. The aglycone part is soluble in organic solvents like benzene, chloroform or ether.

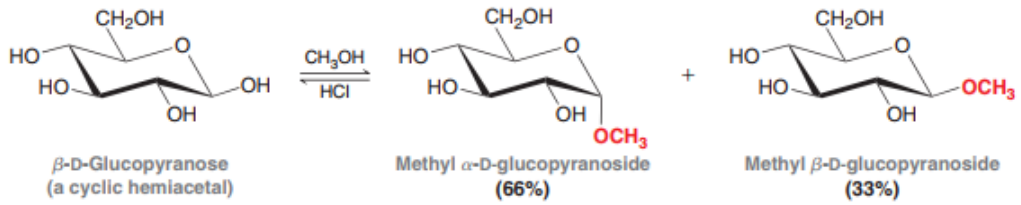
Chemical properties of Glycosides:

a. Glycosides do not themselves reduce Fehling's solution, the simple sugars which they produce on hydrolysis will do so with precipitation of red cuprous oxide.
b. Glycosides are hydrolysed into sugar and another organic compound by boiling with mineral acids (Except the C-Glycoside which requires more vigorous conditions as oxidative hydrolysis).
c. Glycoside are also hydrolysed by enzyme specific for the type of glycosidic linkage example: emulsin of almond kernels, and myrosinase of the black mustard.

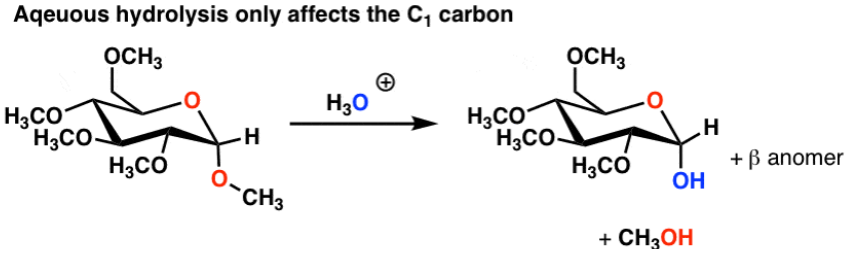
Extraction and Isolation of Glycosides

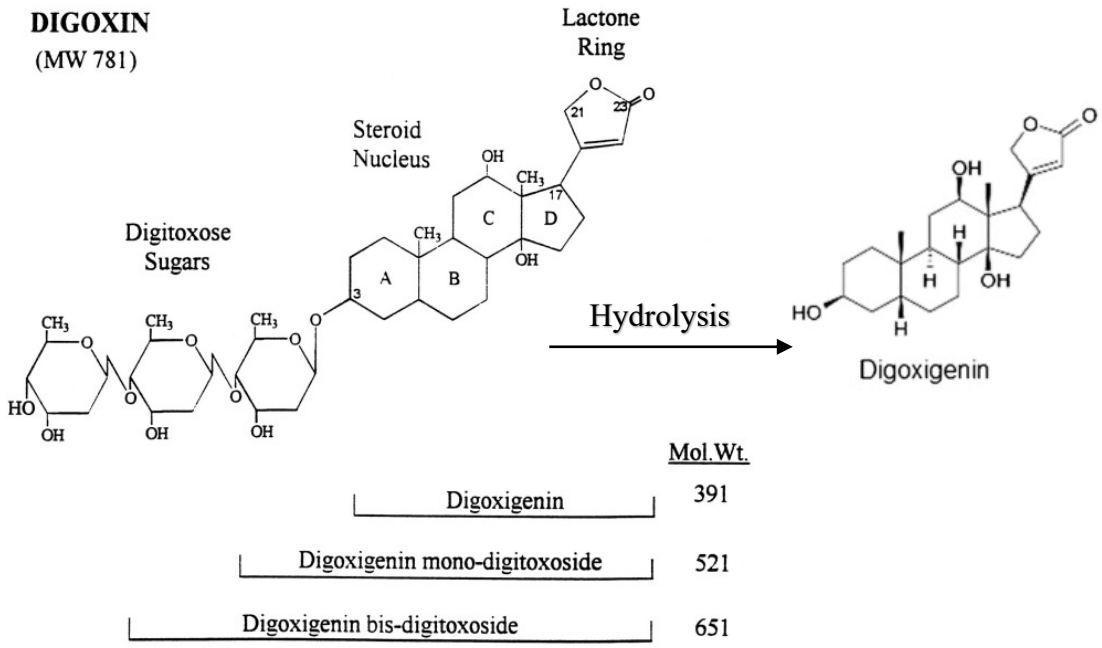
a. Polar solvent, usually alcohol, is used in the isolation and extraction of glycosides.
b. Various enzymes present in plant parts are deactivated due to the use of heat.
c. The thermolabile glycosides, however, should be extracted at temperature preferably below 45°C.
d. The extract is treated with lead sub acetate to precipitate tannins and thus eliminate non-glycosidal impurities.
e. Neutral pH should be assured before and during extraction because as acidity may result in hydrolysis, and this is done by adding a base as CaCO3.
f. Defatting of fat-rich organs (e.g. seeds) before extraction is an important step as high amounts of lipids hinder glycoside extraction. The defatting process is usually carried out using petroleum ether.

--formed when a solution of glucose in boiling methyl alcohol is treated with 0.5% HCl as a catalyst.



--Hydrolysis of Glycoside





Structure of digoxin

-Digoxin: is a cardiac glycoside (or steroid glycoside)

-found in the **foxglove** plant species: ***Digitalis lanata*** .

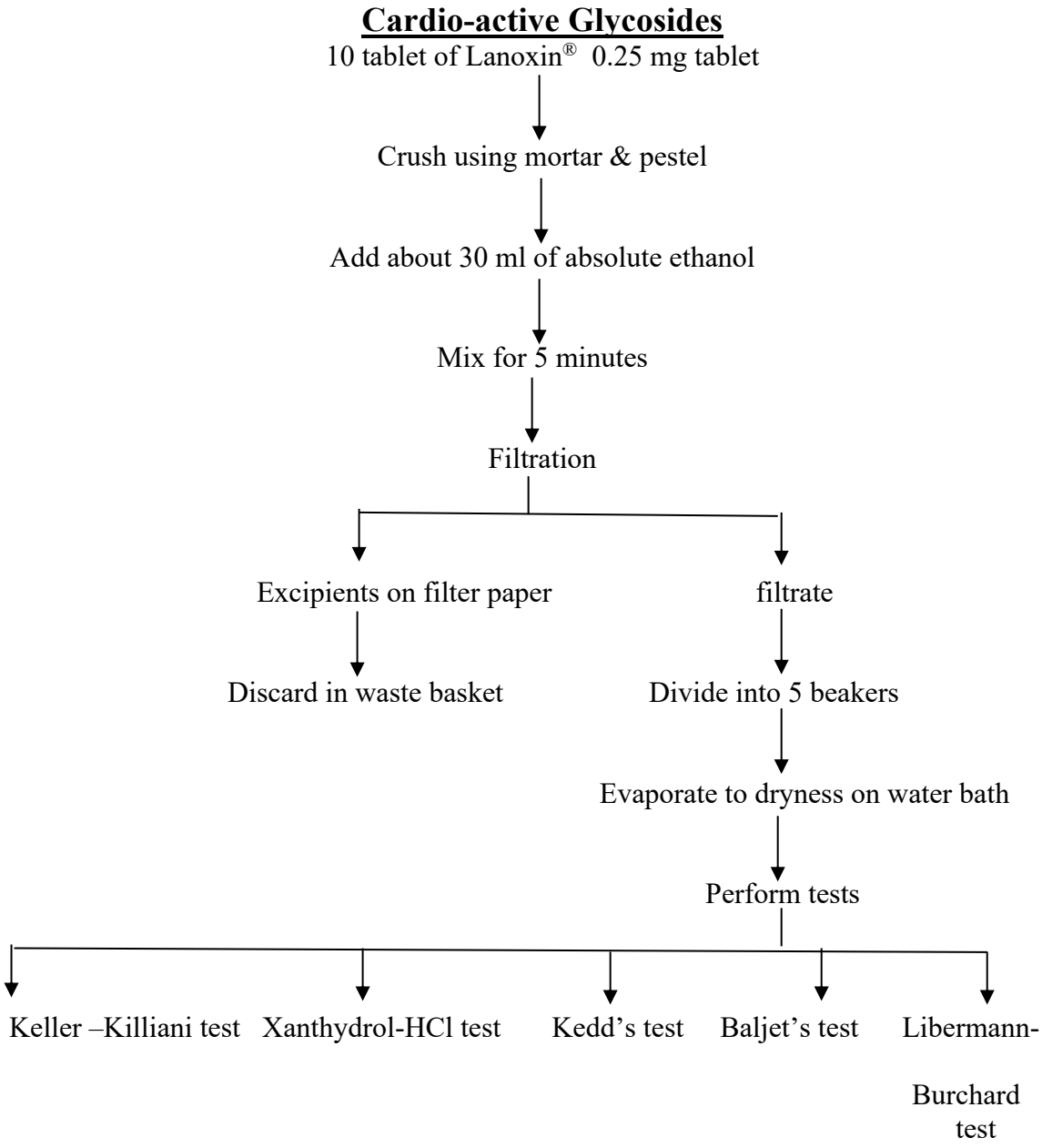
-Digoxin's involves inhibition of the Na+/K+ ATPase in the heart. It is a potent drug used in the treatment of congestive heart failure CHF.

-Structure of Digoxin:

Digoxin is made up of 2 parts:

1. An **aglycone** part: a steroid nucleus (**digoxigenin**).
2. A glycoside part consisting of **three molecules of digitoxose sugar**.
3. The glycosides are composed of **two** portions: a **sugar** and **cardenolide**

First: Isolation (extraction) of digoxin from drug:



Second: chemical identification of digoxin

a. <u>Baljet test:</u>

***Procedure**

1. Evaporate 2 ml of cardiac glycoside extract to dryness.
2. Add 5 ml of methanol.
3. Add 1 dropml of picric acid solution.
4. Add 1 ml of 10% of sodium hydroxide (NaOH) solution & detect the color

***Results**

1. +ve →Orange color; with cardenolides (5-membered ring, butenolide ring).
2. –ve →no color change; with bufadenolides (6-membered ring).
   N.B: You can compare your result with the color of a control sample (1mL dichloromethane + 1 mL 10% NaOH + 1 drop picric acid).

b. <u>Kedd's test</u>

***Procedure**

1. Evaporate 2 ml of cardiac glycoside extract to dryness.
2. Add 2 ml of 3,5-dinitrobenzoic acid/methanol solution.
3. Add 2 ml of 10% NaOH solution.
4. Allow to stand and detect the color.

***Results**

1. +ve →violet color; with cardenolides (5-membered ring,butenolide ring).
2. –ve →no color change; with bufadenolides (6-membered ring).

c. <u>Keller-killian's test: (for the glycoside part identification)</u>

***Procedure**

1. Evaporate 2 ml of cardiac glycoside extract to dryness.
2. Add 3ml of $FeCl_3$/Glacial acetic acid solution.
3. Pour the mixture from step 2 on the inner wall of **<u>dry</u>** test tube containing (2ml) of H_2SO_4 without mixing.
4. Allow to stand and detect the color.

***Results**

1. +ve →reddish-brown ring; with deoxy sugars (digitoxose sugar).
2. –ve →no color change; in the absence of deoxy sugars.

d. <u>Xanthydrol-HCl test: for the glycoside part identification)</u>

***Procedure**

1. Evaporate 2 ml of cardiac glycoside extract to dryness.
2. Add 3ml of Xanthydrol-HCl solution.
3. Heat for 3 minutes using water bath.
4. Allow to stand and detect the color.

