

A study on essential oil of *Peganum harmala* L.: Antioxidant and antibacterial activities

Tahreem Saleem¹, Khurram Shahzad Munawar^{2,3*}, Zafar Iqbal^{4*}, Jamila Javid⁵

¹ Department of Chemistry, University of Management and Technology, Lahore, Pakistan

² Institute of Chemistry, University of Sargodha, Sargodha, 40100, Pakistan

³ Department of Chemistry, University of Mianwali, Mianwali, 42200, Pakistan

⁴ Applied Chemistry Research Centre, PCSIR Laboratories Complex, Lahore, Pakistan

⁵ Department of Chemistry, University of Sialkot, Sialkot, 51040, Pakistan

*Corresponding author's email: khurramchemist@gmail.com,
zafarmayo2000@yahoo.com

Abstract

This study investigates the extraction and characterization of the essential oil from *Peganum harmala* L., focusing on its antioxidant and antibacterial properties. The essential oils were extracted from the plant's seeds and leaves using the hydro-distillation method, and nine major volatile constituents were identified via GC-MS analysis. The antioxidant potential of the sample was evaluated using the DPPH radical scavenging method, achieving a 98.085% scavenging rate at a concentration of 100 µg/mL. The antibacterial effectiveness was assessed through the well diffusion method, demonstrating strong antibacterial efficacy against *Escherichia coli* and *Staphylococcus aureus*. These results highlight the chemical composition and significant antioxidant and antibacterial properties of *Peganum harmala* L. oil, suggesting its potential use in traditional medicine and recommending further exploration of its therapeutic applications.

Keywords: *Peganum harmala* L., Antibacterial, Antioxidant activities, Essential Oil

Article History: Received: 12 Mar 2024, Revised: 10 Apr 2024, Accepted: 29 Jul 2024, Published: 8th1 Aug 2024.

Creative Commons License: NUST Journal of Natural Sciences (NJNS) is licensed under Creative Commons Attribution 4.0 International License.



Introduction

Plant materials are the perfect enriched gift of nature for humanity, containing diverse chemicals that represent the most important arenas of traditional drugs worldwide [1-2]. For example, aspirin (the first pain reliever) was made from salicylic acid, which is an active constituent in the bark of the *Salix* species (willow tree) [3]. Roots and the aerial parts of *Echinacea purpurea* possess antiviral and anti-

inflammatory activities [4]. *Taxus brevifolia's* bark synthesizes taxol, which helps treat breast, ovarian, and lung cancer [5]. Leaves of *Acacia modesta* revealed anti-inflammatory, hypoglycemic, ant-platelet, and analgesic effects [6]. *Zingiber officinale* (ginger) has extensively been used in tea, herbal medicine, and vegetables [7]. *Peganum harmala* L., frequently known as "harmal," belongs to the *Zygophyllaceae* family, which includes 30 genera and approximately 250 species [8].

Peganum harmala L. is a perpetual greenish herb that is 30-60 cm in height, with dark brown seeds in an angular shape; leaves are alternate, bright green and two inches long; woody root; many-branched green stem; conspicuous five-petaled white or pale-yellow flower; and green/orange-brown fruit (Figure 1) [9-11]. *Peganum harmala* L. is found worldwide and is mostly native to Pakistan, Temperate Asia, Mongolia, India, Turkey, the Mediterranean, Europe, China, the Middle East, and the southern splits of Iran [12]. It is present in semi-barren, steppe areas, and sandy soils [13].



Figure 1: *Peganum harmala* L. plant.

