



Pharmacognosy

Third year

Practical sessions



Performed by

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Head of department

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Semester I

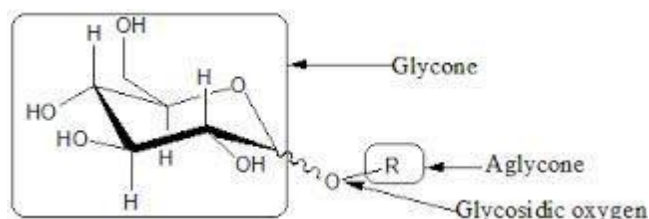
Introduction: Glycosides

Glycosides are compounds that yield on hydrolysis, one or more sugar part and another non-sugar part. The sugar part is known as **glycone**, and the non-sugar part is the **aglycone**. In general there are four basic classes of glycosides: C- glycosides, in which the sugar is attached to the aglycone through C-C bond, and the O-glycosides in which the sugar is connected to the aglycone through oxygen –carbon bond, S-glycosides and N-glycosides.

Chemically the glycosides are acetals in which the hydroxyl group (OH) of the glycone is condensed with the hydroxyl group of aglycone. More simply the glycosides may be considered as sugar ether. Two forms of glycosides are present, the α -form and the β -form, but the β -form is the one that occurs in plants, even the hydrolytic enzymes act on this type.

Inside the body the glycosides will be cleaved to glycone and aglycone parts, the glycone part confers on the molecule solubility properties, thus is important in the absorption and distribution in the body, while the aglycone part is responsible for the pharmacological activity.

Generally all glycosides are hydrolyzed by boiling with mineral acids, on the other hand the presence of specific enzyme in the plant tissue, but in different cells from those that contain the glycosides, are able to hydrolyze the glycosides, such as the emulsin enzyme which is present in the almond kernel, and the myrosin enzyme which is found in the black mustard seeds.



Generally in the extraction of glycosides we have to consider the following points:

1. A polar solvent , which is mostly alcohol ,but not water ,since water may induce fermentation , in addition water need high temperature due to its high boiling point.
2. Neutralization of the extract with base, since the presence of acid lead to hydrolysis of the glycoside.
3. Use of heat is to inhibit the activity of hydrolytic enzymes that present in the plant cell.

The glycosides are classified according to the aglycone chemical structure to

1. Cardioactive glycosides.
2. Anthraquinone glycosides .
3. Saponin glycosides.
4. Cyanophore glycosides.
5. Isothiocyanate glycosides.
6. Flavonoid glycosides.
7. Alcohol glycosides.
8. Aldehyde glycosides.
9. Lactone glycosides.
10. Phenol glycosides.
11. Miscellaneous glycosides.

LAB No.1

Cardioactive Glycosides

Definition: they are type of glycosides which exert into the failing heart a slowing and strengthening effect.

The main chemical structure consists of three areas: unsaturated lactone ring, steroidal skeleton and a sequence of sugars with a notice that the sugar molecule which is attached to the steroid is a de-oxy sugar (i.e. digitalose).

The lactone ring is attached to C-17 of steroid, while the sugar sequence is attached to C-3 site.

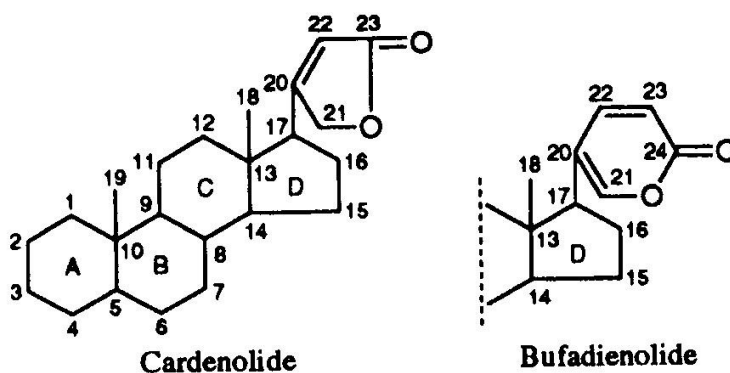
-According to the lactone ring, cardiac glycosides are divided into:

Cardenolides: they contain a five membered unsaturated ring. Therefore, they are C-23 glycosides.

Medicinally, cardenolides are the most important. They could be found in several medicinal plants (i.e. *Digitalis SP.* , *Convallaria majalis* , *Nerium oleander*).

Bufadienolides: they contain a six membered unsaturated ring. Therefore, they are C-24 glycosides.

An example of bufadienolides is the glycosides found in squill (*Urginea maritima*).



Solubility:

They are soluble in water and aqueous alcohol, but usually insoluble in fat solvents with exceptions of chloroform and ethyl acetate.

Detection of cardiac glycosides:

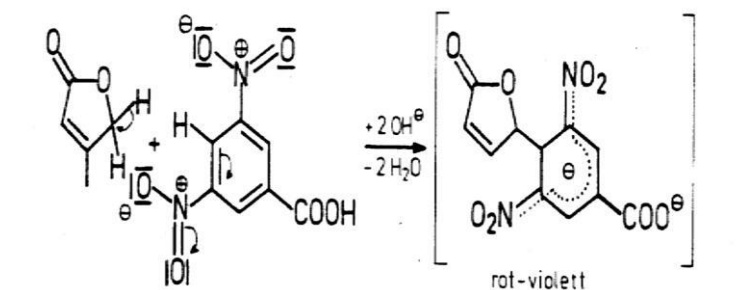
There are several reactions which detect each part the cardiac glycoside, so there are reactions that detect the unsaturated lactone ring, reactions that detect the steroid and reactions which are specialized in the detection of de-oxy sugars.

The principle of extraction:

1. Cardiac glycosides are extracted with aqueous alcohol via heating the dried plant with ethanol 50% and with the addition of lead acetate solution (to remove tannins and other polyphenols which may disrupt detection).
2. A liquid-liquid extraction is done twice with chloroform and the chloroform layer is then obtained and heated until dryness. Finally, several reaction could be applied.

The detection of the lactone ring:

The five membered unsaturated lactone ring in cardinolides reacts with polynitro aromatic compounds in a basal medium and lead to the constitution of colorful complex (Meisennheimer salt).



Kedde reaction: a reaction between 3,5 dinitrobenzoic acid and cardinolides gives an unstable red violet color that disappears after a while.

Baljet reaction: a reaction between picric acid and cardinolides gives an orange color.

Raymond test: a reaction between 1,3 dinitrobenzol and cardinolides gives a red violet to blue violet color.

The detection of the de-oxy sugars:

Xanthydrool reaction:

The 2-de-oxy sugars or 2,6- de-oxy sugars react with Xanthydrool solution giving a colorful compounds.

(Xanthydrool solution is composed of xanthydrool in a solution of methanol, acetic acid and hydrochloric acid).

Killer-killiani test:

A De-oxy sugar is dissolved in acetic acid containing traces of ferric chloride and transferred to the surface of concentrated sulfuric acid. At the junction of the liquids a reddish-brown color is produced which gradually becomes blue refers to the deoxy sugar. The layer of acetic acid is colored with green or blue referring the steroidal skeleton.

The detection of steroidal skeleton:

There are several reaction that can detect the steroidal part of the cardiac glycoside aglycone.

Lieberman Buchard's test: the reaction with anhydrous acetic acid and sulfuric acid gives a colored complex

Rosenheim test: steroids that contain double bonds react with trichloric acetic acid giving a colored compounds (usually pink that turns into violet).

Jensen Kny test:

The reaction between the steroid that contains double bonds, chloramine T and trichloric acetic acid gives a yellowish green to blue florescence under the wavelength 365 nm.

This reaction gives positive result with either cardinolides or bufadienolides.

Experiment:

Equipments and reagents:

- Beakers 250 ml, 100ml, 2 (50ml).
- Conical flasks 2 (100ml).
- Centrifuge tubes.
- Separatory funnel.
- Rotatory evaporator or water bath.
- 70% ethanol.
- Lead sub acetate.
- 10% Sodium phosphate solution.
- Chloroform-ethanol (3:1 v/v).
- Anhydrous sodium sulphate.
- 4N HCl.
- Chloroform.

Procedure:

10g of dried powder of the leaves was weighed and transferred into a conical flask and it was macerated with **50ml** of **70% ethanol** for 24 hrs. The whole mixture was filtered and the filtrate was collected. Place **30ml** of the alcoholic extract in a conical flask and then add **13ml** of lead sub acetate. Mix thoroughly and allowed standing for two minutes. Placing the mixture in the centrifuge tubes and centrifuging for two minutes. Decant and take the supernatant into a flask and add **20ml** of **10%** sodium phosphate solution.

Mix thoroughly and filter. Now divide the mixture you had into two divisions.

FRACTION A:

Take one of the two divisions you made above and put in a separatory funnel, then shake it three quantities each of **20ml** of Chloroform-ethanol (**3:1 v/v**). combine the organic extracts, then dry by the addition of a small quantity of anhydrous sodium sulphate and allowed standing for two minutes. Decant the Chloroform-ethanol extract and reduce the volume to about **1ml** on a rotatory evaporator or (water bath).

FRACTION B:

Place the other division of the extract in a conical flask, and then add **3ml** of **4N HCl** and place in boiling water bath for **20** minutes. Cool the hydrolysate and transfer to separatory funnel, then partition with three quantities each of **25ml** of chloroform. Combine the chloroform extracts and dry with a small quantity of anhydrous sodium sulphate. Decant the chloroform and reduce the volume to about **1ml** on a rotary evaporator or (water bath).