***Results**

1. +ve →red color; with deoxy sugars (digitoxose sugar)
2. –ve →no color change; in the absence of deoxy sugars.
3.

e. <u>Lieberman's test (This reaction is due to the steroid part of the molecule.)</u>

***Procedure**

1. Evaporate 2 ml of cardiac glycoside extract to dryness.
2. Cool and add 2ml of acetic anhydride.
3. Add only 1 drop of concentrated H_2SO_4 without mixing.
   Allow to stand and detect the color. (observe the change in color from rose, through **red**, violet and blue to green.)

***Results**

1. +ve →rose color disappears after awhile; with steroidal nucleus having double bond at carbon number 4 (between C4 and C5), example with **<u>diosgenin.</u>**
2. –ve →no color change; in the absence of one of the previously mentioned conditions.

Faculty of Pharmacy كلية الصيدلة	**Phytochemistry Laboratory Report**	Al-Ahliyya Amman University جامعة عمان الأهلية

Lab Report No.:	**Student's name:**
Experiment name:	**Group number:**

I. Objective:

……..

II. Observations:

-Definition of Cardiac glycosides: ……

……..

-Botanical Plant name: ……

-Tablet used for the extraction: ……

-The chemical class of the isolated secondary metabolite: …….

-Pharmacological uses: …….

III. Data and Calculations

a. Chemical principle (draw chemical structure and name of AI)

b. Chemical test used for identification and their results:

Chemical test	Result (color, change,etc).	Part of structure detected

IV. Conclusion

Q: Name one test for identification of steroid nucleus of cardioactive glycosides?

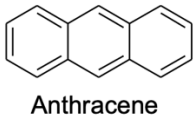
…….

Lab 4 ISOLATION and identification of anthraquinone glycosides

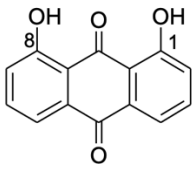
Objective: 1. To study the microscopic characters of Senna
2. To extract and identify anthraquinone Glycoside

The drugs in this group are characterized by the presence phenolic and glycosidic compounds, derived from anthracene and have variable degree of oxidation (**anthrones, anthronols, athraquinones**): They are the **anthraquinone glycosides**. These molecules have in common a double hydroxylation in the C(1)- and the C(8)-positions.

Anthranoid derivatives are used all over the world as a treatment for constipation. These compounds are present in several drugs of plant origin, especially as O- or C-glycosides. Besides featuring different substituents, the aglycone might consist of an anthraquinone, an anthrone or a dianthrone.

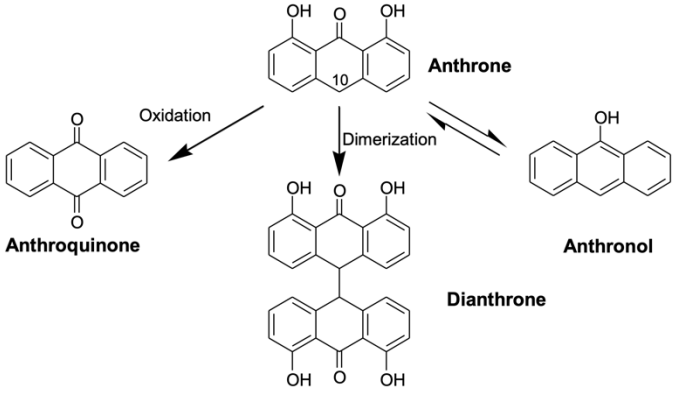


Anthracene



1,8-dihydroxy-anthraquinone
(Danthron)

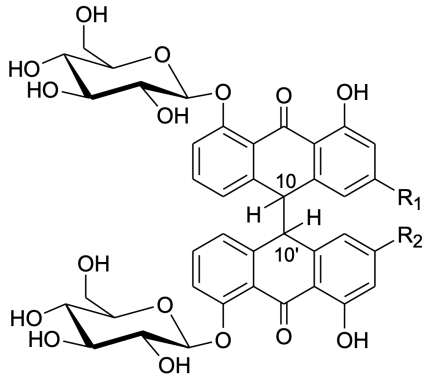
- Many of the purgative drugs such as senna, rhubarb, cascara, frangula and aloes contain anthracene at different oxidation levels.
- Senna pods and senna leaves contain glycosides which are derivatives of anthracene.
- The aglycones are all oxygenated derivatives of anthracene and are normally found in the drug both as the free aglycone and in combination with sugars, i.e. glycosides.
- The total amount of these compounds (both free and as glycoside) usually 3-5% of the dry weight of the drug, although in the case of Aloe which is a plant extract, the proportion is much higher.



- **Anthraquinone glycosides in senna are called Senna glycosides** or **sennosides** and are used to treat constipation or empty the large intestine before surgery.
- Anthraquinone glycosides in senna : known as **Sennosides A, B, C and D**.

➔ A & B are dianthrone of Rhein. C-C bridge connects the 2 anthranol (Rhein)units.
➔ C & D are dianthrone of Rhein and Aloe emodin with a C-C bridge connecting the 2 anthranol units .

- **Free Anthraquinones** (aglycones) in senna are: Rhein, Aloe emodin.



Sennoside A: R_1, R_2 = COOH (+)-form
Sennoside B: R_1, R_2 = COOH Mesoform
Sennoside C: R_1 = COOH R_2 = CH_2OH (+)-form
Sennoside D: R_1 = COOH R_2 = CH_2OH Mesoform

Part 1. Senna Characters

Senna leaf
Botanical name; *Cassia senna, Cassia angustifolia*
Biological Source: It consists of dried leaflets of *Cassia angustifolia* belonging to family Leguminosae.

Macroscopic Characters:

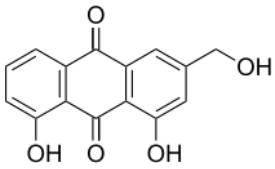
- Colour-Yellowish-green
- Odour-Slight
- Taste- Mucilagenous, bitter and Characteristic.
- Size- 7-8mm in width and 25-60mm in length.
- Shape-Leaves are lanceolate, entire, apex is acute with spine at the top.

Powder Microscopy of Senna:

- Mount: using chloral hydrate a mount.
- Epidermis with paracytic stomata.
- Trichomes.
- Palisade and spongy cells.
- Xylem vessels with annular thickening.
- Crystal sheath or Calcium oxalate prisms
- Non-glandular, unicellular, warty cuticle (covering) trichomes.

Pharmaceutical Uses:

1. It is useful as Purgative, in habitual constipation.
2. It also acts as a Carminative and causes therapeutic action.
3. It also shows gripping action due to presence of aloe-emodin.



Aloe-emodin

Materials and methods

1. Macroscopical identification:

Examine a small amount of (Senna, peppermint and thyme leaves), notice the organoleptic characteristics (taste, colour, odor and texture).

2. Microscopical identification:

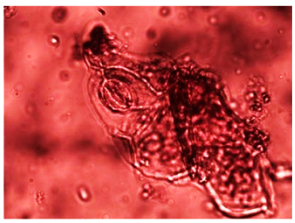
Prepare amount of Senna leaves powder using **chloral hydrate** (clarifying reagent) then put one drop of **phloroglucinol** reagent,afterone minute add one drop of **HCI con** and examine under microscope the key elements. Draw and label

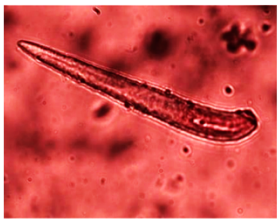
Repeat the previous steps using the peppermint and thyme powder without using **phloroglucinol+HCI con**).

- **Cross section: (Senna leaf)**

a. Cut the Senna leaf_with blade - on slide & make it very thin.
b. Choose three or four cutting appear right & thin.
c. Put on each part **chloral hydrate** to clarify the cross section:
d. Heat the slide quickly to accelerate the reaction..
e. Put one drop of **phloroglucinol** reagenton slide and wait one minute.
f. Put HCl_con.to slide which **(phloroglucinol+HCI** con give pink color with lignin structure).
g. Put the cover slide & examine under microscope layers of Senna leaf.

Fig.2 Powder microscopy of *C. angustifolia* showing a wavy epidermal cells, stomata, trich and mid rib Portion


Stomata


Trichome

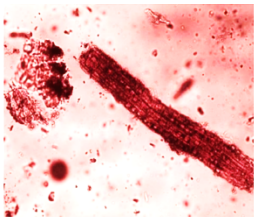
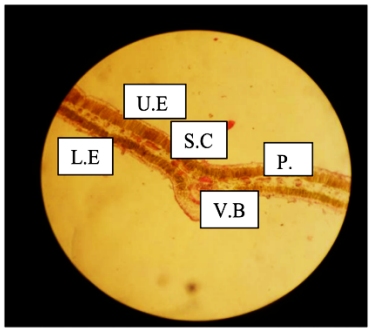
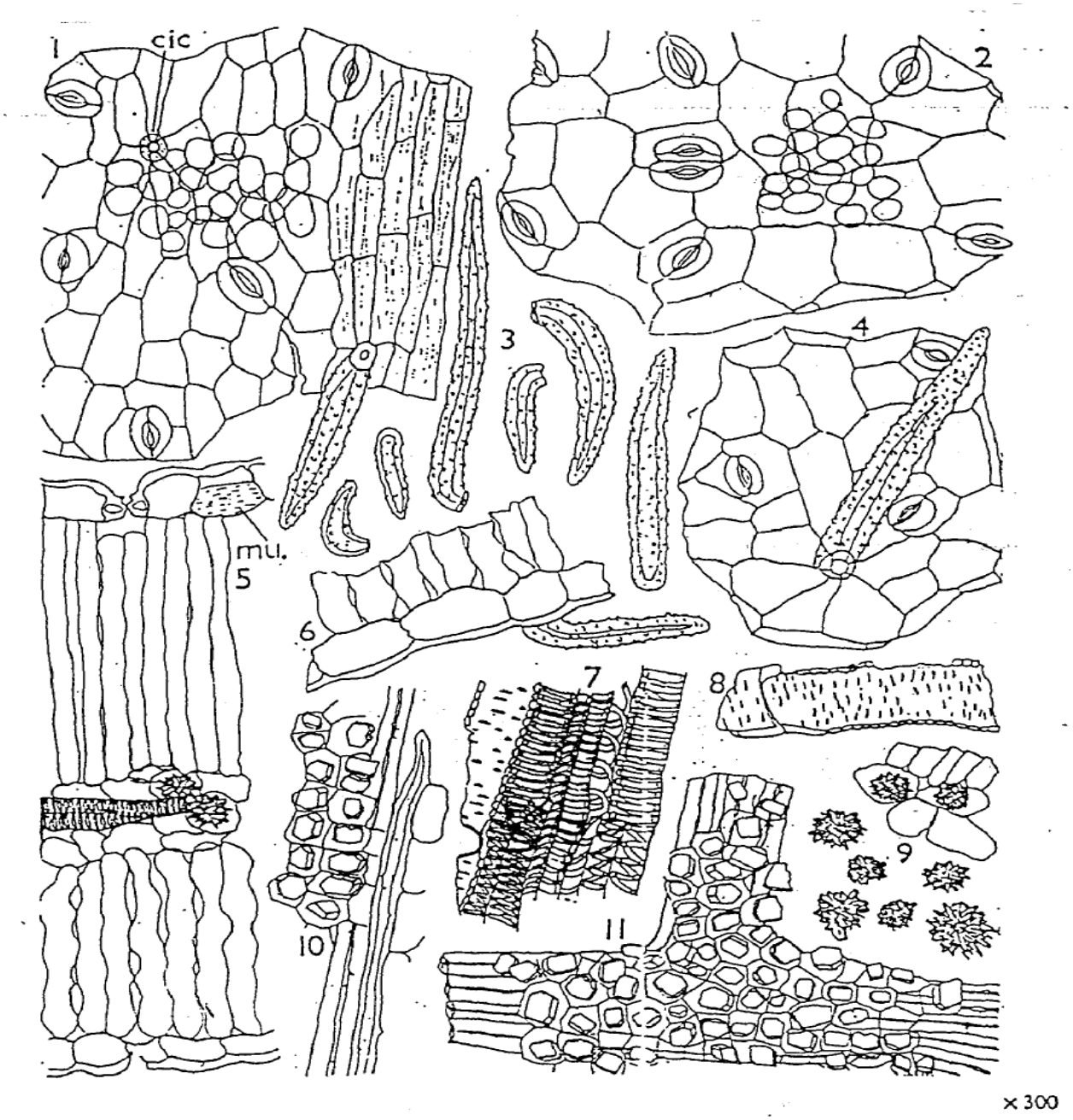

