



Lab.5 Indole Alkaloids

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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.

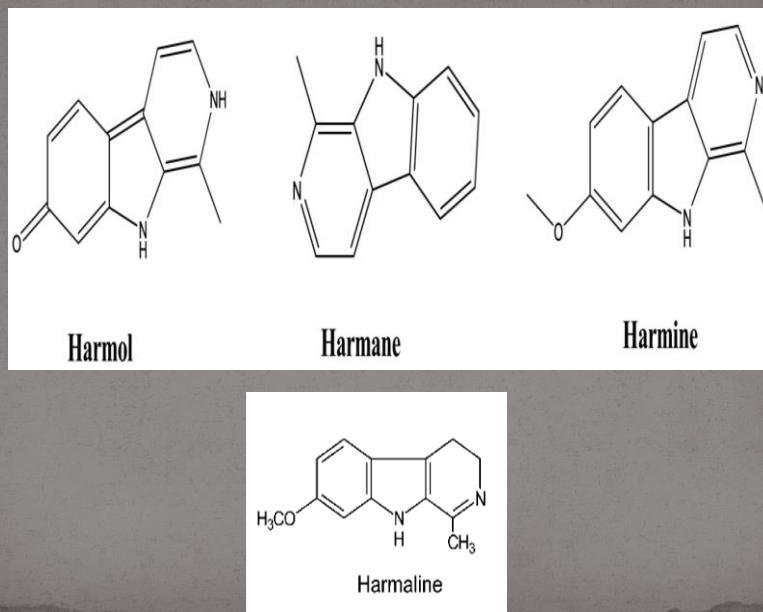


The Traditional and Medical Uses:

- The traditional uses including the *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.

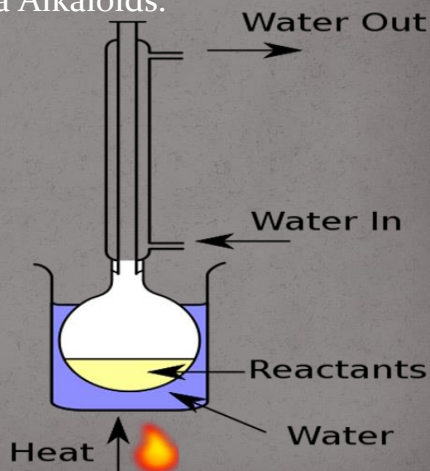
- This work was designed to investigate some aspects of the anti neoplastic properties of Peganum harmala in that the active principle at a dose of 50 mg / kg given orally to mice for 40 days was found to have significant anti tumor activity.
- Peganum harmala alkaloids thus possess significant anti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



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.*

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

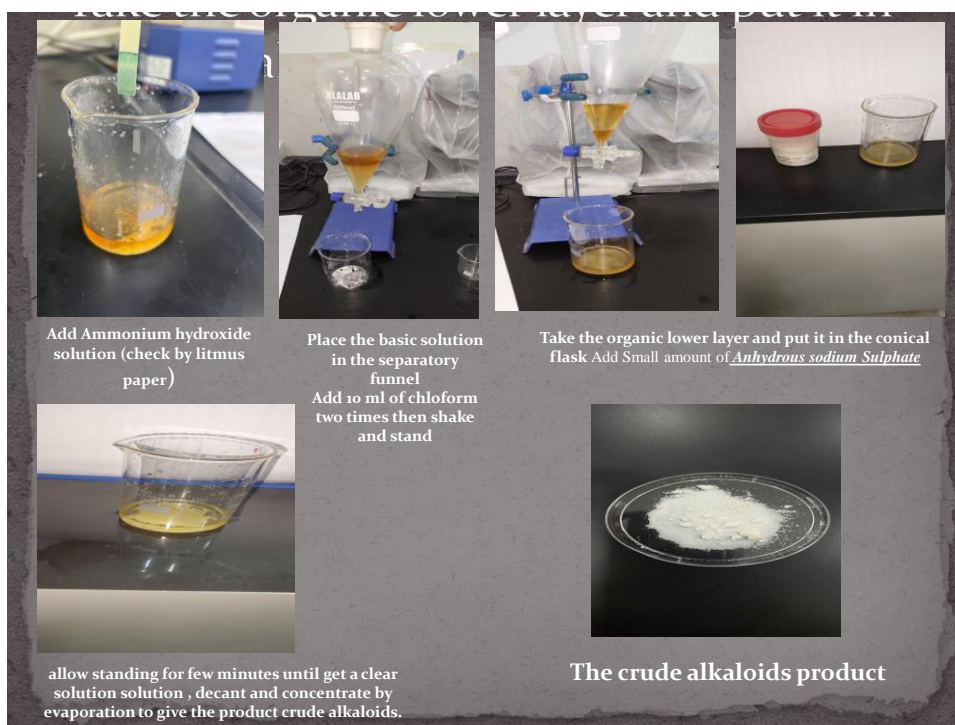
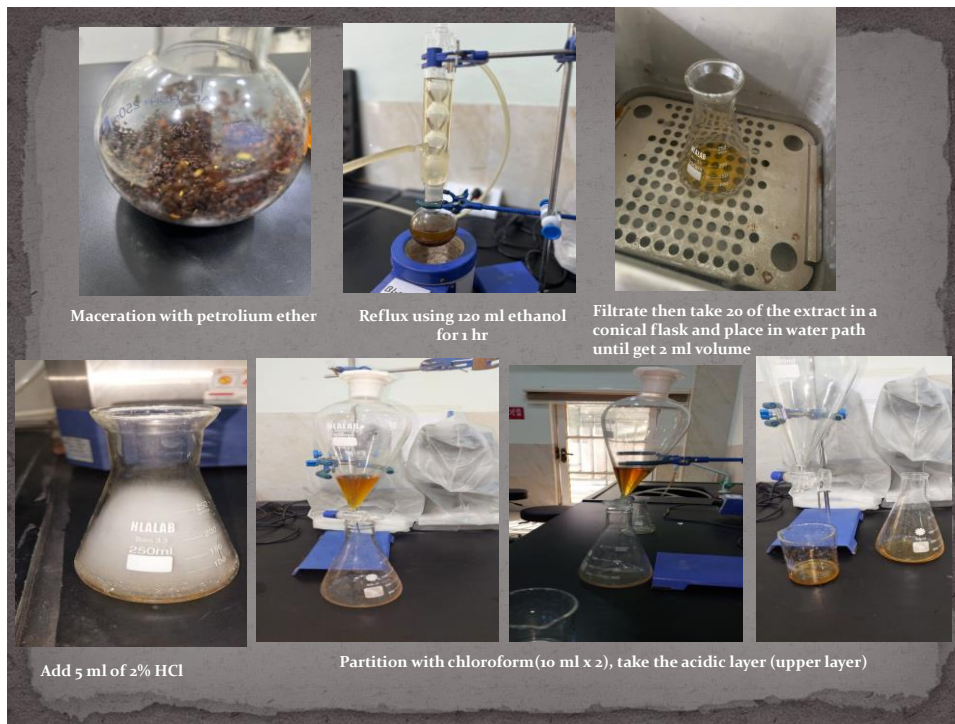
10 ml of chloform two times



Shake and 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.



Identification of Harmala alkaloids

1- **Quantitative Analysis:** By weighing the residue obtained.

- 2- **Qualitative Analysis:**
- A- The General Chemical Tests :
- B- 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 R_f values.
- Results:
- Fluorescence spot appears under the U.V. while an orange spots are seen when sprayed with the sprayer.

