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PHYTOCHEMICAL AND ANTIBACTERIAL ACTIVITY OF THE *PEGNUM HARMALA* SEEDS AND ITS ALKALOIDS

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Abstract

Pegnum harmala is belong to Zygophyllaceaee family. It is a wild growing flowering plant which is possess antimicrobial functions and an essential component in commercial medicine, Traditionally revealed the smoke of its seeds is used as antiseptic.

The aim of this study: harmala alkaloids (Harmaline, Harmalol, Harmol, Harmane, Harmine, tetra hydroharmine, acisine, acisinone) were isolated and chemically identified from *Pegnum harmala* seeds.

Phytochemical screening of *Pegnum harmala* seeds showed the absence of flavonoids, Coumarin and resins and presence of alkaloids, saponins, tannins, glycosides, anthraquinons, terpenoids and steroids.

In vitro antibacterial activity results were summarized in table 3 of *Peganum harmala* and its alkaloids against some pathogenic bacterial strains isolated from patients *Streptococcus, Staphylococcus, Aeromonas*, *E.coli, klebsiella, Acinetobacter*.

Key words *: Pegnum harmala*, Alkaloids, Phytochemical, Antibacterial Activity and Isolated.

Introduction

Phytochemistry or the chemistry of plants is important branch of chemistry concerned with plants and plant products one of the early subdivisions of organic chemistry, has been of great importance in the identification of plant substances of medicinal importance (Harborne *et al.,* 1999).

Many naturally product occurring compounds found in plants herbal and spices have been a rich of bioactive compounds. Some of these shown to possess antimicrobial functions. Medicinal plants (Herbal) were the first medicines have been used since ancient times and they continue to be used by many cultures around the. world (Fereshteh *et al.,* 2014).

Harmala plant botanical name is *Peganum harmala* belongs to the family of Zygoephyllaceae. It is a wild growing flowering plant. It is also called Syrian rue. African rule, wild rue, harmala in Iraq and Algeria. The plant is widely, distributed in predesertic regions of North Africa, Southeast. Morocco and the Middle East (Momtaz *et al.,* 2013). *Peganum harmala* contains. Up to 4% total. Alkaloids, in seeds and the roots Muhi-eldeen *et al.,* (2008) like Harmaline, Harmalol, Harmol, Harmane, Harmine, Tetrahydroharmine, Vacisine, vacisinone as shown in table 1.

Number of researchers. revealed the smoke, of its *Peganum harmala* seeds used traditional antiseptic (Shahverdi *et al.,* 2008). In addition also showed various pharmaco-logical activities such as antioxidant (Dickson *et al.,* 2006), antitumor (Kaskoos, 2014). Antispasmodic, anti-histaminic, vasorelaxant effects (Asghari and Lockwood, 2002), wound healing immuno modulation properties, leukemia healing (Zaker *et al.,* 2007), antibacterial and antitubercular activities (Shahverdi *et al.,* 2008) and antimicrobial (Al-Jiffri *et al.,* 2011; Dogruoz *et al.,* 2008)

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The present study revealed extracted, isolated, phytochemical screened, chemical, identification and vitro antibacterial studied of Harmala alkaloids like (Harmaline, Harmalol, Harmol, Harmane, Harmine, tetrahydroharmine, vacisine, vacisinone) as showed in table 2 against some pathogenic bacterial strains isolated from patients (*Streptococcus, Staphylococcus, Aeromonas*, *E.coli, klebsiella, Acinetobacter*). Results were summarized in table 3.

Materials And Methods

Materials

Peganum harmala seeds were purchased from the local market of Nasiriya and grounded to a powder then kept in dry container.

All chemicals obtained from the college laboratory. The work was performed at the organic chemistry laboratory.

Preliminary phytochemical screening

Preparation of ethanolic extract by soxhaleted 50 grams of *Pegnum harmala* seeds powder in 250 mL of ethanol for 1hrs. The extract solution was filtered and ethanol was evaporated on a rotator evaporator under vacuum at a temperature of 450^{0}C one to fifth. The filtrate was used for phytochemical screening to confirm the phytochemicals present by the following test.

Alkaloids (dragendorff's tests), flavonoids (shinoda), glycosides (molish tests), tannis acid (10% $fecl_3$ test), saponins (foam tests), sterols (liberman-burchard test), coumarin (test of filter paper soaked by diluted naoh), anthraquinons (borntrager's test) were carried out (Behidj-Benyounes *et al.,* 2014)

Results: Phytochemical screening of *P. harmala* seeds showed the absence of flavonoids, Coumarin and resins and the presence of alkaloids, saponins, tannins, glycosides, anthraquinons, terpenoids and steroids. As showed in table 1.