Midrib

Fig.2: *Cassia angustifolia* leaf T.S



Note:
U.E=Upper Epidermis,
L.E=Lower Epidermis,
P.T=Palisade Tissue,
P=Phlome,
S.C=Sclerrrren chyma,
C.C=Collen chyma

Senna Leaves

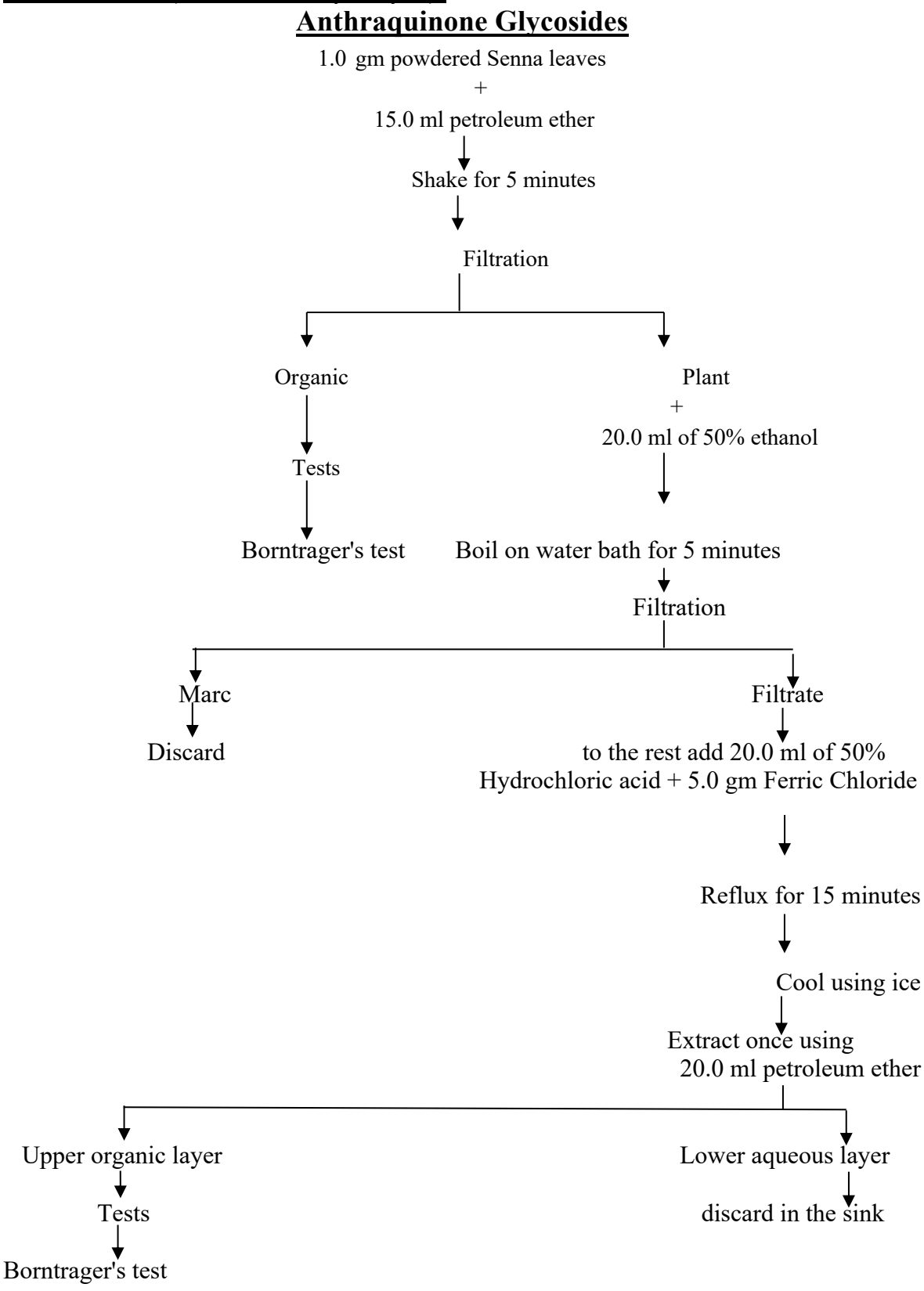


1.Epidermis in surface view showing paracytic stomata, a cicatrix (cic.), underlying palisade cells and the elongated cells over a vein with striated cuticle and an attached trichome.
2.Epidermis in surface view showing paracytic stomata and underlying palisade cells.
3.Covering trichomes.
4.Epidermis in surface view with paracytic stomata and an attached trichome.
5.Part of the lamina in sectional view showing the upper epidermis containing mucilage (mu.), the upper and lower palisade, spongy mesophyll cells containing cluster crystals of calcium oxalate and the lower epidermis.
6.Part of the 1arnina in sectional view with a trichome attached to the lower epidermis.
7.Xylem elements from one of the larger veins.
8.Part of a pitted vessel from one of the larger veins.
9.Cluster crystals of calcium oxalate.
10.Part of a group of fibers with calcium oxalate prism sheath.
11.Groups of fibers with calcium oxalate prism sheaths at the junction of two small veins.

Part 2. Extraction and identification of Anthraquinone

Procedure:

First: Isolation (extraction & hydrolysis):



Chemical Identification:

a.Borntrager's Test

***Procedure**

1. Take the etheric layer from extraction in a beaker.
2. Add to it 5ml of diluted ammonia (NH_4OH).
3. Shake and allow to stand and detect the color.

***Results**

1. +ve →pink to purple color; with free anthraquinone.
2. –ve →no color change; in the absence of free anthraquionone.

b.Bromine/Water Test

***Procedure**

1. Place 1ml of aqueous extract of anthraquinone glycoside.
2. Add 1ml of bromine/water solution.
3. allow to stand and detect the result.

***Results**

3. +ve →formation of puffy precipitate; with anthraquinone glycoside.
4. –ve →no precipitate formation; in the absence of anthraquionone glycoside.

Fig.1 Different parts of *Cassia angustifolia* plant

Potted Plants **Flowering Plants** **Dry leaves**



Faculty of Pharmacy كلية الصيدلة	**Phytochemistry Laboratory Report**	Al-Ahliyya Amman University جامعة عمان الأهلية

Lab Report No.:	**Student's name:**
Experiment name:	**Group number:**

I. Objective:

……..

II. Observations:

-Definition of Anthraquinone glycosides:

……..

-Botanical Plant name: ……

-The chemical class of the isolated secondary metabolite: …….

-Pharmacological uses: …….

-Drug-Plant interaction: ……

III. Data and Calculations

a. Chemical principle (draw chemical structure and name of AI)

b. Chemical test used for identification and their results:

Chemical test	Before hydrolysis	After hydrolysis

IV. Conclusion

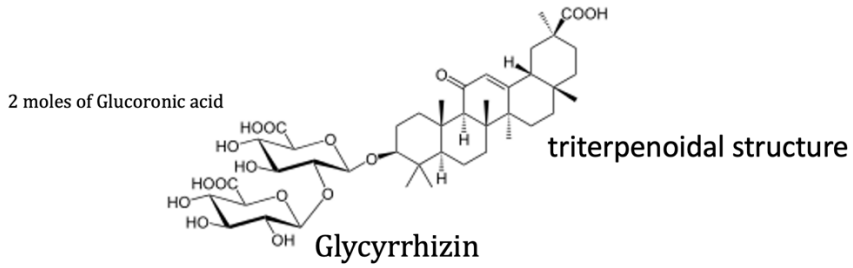
Q: Give one reason for the use of ferric chloride and HCl in the extraction procedure of anthraquinone glycoside

…….

Lab 5 Extraction & Identification of saponins from Liquorice

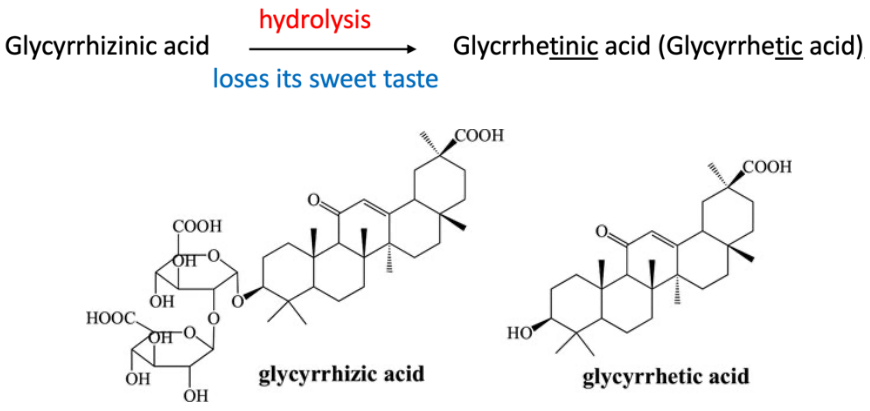
Objective: To extract and identify saponin from liquorice.

Botanical name: *Glycyrrhiza glabra*
Family name: Leguminosae
Part used: root
Therapeutic use: Demulcent, expectorant, laxative



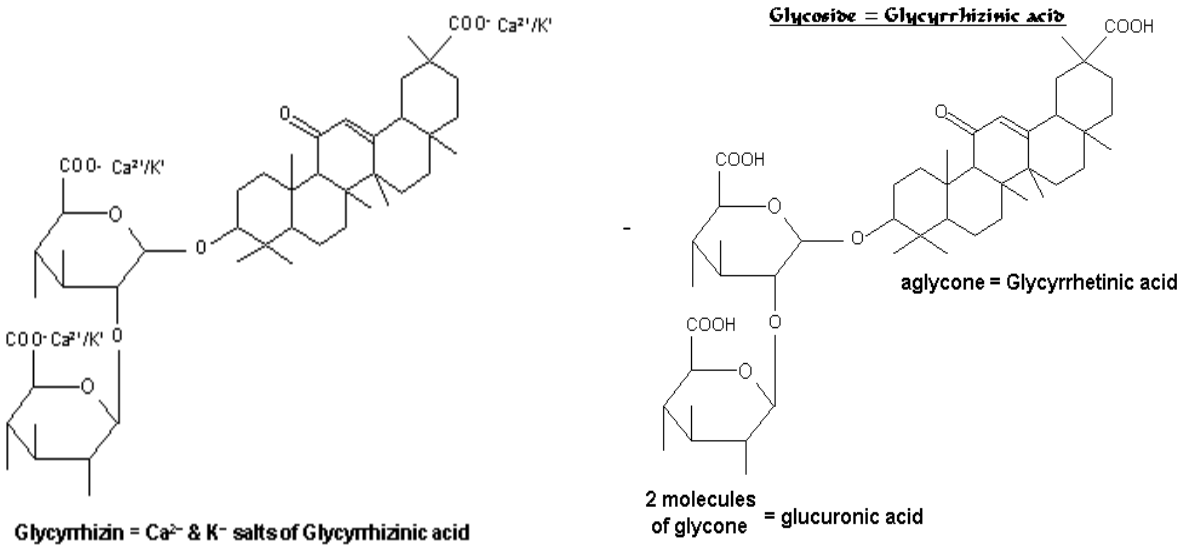
- Saponins:
  - Are plant amphipathic glycosides that derive their name from their soap-like properties.
  - Are steroid or triterpenoid glycosides, common in a large number of plants and plant products that are important in human and animal nutrition. Several biological effects have been ascribed to saponins.
  - Saponins on **hydrolysis** yield an **aglycone** known as "**sapogenin**" and a **sugar moiety**.