The phytochemicals identified in *Peganum harmala* L. include flavonoids, alkaloids, tannins, saponins, anthraquinones, and cardiac glycosides [14-15]. Beta-carboline and quinazoline alkaloids are more pronounced in the roots and seeds of harmal than in the leaves and stems. Notably, these alkaloids are not found in the flowers of the plant. The total alkaloids present in harmal are 2-5%. Harmaline, harmol, harmane, harmalol, harmine, and tetrahydroharmine are the major β -carboline alkaloids, while vasicinone and vasicine are the primary quinazoline alkaloids of *Peganum harmala* L. [16]. Harmine showed antibacterial, antiviral, antioxidant, antidiabetic, antitumor, antiparkinson, osteogenic, angiogenic, drug interaction, and cardiovascular effects [17-19]. Harmaline is involved in drug interaction, antiparkinson, CNS stimulant, hallucinogenic, reversible inhibitor of MAO-A, antibacterial, and antioxidant activities [20-22]. Harmane changes their mood and is involved in anti-depressant

activity [23]. Vasicinone revealed abortifacient, antiprotozoal, and vasorelaxant effects [24-25]. Vasicine also showed abortifacient effects [26].

Several reports in the sources discuss the chemical constituents of the seeds and leaves of *Peganum harmala* L., as well as the antibacterial activity and various uses of its essential oils [27-38]. In previous years, synthetic antibacterial drugs were manufactured because bacteria were the main cause of animal, plant, and human deaths. Soon, these synthetic antibacterial drugs were replaced by naturally produced antibacterial medicines due to their severe aftereffects [39]. Active oxidative species are continuously generated in the body in response to metabolism, which leads to diabetes mellitus, immune dysfunction, malignant tumours and cell ageing. So, synthetic antioxidants like butylated hydroxytoluene (BHT) were synthesized in previous years to prevent the stress of free radicals in the body. Consequently, these synthetic antioxidants were replaced by natural antioxidants because humans were at risk of the harmful effects of synthetic antioxidants [40-44].

Despite the potential therapeutic benefits of *Peganum harmala* L. essential oil, the research data available from various regions in Pakistan still needs to be improved. This article examines the components extracted from the leaves and seeds of *Peganum harmala* L. and assesses their antioxidant and antibacterial properties. The primary goal of this study is to identify novel botanical drugs that can effectively combat free radicals and bacterial infections.

Materials and Methods

In February 2017, leaves and seeds of *Peganum harmala* L were gathered from Chakwal, Punjab, Pakistan. The leaves and seeds underwent a two-week air-drying process at room temperature. Subsequently,

the leaves were meticulously cut into small pieces using heavy-duty scissors, while the seeds were finely ground into a powder.

Materials and Methods

175 g of dried leaves of *Peganum harmala* L. were subjected to hydro-distillation in the Dean-Stark apparatus for 15 hours. Also, the 1.5 kg of seeds were ground into powder, put into a water bowl, and left for three days. Then, this material was subjected to hydro-distillation in the Dean-Stark apparatus for 18 hours. The oil obtained from seeds and leaves was in the form of an emulsion, which was extracted thrice with the help of diethyl ether (3×150 mL) in a separating funnel. Using anhydrous sodium sulphate, the organic layer was dehydrated. The ethereal layer evaporated at room temperature and pressure (25 °C and 1 atm). The essential oil acquired was carefully preserved in a refrigerated environment at a temperature of 4 °C. It was stored in an amber-colored bottle to ensure its integrity and suitability for future research.

GC-MS analysis

The essential oil extracted from the seed and leaves of *Peganum harmala* L. was analyzed by PCSIR Laboratories in Lahore, Pakistan, using Agilent 6890 gas chromatography-mass spectrometry (GC-MS) instruments [45]. The HP-5MS capillary column was 30 metres long, and helium was utilized as the carrier gas; 0.25 millimetres, 0.25 μm in diameter, and 0.1 micrometres in pore size were used for separation. The temperatures and flow rates for the different stages were as follows: The first stage could reach temperatures of 50–100 °C at a pace of 5 degrees Celsius per minute, while the last stage could reach temperatures of 100–250 °C at a rate of 3 °C per minute. The last step was a 20-minute hold at 260 °C. Approximately 2 microliters (μL) of the sample were injected into the column using split less and manual modes.

The successful separation of constituents from the essential oil was achieved by setting the column temperature to 200 °C. Their identities were established by comparing the essential oil's chemical components' mass spectra and retention indices with those already in the NIST collection.

