



University of Baghdad

College of Pharmacy

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Practical Pharmacognosy

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The Alkaloids

Alkaloids (alkali-like) are defined as organic nitrogenous compounds of plant origin that are physiologically active, ending in the suffix "*ine*".

Plants have been a rich source of alkaloids but some are found in animals, fungi, and bacteria. Among the plants, the *angiosperms* are rich in alkaloids.

The following families represent good examples of plants which contain alkaloids:

- Leguminosae.
- Papaveraceae.
- Ranunculaceae.
- Rubiaceae.
- Solanaceae.
- Berberidaceae.

The Labiatae and Rosaceae are almost free of alkaloids; the *gymnosperms* only rarely contain alkaloids.

The names of alkaloids are obtained in various ways:

- ✓ From the *generic* name of the plant yielding them as *atropine*.
- ✓ From the *specific* name of the plant yielding them as *cocaine*.
- ✓ From the *common* name of the drug yielding them as *ergotamine*.
- ✓ From their *physiologic activity* as *emetine*.
- ✓ From the *discoverer* as *pelletrine*.

Alkaloids usually contain one nitrogen atom, but some may contain up to 5.

The nitrogen may exist as a primary amine (RNH₂), as secondary amine (R₂NH), or as a tertiary amine (R₃N).

As the nitrogen atom bears an unshared pair of electrons, such compounds are basic and resemble ammonia's chemical properties. The degree of basicity varies greatly, depending on the structure of the molecule and the presence and location of

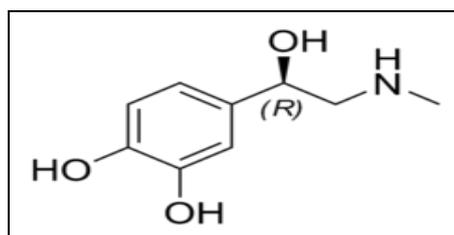
Like ammonia, the alkaloids are converted into their salts by aqueous mineral acids, and when the salt of an alkaloid is treated with hydroxide ion, nitrogen gives up a hydrogen ion and the free amine is liberated. The quaternary ammonium compound has no proton to give up thus is not affected.

The alkaloids, like other amines, form double salts with the heavy metals appear as precipitates, and are used in their identification. These reagents include:

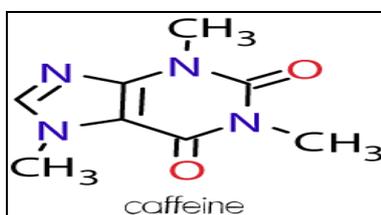
- 1) Wagner's reagent (iodine in potassium iodine).
- 2) Mayer's reagent (potassium mercuric iodide).
- 3) Dragendroff's reagent (potassium bismuth iodide).
- 4) Hagger's reagent (saturated solution of picric acid).

Types of alkaloids:

1. **True alkaloids:** these characterized by contain nitrogen atom in the heterocyclic ring and derived from amino acids. Example is *Atropine*.
2. **Proto alkaloids:** which contain nitrogen atom without a heterocyclic ring and also derived from amino acids. Examples include *Adrenaline* and *Ephedrine*.



3. **Pseudo alkaloids:** These characterized by heterocyclic ring with a nitrogen atom, but are not derived from amino acids. Example Caffeine.



In General the Alkaloids are Classified According to Chemical Structure in to two Broad Divisions:

- A. Non-heterocyclic or atypical alkaloids or biological amines.
- B. Heterocyclic or typical alkaloids, divided in to 14 groups according to their ring structure ,as follows:
 - 1. Pyrrol and pyrrolidine.
 - 2. Pyrrolizidine.
 - 3. Pyridine and piperidine.
 - 4. Tropine.
 - 5. Quinolone.
 - 6. Isoquinolone.
 - 7. Aporphine.
 - 8. Norlupinane.
 - 9. Indole.
 - 10. Indolizidine.
 - 11. Imidazole.
 - 12. Purine.
 - 13. Steroids.
 - 14. Terpenoids.

Exp. No.1

[Lab.1] Black Pepper

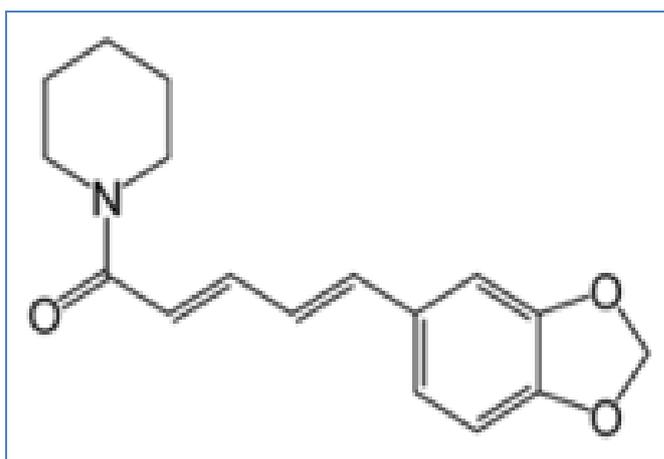
The botanical name: **Pipper nigrum** of the family **Piperaceae**. It belongs to the third group of the typical alkaloid, which is the *pyridine* and *piperidine* group.