Methods of Extraction and Isolation of Harmala Alkaloids from *Peganum harmala*

In the first step, 10 gm of *Peganum harmala* seeds powder macerated in 100 ml of petroleum ether 48 hrs. then filtrated to removed the non polar components (defatted process) like aromatic oil, fatty acid and waxes.

In the second step, The residue was dried then dissolved in 100 mL of 90% ethanol for soxhaleted 2 hrs to extract the polar components like alkaloids and anthocyanin glycoside etc. alcoholic extract was evaporated to one fifth of the initial volume by rotary evaporator.

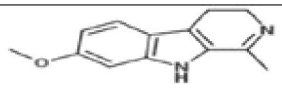
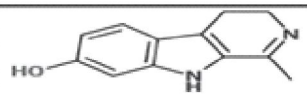
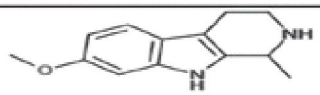
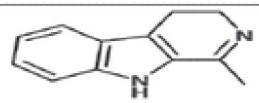
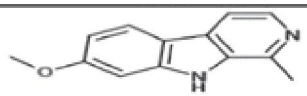
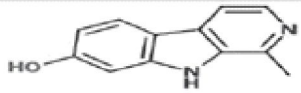
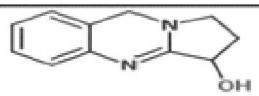
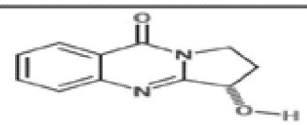
In the third step, the latter was treated twice with 5 ml of 2% hydrochloric acid HCl to form alkaloidal salt as shown in the (scheme No 1), then separated by treated with 20 ml of chloroform twice extracted in a separation funnel through formation two layer the aqueous layer contained alkaloidal salt (acidic layer) while the organic layer (chloroform) contains all other polar constituent.

In the fourth step, Added 5 ml of NH_4OH until the pH became (9) (check by litmus paper) to produce the free alkaloids and ammonium chloride, chloroform was added to the basic solution for extracted the free alkaloid harmala by classical method mention above, as shown in the (scheme No 1) (Benboltt *et al.,* 2012).

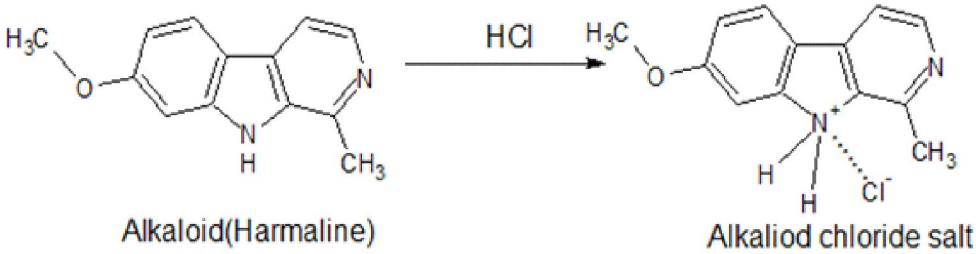
Table 1: Phytochemical screening (where, – absent and + present)The structure formula of major alkaloids in *Pegnum harmala* seeds (Harmaline , Harmalol, Harmol, Harmane, Harmine, tetrahydroharmine, vacisine, vacisinone) showed below in table No 2.

Code No.	Chemical structure	Chemical test	Test result	Chemical note
1	Alkaloids	Dragendroff reagentWagner reagent	+ve+ve	Orange ppt Brown ppt
2	Flavonoids	Shinoda test	-ve	No formation of pinkish violet color
3	Carbohydrate	Molish test	+ve	Violetcolor ring formation
4	Glycoside	Fehling's test	+ve	Blue color formation
5	Tannin	Fecl3	+ve	Bluish black color Formation
6	Saponin	Shaken of the extraction	+ve	Formation of foam
7	Sterols	Liebermann burchard	+ve	Formation green-blue color
8	Coumarine	Filter paper soaked by diluted NaoH	-ve	No formation of yellowish green color on filter paper
9	Terpenoids	Salkowski reaction	+ve	Formation of reddish brown color
10	Resins	Ethanol 95% +boiling + 4% hcl	-ve	No formation of turbidity
11	Anthraquinons	Borntrager's test	+ve	Formation of red rose color