Determination of antioxidant activity

Peganum harmala L. essential oil was tested for its antioxidant properties utilizing 2,2-Diphenyl-1-picrylhydrazyl (DPPH) as a radical scavenger [46]. Various concentrations of samples, such as 20 μL , 40 μL , 60 μL , 80 μL , and 100 μL , were combined with DPPH dissolved in methanol. The incubation of samples was made light-free under room temperature for 30 minutes. Later, the samples' absorbance values were measured using a 517 nm wavelength relative to the blank with a DPPH solution. Ascorbic acid was the positive control, whereas water was the negative control for comparison purposes. The absorbance of the DPPH solution was set after being diluted 6-folds and then read at 517 nm using the UV-Vis spectrometer to determine the percent decrease. To calculate the degree of DPPH inhibition, the researchers employed Equation 1.

$$\% \text{ DPPH inhibition} = A_0 - A_1 / A_0 \times 100$$

Where, A_0 denotes the absorbance of the control, whereas A_1 represents the absorbance of the samples.

Determination of antibacterial activity

An antibacterial assay was conducted using four bacterial species: *Klebsiella pneumoniae*, *Escherichia coli*, *Pseudomonas aeruginosa* (all gram-negative), and *Staphylococcus aureus* (a gram-positive bacterium). The study employed the excellent diffusion method to evaluate the antibacterial action of the

essential oil isolated from the leaf and seed, following the guidelines established by the National Committee for Clinical Laboratory Standards (1993) [47-48]. Before injection, bacterial strains were sub cultured three times on nutrient agar media. The stock cultures were heated at 37 °C for 24 hours, and Perforation was made in the Petri plates with the help of a 9 mm corn borer. The essential oil of the seeds and leaves occupied the wells. Then, Petri dishes were enclosed by the lid and left for 30 minutes. These plates spent 24 hours in a 37 °C incubator. Inhibition zones surrounding the wells were quantified in millimeters.

Statistical Analysis

The antioxidant potential of *Peganum harmala* L. was compared to that of ascorbic acid, and the standard deviation and mean were calculated using traditional statistical techniques. The significance level ($p < 0.05$) was determined using an unpaired sample t-test. The result showed a statistical significance of $p = 0.0074$ ($p < 0.05$). Two-way analysis of variance (ANOVA) was used to assess the effectiveness of antioxidants, with

concentration and essentiality serving as independent variables.

Mean, standard deviations and statistical significance calculated the variation between experiments. The student's t-test estimated the difference in inhibition potential between the tested samples and the standard. The results of antibacterial activity were statistically significant because of the probability of $P=0.0039$ ($P \leq 0.05$). The mean and standard deviation of ampicillin and essential oil were 23.1 ± 0.8165 and 15.075 ± 3.434 , respectively.

Results and Discussion

Chemical composition of the essential oil

Peganum harmala L. extract was analyzed using GC-MS. Five essential oil elements were isolated from tseeds and four from the leaves, for around nine constituents in the volatile oil of the leaves and seeds. The identified essential oil components were initially confirmed by comparing their spectra with the spectra of the NIST library. Then, it was further confirmed by observing their mass fragmentation pattern. The results are given in Tables 1 and 2.

Table 1: Major volatile components of commercial oil of seeds of *Peganum harmala* L. by GC-MS studies

S.No.	Compounds	Retention time (Minutes)
1	n-Hexadecanoic acid	10.811
2	9-Octadecenoic acid, methyl ester, (E)	11.669
3	9,12-Octadecadienoic acid (Z, Z), methyl ester	11.735
4	cis-Vaccenic acid	12.163
5	Harmine	14.591

Table 2: Major volatile components of commercial oil of leaves of *Peganum harmala* L. by GC-MS studies

S.No.	Compounds	Retention time (Minutes)
1	Hexadecanoic acid, methyl ester	10.416
2	11-Octadecenoic acid, methyl ester	11.681
3	9-Octadecenoic acid, methyl ester, (E)	11.669
4	9,12-Octadecadienoic acid (Z, Z), methyl ester	11.735

To the best of our understanding, the chemical makeup of *Peganum harmala* L. essential oil has only been reported in a few scientific papers [49]. In all these reports, different essential oil constituents were abundant because of the different pH of soils, nutrient contents, air, water content, and harvesting season. Tahrouch et al [27]. found essential oil of both dried and fresh *Peganum harmala* L. plant parts contain mostly =2,3-dihydrobenzofurane and propyllic acid.