This plant is a perennial plant producing berry –like and aromatic pungent fruits, that are green when unripe and become red at mature, then the dried berries become **black** and wrinkled producing **black pepper**.

The pepper yields both, black and white pepper according to the method of drying .In that when the ripe and unripe fruit are dried directly under the sun, **black pepper** is the result. While if the fruit is first soaked, and then removed the outer skin, before drying, and then the result is **white pepper**.

The alkaloid extracted from the black pepper is **piperine**.

Piperine alkaloid is a solid substance essentially insoluble in water. It is a weak base that is tasteless at first, but leaves a burning aftertaste. The molecular formula is $C_{17}H_{19}NO_3$,and the chemical Chemical structure of piperine is:



Piperine

The Pharmacological Activity of Piperine :

- 1) Piperine aid in the digestion of food due to its stimulation to the digestive enzymes.
- 2) There is some evidence that it has an anticonvulsant activity in the treatment of epilepsy.
- 3) There is some evidence that it has an anticancer and anti-inflammatory activity due to its antioxidant property.

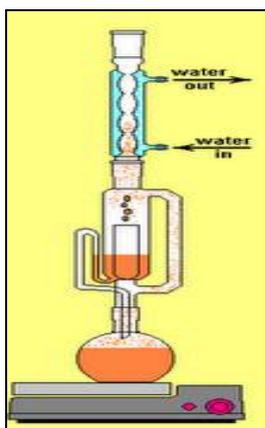
The Isolation and Identification of Piperine from Black Pepper:

1. Extraction:

Aim: to isolate the piperine alkaloid from black pepper. .

Equipments:

- ❖ Large beaker & medium size beakers.
- ❖ Soxhlet instrument.
- ❖ Funnel & filter paper.
- ❖ Water bath.



Soxhlet

Reagents:

- ❖ 90% ethanol.
- ❖ 10% alcoholic potassium hydroxide.

Procedure:

Method of extraction: Decoction.

Plant used: Piper nigrum.

Part used: Seeds.

10 gm fine powdered of black pepper in **150 ml** of 90% ethanol for **2 hrs**. In Soxhlet extractor

↓
Hot filtration

30 ml of extract solution concentrated on water bath at **60⁰C**

↓
Add

2 ml of 10% Alcoholic KOH

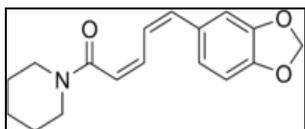
↓
Filtrate & alcoholic extract is left over night

Results:

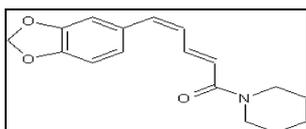
Yellow needles with melting point of 125°C are deposited. Yielding 0.3 gm of piperine alkaloids.

Discussion:

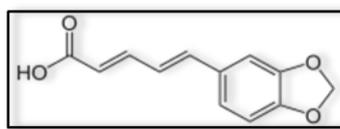
1. The plant is affected by heat; therefore Soxhlet apparatus is used in its extraction.
2. The use of **90% ethanol** is to extract both, the alkaloid and the alkaloidal salt that might be present. (This is true for the extraction of most alkaloids).
3. The use of **alcoholic KOH** is to precipitate the isomers of piperine that are **chuvacine**, **isochuvacine** and **piperic acid**.



Chuvacine

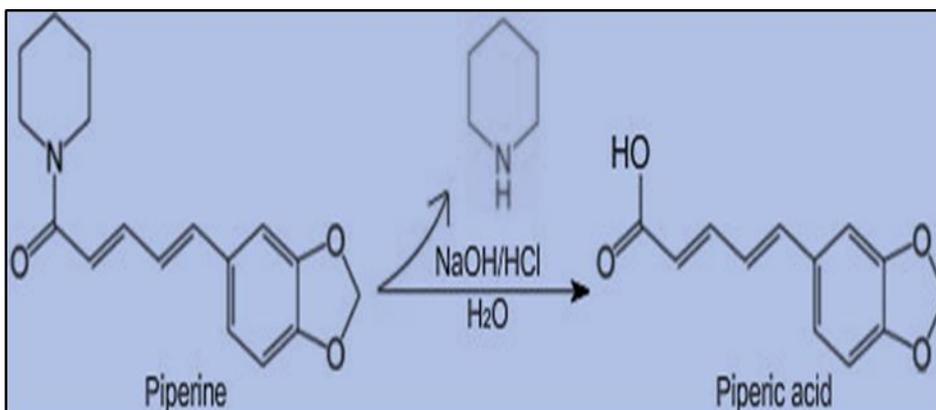


isochuvacin



Piperic acid

4. Alcohol was used in the preparation of KOH instead of water, since water will hydrolyze piperidine and piperic acid as shown below:



Quantitative and Qualitative Analysis of Piperine Alkaloid:

Quantitative Analysis:

This is done by weighing the crystals of piperine alkaloid.