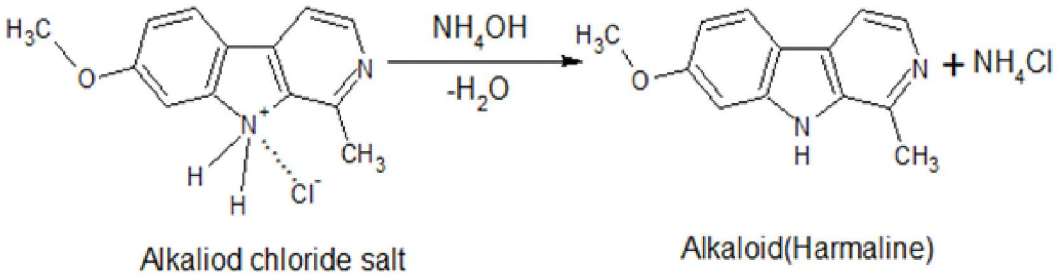
Table 2: Molecular structure of major alkaloids of *Peganum harmala*[15].

Harmaline	
Harmalol	
Tetrahydroharmine	
Harmane	
Harmine	
Harmol	
Vasicine	
Vasicinone	

Third step



Forth step



Scheme No 1 : Third steps Chemical reaction between alkaloids and hydrochloric acid . while the fourth steps explain the reaction between the alkaloids chloride salts and ammonium chloride to produce the alkaloids .

In the fifth steps, the alkaloids purified by added Small amount of Anhydrous sodium Sulphate & allow standing for few minutes until get a clear solution, decanted and concentrated the chloroform layer by evaporation to dryness to give the product alkaloids.

The essential chemical reaction in (third and fourth step) of methods of extraction and isolation of harmala alkaloids *a Pegnum harmala*as shown in the (scheme No 1). Harmala inetaked as example of alkaloids in the scheme No 1.

Scheme No 1: Third steps Chemical reaction between alkaloids and hydrochloric acid while the fourth steps explain the reaction between the alkaloids chloride salts and ammonium chloride to produce the alkaloids.

Results and Discussion

Chemical Identification of Pure Harmala Alkaliods

Quantitative Analysis: was done by weighing the crystals of Harmala Alkaliods.

Results: brown needles Yielding 1.2 gm of Harmala alkaliods.

Qualitative Analysis (Chemical Identification)

After Harmala Alkaliods isolated from Pegnum harmala seeds by extraction and isolation method shown above. It was identified chemically to conform the isolated alkaloids by the following test.

Harmal inetaked as example of alkaloids in the shown scheme No2 and scheme No. 3.

Mayer's Test

Taken few crystals of alkaloids and dissolved in few ml of ethanol, in test tube then added 2 drops of HCl. Then added 2 drops of reagent.

Result: A yellowish white precipitate formed.

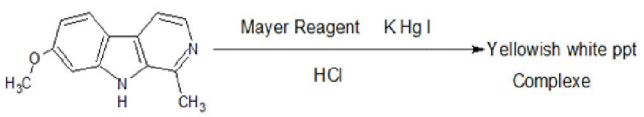
Wagner's Test

Procedure: Taken few crystals of alkaloid and dissolved in few ml of ethanol, in test tube then added 2 drops of (HCl). Then added 2 drops of Wagner's reagent.

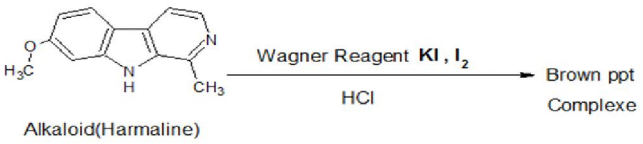
Result: Brown precipitate formed.

Stock solution preparation

The stock solution of *Pegnum harmala* and the active compound of its Alkaloids was done by dissolving dissolving



Scheme No 2: Mayer 's Test



Scheme No 3: Wagner test

Table 3: *In vitro* antibacterial activity of *Peganum harmala* against some pathogenic bacterial strains isolated from patients.