Tests:

Baljet test:

Take 5 ml of the previous chloroform extract and dry it completely on a water bath. Add 2 ml of picric acid solution and 1 ml of NaOH 1N solution.

Killer-Killiani:

Take 5 ml of the previous chloroform extract and dry it completely on a water bath. Add 1 ml of glacial acetic acid, 2 drops of alcoholic FeCl_3 solution, then add slowly add 1ml of conc sulfuric acid on the tube wall without shaking.

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Preparation of solutions:

FeCl_3 solution: dissolve 10 g of ferric chloride in 100 ml ethyl alcohol.

NaOH solution (1N): dissolve 45 g of sodium hydroxide in 1 liter distilled water, then filtrate after 24 hr.

Picric acid Solution: dissolve 1 gr of picric acid in an a little volume of ethyl alcohol, then complete to 10 ml with ethanol 50%.

Lead acetate solution: dissolve 10 g of lead acetate in 100 ml distilled water.

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LAB No.2

Saponin glycosides

Definition:

This group of glycoside is widely distributed in higher plants. Saponin glycosides form colloidal solution in water that foam upon shaking, this is due to a decrease in the surface tension action done by saponin glycosides, as a result of the hydrophobic/hydrophilic characteristics of the saponin, and due to this property, saponins are used in the manufacturing of beer, and soap. Moreover, saponins stabilize suspensions and emulsions.

Saponins have a bitter, acrid taste, and drugs containing them are usually sternutatory and otherwise irritating the mucus membrane.

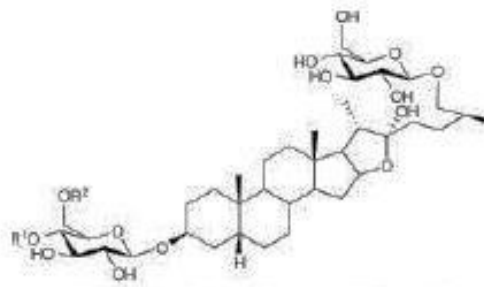
They destroy red blood corpuscles by hemolysis and are toxic especially to cold blooded animals therefore many saponins are used as fish poisons. The more poisonous saponin is often called sapotoxin, many are toxic to insects and mollusks, and some are used to control schistosomiasis snails.

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

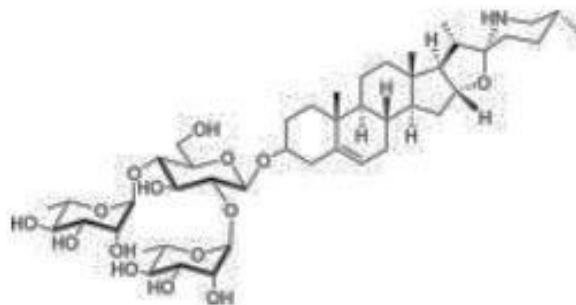
According to the structure of the aglycone, three kinds of saponin are recognized:

1. **Pentacyclic triterpenoid saponins** (acidic, and the C-atom is C30): they could be found in licorice (*Glycyrrhiza glabra*) and English ivy (*Hedera helix*).
2. **Steroid saponins** (neutral C-atom is C27): such an example of plants that contain this type of saponins is fenugreek (*Trigonella foenum graecum*).
3. **Steroid alkaloid saponins**: they contain a nitrogen atom in the skeleton of aglycone: an example is Solanoin compound which is found in several plants that belongs to solanaceae family (unripe tomatoes and potatoes).

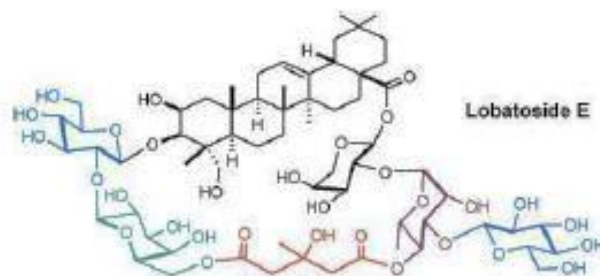
-Aglycones usually can be obtained by acid hydrolysis.



Steroidal saponin



Steroidal alkaloid saponin



Triterpene saponin

Solubility: they are soluble in water and aqueous ethanol, but slightly soluble in ethanol 96%.

They are precipitated in nonpolar solvents such as chloroform and diethyl ether.

Foam test and foam index:

The method is based upon the property of saponin to form a stable foam when shaken with water.

The method is based upon the property of saponin to form foam when shaken with water. The foam index signifies the dilution of the substance or drug to be tested which gives a layer of foam 1cm high if the aqueous solution is shaken for 15 seconds, and then allow standing for 15 minutes before reading is made.

Note: foam test is not specific to saponins since several compounds such as tannins and emulsifiers give a positive results, also.

Method of foam test:

Take 0.5 g of drug and add it to a test tube which contain 10 ml hot distilled water. Leave it to be cooled then shake it.

A column of foam will constitute (the height should be known).

The hemolytic Test (specific):

Procedure:

Take two test tubes and place in each one 5ml of a 10% solution of blood in normal saline. To one of them, add 5ml of normal saline solution and to the other one add 5ml of the saponin extract. Shake both tubes gently and notice the result. The test tube containing 5ml of the extract will cause blood hemolysis.

Coloring reagents:

Zlatkis- Zak reagent: triterpenes and steroids that contain hydroxyl groups give a stable colors when they react with an oxidizing agent (salts of heavy metals).

Method of Zlatkis- Zak reagent:

Take 0.5 g of drug and extract with 15 ml ethanol 50% for 10 minutes with shaking, then filtrate.

In a test tube, add 2 ml of the extract and 3 drops of either ferric chloride solution (10%) (it gives yellow solution) or copper sulfate (10%) (it gives green solution).

Notice: the plants which may be used in this experiment are: *Glycerrhiza glabra*, *Hedera helix* , *Trigonells foenum-graecum* and *Saponaria officinalis*

LAB No.3

Anthraquinone glycosides

Introduction: Anthraquinones and related glycosides, are stimulant cathartics, and exert their action by increasing the tone of the smooth muscle in the wall of colon and stimulate the secretion of water and electrolytes into the large intestine.

After the oral administration, the anthraquinone glycosides are hydrolyzed in the colon by the action of enzymes of the micro flora, to the pharmacologically active free aglycones which usually produce their effect in 8 -12 hrs. after administration, these agents are indicated for constipation in patient who do not respond to milder drugs and for bowel evacuation before investigational procedure or surgery.

Stimulant laxative are habit forming so the long-term use may result in laxative dependence and loss of normal bowel function.

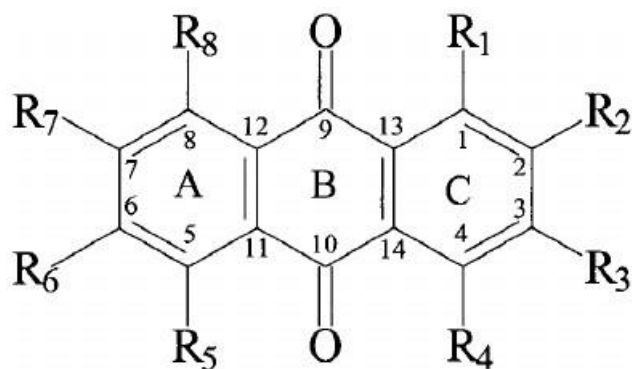
The glycosides of anthranols and anthrones elicit a more drastic reaction than do corresponding anthraquinone glycosides and cause discomforting and gripping action.

The drugs mostly used are cascara barks ,frangula and Senna.

Aloe and Rhubarb are not recommended due to their irritating actions which increase the chance for gripping effect.

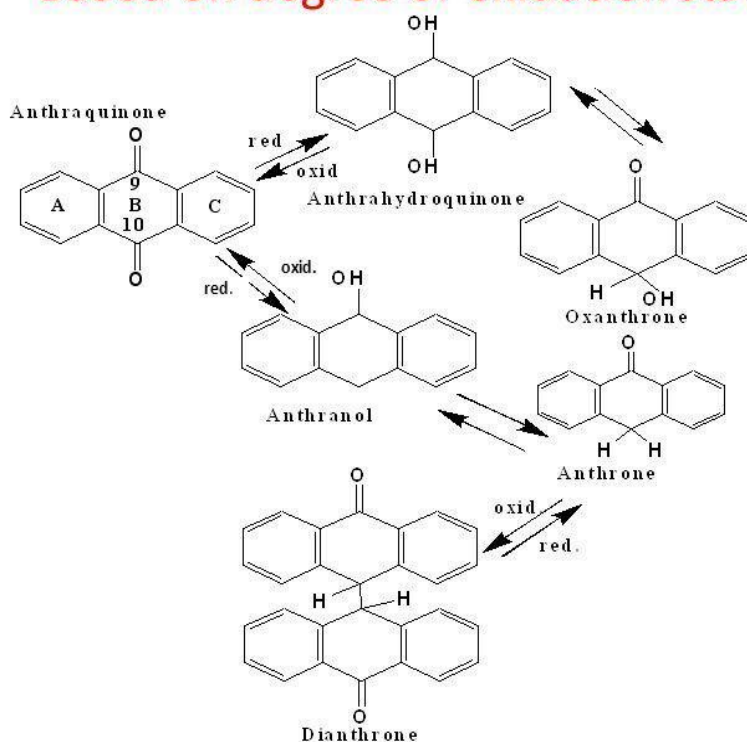
The anthraquinone hydrolyzed to give aglycone which are di, tri, or tetra – hydroxyanthraquinone .Also there are anthrone, dianthrones and oxanthrones.