Qualitative Analysis:

The General Chemical Tests :

1. Mayer 's Test:

Aim: to indicate in *general* the alkaloid as other alkaloids.

Equipments and Reagents:

- ✓ Petri dish.
- ✓ Ethanol.
- ✓ HCl.
- ✓ Mayer's reagent.

Procedure:

Take few crystals of *piperine alkaloid* and dissolve in few ml_s of *ethanol*, in Petri dish then add **2 drops** of *HCl*. Then add **2 drops** of *Mayer's reagent*.

Result:

White precipitate will occur.

2. Wagner 's Test:

Aim: to indicate in *general* the alkaloid as other alkaloids.

Equipments and Reagents:

- ✓ Petri dish.
- ✓ Ethanol.
- ✓ HCl.
- ✓ Wagner's reagent.

Procedure:

Take few crystals of *piperine alkaloid* and dissolve in few ml_s of *ethanol*, in Petri dish then add **2 drops** of *HCl*. Then add **2 drops** of *Wagner's reagent*.

Result:

Brown precipitate will occur.

3. Dragendorff's Test:

Aim: to indicate in *general* the alkaloid as other alkaloids.

Equipments and Reagents:

- ✓ Petri dish.
- ✓ Ethanol.
- ✓ HCl.
- ✓ Dragendorff's reagent.

Procedure:

Take few crystals of *piperine alkaloid* and dissolve in few ml_s of *ethanol*, in Petri dish then add **2 drops** of *HCl*. Then add **2 drops** of *Dragendorff's reagent*.

Result:

Orange precipitate will occur.



Black pepper

The Identification of Piperine Alkaloid By

Chromatography (TLC) :

- ❖ By the use of thin layer chromatography (T.L.C)
- ❖ The stationary phase = *Silica gel G*.
- ❖ The mobile phase = ***Toluene: Diethylether: Diaxon (62.5:21.5:16)***.
- ❖ The standard compound =
- ❖ The spray reagent = ***Dragendorff's reagent***.
- ❖ Mechanism of separation = *Adsorption*.
- ❖ Developing = *Ascending*.
- ❖ ***Other mobile phases :***
Toluene: Ethyl acetate (70:30), Acetone Water: Ammonia (90:7:3).

Procedure:

- 1) Prepare ***100ml*** of mobile phase, and place it in the glass tank.
- 2) Cover the tank with glass lid and allow standing for ***45 minutes*** before use.
- 3) Apply the sample spots, and the standard spot on the silica gel plates, on the base line.
- 4) Put the silica gel plate in the glass tank and allow the mobile phase to rise to about *two-third* the plate.
- 5) Remove the plate from the tank, and allow drying, and then detecting the spots by the use of the spray reagent.

Result:

Orange spot will appear.

Study problems:

- Q1.** Give the botanical name of black pepper and mention its alkaloids?
- Q2.** What is the pharmacological activity of piperine alkaloids?
- Q3.** Give the reasons off the use of alcoholic KOH in the extraction procedure of black pepper?
- Q4.** How can you identify an extract containing piperine alkaloids?

Exp.No. 2

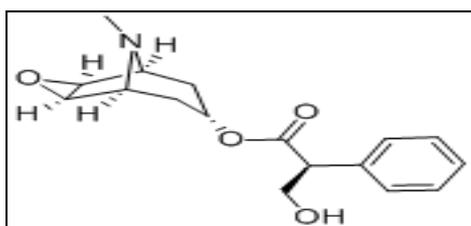
[Lab.3] Tropane Alkaloids

Datura stramonium, known by the common names **Jimson weed** or **datura**, is a plant in the *Solanaceae* (nightshade) family. For centuries, datura has been used as a herbal medicine to relieve asthma symptoms and as an analgesic during surgery or bone setting. It is also a powerful hallucinogen and deliriant, which is used spiritually for the intense visions it produces. However, the tropane alkaloids which are responsible for both the medicinal and hallucinogenic properties are fatally toxic in only slightly higher amounts than the medicinal dosage, and careless use often results in hospitalizations and deaths.

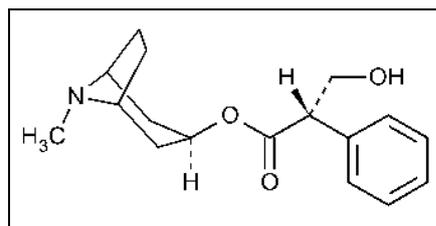
Constituents of datura are:

Hyoscyamine and its isomer **atropine**, which is formed during extraction procedure. Also it contains **hyoscine** (scopolamine) alkaloid, which is found in trace amounts.

