University College

Department of Pharmacy

Pharmacognosy Lab.

Practical Pharmacognosy

Third year

2nd term

Done by:

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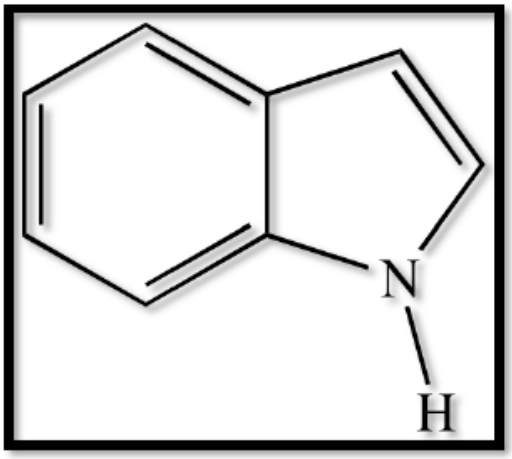
[Lab.2] Indole Alkaloids

Indole alkaloids have a bicyclic structure, consisting of a six-membered benzene ring fused to a five-membered nitrogen-containing pyrrole ring. This pyrrole ring with nitrogen atom gives rise to the basic properties of indole alkaloids that make them particularly pharmacologically active.



Chemical structure of indole

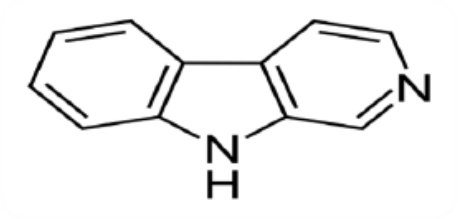
Harmala Alkaloids

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 pharmacologically active compounds of ***Peganum harmala*** are several alkaloids, which are found especially in the seeds (2-7% total) and the roots.

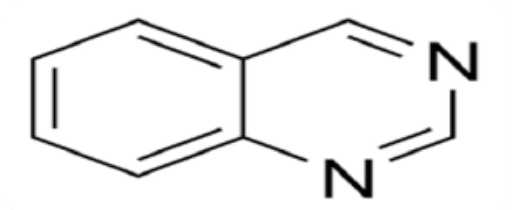
Alkaloids in *Peganum harmala*

β-Carboline: β-Carboline belongs to the group of indole alkaloids and consist of pyridine ring that is fused to an indole skeleton. These include: harmine, harmaline, harman, harmol and others.

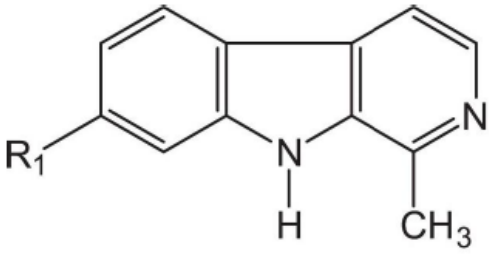


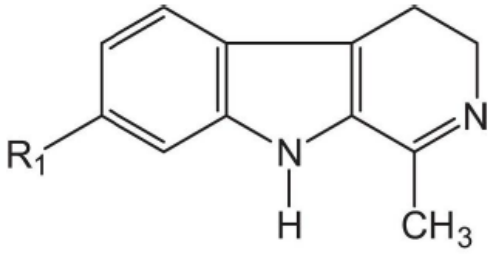
β-Carboline alkaloid

Quinazoline: It is an aromatic heterocycle with a bicyclic structure consisting of two fused six-membered aromatic rings, a benzene ring and a pyrimidine ring. quinazoline derivatives in harmel are **vasicine** and **vasicinone.**