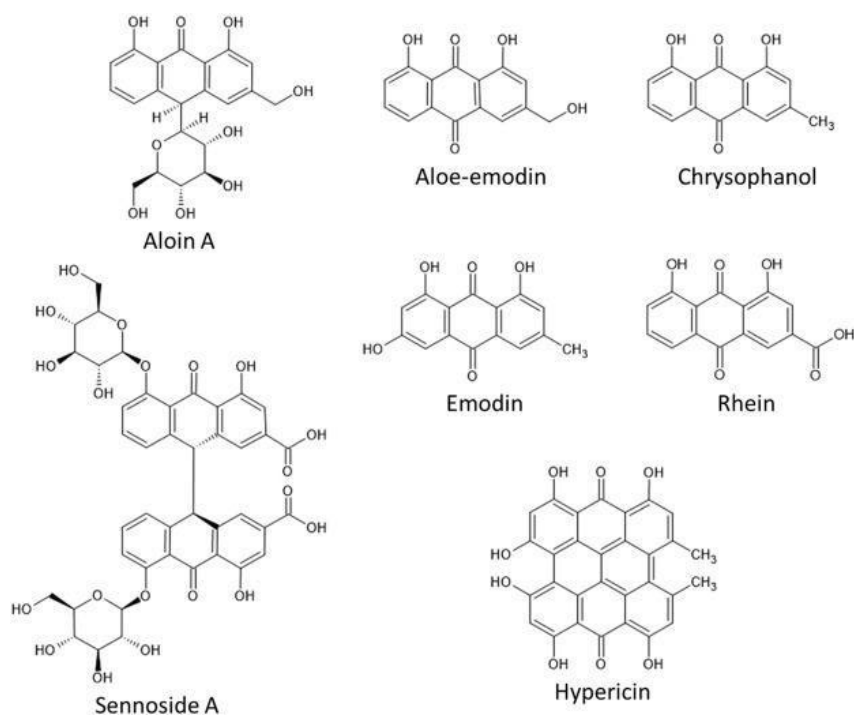
Anthraquinone glycosides are usually C-O glycosides while some of them are C-C glycosides such as aloe glycosides.



Classes of Anthraquinones

Based on degree of oxidation status



**Solubility:**

Anthraquinone glycosides are soluble in water, methanol and ethanol. On the other hand, aglycones are soluble in chloroform and diethyl ether.

Identification:**Boratrager test:**

Free anthraquinones (aglycones) are extracted with nonpolar solvent and the addition of an alkaline turns the color into red.

Materials:

Diethylether, Ammonia solution (10%).

Method:

Rhubarb (*Rheum palmatum*) free anthraquinones are extracted and identified as follow:

- Take 150 mg of drug and mix with 7 ml diethyl ether.
- Shake for about 2 minutes then filtrate.

- add 3 ml of the extract to a test tube then add 2 ml of ammonia solution.
- Shake until the red color in the ammonia layer appears.

Shouteten reaction:

Anthraquinone glycosides are extracted with hot water. The addition of talc removes impurities. Then the addition of sodium tetrahydridoborate will lead to the formation of a green inflorescent complex.

This reaction is not selective due to the fact that some compounds like coumarins possess a spontaneous florescence under the UV light.

LAB No.4

Extraction and detection of Senna anthraquinone glycosides

Preface:

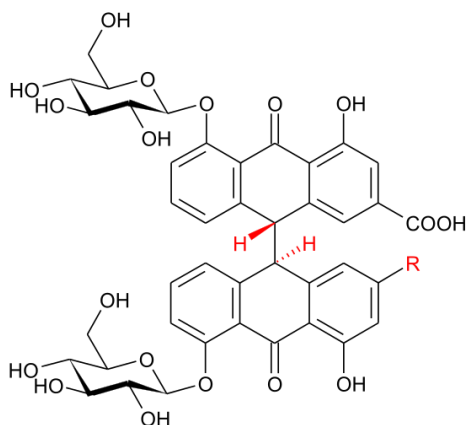
The genus Cassia comprises shrubs, subshrubs, and herbaceous perennials with paired-pinnate leaves. It belongs to the Fabaceae family.

The British pharmacopeia points out that two species can be used as medicine which are, *Cassia angustifolia* and *Cassia acutifolia*.

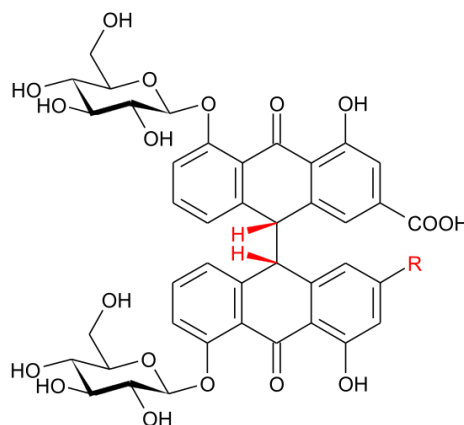
The leaves and pods (fruits) are used as medicine.

Anthraquinones of senna leaves and pods:

Anthraquinone derivatives are existed as dianthone glycosides which are called, Sennosides (Sennoside A, Sennoside B, Sennoside C and Sennoside D).



Sennosid A: R = COOH
Sennosid C: R = CH₂OH



Sennosid B: R = COOH
Sennosid D: R = CH₂OH

Extraction and detection of sennosides:

Since Borantrager test detects only free anthraquinones, hydrolysis should be done to obtain the aglycones of sennosides, and the addition of ferric chloride lead to get monoanthrones which can be detected by Borantrager test and lead to a pale pink color after the addition of ammonia solution. The previous method is called modified Borantrager test.

Method of extraction:

A decoction for the drug is prepared with acidic water and ferric chloride solution.

The free monoanthrones are extracted with chloroform twice.

The addition of ammonia solution (10%) to the chloroform layer lead to a pale pink color.

Steps of the experiment:

1. Place 1 gm of powdered senna leaves in a beaker and add 100 ml of water, boil gently for 15 minutes, cool and centrifuge or filter it.
2. Place the filtrate in a separatory funnel and extract by shaking with two quantities of (2*40 ml) chloroform.
3. Combine the chloroform extracts, and then concentrate to about 1ml, (Fraction B).
4. The aqueous extract will be divided in two parts, one will be placed in a small flask and evaporate carefully almost to dryness on a rotary evaporator, (Fraction A).
5. The other part is placed in a 250 ml round-bottomed flask and adding to it 7 ml of ferric chloride solution (5% w/v). Reflux for 20 minutes, and add 4ml of conc. HCl, continue heating for further 20 minutes, shaking the flask occasionally to dissolve as much as possible of the precipitate, and allow cooling.
6. Place the hydrolysate in a separatory funnel and extract by shaking with two quantities of (2*40 ml) chloroform.
7. Concentrate the bulked chloroform extract to about 3ml, (Fraction C).

IDENTIFICATION BY CHROMATOGRAPHY:

Thin-layer chromatography using specific solvent systems and spray reagents indicated the presence of anthraquinones

The stationary phase of TLC is silica gel

The mobile phase of TLC is **(n-propanol: ethyl acetate: water)(40:40:30)**.

Spray reagent is alcoholic KOH (**5% w/v**). (for better result spray first with nitric acid 25% then heated in the oven, then sprayed with alcoholic KOH reagent. This step is done to intensify the color of the spots).

Procedure:

Prepare the solvent system and place it in the glass tank, leave it for 45 minutes for saturation. Apply the sample fraction A, B, C and standard spot on a TLC plate. Then place the TLC plates in the tank and allow the mobile phase to rise to about two –third the plates. Let to dry at room temperature, then spray first with 25% nitric acid solution and heat for 10 minutes at 110 °C. Allow to cool, and then spray with alcoholic KOH (**5% w/v**). Detect the spots and calculate R_f value .

LAB No.5

Tannins

Definition:

Tannin is a naturally occurring **polyphenol** found in plants, seeds, bark, wood, leaves and fruit skins. The tannin compounds are widely distributed in many species of plants, where they play a role in protection from predation, and perhaps also as pesticides, and in plant growth regulation.

Tannin tastes dry and astringent and you can feel it specifically on the middle of your tongue and the front part of your mouth.

Tannins precipitate proteins from solutions and can combine with proteins, rendering them resistant to proteolysis' enzymes.

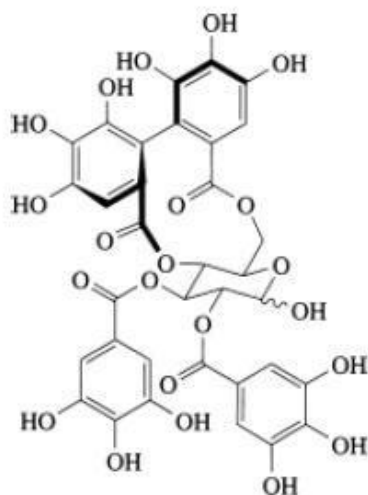
Unsweetened black tea is a great example of nearly pure tannin dissolved in water.

There are three major types of tannins: condensed, hydrolysable and complex tannins.