The medicinal use is mostly due to the hyoscyamine (atropine), used as mydriatic, antispasmodic, antidote to the toxicity of cholinergic compound, decrease in the secretion (upper and lower respiratory tract) before surgery. While the use of scopolamine mostly in motion sickness. The tropane alkaloids (hyoscyamine and hyoscine) have the following structures:



Hyoscine (scopolamine)



Hyoscyamine

These alkaloids are also present in other plants as **Hyoscyamus niger** of the family Solanaceae, **Atropa belladonna** of the same family, and others.

Isolation and Identification of the Datura Alkaloids:

2. Extraction:

Aim: to isolate datura alkaloids.

Equipments:

- ❖ *Reflex apparatus.*
- ❖ *Conical flasks.*
- ❖ *Stirrer.*
- ❖ *Funnel.*
- ❖ *Separatory funnel.*
- ❖ *Water bath.*
- ❖ *Filter paper.*
- ❖ *Litmus paepr.*

Reagents:

- ❖ *90% ethanol.*
- ❖ *2% HCl.*
- ❖ *Ammonium hydroxide solution.*

❖ Chloroform.

Procedure:

Method of extraction:.

Plant used: Datura stramonium.

Part used: fruits.

Extract **50 gm** of the datura fruits in **150 ml** of 90% ethanol under
Reflex condenser for **1 hrs.**

↓
Filtration

Take **20 ml** of alc. Extract in conical flask and concentrate on the
water bath to about **2 ml** to remove all of ethanol

↓

Pour the concentrated in to **10 ml** of 2% HCl

↓

Heat gently
(**5 min_s**)

Cool and filter the Acidic extract and place in a separatory funnel

↓

[Wash with **5 ml** of Chloroform] **two times**

↓

Take supernatant (upper layer) and made alkaline by addition of

↓

Ammonium hydroxide solution (check by litmus paper)



[Partition with **5 ml** of Chloroform] **two times**



Take the lower layer, dehydrate by adding anhydrous sod.

Sulphate filter (or decant) , evaporate to dryness

2. Results:

Product containing the mixture of the alkaloids.

The Identification of Datura Alkaloids:

Qualitative Analysis:

A. The specific tests for tropane alkaloids:

1. Vitalli Marine Bu Test:

Aim : to identify the *tropane alkaloids* from other alkaloids.

Equipments and Reagents:

- ✓ Small beaker.
- ✓ Fuming nitric acid.
- ✓ Alcoholic KOH.

Procedure:

Take few ml_s of the extract, add to it drops of fuming nitric acid and evaporate, then add **2 ml** of alc. KOH.

Result:

A *violet* color will be developed.

2. *Gerhard's Test:*

Aim: to identify the *tropane alkaloids* from other alkaloids.

Equipments and Reagents:

- ✓ Small beaker.
- ✓ 2% HgCl₂ in 50% aqueous ethanol.

Procedure:

Add **2%** HgCl₂ in **50%** aqueous ethanol to **0.0006 g** of atropine.

Result : A **deep red** color will be developed.

B. General tests for tropane alkaloids:

All reagents used for tests of alkaloids could be applied on tropane alkaloids since they are true alkaloids.

Identification of Datura Alkaloids By

Chromatography:

- ❖ By the use of thin layer chromatography (T.L.C)
- ❖ The stationary phase = *Silica gel G*.
- ❖ The mobile phase = ***Butanone: Methanol: Ammonia (60:70:10)***

Or Acetone: Water: Ammonia (90:7:3).

- ❖ The standard compound = atropine or hyoscine.
- ❖ The spray reagent = ***Dragendorff's reagent***.
- ❖ Mechanism of separation = *Adsorption*.
- ❖ Developing = *Ascending*.
- ❖ ***Other mobile phases :***

Chloroform: Acetone: Diethyl amine (50:40:10),

Chloroform: Diethyl amine (90:10).

Procedure:

- 1) Prepare *100ml* of mobile phase, and place it in the glass tank.
- 2) Cover the tank with glass lid and allow standing for *45 minutes* before use.
- 3) Apply the sample and the standard spots on the silica gel plates, on the base line by the use of capillary tube.
- 4) Put the silica gel plate in the glass tank and allow the mobile phase to rise to about *two-third* the plate.
- 5) Remove the plate from the tank, and allow drying, and then spray with the spraying reagent.



Datura stramonium

Study problems:

Give the reason of :

1. Using the reflux in extraction the tropane alkaloid from datura stramonium.
2. Making the evaporation step after filtration?
3. Addition of 2% HCl to the alcoholic extract?
4. Addition of ammonium hydroxide?
5. Using of chloroform in partitioning step?