- Glycyrrhizinic acid is 50 times sweeter than sugar (sucrose). Upon hydrolysis, the glycoside loses its sweet taste and is converted to the aglycone glycyrrhetinic acid plus two molecules of glucuronic acid



- **Liquorice** root of *Glycyrrhiza glabra* plant contains saponins:
- The sweet principle glycyrrhizin
- The Potassium calcium salt as glycyrrhizinic acid.

A saponin glycoside called Glycyrrhizin (glycyrrhizinic acid).



Glycyrrhizin is the Ca^{2+} and K^{+} Salt of glycyrrhizinic acid.

Medical uses of glycyrrhetinic acid:

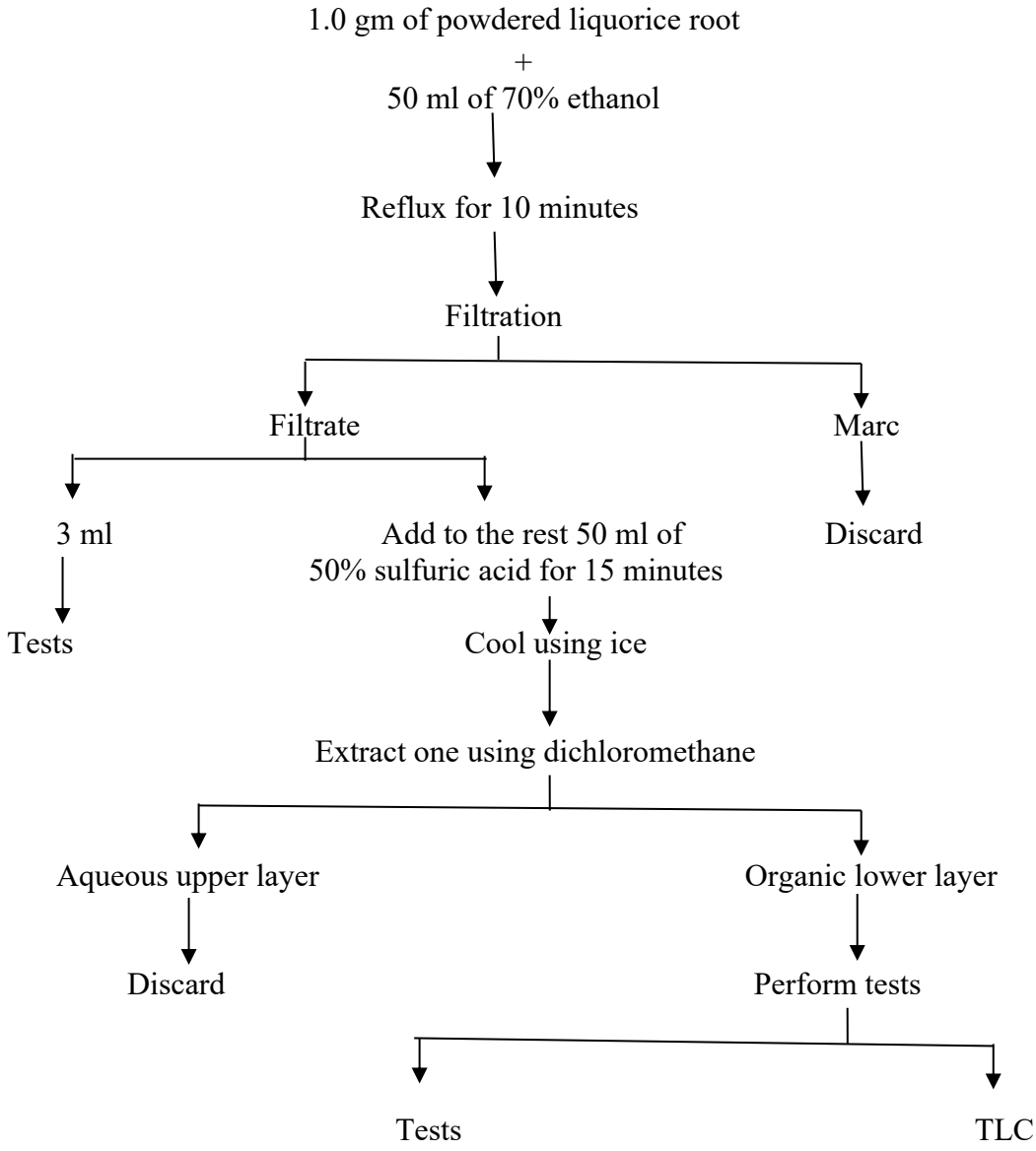
1. -It has expectorant and antitussive properties. Expectorants are used to decrease the viscosity of tenacious mucus, or to increase the secretion of mucus in dry irritant unproductive cough, thereby, lubricating the air passages and making coughing more productive.
2. -It is used considerably as a flavoring agent and is frequently employed to mask the taste of bitter drugs such as aloe, quinine etc.
3. Has potential in treating peptic ulcer.
4. Can cause abortion; therefore, licorice should not be taken during pregnancy.

❖ Identification of Saponins:

1. Physical identification: is done by frothing test.
2. Chemical identification: is done by precipitation test.

First: Extraction & hydrolysis

Saponin Glycosides



Tests include: (done twice before hydrolysis & after hydrolysis).

1-Froth formation test.
2-Precipitation by heavy metals.
3-Emulsion formation test.
4-Blood hemolytic test.

Second: Identification tests of saponins

A. Frothing test

***Procedure**

1. In two different test tubes put 2ml of saponin and 2ml of sapogenin extract.
2. Add 5 ml distilled water.
3. Shake well for 2 minutes.

***Results**

1. +ve →formation of froth which persist for a long time with a tall height; with saponin glycoside.
2. –ve →no froth formation; with sapogenin or other compounds.

B. Precipitation by heavy metals:

***Procedure**

1. In two different test tubes put 2ml of saponin and 2ml of sapogenin extract.
2. Add few drops of lead acetate solution.

***Results**

1. +ve →formation of precipitate; with saponin glycoside.
2. –ve →no precipitate formation; with sapogenin or other compounds.

C. Emulsion formation test:

***Procedure**

1. In two different test tubes put 2ml of saponin and 2ml of sapogenin extract.
2. Add 1 drop of castor oil.
3. Shake well.

***Results**

1. +ve →formation of emulsion (one phase); with saponin glycoside.
2. –ve →no emulsion formation (two phase); with sapogenin or other compounds.

D. Blood hemolytic test:

***Procedure**

1. In two different beakers put 2ml of saponin and 2ml of sapogenin extract.
2. Evaporate to dryness.
3. Add 2ml of normal saline to the saponin residue.
4. Pour into a disposable test tube containing 5ml of blood cell suspension.

***Results**

1. +ve →blood hemolytic; with saponin glycoside and sapogenin.
2. –ve →no blood hemolytic; with other compounds.

Faculty of Pharmacy كلية الصيدلة	**Phytochemistry Laboratory Report**	Al-Ahliyya Amman University جامعة عمان الأهلية

Lab Report No.:	**Student's name:**
Experiment name:	**Group number:**

I. Objective:

……..

II. Observations:

-Definition of Saponin glycosides:

……..

-Botanical Plant name: ……

-The chemical class of the isolated secondary metabolite: …….

-Pharmacological uses: …….

-Drug-Plant interaction: ……

Toxicity& overdose: …….

III. Data and Calculations

a. Chemical principle (draw chemical structure and name of AI)

b. Physical and Chemical test used for identification and their results:

Chemical test	Before hydrolysis	After hydrolysis

IV. Conclusion

Q: What are the properties of the saponin glycosides

…….

Lab 6 Extraction of Tannins

Objective: To extract and identify Tannins.

Plant used:

1. **Botanical name:** *Quercus infectoria*, (Nutgall) **Family name**: Fagaceae
2. **Botanical name:** *Camellia sinensis*, (Tea) **Family name**: Theaceae

Therapeutic use: Astringent, cold sore, antidote

Taninns is a natural compounds have high molecular weight and contain large number of phenolic hydroxyl or other groups that enable them to form cross link between proteins of the animal hides and prevent their putrefaction and convert them to leather.

Classification:

1) Hydrolysable tannins: may be hydrolyzed by acids or enzymes. They also called pyrogallol tannins. With ferric chloride will give bluish- black color or ppt.
2) Condensed tannins: they can't be hydrolyzed by enzymes or acids. They also called catechol tannins. With ferric chloride will give greenish-black color or ppt.

General Characteristics of Tannins

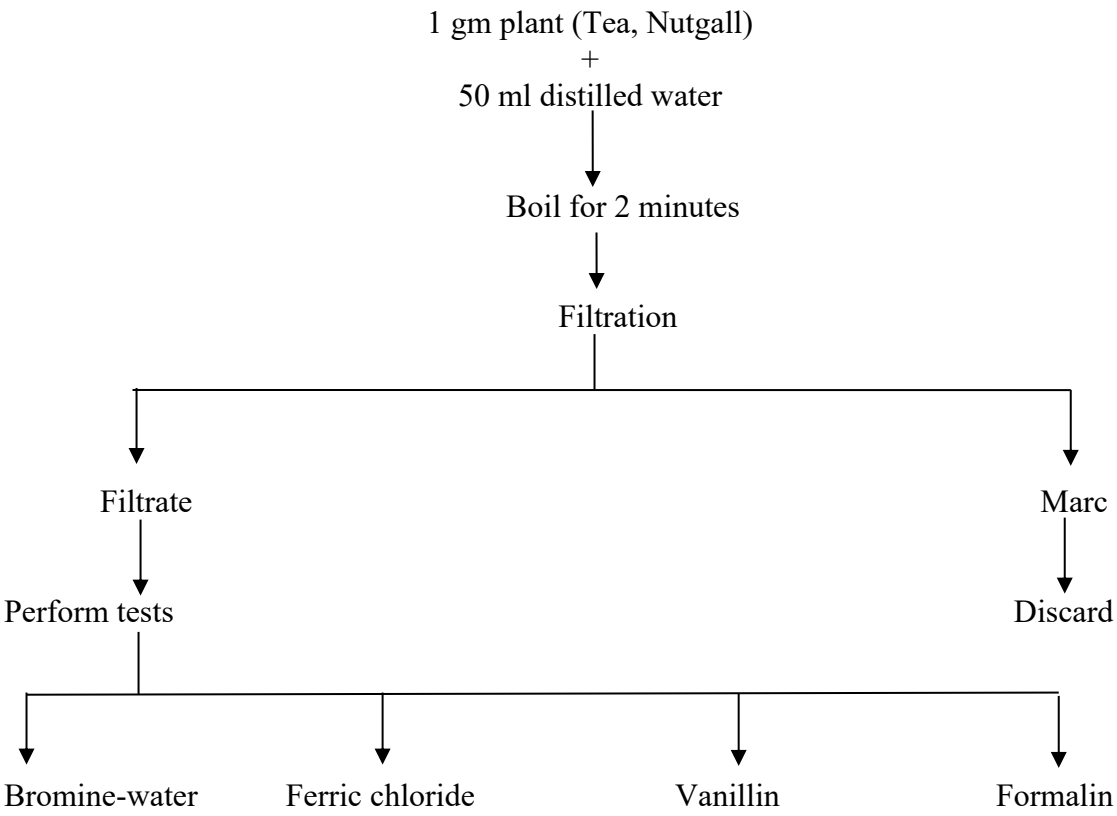
a. Tannins are colloidal solutions with water.
b. Non crystalline substance.
c. Soluble in water, alcohol, dilute alkali, and glycerin.
d. Sparingly soluble in ethyl acetate.
e. Insoluble in organic solvents, except acetone.
f. Can bind with proteins and form insoluble or soluble tannin—protein complexes.
g. They cause precipitation of solution of gelatin as well as alkaloids
h. They form dark blue, greenish-black soluble compounds with ferric salts.
i. They are precipitated by salts of copper, lead and tin