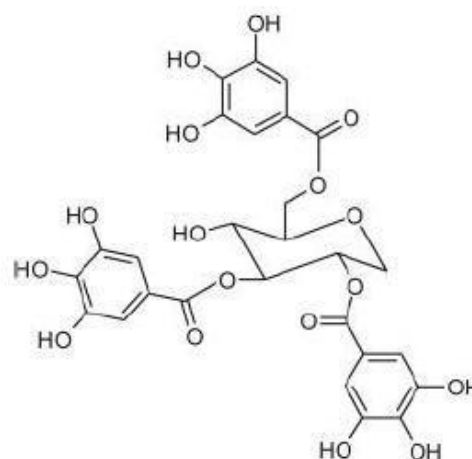
1. Hydrolysable tannins

Hydrolysable tannins are derivatives of **Gallic acid** (3, 4, 5 - trihydroxyl benzoic acid) and so called gallotannins or **ellagic acid** and so called ellagitannins. Gallic acid or ellagic acid esterifies to a core polyol (glucose), and the galloyl or ellagic groups may be further esterified or oxidatively cross linked to yield more complex hydrolysable tannins.

Gallotannins can be found in *Quercus infectoria*, while ellagitannins can be found in pomegranate (*Punica granatum*) fruit skin.



(106)
Ellagitannin

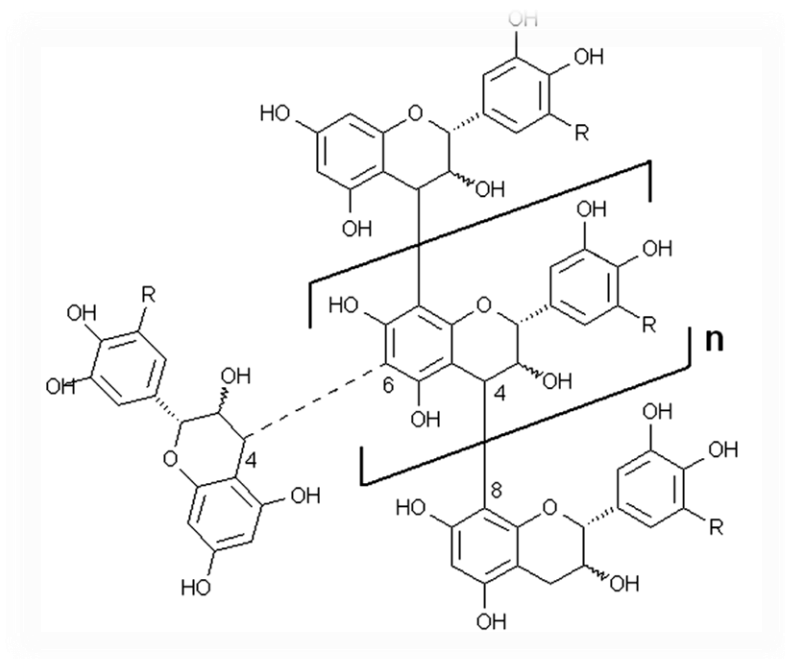


Gallotannin

2. Nonhydrolysable tannins or condensed tannins:

The condensed tannins (proanthocyanidins) are derived from flavonoid monomer (flavan-3-ol or Flavn3,4-diol). They are much more resistant to decomposition and merely yield polymers or amorphous precipitates under the influence of acids. They do not contain sugar residues. They are called proanthocyanidins as they yield anthocyanidins when depolymerized under oxidative conditions. These compounds when treated with acid or enzymes, are converted into red insoluble compounds known as phlobaphenes.

Condensed tannins are existed in tea (*Camellia sinensis*).



Condensed tannins

3. Complex tannins:

Which are composed of hydrosable tannins and condensed tannins.

Uses of tannins:

1. They are tightening, healing and drying, reducing irritation and inflammation and creating a barrier against infection that is helpful in wounds and burns.
2. Tannins are an important ingredient in the process of tanning leather
3. Iron gall ink is produced by treating a solution of tannins with iron(II) sulfate.
4. Antidote treatment of alkaloid poisoning.

Extraction and detection of tannins:

Boil 1 g of plant coarsely powdered of (*Punica granatum* or *Camelia sinensis*) with 50 ml of water. Cool and filter.

Chemical tests:**1. Ferric chloride solution:**

Ferric chloride in an alcoholic medium gives a colored complex compounds with phenols, in general. It differentiates between the two main types of tannins.

Condensed tannins give a green black color ; hydrolysable tannins give a blue black color.

Use 1 ml of tannin extract and dilute with ethanol until the color appears yellow. After that add carefully 1 drop of ferric chloride solution (5% in absolute ethanol).

2. Lead acetate precipitation:

Phenolic compounds in general have the ability to precipitate the salts of heavy metals such as lead acetate.

Use 1 ml of extract, then add 2 drops of lead acetate solution (10%).

3. Sodium nitrite Test:

This test differentiates ellagitannins from others; the addition of sodium nitrite gives a red brownish color turned into a green after a while in the presence of ellagitannins.

Use 0.5 ml of tannin extract and dilute with ethanol until the color appears yellow, then add few crystals of sodium nitrite. Shake and wait.

4. Gelatin Test:

True tannins have the ability to precipitate gelatin (in a solution).

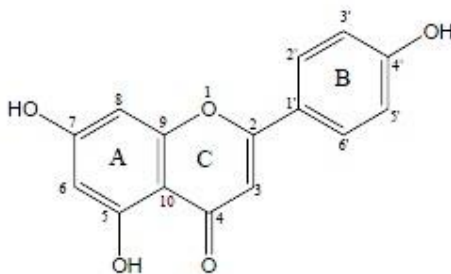
Use 1 ml o tannin extract and add 1 ml of 1 % gelatin solution, add little 10 % sodium chloride to rise the sensitivity of reaction.

LAB No.6

Flavonoid glycosides

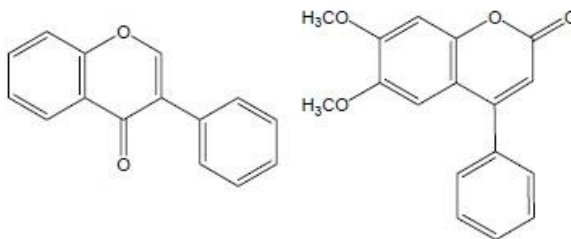
Flavonoids (from the Latin word flavus meaning yellow, their colour in nature) are polyphenols of plant origin that are among the most important compounds in human diet due to their widespread distribution in foods and beverages.

Flavonoids have the general form of C₆-C₃-C₆. They have the skeleton of phenyl benzo pyran. They are derived from Chalkones which are yellow pigments existed in plants.



General structure of flavonoids

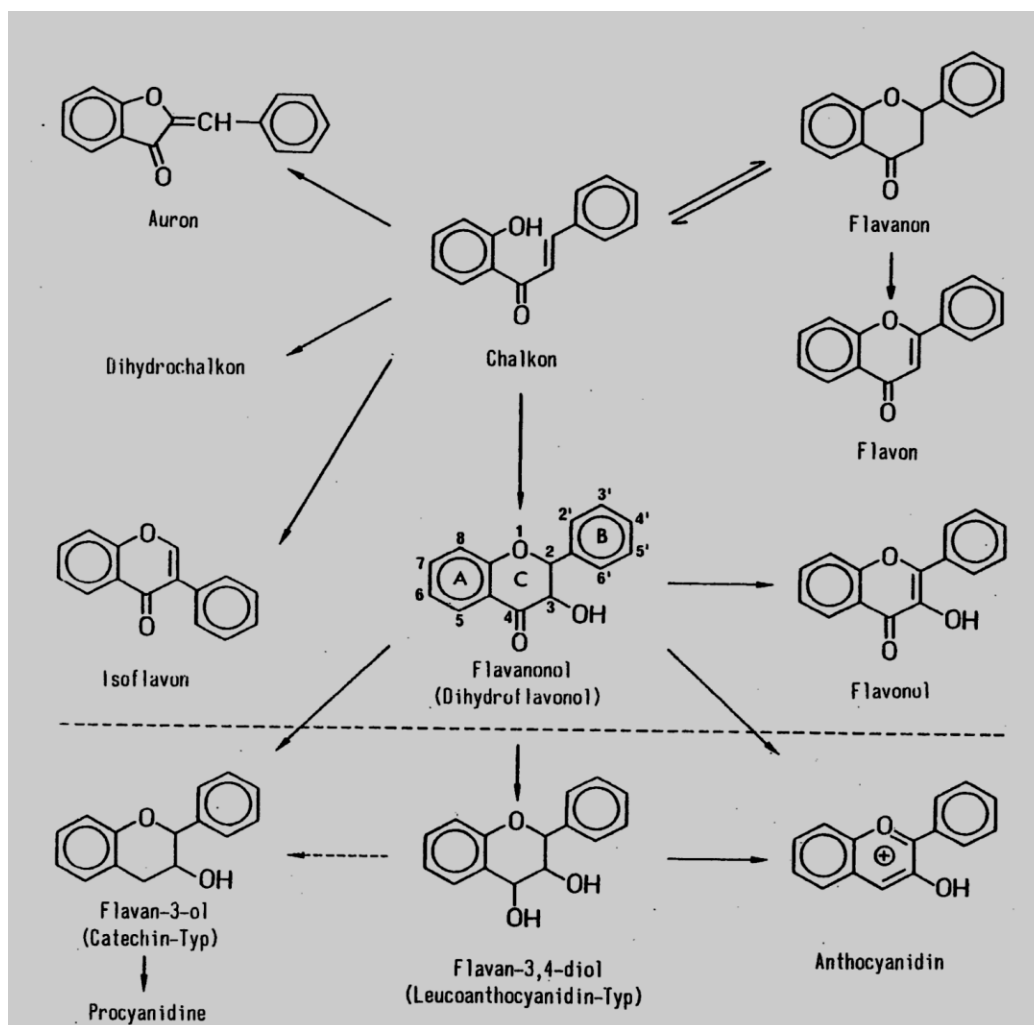
Most of flavonoid classes are 2-phenyl benzo pyran, while isoflavonoids are 3-phenyl benzo pyran and neoflavonoids are 4-phenyl benzo pyran.