Exp.No. 3

[Lab.4]

Indole Alkaloids

Harmala Alkaloids

Peganum harmala of the family Zygophyllaceae.

It is a woody, perennial, succulent shrub native to arid regions. The leaves are bright green, finely divided and about 1 cm long. Both the roots and seeds contain significant quantities of Beta- carbolines (indole) alkaloids, which are absent in the rest of the plant.

The Traditional and Medical Uses:

The traditional uses including as *the dye "turkey red"*, and as *incense* from ancient times.

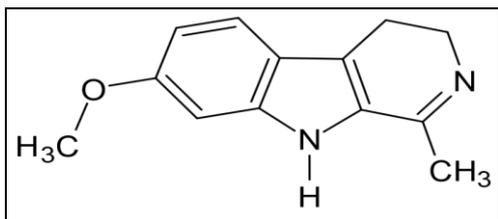
Peganum harmala was claimed to be an important medical plant. Its seeds were known to possess hypothermic and essentially hallucinogenic properties since it is MAO inhibitor agent .

Various authors have under taken studies on the antibacterial, anti fungal and antiviral effects of **Peganum harmala** seeds. In Moroccan traditional medicine , seed powder is sometimes used on skin and subcutaneous tumors.

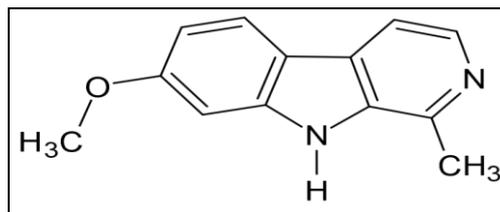
This work was designed to investigate some aspects of the anti neoplastic properties of **Peganum harmala** in that the active principle at a dose of 50 mg / kg given orally to mice for 40 days was found to have significant anti tumor activity. **Peganum harmala** alkaloids thus possess significant anti tumor potential, which could prove useful as novel anticancer therapy. The pharmacologically active compounds of **Peganum harmala** are several alkaloids ,which are found especially in the seeds (2-7% total) and the roots.

These include beta-carbolines such as: **harmaine** , **harmaline** and **Harman**.

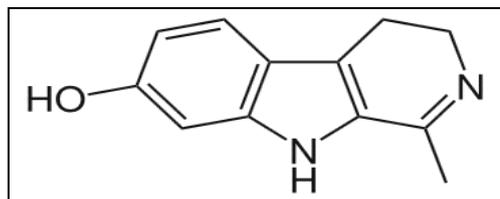
Peganum harmala also contains the quinazoline derivatives **vasicine** and **vasicinone**. It is believed that these quinazoline alkaloids are responsible for the abortifacient activity of **Peganum harmala** extracts. It has been reported that these chemicals have a uterine stimulatory effect, apparently through the release of prostaglandin. **Peganum harmala** alkaloids are characterized by the fluorescence property.



Harmaline



Harmine



Harmalol

Isolation of The Harmala Alkaloids:

Extraction:

Aim: to isolate the Harmala Alkaloids.

Equipments:

- ❖ Large beaker.
- ❖ Small conical flask.
- ❖ Reflux apparatus.
- ❖ Separatory funnel.
- ❖ Water bath.
- ❖ Litmus paper.
- ❖ Funnel.
- ❖ Filter paper.

Reagents:

- ❖ Petroleum ether.
- ❖ 90 % Ethanol.
- ❖ Ammonium hydroxide solution.
- ❖ 2% HCl.
- ❖ Chloroform.
- ❖ Methanol.

Procedure:

Method of extraction: Reflux.

Plant used: Peganum harmala

Part used: Seeds.

Maceration **50 gm** of the harmala seeds in **500 ml** of petroleum ether for **24 hrs (over night)**.

Filter

Reflux with **90%** ethanol for **1 hr**.

Cool & Filter

Take **20 ml** of Extract in conical flask

Evaporate the filtrate on water bath to about **2 ml**

Add

5ml of **2%** HCl
(Filter if necessary.)

Partition with Chloroform (**10 ml** x 2), take the acidic layer (upper layer)

Add

Ammonium hydroxide solution (check by litmus paper)

Place the basic solution in the separatory funnel

↓
Add

[10 ml of Chloroform] two times

↓
(Shake & stand)

Take the organic lower layer and put it in the conical flask

↓
Add

Small amount of Anhydrous sodium Sulphate & allow standing for few minutes until get a clear solution , decant and concentrate by evaporation to give the product crude alkaloids.

[Lab.5]

Identification of Harmala Alkaloids

Quantitative Analysis:

By weighing the residue obtained.

Qualitative Analysis:

The General Chemical Tests :

The same as for other alkaloids.