Uses:

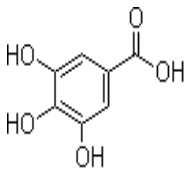
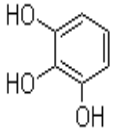
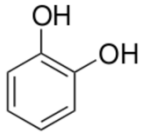
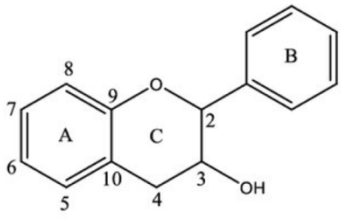
a. Tannins as **astringents** can be used internally for the relief of diarrhea and also can be used externally for acne and also relieve minor skin irritations which results from superficial cuts, insect bites and fungal infection.
b. Tannins used in the **treatment of burns** as the proteins of the exposed tissue are precipitated and form a mildly **antiseptic**, protective coat under which the regeneration of new tissues may take a place.
c. Tannins also have been employed as **antidotes** in poisoning by heavy metals, alkaloids and glycosides
d. Commercial tannins used in ink and leather industry.

First: Extraction:

Tannins



*Compare the results of your tests on Tea & Nutgall with the results of Gallic acid standard.

Hydrolysable Tannins	Condensed Tannin
 Gallic acid  Pyrogallol  Catechol	 Flavan-3-ol

Second: Identification tests:

a. Ferric chloride test

***Procedure**

1. In three different test tubes put 1ml of tea extract and 1ml of nut gall extract and 1 ml of gallic acid (standard hydrolysable tannins).
2. Add 1 drop of 3% FeCl3 solution to each test tube.
3. Detect the result.

***Results**

+ve →green-black color; with condensed tannins
And blue-black color; with hydrolysable tannins.

N.B: It is not a specific reagent for tannins, as other phenolic compounds will also give a positive result. So it indicate if the compound is phenolic or is not phenolic compound.

b. Vanillin-Hcl reagent

***Procedure**

1. In three different test tubes put 1ml of tea extract and 1ml of nut gall extract and 1 ml of gallic acid (standard hydrolysable tannins).
2. Add 2ml of vannilin/HCl solution drop wise to each test tube.
3. Detect the result.

***Results**

1. +ve →crimson color; with condensed tannins.
2. –ve →no color change; with hydrolysable tannins.

c. Bromine-Water Test

***Procedure**

1. In three different test tubes put 1ml of tea extract and 1ml of nut gall extract and 1 ml of gallic acid (standard hydrolysable tannins).
2. Add 1ml of bromine/water solution to each test tube.
3. Allow to stand for 5 minutes and detect the result.

***Results**

1. +ve →puffy colored precipitate formation; with condensed tannins.
2. –ve →no precipitate formation; with hydrolysable tannins.

d. Formalin Test

***Procedure**

1. In three different test tubes put 1ml of tea extract and 1ml of nut gall extract and 1 ml of gallic acid (standard hydrolysable tannins).
2. Add 3drops of formalin solution to each test tube.
3. Add 1ml of 10%HCl solution to each test tube.
4. Heat on water bath for 3 minutes.
5. Detect the result.

***Results**

1. +ve →red precipitate formation; with condensed tannins.
2. –ve →no precipitate formation; with hydrolysable tannins.

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Lab Report No.:	**Student's name:**
Experiment name:	**Group number:**

I. Objective:

……..

II. Observations:

-Definition of Tannins:

……..

-Botanical Plant name: (1)……. (2) ……

-The chemical class of the isolated secondary metabolite: …….

-Pharmacological uses: …….

Toxicity& overdose: …….

III. Data and Calculations

a. Chemical principle (draw chemical structure and name of AI)

b. Physical and Chemical test used for identification and their results:

Chemical test	Hydrolysable tannins	Condensed tannins

IV. Conclusion

Q: Tannins are divided according to the phenolic nuclei into two classes. Mention them in details

…….

Lab 7 Identification of flavonoids

Objective: To isolate and identify Flavonoids.

Botanical name of used plant: *Citrus sinensis* (Orange), **Family name**: Rutaceae
Therapeutic use: Increase elasticity of blood vessels

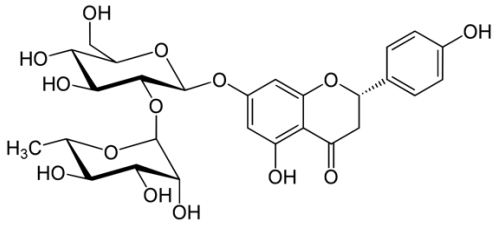
They are the largest group of natural chemical class, they may found in free state or as glycoside form. Also They are highly polar so soluble in water and alcohol but insoluble in organic solvents.

They are often yellow in color. (The yellow color depend on the number of hydroxyl group in the structure).

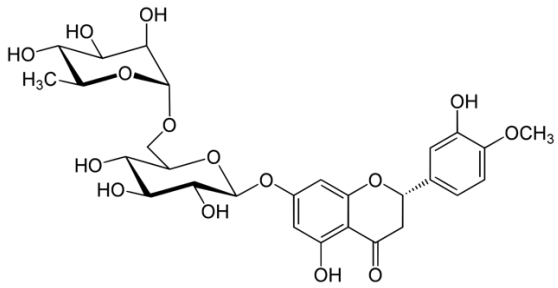
Uses:
- it has anti-inflammatory and antiallergic effect.
- it has vasoprotective properties.
- it have protection effect against gastric mucosa.
- it have diuretic , antimicrobial, antifungal and antispasmodic properties.

Citrus peels described as rich source of unique phenolic compounds to citrus, especially the characteristic flavanone glycosides (mainly naringin, hesperidin, narirutin, and neo hesperidin).
Lemon peels were applied for pectin and flavonoids (narirutin) production. Orange peels were also employed for recovery of flavonoids e.g. hesperidin, essential oils, and carotenoids.

Hesperidin is a flavanone glycoside found primarily in the rind (peel) of citrus fruits.



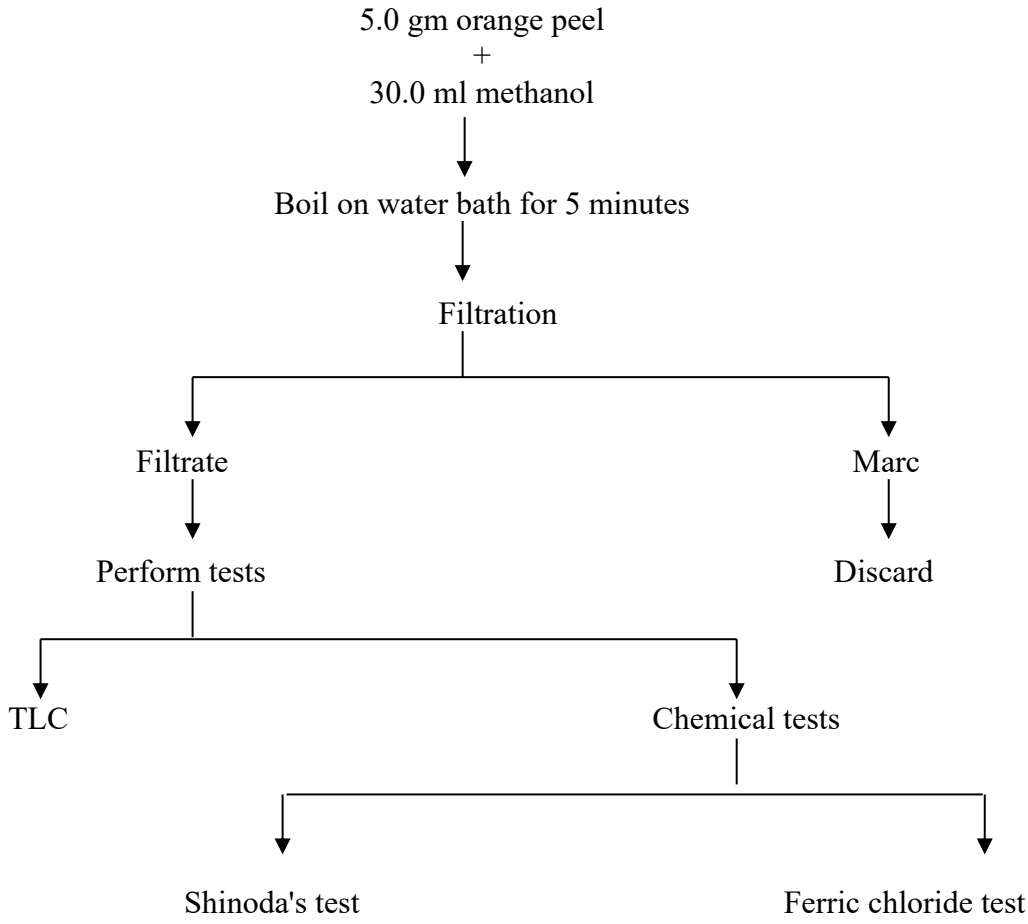
Naringin



Hesperidin

First: Extraction:

Extraction of Hesperidin



TLC:
Mobile phase Solvent system:
Formic acid: Glacial acetic acid: water: ethyl acetate/ ratio (11:11:24:100) respectivily in 146 ml volume

*In 20 ml (Jar) add the following amounts:
Formic acid: 20 x 11/146= 1.507 ml
Glacial acetic acid: 20 x 11/146= 1.507 ml
H_2O: 20 x 24/146= 3.29 ml
EtOAc: 20 x 100/146= 13.70 ml

Second: Identification tests:

1. Shinoda's test

***Procedure**

1. Put 1ml of flavanoide extract (orange extract) in a test tube.
2. Add few pieces of magnesium (Mg) metal.
3. Add 1ml of concentrated HCl.
4. Detect the result.
   NB: repeat the test on **Naringin** and **Hesperidin** standards.

***Results**

3. +ve →pink to red color; with **all flavanoides.**
4. –ve →no color change; with other compounds.

2.Ferric chloride test:

***Procedure**

1. Put 1ml of flavanoide extract (orange extract) in a test tube.
2. Add 1 drop of 3% $FeCl_3$ solution.
3. Detect the result.
   NB: repeat the test on **Naringin** and **Hesperidin** standards.

***Results**

+ve → green color; with **Naringin**

And → brown color; with **Hesperidin.**

Thin Layer Chromatography (TLC)

TLC is based on the same fundamental principles as CC.

The stationary phase is prepared as a thin layer (about 0.2mm thickness) on a glass, plastic or aluminum plate.

A solution of the mixture to be test is applied to the bottom of the plate.

The mobile phase travels up the layer of adsorbent by capillary action.