A study was done in North Africa to show the composition of commercial oil seeds of *Peganum harmala* L., which demonstrated that the most abundant constituents were eugenol and thymol [16]. These discoveries are not based on the outcomes displayed here. Changes in essential oil composition may have emerged from a few contrasts, such as seasonal, occasional, topographical, and geogra-phical.

Antioxidant activity of *Peganum harmala* L.

Peganum harmala L. has therapeutic properties utilized in traditional and modern drugs to treat various diseases in certain regions. Plant seed and leaf extracts are rich in multiple flavonoids and phenolics, which possess antioxidant properties and effectively inhibit the generation of oxidative species within the human body. DPPH is often used as an indicator to estimate the free-radical-scavenging activity of both synthetic and natural antioxidants. When both natural or synthetic antioxidants interact with DPPH, the stability of free radicals is compromised, and a yellow-colored form of diphenyl picrylhydrazine is formed. The hydrogen-donating antioxidant activity radical was displayed on the DPPH radical [50].

In this work, the antioxidant capacity of *Peganum harmala* L. essential oil was evaluated by its ability to slake the DPPH

radical. Essential oil derived from seeds exhibited a significant inhibition rate of 88.085% at a concentration of 100 $\mu\text{g}/\text{mL}$ compared to ascorbic acid, which served as the standard and demonstrated an inhibition rate of 92%. Water (a negative control) does not show any kind of inhibition. The essential oil extracted from the seeds is more antioxidant than the plant's leaves. The observed inhibitory effects of the volatile oil derived from *Peganum harmala* L. indicate the presence of phenolic compounds within the plant. The efficacy of commercial oil in reducing power is determined by the dosage, which shows varying chemical components and the synergistic impact of minor constituents. Tables 3 and 4 represent the data on the antioxidant properties of *Peganum harmala* L. essential oil. Results indicate that the volatile oil of *Peganum harmala* L. exhibits a limited inhibitory effect, suggesting the presence of a low concentration of phenolic constituents. The antioxidant activity of commercial oil is influenced by various factors, including its structural features, dosage, temperature, concentration, light exposure, and more.

Table 3: Antioxidant activity of essential oil seeds of *Peganum harmala* L.

S.No.	Concentration of sample (μL)	Absorbance (nm)	%age inhibition
1	20	0.549	45
2	40	0.451	55
3	60	0.349	64
4	80	0.248	75
5	100	0.113	88

Table 4: Antioxidant activities of essential oil of leaves of *Peganum harmala* L.

S.No.	Concentration of sample (μL)	Absorbance (nm)	%age inhibition
1	20	0.589	41
2	40	0.509	49
3	60	0.459	54
4	80	0.341	65
5	100	0.248	71

The health benefits and nutritional functions of antioxidants derived from therapeutic plants and nutritious foods have been the subject of ongoing investigation. In biological settings, DNA damage and oxidation of proteins and lipids may be triggered by exposure to reactive nitrogen species (RNS) and reactive oxygen species (ROS), such as hydroxyl, nitric oxide, and superoxide [51]. The most antioxidant process is with the natural assortment of the human body, which scavenges the free radicals, thereby maintaining the balance between anti-oxidation and oxidation. On the one hand, radiation, alcohol, cigarette smoking, or environmental pollutants are the factors that cause overproduction of RNS and ROS. This imbalance between oxidation and anti-oxidation processes triggers the manifestation of degenerative and chronic illnesses [52].