The Identification of Harmala Alkaloids By Chromatography :

- ❖ By the use of thin layer chromatography (T.L.C)
- ❖ The stationary phase = *Silica gel GF₂₅₄*.
- ❖ The mobile phase = ***Chloroform : Methanol: Acetone (35:15:10)***
Or Chloroform: Methanol: 10% Ammonium hydroxide (80:20:15).
- ❖ The standard compound = any harmala alkaloids.
- ❖ The spray reagent = ***Dragendorff's reagent.***
- ❖ Mechanism of separation = *Adsorption.*
- ❖ Developing = *Ascending.*
- ❖ ***Other mobile phases :***
Chloroform: Acetone: Diethyl amine (50:40:10),
Chloroform: Diethyl amine (90:10).
- ❖ UV instrument.

Procedure:

- 1) Prepare mobile phase, and place it in the glass jar.
- 2) Cover the jar with glass lid and allow standing for **45 minutes** before use.
- 3) Apply the sample and the standard spots on the silica gel plates, on the base line by the use of capillary tube.
- 4) Put the silica gel plate in the glass jar and allow the mobile phase to rise to about *two-third* the plate.
- 5) Remove the plate from the jar, dry and identified first by U.V. 254 ,366 nm.
- 6) Spray the plate with spraying reagent (***Dragendorff's reagent***) and then calculate the Rf values.

Results:

Fluorescence spot appears under the U.V. while an orange spots are seen when sprayed with the sprayer.



Peganum harmala

Exp.No. 4

[Lab.5]

Purine Bases

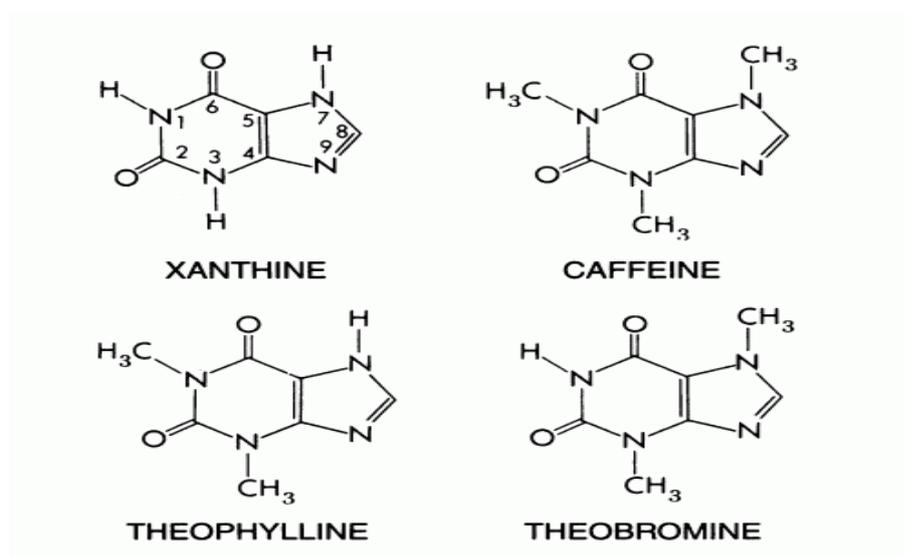
Purines nucleus is a heterocyclic nucleus consisting of pyrimidine ring fused to 5-membered imidazole ring known as xanthine.

Purines unlike other alkaloids don't give positive results with the general tests of alkaloids, instead murexide test is used in its identification.

Purines are present as methylated compounds, which are:

1. Caffeine (1, 3,7- tri methyl xanthine).
2. Theophylline (1,3-di methyl xanthine).
3. Theobromine (3,7- di methyl xanthine).

The following are their structures:



Generally the pharmacological activities of these methylated compounds are:

1. Stimulation of the CNS.
2. Diuretic effects.
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 in this group are :

1. **Coffee** (Coffea arabica of the family Rubiaceae).

Contain about 1-2 % of caffeine.

2. **Tea** (Camellia sinensis of the family Theaceae).

Contain about 1-4 % of caffeine.

3. **Cola** (Cola nitida of the family Sterculiaceae).

Contain about 3.5 % of caffeine.

Isolation of The Caffeine From Tea:

1. Extraction:

Aim: to isolate caffeine from tea leaves.

Equipments:

- ❖ Large beaker & two medium size beakers.
- ❖ Two conical flasks.

- ❖ Centrifuge tubes & Centrifuge.
- ❖ Separatory funnel.
- ❖ Water bath.
- ❖ Muslin.
- ❖ Conical flask.

Reagents:

- ❖ Sulphuric acid.
- ❖ Basic Lead acetate.
- ❖ Chloroform or methylene chloride.
- ❖ Hot 60 °C ethanol.

2. Procedure:

Method of extraction: decoction.

Plant used: Camellia sinensis

Part used: dry leaves.