Isoflavone

neoflavone

Flavonoids are existed in free form (aglycones) and as glycosides (C-O or C-C glycosides).



Pharmacological activity of flavonoids:

A number of flavonoid-containing herbs have now been included in the BP/ EP, examples are Birch leaf (*Betula* SP), *Calendula officinalis* Flower and Elder flower (*Sambucus nigra*). The group is known for its anti-inflammatory and anti-allergic effects, for antithrombotic and vasoprotective properties, for inhibition of tumor promotion and as protective for the gastric mucosa. Some of these pharmacological properties can be explained on the bases of antioxidant activity. Many flavonoid containing plants are diuretic (e.g. *Betula* and *Ruscus aculeatus*) or antispasmodic

(e.g. liquorice and parsley). Some flavonoids have antitumour, antibacterial or antifungal properties.

Solubility of flavonoids:

Flavonoid glycosides are soluble in water, ethanol and methanol. On the other hand, aglycones are soluble in non-polar organic solvents such as ether and ethyl acetate.

Detection of flavonoids:

Extraction:

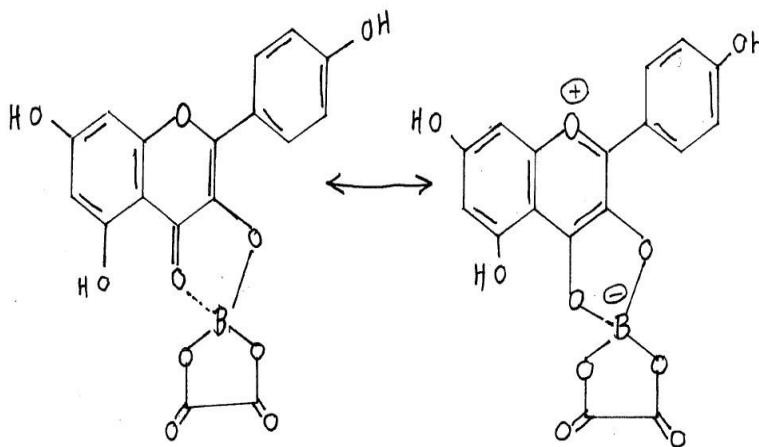
Flavonoids are extracted with alcohol and then petroleum ether is added to remove chlorophyll.

Reaction with alkaline:

In alkaline solutions flavanones yellow precipitates, that for some time become brightly yellow or yellow (isomerization to formation of chalcones).

Wilson-taubock test:

5-oxyflavones and 5-oxyflavonoles with Wilson's reagent (boric and oxalic acids in anhydrous acetone) develop yellowish-green fluorescence under the UV 365 nm.



Shinoda test:

Flavone, flavonols and their glycosides can be reduced into anthocyanins, which have a red to violet color. Using magnesium and concentrated HCL.

Steps of experiment:

- Boil 10 gr of plant with 100 ml ethanol 70% for 20 minutes under a condenser.
- Cool and filter.

Reaction with alkaline:

Take 3-4 drops of the extract into a test tube and add dilute with 2 ml distilled water, then add 1 ml of Sodium hydroxide 10% solution and shake well.

Wilson-taubock test:

- Take 5 ml of the extract and boil it until dryness.
- Add a few amount of oxalic acid and boric acid.
- Add 3 ml of acetone
- Boil the mixture until dryness.
- Check the florescence under the UV light.

Shinoda test:

- Take 3 ml of extract and evaporate until dryness.
- Dissolve the dry extract with 2 ml ethyl alcohol (Absolute).
- Add 0.1 g of magnesium and few drops of HCL Conc.

Note: the plants which are used in this experiment are:

Crataegus lavigata aerial parts/ *Hypericum perforatum* aerial parts/ *Glycyrrhiza glabra* roots.

Identification of Flavonoids By Chromatography:

1-By the use of Paper chromatography (P.C):

The stationary phase = Filter paper (Whatman no.1).

The mobile phase = n-BuOH:HOAc:H₂O (4:1:5).

The standard compound = Rutin.

The spray reagent = 5% alcoholic KOH.

Mechanism of separation = Partition.

Developing = Ascending.

2-By the use of Thin layer chromatography (T.L.C):

The stationary phase = Silica gel G.

The mobile phase = Ethyl acetate -formic acid - glacial acetic acid - water(100:11:11:26).

The standard compound = Rutin.

The spray reagent = flavonoids spot on TLC plates produce a yellow-brown

Spots when reacted with Iodine vapor.

Mechanism of separation = Adsorption.

Developing = Ascending.

Detection: Flavonoids may appear as dark spots on a green background fluoresce when observed in UV light at 254 nm UV-plates containing fluorescent indicator (such as silica gel F254). If under 365 nm UV light, spot colors depending on the structure of flavonoids, can be yellow, green or blue fluorescent. It would be more clear and intense after being sprayed with the reagent.

Colors can be observed at 365 nm UV light are as follows:

Quercetin, myricetin, and 3 & 7-O-glycosides: orange-yellow

kaempferol, isorhamnetin, and 3 & 7-O-glycosides: yellow-green

Luteolin and 7-O-glycosides: orange

Apigenin and 7-O-glycosides: yellow-green

LAB No.7

Volatile Oils

They are odorous principles found in various plant parts. Because they evaporate when exposed to the air at room temperatures, they are called volatile oils; they are also called essential or etherical oils.

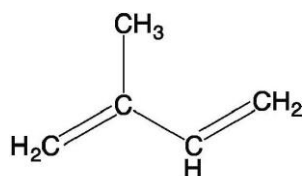
Volatile oils are usually colorless, even though some are yellow to pale yellow particularly when they are fresh, but on long standing they may oxidize and resinify, thus darkening in color, to prevent this darkening, they should be stored in a cool, dry place in tightly, preferably full, amber glass containers.

Note: German chamomile essential oils is naturally dark blue

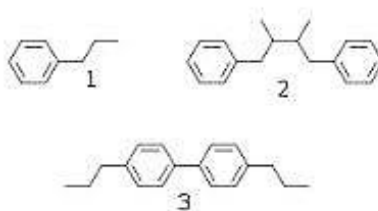
Solubility: volatile oils are immiscible with water, but they are sufficiently soluble to impart their odor to water. They are soluble in ether, alcohol and most organic solvents.

Chemical composition: many volatile oils consist largely of terpenes (terpenes are natural products whose structures may be divided into isoprene units).

Another major group of volatile oil constituents are the phenylpropanoids. (These compounds contain the C₆ phenyl ring with an attached C₃ propane side chain).



Isoprene



Phenyl propane

Generally volatile oils and volatile oil-containing drugs are divided in to the following classes:

1. Hydrocarbons (a-pinene, Limonene).
2. Alcohols (Menthol, Geraniol, Linalool).
3. Aldehydes.
4. Ketones (Fenchone, Carvone).
5. Phenols (Thymol).
6. Phenolic ethers (Anethole).
7. Oxides (1,8-cineol).
8. Esters (Linalyl acetate).

Essential oils in plant parts:

Essential oils can be existed in:

- Leaves- Rosemary, Basil, Eucalyptus.
- Flowers- Rose, Lavender, Clove.
- fruits and seeds: Fennel, Anise, cumin, Black pepper.
- Bark: Cinnamon.
- Rhizome: Ginger and turmeric.

Isolation and Identification of the Volatile Oils:

Determination of the volatile content of crude drugs is done by water distillation method using Clavenger type as an apparatus.



Clavenger apparatus (oil heavier than water) Clavenger apparatus (oil lighter than water)

The principle of isolation: volatile oil compounds are light so they can be carried from the plant with water steam. Then after condensing the steam volatile oils can be separated from water.

Most of volatile oils are lighter than water (have a density lower than water's), but some of them are heavier (Clove oil, for instance).

Procedure:

- 1) Weigh out 100 g of the plant material and place into a distilling flask.
- 2) Add 200 ml distilled water to the flask and shake well. Add another 200ml of water by rinsing the neck of the flask.
- 3) Connect the distilling flask with the still head of the apparatus. By the means of the pipette or washing bottle, fill the receiver with water until over flows.
- 4) Connect the condenser of the apparatus with the cooling water (from the tap). 5) Heat the distilling flask until the boiling starts. Record the time of the beginning of distillation, and continue the distillation for 1-2 hours.

- 6) Switch off heating. Allow the graduated receiver to cool. Read off the volume of the volatile oil (count all small divisions in the receiver of the layer of oil).
- 7) Calculate the %v/w of the volatile oil content of drug.

Note 1: distillation rate should be about 2-3 ml/minute.

Note 2: volatile oil isolation can be done using dried or fresh herbs (prefer herbs which are freshly collected).