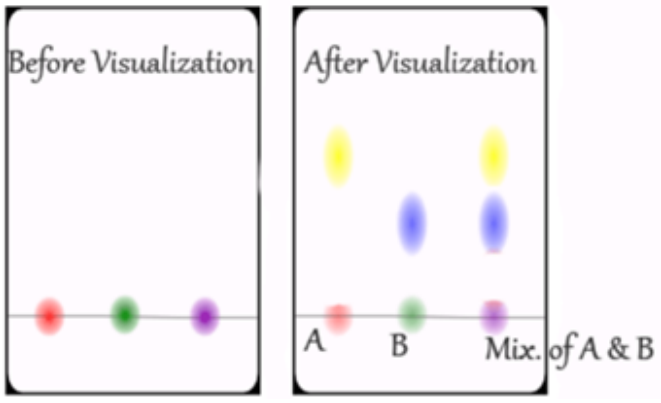
The mobile phase move relative to the stationary phase and transports the components with it.

Note that: the less polar solute moves up faster than the more polar one.

Lab practice of TLC:

1. Plate preparation.
2. Sample application: a micropipette is used.
3. Running the preparation :
   - Chose the suitable size of chamber or jar.
   - The plate is placed in the bottle with spotted end in the bottom and above the surface of the solvent.
   - Remove the plate when the solvent reach about 1 cm from the upper end of plate.
4. Detection of spot:
   - Ultraviolet light (UV)
   - Iodine (I_2) vapor: forms a brown spot color on the TLC.
   - Spraying with certain reagent like dragendorf to detect the alkaloid.

$$RF = \frac{\text{Distance travelled by substance}}{\text{Distance travelled by solvent}}$$



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Lab Report No.:	**Student's name:**
Experiment name:	**Group number:**

I. Objective:

……..

II. Observations:

-Definition of Flavonoids:

……..

-Botanical Plant name: …….

-The chemical class of the isolated secondary metabolite: …….

-Pharmacological uses: …….

-Pharmaceutical preparation available in market: ……...

III. Data and Calculations

a. Chemical principle (draw chemical structure and name of AI)

b. Physical and Chemical test used for identification and their results:

Chemical test	Hesperidin	Naringin

Stationary Phase:	
Mobile Phase:	
Spraying agent:	
Spots:	
Results:	

IV. Conclusion

Q: Give one pharmacological action of hesperidin

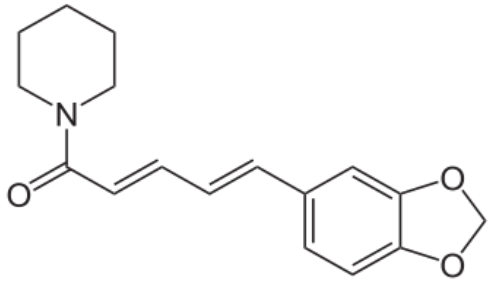
…….

Lab 8 Isolation and identification of PIPERINE

Objective: To isolate and identify Piperine from black pepper.

Botanical name of used plant: *Piper nigrum* (Black pepper), **Family name**: Piperaceae

Therapeutic use:
-Bioavailability-enhancing activity for some nutritional substances and for some drugs.
-Anti-inflammatory activity and may have activity in promoting digestive processes.



Structure of Piperine

General characteristics of alkaloids:

1. They are secondary metabolites derived from plant sources.
2. Basic in nature.
3. Nitrogenous compound (contain one or more nitrogen atoms so they will classified according to the substitutions on nitrogen atoms as 1^o, 2^o and 3^o alkaloids; those are water insoluble, and quaternary alkaloids; those are water soluble.
4. Have different physical properties :
   crystalline solid like (most of them) and liquid like nicotine.
   Their taste are bitter.
5. The solubility of them depend on their basic properties, in genral they are insoluble in water but soluble in ethanol, methanol and Chloroform.
6. They are considered as toxic compounds but they have a marked physiological action and an important therapeutic activities.
7. An examples:
   Morphine (narcotic and analgesic)
   Strychnine (central stimulant)
   Atropine (mydriatic)
   Ephedrine (arises blood pressure)
   Reserpine (decrease blood pressure)
8. Found in many plant families such as Solanacaealeguminosae, papaveracea, rubicaceae and others.
9. Rosaceae and labitateae families don't have alkaloids.

Reagent used for detection of alkaloids:

1. Mayer's reagent (potassium mercuric iodide: $HgCl_2$/KI).
   Give (Creamy ppt) result.
2. Wagner's reagent (solution of iodine in potassium iodide KI/I_2) Give (Reddish brown ppt) result.
3. Dragendorff's reagent (solution of potassium bismuth iodide)
   Give (Red-orange ppt) result.
4. Solution of tannic acid. Give different colored ppt with different alkaloids result
5. Hagers reagent (solution of picric acid). Give (yellow ppt) result.

Piperine: is an alkaloid found in the fruits and roots ofpipernigrum and Piper longum species of Piperaceae family.

Uses and applications of piperine:
-Piperine, along with its isomerchavicine, is the alkaloid responsible for the pungency of black pepper and long pepper.
-It can dramatically increase the absorption of selenium, vitamin B and β-carotene as well as other nutrients.
-It can stimulate pancreatic and intestinal digestive enzymes and also increases biliary bile acid secretion when orally administrated.
-In addition to its involvement in increasing the absorption of other nutrients in the body, piperine has other novel applications like helping to fight against colon cancer.
-It has anti-inflammatory, thermogenic, growth stimulatory, anti-thyroid, antipyretic, analgesic, insecticidal, immunomodulatory, antitumor, anti-depressant and anti-apoptotic activities.

Physicochemical properties:
-Piperine is slightly soluble in water (1 g/25 L (18 °C)) and is highly soluble in alcohol (1 g/15 mL), ether (1 g/36 mL) and chloroform (1 g/1.7 mL).

-This alkaloid is responsible for the pungency taste. The pungency of piperine is caused by the activation of the heat and acidity sensing Transient receptor potential vanilloid (TRPV) ion channel TRPV1 on nociceptors (painsensing nerve cells).

Procedure:

First: Isolation of piperine from Black pepper:

Isolation of Piperine from Black Pepper

10 gm of grounded black pepper

↓

Extract using 200 ml of absolute ethanol using Soxhlet apparatus

↓

Concentrate the extract to 5 ml using rotary evaporator

↓

Add to the concentrated extract 5 ml of 10% **alcoholic Potassium hydroxide (KOH)**

↓

Decant the supernatant from the insoluble residue in a pre clean, dry, weighed beaker

↓

Leave the soluble residue over night where upon Yellow needles of melting point 125^{o}C

↓

Perform test

↓

Piperine forms a solid complex with 1,3,5-Trinitrobenzene in a ratio 1:1 in the form of red needle; with melting point of 130^{o}C

Second: Identification of piperine:

1-General Test For Alkaloids (-Mayer's Test)

***Procedure**

1. Place 1 drop of alkaloid solution on a clean watch glass.
2. Place 1 drop of Mayer's reagent.
3. Wait for 1 minute and detect the formation of precipitate.

***Results**

1. +ve →formation of white precipitate; with **true alkaloids**.
2. –ve →no precipitate formation; with **pseudo alkaloids**.

2-General Test For Alkaloids (Dragendorff's Reagent)

***Procedure**

1. Place 1 drop of alkaloid solution on a clean watch glass.
2. Place 1 drop of Dragendorff's reagent.
3. Waite for 1 minute and detect the formation of precipitate.

***Results**

1. +ve →formation of orange precipitate; with **true alkaloids**.
2. –ve →no precipitate formation; with **pseudo alkaloids**.

3-Specific Chemical Test For Piperine

***Procedure**

1. Take few crystals of piperine alkaloid and dissolve in few ml.s of ethanol in a petri dish.
2. Add few drops of 1,3,5 trinitrobenzen.

***Results**

1. +ve →formation of red needle shape crystals with melting point of 130°c; with piperine.
2. –ve →no precipitate formation; in the absence of piperine

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Lab Report No.:	**Student's name:**
Experiment name:	**Group number:**

I. Objective:

……..

II. Observations:

-Definition of Alkaloid:

……..

-Botanical Plant name: …….

-The chemical class of the isolated secondary metabolite: …….

-Pharmacological uses: …….

-Toxicity& overdose: ……...

III. Data and Calculations

a. Chemical principle (draw chemical structure and name of AI)

b. Yield of Piperine:

c. Physical and Chemical test used for identification and their results:

Chemical test	Result

IV. Conclusion

Q: Give the reason for the use of alcoholic KOH in the extraction procedure of black pepper

…….

Lab 9 Isolation and Identification tests of Caffeine

Objective: To isolate and identify Caffeine from Coffee and Tea.

Plant used:

1. **Botanical name:** *Coffea arabica,* (Coffee) **Family name**: Rubiaceae
2. **Botanical name:** *Camellia sinensis*, (Tea) **Family name**: Theaceae

Therapeutic use: CNS stimulant, diuretic

Caffeine is a bitter, white crystalline purine, a methylxanthine alkaloid, and thus closely related chemically to the adenine andguanine .

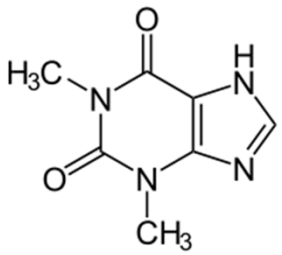
Caffeine is a central nervous system (CNS) stimulant of the methylxanthine class of psychoactive drugs.

-It is the world's most widely consumed psychoactive drug, but unlike many other psychoactive substances, it is legal and unregulated in nearly all parts of the world.

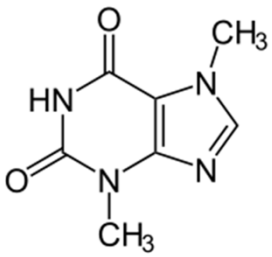
-It is found in the seeds, nuts, or leaves of a number of plants native to South America and East Asia. The most well known source of caffeine is the seed (commonly incorrectly referred to as the "bean") of Coffea plants.

-Beverages containing caffeine are ingested to relieve or prevent drowsiness and to increase one's energy level. Caffeine is extracted from the plant part containing it for making beverages by steeping it in water, a process called infusion.

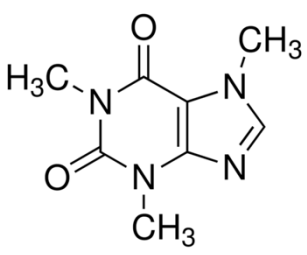
-Caffeine is classified by the Food and Drug Administration as "generally recognized as safe" (GRAS). Toxic doses, over 10 grams per day for an adult, are much higher than typical dose of less than 500 milligrams per day.