Place **10 gm** of the powdered tea leaf in **50 ml** of water

↓
Boil for **(15 min_s)**
(Constant stirring)
↓

Strain the resulting hot extract through muslin, express well

↓

Wash the mass remains on the muslin with **10 ml** of boiling water and express again

↓

Add (carefully)

5drops of lead sub acetate
(Heat the mixture to boiling)

↓

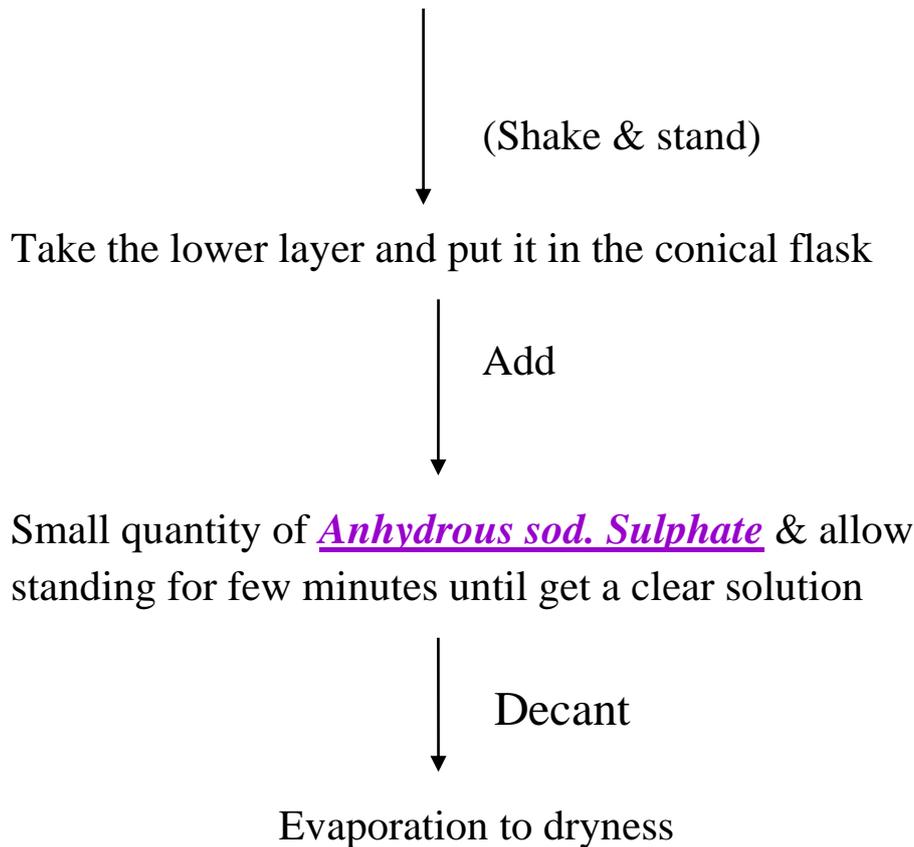
Centrifuge
(5 min_s.)
↓

Decant and take the supernatant (upper layer)

↓

Cool & transfer to separatory funnel

[Extract with **15 ml** of methylene chloride OR chloroform] two times



1. Results :

Pure crystals with a *white* color will be obtained.

2. Discussion:

1. Purines differ from other alkaloids in that they are soluble in hot water, which is used in its extraction, and this is the cause why all the time you should heat the mixture (to prevent the precipitation of caffeine in cold water).
2. *Lead sub acetate* is added to precipitate tannins and other unwanted material.
3. The *centrifugation* is used to isolate the decant containing unwanted materials from the desired supernatant.
4. Use of *methylene chloride* is to extract the caffeine from other component of the mixture.

Quantitative and Qualitative Analysis of caffeine Crystals :

Quantitative Analysis:

This is done by weighing the crystals of caffeine alkaloid.

Qualitative Analysis:

The Specific Chemical Tests :

The Murexide Test:

Aim: to identify the caffeine alkaloid (purine alkaloids) from other alkaloids.

Equipments and Reagents:

- ✓ Porcelain dish.
- ✓ Water bath.
- ✓ Potassium chlorate ($KClO_3$).
- ✓ Conc .HCl.
- ✓ Ammonia vapor.

Procedure:

Take few crystals of *caffeine alkaloid* in porcelain dish and add small amount of *potassium chlorate* ($KClO_3$), then add **2 drops** of *conc. HCl* . Evaporate to dryness, then expose to *ammonia vapor*.

Result:

Purpule color is produced with caffeine and other purine derivatives .

H.W/ What is the mechanism of murexide test?

The Identification of purine (caffeine) Alkaloids

By Chromatography:

- ❖ By the use of thin layer chromatography (T.L.C)
- ❖ The stationary phase = *Silica gel GF₂₅₄*.
- ❖ The mobile phase = *ethyl acetate : Acetic acid (95:5)*

Or Acetone: Water: Ammonia (90:7:3).

- ❖ The standard compound = *caffeine*
- ❖ Mechanism of separation = *Adsorption*.
- ❖ Developing = *Ascending*.
- ❖ Detection: by **UV** instrument.