Note2: some volatile oils cannot be isolated from flowers due to the decomposition of the fragrance by water and heat (such as jasmine oil), therefore, these oils can be extracted using organic solvents or fatty materials (enfleurage method).

Semester II

LAB No.8

Alkaloids

‘Alkaloid’ is any of a class of naturally occurring organic nitrogen-containing bases. Typical alkaloids are derived from plant sources, they contain one or more nitrogen atoms (usually in a heterocyclic ring) and they usually have a marked physiological action on man or other animals. Well-known alkaloids include morphine, strychnine, quinine, ephedrine, and nicotine.

Alkaloids distribution in plants kingdom:

Alkaloid distribution in the angiosperms is uneven, they are commonly found in families such as Solanaceae (*Atropa belladonna*, *Datura stramonium* and *Hyocyamus niger*), Papaveraceae (*Papaver somniferum*), Rubiaceae (*Cinchona* SP), Fabaceae (*Spartium junceum*) and Loganiaceae (*Strychnox nux-vomica*). In Monocotyledon, alkaloids may exist in Colchicaceae (*Colchicum autmnale*). Alkaloids are rarely found in other angiosperm families (in Lamiaceae), for instance. They may be also presented in fungi such as Ergot alkaloids.

Alkaloids nomenclature:

Alkaloid names generally end in the suffix -ine, are obtained in various ways:-

- 1- From the generic name of plant yielding them as atropine.
- 2- From the specific name of plant yielding them as cocaine.
- 3- From the common name of the drug yielding them as ergotamine.
- 4- From their physiological activity as emetine.
- 5- From the discoverer as pelletrine.

Alkaloid classifications:

Many different systems of classification are possible. There are three main types based on chemical structures and derivation:

I . Non-heterocyclic or atypical alkaloids, sometimes called ‘proto-alkaloids’ or biological amines. They are derived from amino acids such as Ephedrine and Capsaicin.

II . Heterocyclic or typical alkaloids: they are derived from amino acids and divided into groups depending on their ring structure and amino acid derivation: to their ring structure as follows:- 1.

Pyrrole and pyrrolidine : (Hygrines).

2. Pyrrolizidine :(Symphitine).

3. Pyridine and piperidine : (Piperine).

4. Tropane : (Hyoscyamine, atropine). 5. Quinoline

6. Isoquinoline : (Morphine, codeine).

7. Aporphine (reduced isoquinoline): (Boldine).

8. Quinolizidine : (Sparteine).

9. Indole.

10. Indolizidine.

11. Imidazole.

III. pseudoalkaloids: these are not derived from amino acids such as purine alkaloids (Caffeine) and steroidal alkaloids (Solanine).

Alkaloids chemical properties:

Plant rarely contain free alkaloids, in other words, alkaloids are existed in plants in a form of salts for organic acids or tannins.

Alkaloids are weak bases and the basicity depends on the lone pairs of electrons on their nitrogen atoms. The nitrogen atoms may exist as primary amine (RNH_2), as secondary amine (R_2NH), or as tertiary amine (R_3N).

As organic bases, alkaloids form salts with aqueous mineral acids and when these salts treated with hydroxide ion the free amine is librated.

The alkaloids, like other amines , form double salts (contains more than one cation or more than one anion) these salts are descriptive color precipitates, moreover this property helps in the general identification of typical alkaloids.

Most alkaloids are precipitated from neutral or slightly acid solution by Mayer's reagent (potassiomeric iodide solution), by Wagner's reagent (solution of iodine in potassium iodide), by solution of tannic acid, by Hager's reagent (a saturated solution of picric acid), or by Dragendorff's reagent (solution of potassium bismuth iodide). These precipitates may be amorphous or crystalline and are of various colours: cream (Mayer's), yellow (Hager's), reddish-brown (Wagner's and Dragendorff's).

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LAB No.9

Extraction methods of alkaloids & general detection (Berberine type alkaloids and others) Harmala alkaloids

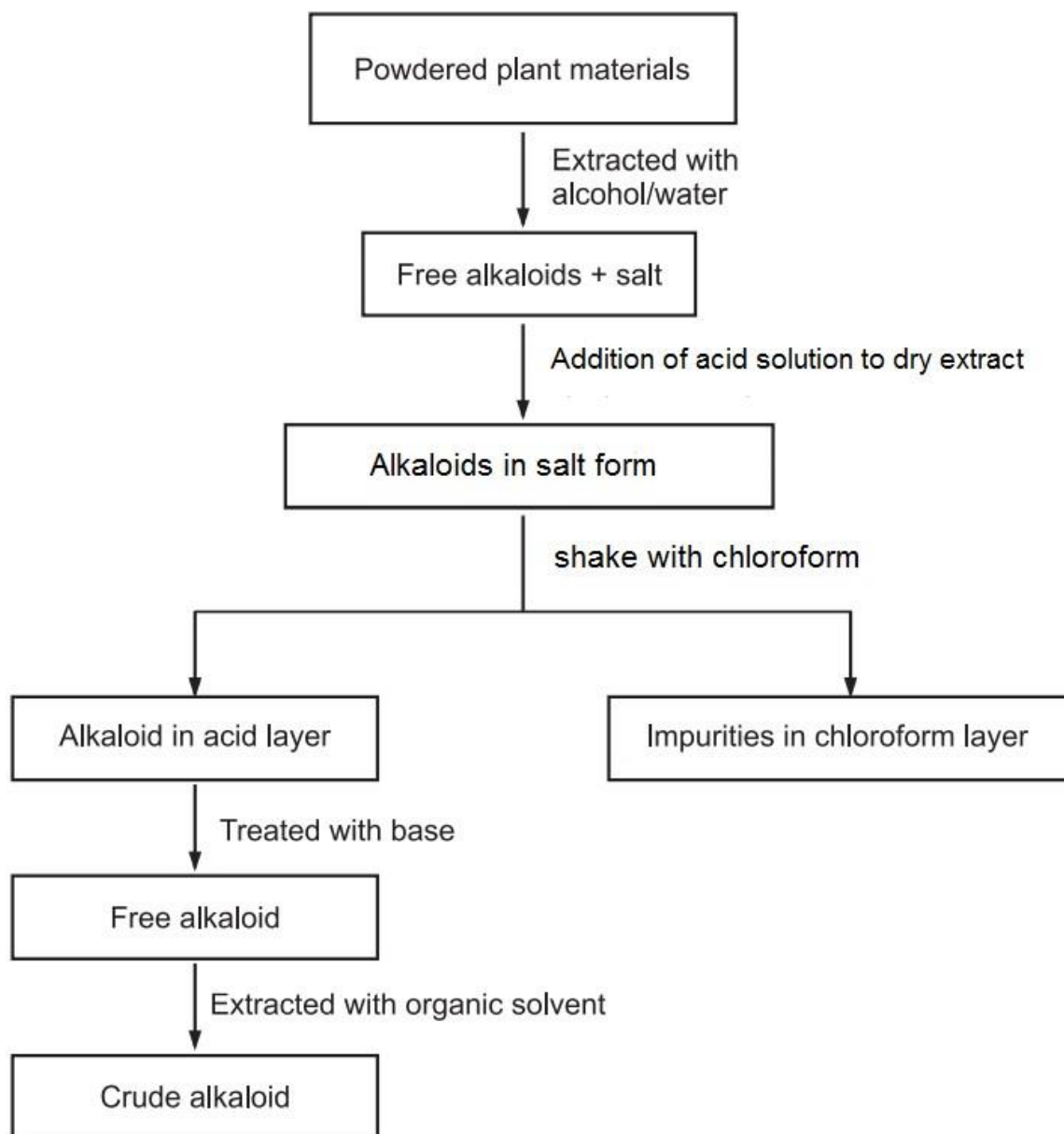
Extraction of alkaloids:

For quantitative measurement of alkaloids, they are generally extracted using ethanol or methanol as solvents which dissolves alkaloids either in the form of salts or the free ones.

There are then two main methods which are used to obtain a crude alkaloids extract: the acid and basic methods.

The acidic method of alkaloid extraction:

1. An acidic water solution (HCl, H₂SO₄...) is added to the dried crude extract of the drug and **this procedure transforms the free alkaloids into a salt form and release the alkaloids which are conjugated to organic acids to be a base for a mineral acid.**
2. A liquid - liquid extraction with chloroform or any other non-polar organic solvent is used to **remove the lipid impurities for the acidic water phase.**
3. A base- like ammonia – is then added to transform the alkaloids into the free form, this will **lead to alkaloid precipitation and enable you to separate alkaloids** from the other compounds dissolved in the aqueous phase.
4. A liquid –liquid extraction with non-polar solvent is used to **pick the free alkaloids form the aqueous solution.**
5. Again if you need to transform alkaloid into a salt form you can do a liquid-liquid extraction with an acidic aqueous solution.

**General tests of alkaloids:**

Most of alkaloids in acid solution are precipitated by heavy metals salts to give a precipitate (non-soluble in water) with different colors.

This depends on the interaction between the cationic alkaloids and the anions in the structure of the heavy metal salt.

Name of reagent	Composition	Color of precipitate
Mayer	K ₂ [HgI ₄]	creamy
Dragendorff	K[BiI ₄]	Reddish brown
Wagner	Iodide solution	Brown
Hager	Saturated picric acid	Yellow
Phosphotungestic	Phosphotungestic acid	Yellow to white

Practical experience:

Take 0.5 g of a plant contains alkaloids then add diluted HCl (1:10) with distilled water.