Bacterial	Mean inhibition zones (mm)of antimicrobial strains activity of plant extracts against test microbes	
	Crude Harmala (*Peganumharmala*)	Harmala alkaloids
Streptococcus	18 mm	20 mm
Aeromonas	0 mm	32 mm
E.coli	14 mm	23 mm
Staphylococcus	12 mm	14 mm
Acinetobacter	10 mm	0 mm
klebsiella	0 mm	0 mm

0.5 gm of the *Pegnum harmala* extracts and the its (alkaloids) in 10 ml of ethanol to get a 50 mg/ml which was the concentration tested as shown in table 3. Sterilization was done by filtration wares through a Millipore 0.45 mm and 0.22 mm.

Biological activity

In vitro antibacterial activity of *Peganum harmala* and its alkaloids against some pathogenic bacterial strains isolated from patients using agar cup method. (*Streptococcus, Staphylococcus, Aeromonas*, *E.coli, Klebsiella,*, *Acinetobacter*,) (Mohamedeen *et al.,* 2015; Ida Apostolico *et al.,* 2016). The result are summarized in table No 3.

Conclusion

As discussed previously, Phytochemical screening of *Pegnum harmala* seeds showed the absence of flavonoids, Coumarin and resins and presence of alkaloids, saponins, tannins, glycosides, anthraquinons, terpenoids and steroids. Harmala alkaloids were isolated and chemically identified from *Pegnum harmala* seeds by chemical test Wagner's, Mayer's.

Antibacterial activity of *Peganum harmala and* Its alkaloids explained the alkaloids were showed highly inhibitionzone against (*Streptococcus, Staphylococcus, Aeromonas*, *E.coli)*. While the *Pegnum harmala showed good* inhibition zone against *Acinetobacter*. While the test against *Klebsiella* showed no activity of both *Pegnum harmala* and its alkaloids against *Klebsiella* in the 50 mg/ml concentration.

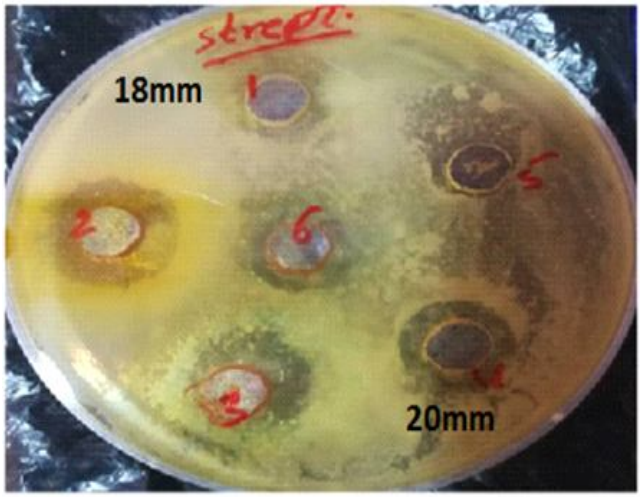


Fig. 1: Mean zone of inhibition (mm) of all extracts of Crude Harmala and harmala alkaloids on *Streptococcus* on Muller Hinton agar.

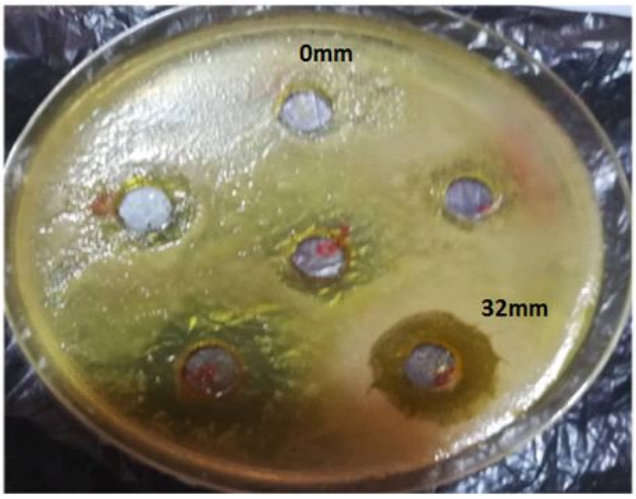


Fig. 2: Mean zone of inhibition (mm) of all extracts of Crude Harmala and harmala alkaloids on *Aeromonas* on Muller Hinton agar.