Theophylline
(1, 3-dimethylxanthine)



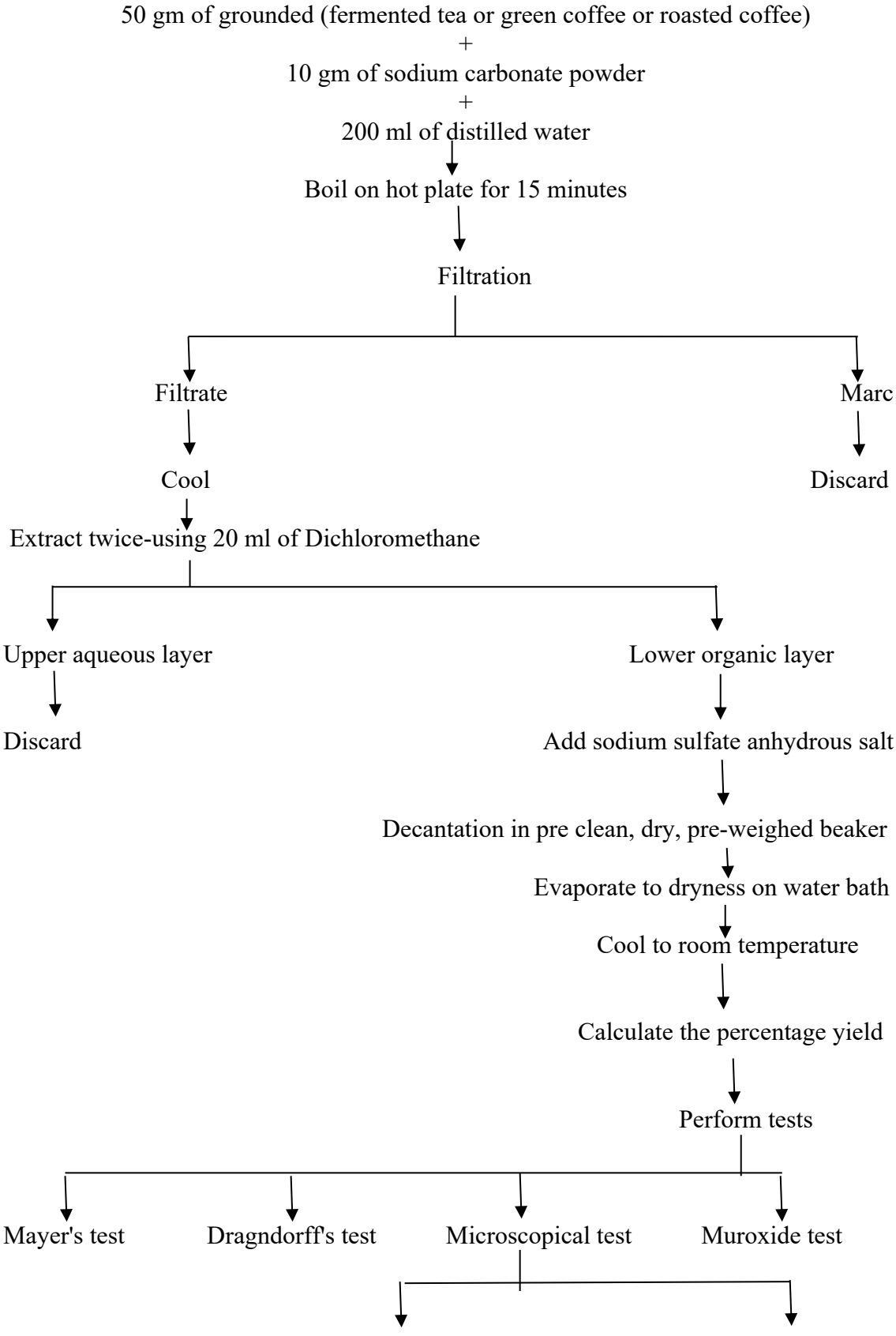
Theobromine
(3,7-dimethylxanthine)



Caffeine
(1,3,7-trimethylxanthine)

First: Isolation of caffeine :

Isolation of caffeine from Tea, roasted coffee, and green coffee



Gold chloride Mercuric chloride

Second: Identification of caffeine:

1-General Test For Alkaloids (-Mayer's Test)

***Procedure**

1. Place 1 drop of alkaloid solution on a clean watch glass.
2. Place 1 drop of Mayer's reagent.
3. Wait for 1 minute and detect the formation of precipitate.

***Results**

1. +ve →formation of white precipitate; with **true alkaloids**.
2. –ve →no precipitate formation; with **pseudo alkaloids**.

2-General Test For Alkaloids (Dragendorff's Reagent)

***Procedure**

1. Place 1 drop of alkaloid solution on a clean watch glass.
2. Place 1 drop of Dragendorff's reagent.
3. Waite for 1 minute and detect the formation of precipitate.

***Results**

1. +ve →formation of orange precipitate; with **true alkaloids**.
2. –ve →no precipitate formation; with **pseudo alkaloids**.

3-Specific Chemical Test For Caffeine (Muroxide Test)

***Procedure**

1. In an evaporating dish add a few milligrams of caffeine powder.
2. Add the same amount of potassium perchlorate crystals.
3. Add 2drops of diluted HCl solution and mix well.
4. Evaporate to dryness on a water bath.
5. Cool and treat it with 2 drops of diluted ammonia (NH_4OH) solution.

***Results**

1. +ve →formation of strong purple color; with caffeine.
2. –ve →no color change; in the absence of caffeine.

4-Microscopical Examination (Mercuric Chloride Test)

***Procedure**

1. Place 1 drop of caffeine solution on a clean glass slide.
2. Add 1 drop of mercuric chloride solution ($HgCl_2$).
3. Observe under microscope using low magnification power without stirring or covering.

***Results**

1. +ve →formation of cluster of long radiating needle shape crystals; with caffeine.
2. –ve →no precipitate formation; in the absence of caffeine.

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Lab Report No.:	**Student's name:**
Experiment name:	**Group number:**

I. Objective:

……..

II. Observations:

-Definition of Alkaloid:

……..

-Botanical Plant name: 1…….

2…….

3……

-The chemical class of the isolated secondary metabolite: …….

-Pharmacological uses: ……

……..

-Toxicity& overdose: ……...

III. Data and Calculations

a. Chemical principle (draw chemical structure and name of AI)

b. Yield of caffein:

c. Physical and Chemical test used for identification and their results:

Chemical test	Result

IV. Conclusion

Q: Q: How can you identify caffeine from other alkaloids?
…….

Lab 10 Isolation and Identification Volatile oil

Objective: To isolate and Identify volatile oil.

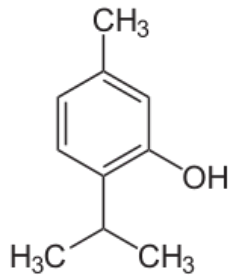
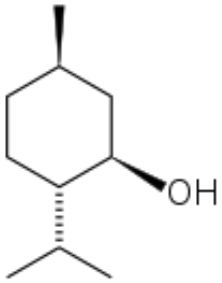
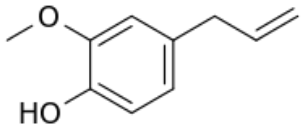
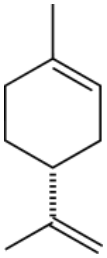
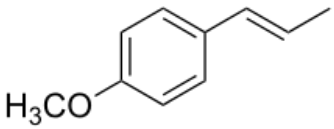
Plant used:

1. **Botanical name:** *Thymus vulgaris,* (Thyme) **Family name**: Lamiaceae
2. **Botanical name:** *Mentha piperita*, (peppermint) **Family name**: Lamiaceae
3. **Botanical name:** *Elettaria cardamomum***, (**Cardamom), **Family name**: Zingibeaceae
4. **Botanical name:** *Pimpinella anisum,* (Anise), **Family name**: Apiaceae

-Volatile oils (also known as essential oils), are oils that are characterized by their volatility and failure to saponify.
They are derived from plant tissues. They are insoluble in water, but soluble with many organic solvents.
Biogenesis: Mevalonic acid, Methylerythritol& Phenylpropanoid pathways

-They evaporate when exposed to air, and thus can be obtained by extraction (by hydrodistillation for pharmaceutical use) from many plants. They should be stored in a cool dry place in a tightly stoppered amber glass containers.

-Contain a complex mixture of aromatic-smelling volatile components with either one or both

		
Thymol	Menthol	Eugenol
		
Cineole	Limonene	Anethole

Procedure:
TLC technique is done for a mixture of two unknown volatile oils Vs a standard volatile oils to figure out the unknown volatile oil

TLC Solvent mobile phase system: Toluene: EtOAc (93:7), **Spraying agent:** vanillin/ H_2SO_4

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Lab Report No.:	**Student's name:**
Experiment name:	**Group number:**

I. Objective:

……..

II. Observations:

-Definition of volatile oil:

……..

-Botanical Plant name: 1…….

2…….

3……..……

4……..

-The chemical class of the isolated secondary metabolite: …….

-Pharmacological uses: ……

……..

-Toxicity& overdose: ……...

III. Data and Calculations

a. Chemical principle (draw chemical structure and name of AI)

b. Physical and Chemical test used for identification and their results:

Stationary Phase:	
Mobile Phase:	
Spraying agent:	
Spots:	
Results:	

IV. Conclusion

Q: How can you determine the volatile oil content of a crude drug?

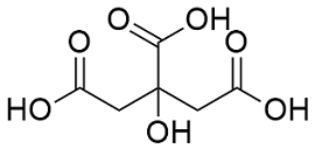
…….

Lab 11 Extraction of Citric acid

Objectives:
To isolate and identify citric acid (as Calcium citrate) from lemon juice

Introduction

citric acid, malic acid, and succinic acid, occur widely in plant tissues in rather high concentrations. Citric acid is one of the most widely distributed plant acids, occurring in cranberries, red currants, strawberries, raspberries, beets, citrus fruit, and animal tissues. It may be isolated from citrus fruits as the comparatively insoluble calcium salt. This compound displays unusual solubility properties in that it becomes less soluble at increased temperatures. This information should be kept in mind when carrying out the following experiment. The formula for citric acid is:



Citric acid

Pharmaceutical uses of citric acid

-Preservative
-Taste enhancement/masking (chewables/syrups)
-Citric acid helps in the removal of dead skin so used for home masks.
-Improves skin tone and skin growth reducing wrinkles, acne scars etc.
-To balance the pH levels citric acid is commonly used the ingredient in cosmetics.
-It is found in hand soap, body wash, nail polish face cleansers, shampoos and some other cosmetics products.

Procedure:

1. Measure 90 mL of thawed frozen lemon juice concentrate into a 250 mL beaker and carefully add a 10% NaOH solution with stirring until the mixture is slightly alkaline.
2. A distinct color change occurs at this point, the solution passing from a clear yellow to a brownish color.
3. Strain the solution through muslin to remove large particles of pulp and then filler through paper in a Buchner funnel. The pores of the filter paper may tend to become clogged by the extract in spite of the previous straining. Should this occur, change the paper in the funnel once or twice as required to complete the filtration.
4. Measure the filtrate and record. Result: _____ mL.
5. Now place the filtrate in a beaker and add 5 mL of 10% $CaCl_2$, stirring constantly for each 10 mL of filtrate.
6. Heat to boiling and filter off the copious precipitate of calcium citrate ($Ca_3C_{12}H_{10}O_{14}$) from the hot solution using a Buchner funnel.
7. Wash the precipitate with a small quantity of boiling water.
8. Now re-suspend it in a minimum quantity of cold water, heat to boiling, and once more collect the insoluble $Ca_3C_{12}H_{10}O_{14}$by filtration.
9. Allow the salt to air dry. Wash and calculate the yield.