Boil the mixture for 5 minutes then filtrate.

Apply 2-3 drops of precipitation reagents to each part and watch the color of the precipitate.

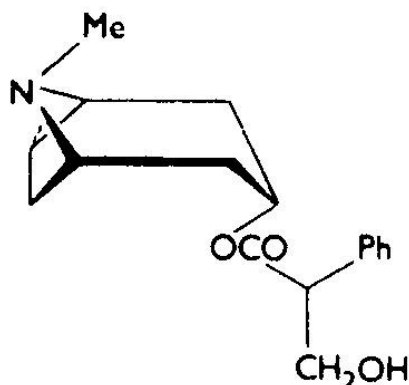
Attention: Psuedoalkaloids such as caffeine does not give a positive result with precipitation reagents.

Attention: the general test of precipitation reagents is not too much selective because it gives positive results with proteins or furanocoumarins.

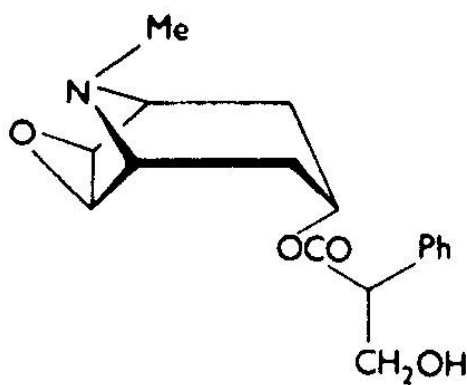
LAB No.10 Tropane alkaloids

They are a class of bicyclic alkaloids which are widely spread in Solanaceae family. The principal alkaloids of medicinal interest in this group are **hyoscyamine** and its more stable racemate **atropine**, besides **hyoscine** (**scopolamine**).

These alkaloids are esters and they contain an aromatic ring, furthermore they are derived from **ornithine** as an amino acid.



Hyoscyamine, Atropine



Hyoscine (Scopolamine)

The previous alkaloids are existed in three famous plant species, they are: deadly nightshade (*Atropa belladonna*), *Datura stramonium* and black henbane (*Hyoscyamus niger*).

These plants are tend to be toxic and hallucinogenic and these effects are attributed to alkaloids.

Medicinal uses:

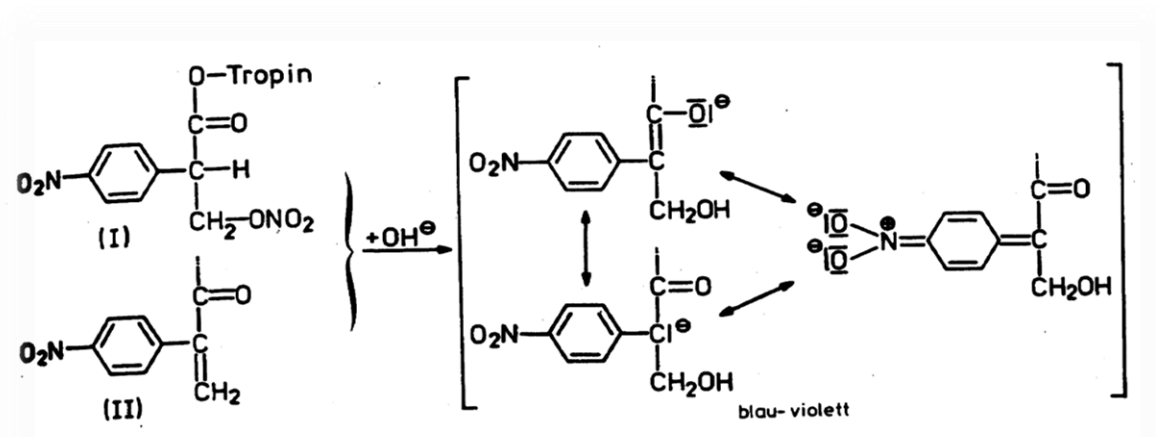
- 1- Atropine is used as an antidote to the toxicity of cholinergic compounds (cholinesterase inhibitors) and hence used as an antidote to treat organophosphate poisoning.
- 2- to dilate the pupil , decrease the salivation and to reduce the gastrointestinal activity.
- 3- Atropine and scopolamine act as anti-muscarinic compounds and act on both CNS and peripheral nervous system.
- 4- Scopolamine is used as an antispasmodic agent to relief abdominal spasms.

Detection of tropane alkaloids:

These alkaloids can be detected by coloring tests such as Vitali-Morine's test and Gerhard's test.

Vitali-Morine reaction:

The principle of this reaction depends on adding nitro groups (via nitric acid) to the molecule of tropane alkaloid. Then the addition of an alkaline (via KOH) will lead to a formation of an unstable violet ionic compound.



Mechanism of Vitali-Morin test

Steps of experiment:

- Take 1 g of Datura leaves and add 10 ml sulfuric acid solution 1N. shake for 2 minutes, then filtrate
- Add 1 ml of ammonia and 5 ml distilled water, then liquid-liquid extraction with 10 ml dichloromethane is done **to obtain the free form of alkaloids..**
- Add a few amount of anhydrous sodium sulfate then filtrate the dichloromethane layer.
- Evaporate on a water bath until dryness.
- Add 7-8 drops of nitric acid then evaporate on a water bath until dryness.
- Add few drops of KOH (3% in ethanol) slowly and an unstable violet color will occur in the presence of tropane alkaloids.

LAB No.11

Black Pepper (PiPerine alkaloid)

Piperidine Amide alkaloids



Scientific names:

Piper nigrum

Family: Piperaceae

Used part: fruits

Active compounds:

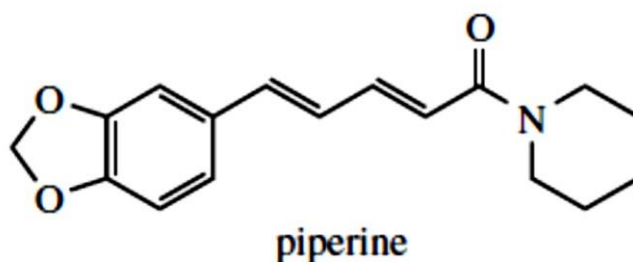
Volatile oil: chief compounds are Sabinene, Limonene and caryophyllene.

Piperidine Amides: the main compound is Piperine which is responsible for the spiciness of black pepper.

Fatty oils.

Pharmacological effects and uses:

- The drug stimulates the thermal receptors .It has an antibacterial and insecticidal effect.
- **The drug aids in digestion of food due to its stimulation to the digestive enzymes.**
(the volatile oils and piperine are responsible for these indications)
- There is some evidence that it has an anticonvulsant activity in the treatment of epilepsy.

**The chemical physiochemical properties of Piperine:**

Piperine forms monoclinic yellow needles. It is slightly soluble in water and is highly soluble in alcohol ,ether and chloroform.

Practical experience:

The isolation of piperine alkaloids:

- Grind 2 g of black pepper.
 - Extract with 20 ml ethanol 90% using the heat (80 C) for 30 minutes.
 - Filter the extract and concentrate the extract to the half on a water bath.
 - Gather all the amounts in one container.
 - Add 10 ml potassium hydroxide solution (10%).
-
- The alcoholic solution is left over night and yellow needles will be formed.

-
- Filter the solution to obtain the crystals.
 - Add 10 ml of diluted HCl solution.
 - Add few drops of Mayer or Dragendorff solution and a precipitate will occur.

Essential notes:

Ethanol is used to obtain the two form of alkaloids: free and salt form.

The use of KOH is to precipitate the isomers of free piperine.

Alcohol was used in the preparation of KOH instead of water , since water will hydrolyze piperine into piperidine and piperic acid.

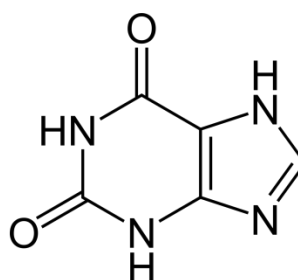
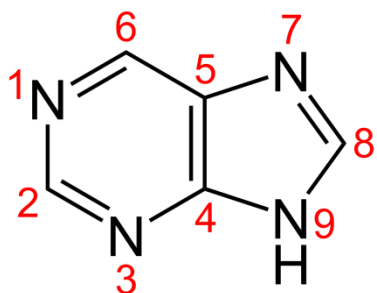
Students should prepare these following solutions:

Lab 12

Purine alkaloids

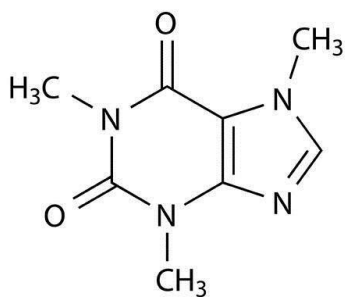
A purine is a heterocyclic aromatic organic compound. It consists of a pyrimidine ring fused to an imidazole ring.

The pharmaceutically important bases of Purine Alkaloids are all methylated derivatives of 2,6 -dioxy-purine (Xanthine).

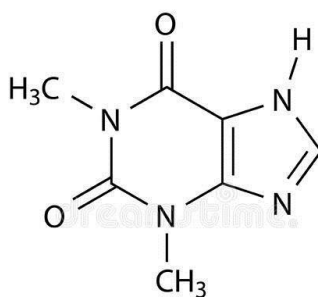


These alkaloids, are weak base ,do not give positive results with the general tests of alkaloids.

