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

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Abstract

Peganum harmala is belong to Zygophyllaceae 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 Peganum harmala seeds. Phytochemical screening of Peganum 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: Peganum 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 Zygophyllaceae. 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).
The present study revealed extracted, isolated, phytochemical screened, chemical, identification and vitro antibacterial studied of Harmala alkaloids like (Harmaline, Harmalol, Harmol, Harmane, Harmine, tetrahydroharmine, vasicine, 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 soxhleted 50 grams of *Peganum 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 45ºC 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% fecl₃ 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, 2014).

#### 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 soxhleted 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 NH₄OH 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, 2012).

### Table 1: Phytochemical screening (where, – absent and + present)

<table>
<thead>
<tr>
<th>Code No.</th>
<th>Chemical structure</th>
<th>Chemical test</th>
<th>Test result</th>
<th>Chemical note</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Alkaloids</td>
<td>Dragendorff reagentWagner reagent</td>
<td>+ve+ve</td>
<td>Orange ppt Brown ppt</td>
</tr>
<tr>
<td>2</td>
<td>Flavonoids</td>
<td>Shinoda test</td>
<td>-ve</td>
<td>No formation of pinkish violet color</td>
</tr>
<tr>
<td>3</td>
<td>Carbohydrate</td>
<td>Molish test</td>
<td>+ve</td>
<td>Violet color ring formation</td>
</tr>
<tr>
<td>4</td>
<td>Glycoside</td>
<td>Fehling’s test</td>
<td>+ve</td>
<td>Blue color formation</td>
</tr>
<tr>
<td>5</td>
<td>Tannin</td>
<td>Fecl₃</td>
<td>+ve</td>
<td>Bluish black color Formation</td>
</tr>
<tr>
<td>6</td>
<td>Saponin</td>
<td>Shaken of the extraction</td>
<td>+ve</td>
<td>Formation of foam</td>
</tr>
<tr>
<td>7</td>
<td>Sterols</td>
<td>Liebermann burchard</td>
<td>+ve</td>
<td>Formation green-blue color</td>
</tr>
<tr>
<td>8</td>
<td>Coumarine</td>
<td>Filter paper soaked by diluted NaoH</td>
<td>-ve</td>
<td>No formation of yellowish green color on filter paper</td>
</tr>
<tr>
<td>9</td>
<td>Terpenoids</td>
<td>Salkowski reaction</td>
<td>+ve</td>
<td>Formation of reddish brown color</td>
</tr>
<tr>
<td>10</td>
<td>Resins</td>
<td>Ethanol 95% +boiling + 4% hcl</td>
<td>-ve</td>
<td>No formation of turbidity</td>
</tr>
<tr>
<td>11</td>
<td>Anthraquinons</td>
<td>Borntrager’s test</td>
<td>+ve</td>
<td>Formation of red rose color</td>
</tr>
</tbody>
</table>
Table 2: Molecular structure of major alkaloids of *Peganum harmala* [15].

<table>
<thead>
<tr>
<th>Molecular Structure</th>
<th>Chemical Structure</th>
</tr>
</thead>
<tbody>
<tr>
<td>Harmaline</td>
<td>![Harmaline]</td>
</tr>
<tr>
<td>Harmalol</td>
<td>![Harmalol]</td>
</tr>
<tr>
<td>Tetrahydroharmine</td>
<td>![Tetrahydroharmine]</td>
</tr>
<tr>
<td>Harmane</td>
<td>![Harmane]</td>
</tr>
<tr>
<td>Harmine</td>
<td>![Harmine]</td>
</tr>
<tr>
<td>Harmol</td>
<td>![Harmol]</td>
</tr>
<tr>
<td>Vasicine</td>
<td>![Vasicine]</td>
</tr>
<tr>
<td>Vasicinone</td>
<td>![Vasicinone]</td>
</tr>
</tbody>
</table>

Scheme No 1: Third step: 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 inaetakd 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 Alkaloids

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

Results: brown needles Yielding 1.2 gm of Harmala alkaloids.

Qualitative Analysis (Chemical Identification)

After Harmala Alkaloids 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

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 Pegnum harmala and Its alkaloids explained the alkaloids were showed highly inhibition zone 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 Acinitobacter on Muller Hinton agar.

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