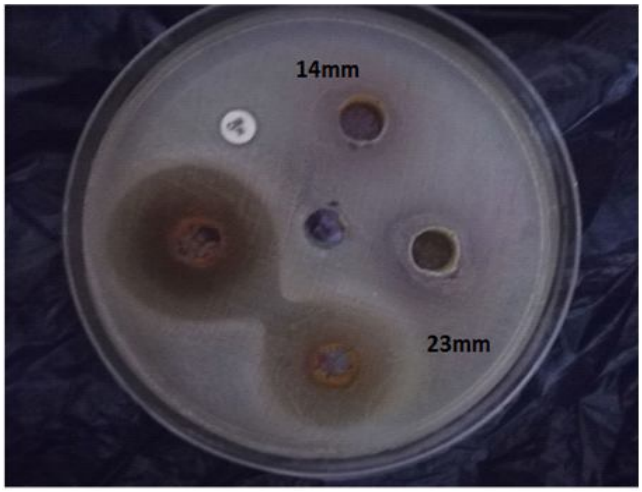


Fig. 3: Mean zone of inhibition (mm) of all extracts of Crude Harmala and harmala alkaloids *E. coli* on Nutrient agar.

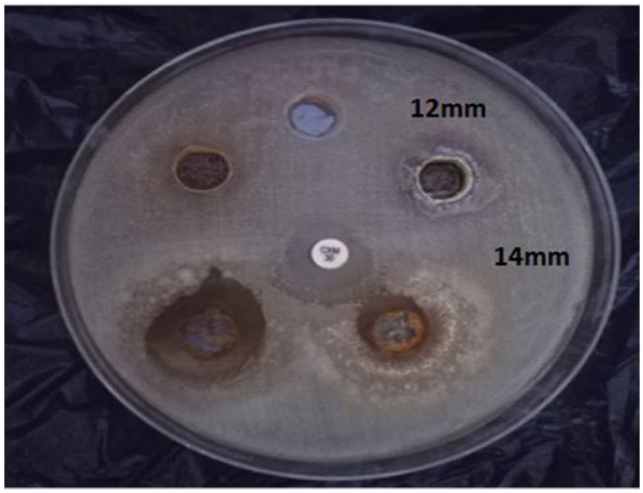


Fig. 4: Mean zone of inhibition (mm) of all extracts of Crude Harmala and harmala alkaloids *Staphylococcus* on Nutrient agar.

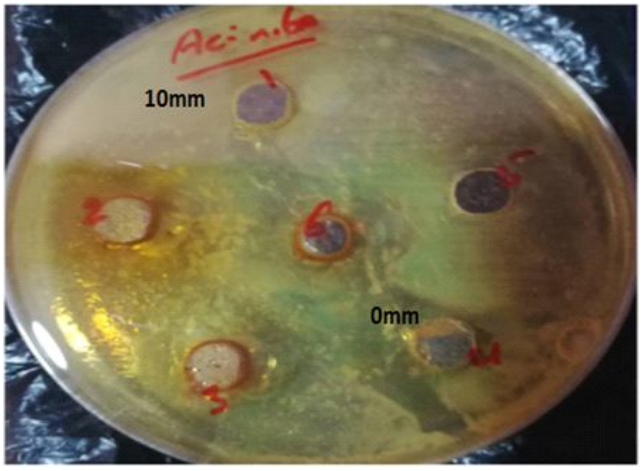


Fig. 5: Mean zone of inhibition (mm) of all extracts of Crude Harmala and harmala alkaloids on *Acinitobacter*on Muller Hinton agar.

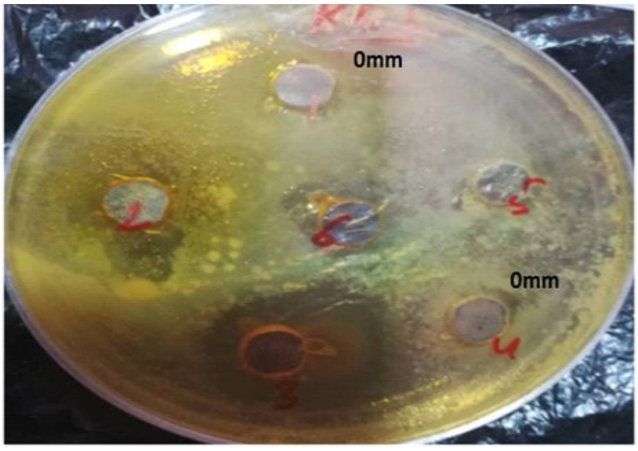


Fig. 6: Mean zone of inhibition (mm) of all extracts of Crude Harmala and harmala alkaloids on *Klebsiella* on Muller Hinton agar.

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