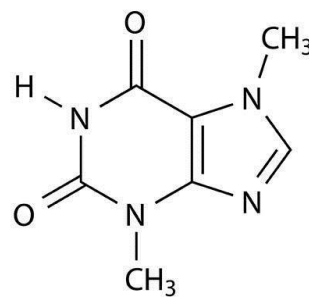
Purine alkaloids includes mainly Caffeine, Theobromine, Theophylline alkaloids.



Caffeine



Theophylline



Theobromine

The pharmacological activities of these methylated compounds are :

1. Stimulation of the CNS.
2. Diuretic effect.
3. Increase gastric acid secretion.
4. Relaxation of the bronchial smooth muscle (Theophylline).

5. Positive inotropic and chronotropic effect on the heart.

The most important plants which contain purine alkaloids are:

Tea leaves (*Camelia sinensis*), Coffee seeds (*Coffee arabica*), cola seeds (*Cola nitida*), Cocoa seeds (*Theobroma cocoa*) and yerba mate(*Ilex paraguariensis*).

Isolation of caffeine from yerba mate leaves:

- 1- Weight 5 g of yerba mate leaves and extract them with 3 ml HCl (2N) and 20 ml distilled water.
- 2- Boil the mixture for 5 minutes then filtrate.
- 3- Add 1 ml of ammonia concentrated solution.
- 4- Extract with 10 chloroform on two steps.
- 5- Obtain the chloroform layer and dry it in a water bath.
- 6- Crystals of caffeine will appear as soon as the chloroform is evaporated.
- 7- Recrystallization can be done by dissolving the residue in hot water then an amount of cold water is added (caffeine is soluble in hot water but insoluble in cold water).

Detection of purine alkaloids in extracts:

Precipitation with Gallic acid:

Take 0.5 ml of filtrate and add 2 drops of gallic acid solution. Notice a formation of white precipitate.

Mayer's test: should be negative

Muvid reaction: evaporate 0.25 ml of filtrate, then add 2 drops of Nitric acid and 2 drops of saturated bromine water. Dry on a water bath then exposure to ammonia smoke until you get a red to violet color.

Lab 13

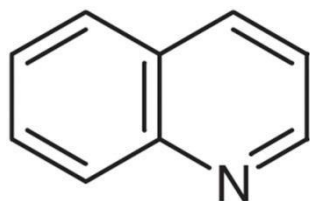
Isoquinoline alkaloids & Quinoline alkaloids

Identification

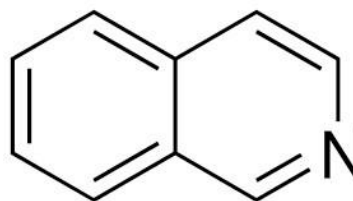
Preface:

Isoquinoline and quinazoline alkaloids, two important classes of *N*-based heterocyclic compounds, have attracted tremendous attention from researchers worldwide since the 19th century. Over the past 200 years, many compounds from these two classes were isolated from natural sources, and most of them and their modified analogs possess significant bioactivities.

Quinoline alkaloids are biogenetically derived from **anthranilic acid** while isoquinolines are derived from **tyrosine**.



Quinoline



Isoquinoline

These two groups of alkaloids are wide spread in plant species and they possess several noticeable pharmacological effects.

Examples of isoquinoline alkaloids:

Alkaloid	Plant source	Pharmacological effect
Morphine	<i>Papaver somniferum</i>	Analgesic, narcotic
Codeine	<i>Papaver somniferum</i>	Analgesic, antitussive
Emetin	<i>Cephaelis ipecacuanha</i>	Anti-emetic

Papaverine	<i>Papaver somniferum</i>	Spasmolytic (smooth muscles)
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Examples of quinoline alkaloids:

Alkaloid	Plant source	Pharmacological effect
Quinine	<i>Cinchona succirubra</i>	Anti-malarial
Quinidine	<i>Cinchona succirubra</i>	Antiarrhythmic activity
Camptothecin	<i>Camptotheca acuminata</i>	Anti-tumor
Galipeine	<i>Galipea officinalis</i>	Anti-tumor/antimalarial

Detection of Cinchona alkaloids:

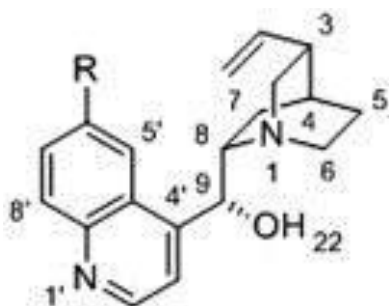
Scientific name: *Cinchona succirubra* /*C. pubescens*

Family: Rubiaceae

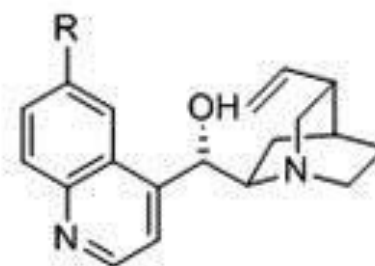
Used part: Barks

Active constituents:

Quinoline type alkaloids: Quinine- Quinidine- Cinchonine- Chinconidine.



Quinine R = OCH₃
Cinchonidine R = H



Quinidine R = OCH₃
Cinchonine R = H

Extract preparation:

- Take 2 g of cinchona barks and add 10 ml sulfuric acid solution 1N. shake for 2 minutes, then filtrate

-
- Add 1 ml of ammonia and 2 ml distilled water, then liquid-liquid extraction with 10 ml dichloromethane is done **to obtain the free form of alkaloids.**
 - Divide the dichloromethane layers into two parts for the identification tests.

Cinchona alkaloids identification tests:

1. Fluorescence under UV spectrum:

Evaporate the dichloromethane extract until dryness. Then dissolve in 5 ml diluted sulfuric acid. A blue fluorescence occurs under UV light 365 nm.

2. Thallioquinine reaction:

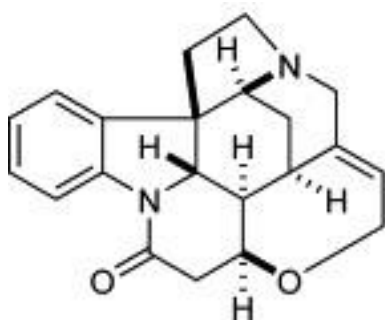
Add to the second part of the dichloromethane extract 5 ml of Conc. Ammonia and 2 drops of water bromine, then a light green color appears.

Lab 14

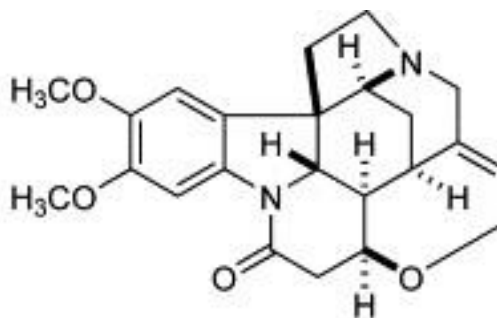
Indole alkaloids Preface:

Indole alkaloids are derived from the tryptophan amino acid. These alkaloids, such as ergot alkaloids found in *Claviceps purpurea*, have various biological effects like reducing blood pressure and inducing abortion. Some of them are found in toxic plants such as Strychnine and Brucine found in Strychnine tree (*Strychnus nux-vomica*). Strychnine is responsible for inhibiting postsynaptic glycine receptors, mostly in the spinal cord, causing painful, involuntary skeletal muscle spasms.

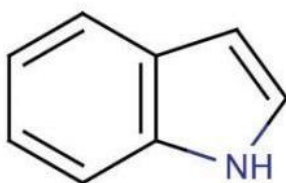
Strychnine is rapidly absorbed after ingestion, inhalation, or intravenous administration. It causes generalized muscle spasms, muscle cramps, stiffness and tightness, agitation, respiratory failure, stimulation sensitive seizures, and possibly death.



Strychnine



Brucine



Detection of indole alkaloids in Strychnine tree:

Scientific name: *Strychnus nux-vomica*

Family: Loganiacea

Used part: seeds.



Method of Extraction:

The Basic method is applied as follows:

- Take 0.5 g of Strychnine tree seeds powder and add 7 ml sulfuric acid solution 1N. shake for 2 minutes, then filtrate
- Add 1 ml of ammonia and 2 ml distilled water, then liquid-liquid extraction with 6 ml dichloromethane is done **to obtain the free form of alkaloids.**

Detection with oxidizing agents:

Take 2 ml of dichloromethane part and evaporate until dryness, then add few drops of concentrated sulfuric acid and one crystal of potassium dichromate. A red to violet color will appear (this reaction detects the indole heterocycle).

Detection with nitric acid:

Take 2 ml of dichloromethane part and evaporate until dryness, then add few drops of Conc. nitric acid, A yellow color will appear in the presence of strychnine, a reddish orange color in the presence of brucine and a yellowish red color in the presence of both alkaloids.

List of Medicinal plants used in the laboratory



Senna

Cassia angustifolia (Fabaceae)



Fennel

Foeniculum vulgare (Apiceae)



Hawthorn

Crataegus azarolus (Rosaceae)



St. John's wort

Hypericum perforatum
(Hypericaceae)



Rosemary

Rosmarinus officinalis
(Lamiaceae)



Oak

Quercus robur (Fagaceae)



Licorice

Glycyrrhiza glabra
(Polygnaceae)



Soapwort

Saponaria officinalis
(Caryophyllaceae)



Rhubarb

Rheum palmatum
(polygnaceae)

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