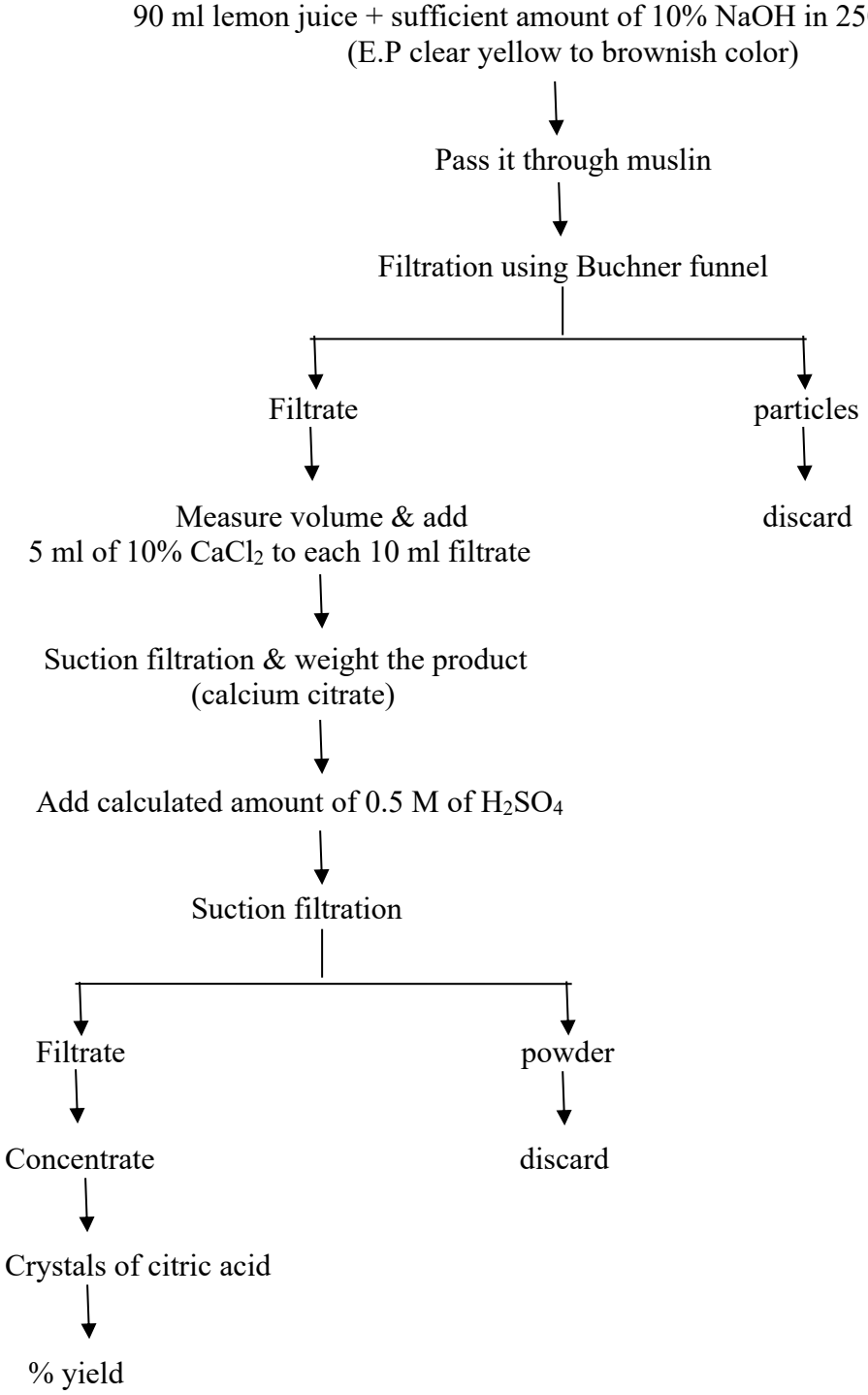
Calculations:

10. Citric acid may be prepared from the citrate salt by weighing the air-dried salt and placing it in a beaker.
11. Add sufficient 1 N H_2SO_4 required to convert the salt to the acid.
12. The equation for the reaction is:

$$Ca_3C_{12}H_{10}O_{14} + 3\ H_2SO_4 \rightarrow C_{12}H_{10}O_{14} + 3\ CaSO_4$$

13. Allow the mixture to stand for a few minutes, filter off the insoluble $CaSO_4$, and concentrate the filtrate to a small volume in a steam bath.
14. Citric acid crystallizes out (small amount of Citric acid is difficult to crystalline because of relatively great water solubility).Filter, dry, and weigh the acid.
15. Calculate the percentage of citric acid in the lemon juice sample you used.

Extraction of Citric acid from lemon juice



Faculty of Pharmacy كلية الصيدلة	**Phytochemistry Laboratory Report**	**Al-Ahliyya Amman University** جامعة عمان الأهلية

Lab Report No.:	**Student's name:**
Experiment name:	**Group number:**

I. Objective:

……..

II. Observations:

III. Calculate the percentage of citric acid in the lemon juice sample you used

IV. Conclusion

Lab 12 Extraction of Pectin

Objectives:
To isolate and identify pectin

Introduction
Pectin is a complex mixture of polysaccharides composed mainly of polymers of D-galacturonic acid whose monomers are joined by (1,4)-linkages. They also contain arabinose and galactose. The carboxyl group of galacturonic acid can be esterified to varying extents with methanol. The hydroxyl groups in the 2nd and 3rd positions menwhile can be acetylated to a lesser extent.
In strong acidic medium, the glycosidic linkages are hydrolyzed. Pectin is most stable at pH 3-4. In basic medium, the ester and glycosidic linkages are split to the same extent. The latter is eliminated because the hydrogen atom in theC-5 position is more acidic compared to the galacturonic acid with free carboxyl groups.

Pharmaceutical uses of pectin
-Pectin has applications in the pharmaceutical industry. It has been reported to help reduce blood cholesterol. Consumption of at least 6 g/day of pectin is necessary to have a significant effect in cholesterol reduction.
-Pectin acts as a natural prophylactic substance against poisoning with toxic cations. It has been shown to be effective in removing lead and mercury from the gastrointestinal tract and respiratory organs.
-Pectin hydrogels have been used in tablet formulations as a binding agent and have been used in controlled-release matrix tablet formulations.

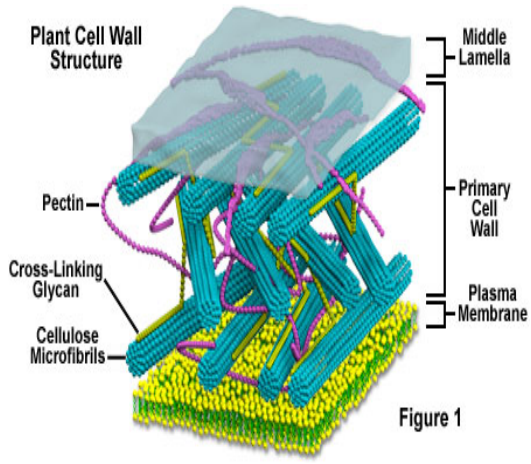
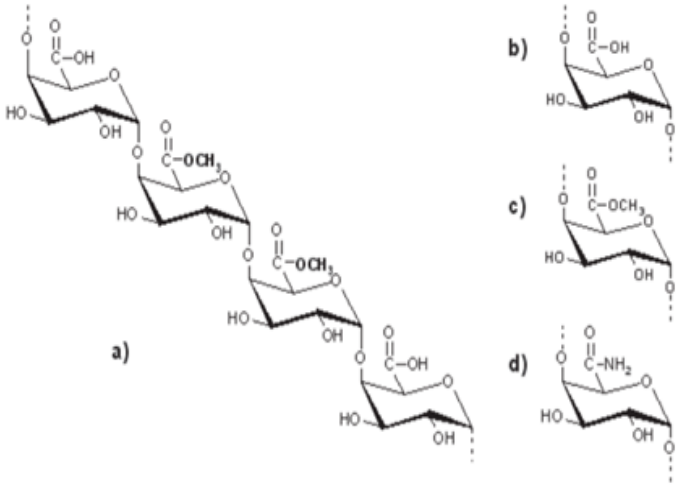
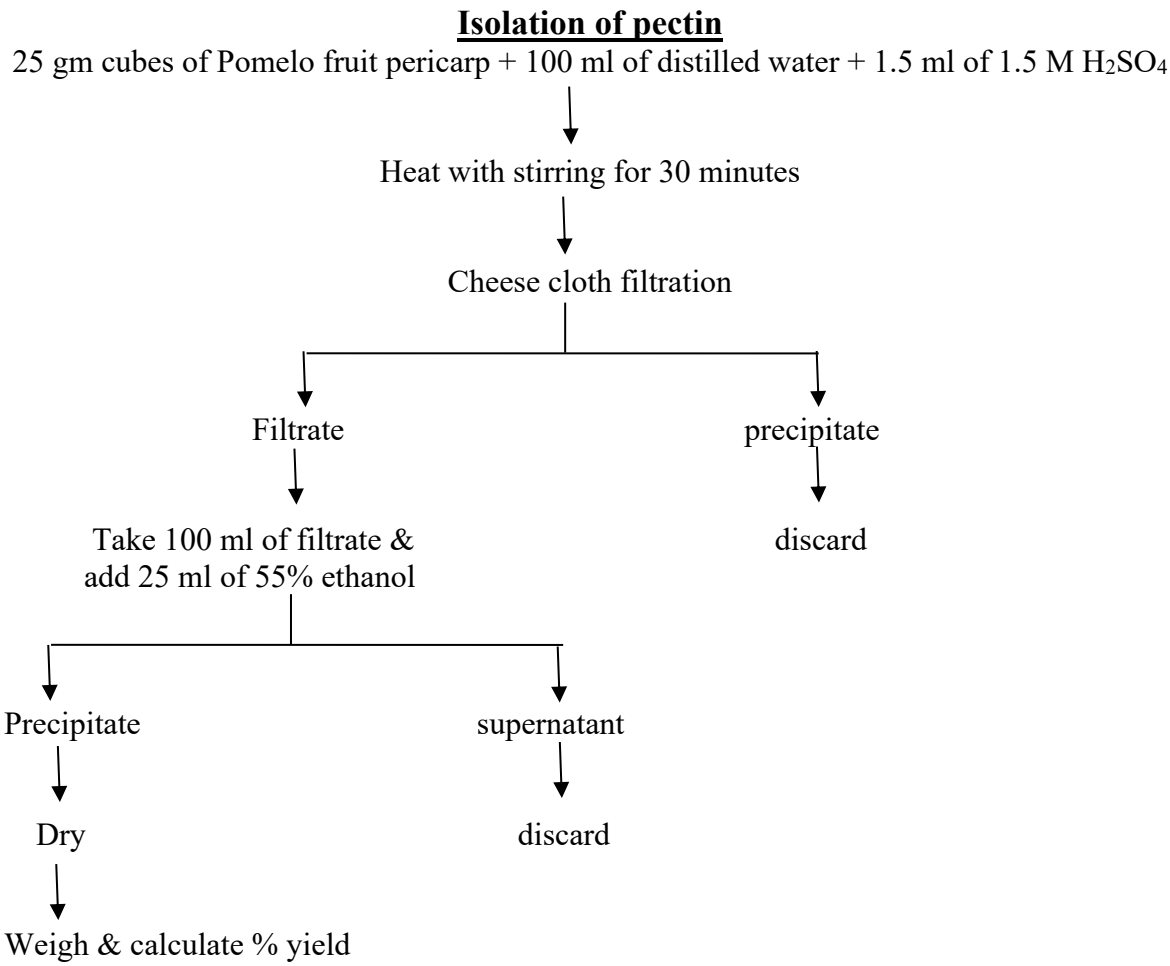


Figure 1



(a) A repeating segment of pectin molecule and functional groups: (b) carboxyl; (c) ester; (d) amide in pectin chain.

Procedure:


Isolation of pectin
25 gm cubes of Pomelo fruit pericarp + 100 ml of distilled water + 1.5 ml of 1.5 M H_2SO_4
Heat with stirring for 30 minutes
Cheese cloth filtration
Filtrate
precipitate
Take 100 ml of filtrate & add 25 ml of 55% ethanol
discard
Precipitate
supernatant
Dry
discard
Weigh & calculate % yield

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Lab Report No.:	**Student's name:**
Experiment name:	**Group number:**

I. Objective:

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II. Observations:

III. Calculate the percentage yield of pectin

IV. Conclusion