Quinazoline type of alkaoid

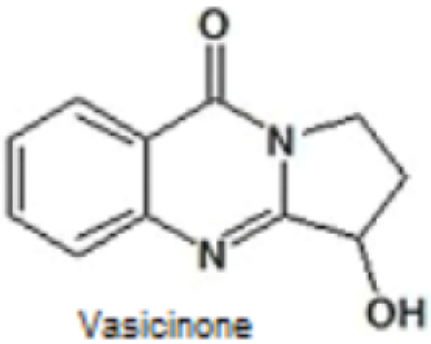




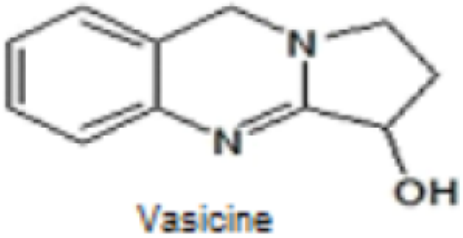
	R_1
1 Harmane	H
2 Harmine	OCH_3
3 Harmol	OH

	R_1
4 Harmaline	OCH_3
5 Harmalol	OH

Beta-carbolines alkaloids



Vasicinone



Vasicine

Quinazoline alkaloids

The Traditional and Medical Uses:

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

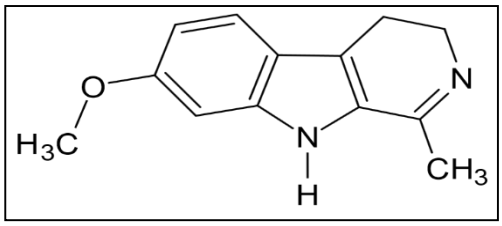
Peganum harmala was claimed to be an important medical plant. Its seeds were known to possess hypothermic and essentially hallucinogenic properties since it is MAO inhibitor agent .

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.

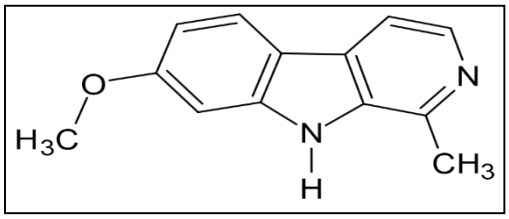
This work was designed to investigate some aspects of the anti neoplastic properties of **Peganum harmala** in that the active principle at a dose of 50 mg / kg given orally to mice for 40 days was found to have significant anti tumor activity. **Peganum harmala** alkaloids thus posses significant anti 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**.

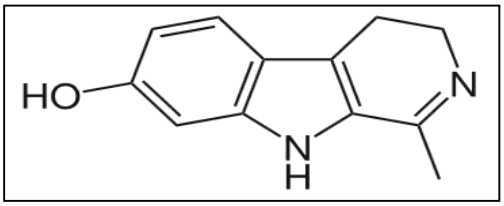
Peganum harmala also contains the quinazoline derivatives **vasicine** and **vasicinone.** It is believed that these quinazoline alkaloids are responsible for the abortifacient activity of **Peganum harmala** extracts. It has been reported that these chemicals have a uterine stimulatory effect, apparently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

Notes regarding extraction and isolation of harmala alkaloids

• The use of petroleum ether for defatting in order to remove the nonpolar compounds as lipids, graces, waxes terpens, pigments and steroidal compounds from the plant parts specially seeds, kernels and leaves. (seeds in our experiment).

• The evaporation step is to get rid of the ethanol. Ethanol is miscible with the 2 other solvents (chloroform and aqueous acidic solution). The partitioning step required two immiscible solvents if ethanol present in a sufficient concentration,

the difference in polarities of the 2 phases decrease and homogenous single phase is produce and no separation will occur.

• By treatment of the extract with dilute acidic solution (2% HCl) the alkaloids are converted to alkaloidal salts and transferred to polar solvent (aqueous layer), while the remaining pigments and other organic secondary metabolites are transferred to organic solvent (chloroform).

• Ammonium hydroxide solution was added to convert the alkaloidal salt back to the alkaloid base. The free alkaloids are soluble in organic solvent.

• Anhydrous sodium sulphate is an inert drying agent, used for removing traces of water from organic solutions.

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* GF_{254}**.**
- 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