Procedure:

- 1) Prepare mobile phase, and place it in the glass jar.
- 2) Cover the jar with glass lid and allow standing for *45 minutes* before use.
- 3) Apply the sample and the standard spots on the silica gel plates, on the base line by the use of capillary tube.
- 4) Put the silica gel plate in the glass jar and allow the mobile phase to rise to about *two-third* the plate.
- 5) Remove the plate from the jar, dry and identified by U.V. 245 ,366 nm. Then calculate R_f values.



Exp.No. 5

[Lab.6]

Isoquinoline Alkaloids

Botanical Name: *Fumaria densiflora* DC

Family name: *Fumariaceae*

Active components: Protopine (% 0.29-0.27), fumariline (% 0.21-0.20), β -allocryptopine (% 0.32-0.30)



Fumaria densiflora DC.

Fumaria species have been used in traditional medicine as antihypertensives, diuretics, hepatoprotectants and laxatives (to treat gastrointestinal disorders), as well as in the treatment of rashes and conjunctivitis. The biological activity of *Fumaria* is mostly associated with the presence of isoquinoline alkaloids in the plant. In the last few

years, a large number of scientific reports have been described the properties of *Fumaria*. There are seventeen wild-growing species belonging to this genus in Turkey.

The extracts of *F. officinalis* L. have been used in traditional medicine for varied purposes treatment of digestive problems, certain metabolic diseases, liver disorders and to purify blood. Phytochemical investigation revealed the presence of several alkaloids such as adlumidicine, copticine, fumariline, perfumine, protopine, fumaranine, fumaritine, paprafumicin and paprarine. The plant has also been evaluated pharmacologically and shown to possess antihelmintic, antipyretic and hypoglycemic properties. *Fumaria densiflora* is annual herb that reaches up to 10-25 cm long Leaves are 2-6 cm. Flowers are with pink and blackish-red tipped petals.

Extraction Method:

Aerial parts of plants (25 g) were dried, powdered and extracted with ethanol in a Soxhlet apparatus until Mayer's test was negative, and then evaporated in vacuum. The ethanolic residue was taken up in % 1 hydrochloric acid (50 ml), filtered and the aqueous acid solution brought to pH 9-9.5 with % 25 ammonium hydroxide and extracted with chloroform (5 x 150 ml). The extracts were dried with anhydrous sodium sulphate and the solvent evaporated to afford a crude extract of alkaloids.

Identification of isoquinoline Alkaloids

TLC: Mobile phase *TLC-densitometry two different solvent systems as:*

- *toluene : chloroform : methanol : % 25 ammonium hydroxide (5:3:1:1)*

- chloroform : methanol (8:2)

Spray reagent: Dragendorff's reagent

Exp.No. 6

[Lab.7]

Citric acid

Botanical Name: *Citrus limonum*

Synonyms and local names: Citrus Limonum, Leemoo, Limoun, Limone.

Family Name: *Rutaceae*

Genus: *Citrus*

The **lemon** is a small evergreen tree native to Asia, and produces a yellow fruit. The fruit is used primarily for its juice, though the pulp and rind (zest) are also used in cooking and baking. The juice of the lemon is citric acid, which gives lemons a sour taste. The distinctive sour taste of lemon juice makes it a key ingredient in drinks and foods such as lemonade.



Therapeutic Uses:

- 1- Aromatherapy, as it boosts the immune system and enhances the mood.
- 2-Strong antibacterial and antiviral agent
- 3-Aids in digestion and liver cleanser: As it eliminate waste more quickly from your body.
- 4-Anticancer properties.
- 5-A rich source for many vitamins and minerals mainly (5-6) % citric acid and offers 80mg of potassium. Also, calcium, vitamin C,

bioflavonoids, pectin and limonene which promotes immunity and fights infections.

Extraction:

Measure 90 ml of lemon juice concentrate it to 250 ml in a beaker and carefully add a sufficient quantity of 10% NaOH solution. Filtrate by using filter paper to remove all the excess large particles and pulp.

Measure the filtrate place in a beaker and add 5 ml of 10% Calcium Chloride solution for each 10 ml of the filtrate. Then wash twice with small quantities of boiling water. Then resuspend in a minimum quantity of cold water, heat to boiling and once again collect the insoluble calcium citrate by filtration. Allow The salt to dry, weight calculate the yield and turn in the product to the laboratory instructor. Citric acid may be prepared from the citrate salt as in the following:

Weight the air-dried salt, place in a beaker and add the calculated amount of 1N sulphuric acid required to convert the salt to acid. Allow the mixture to stand for few minutes, filter off the insoluble calcium sulphate and concentrate the filtrate to a small volume by steam bath. Citric acid crystallization out, because of its relatively great water solubility citric acid is somewhat difficult to recrystallize in small amounts. Collect the crystals, dry and calculate the final product what is citric acid.