Numerous studies in scientific literature have demonstrated the antioxidant

properties of the seed and leaf extracts derived from *Peganum harmala* L. [53-59]. Essential oil is produced from *Peganum harmala* L. seeds and leaves, although its antioxidant activity needs to be better established.

Antibacterial activity of *Peganum harmala* L.

The antibacterial efficacy of *Peganum harmala* L. essential oil was evaluated using the diffusion method against various food-borne bacteria, with results detailed in Table 5 and Figure 2. The study demonstrated that the volatile oil from *Peganum harmala* L. possesses notable antibacterial activity against *Staphylococcus aureus* and *Escherichia coli*. After 24 hours of incubation, the oil produced maximum inhibition zones of 18.1 mm for *Staphylococcus aureus* and 18.0 mm for *Escherichia coli*.

Table 5: Antibacterial activity of essential oil seeds of *Peganum harmala* L.

S. No.	Test Organism	Colony Morphology	Zone of Inhibition for Ampicillin(mm)	Incubation Temp. (°C)	Zone of inhibition for Essential oil(mm)
1	<i>Klebsiella pneumonia</i>	Gram -ve rods	23.1	37	12.1
2	<i>Staphylococcus aureus</i>	Gram -ve cocci	24.1	37	18.1
3	<i>Pseudomonas aeruginosa</i>	Gram -ve rods	22.1	37	12.1
4	<i>Escherichia coli</i>	Gram +ve rods	23.1	37	18

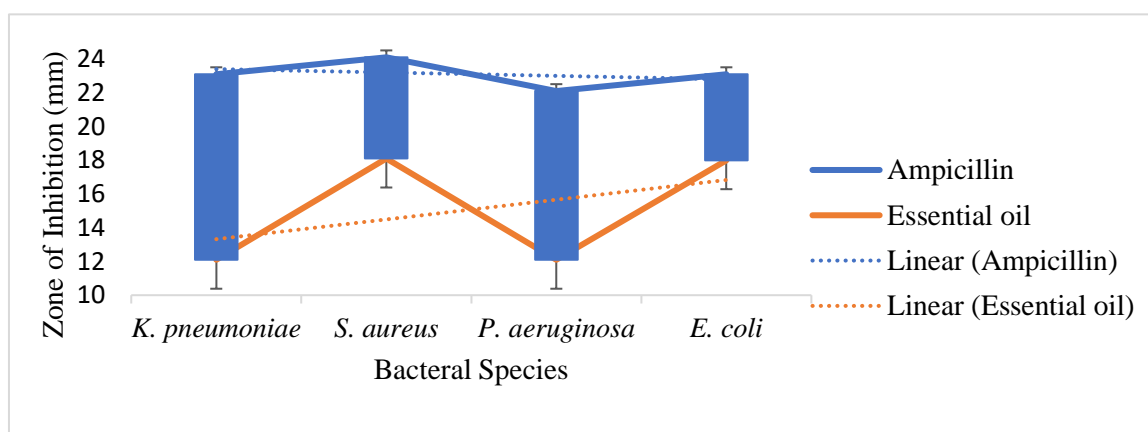


Figure 2: Antibacterial activity of essential oil seeds of *Peganum harmala* L.

Klebsiella pneumoniae and *Pseudomonas aeruginosa* exhibited a minimum area of inhibition measuring 12.1 mm each following a 24-hour incubation period (Figures 3 and 4).

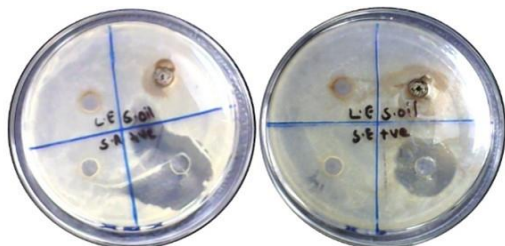


Figure 3: Antibacterial activity of essential oil against *Klebsiella pneumoniae* and *Staphylococcus aureus*.



Figure 4: Antibacterial activity of essential oil against *Pseudomonas aeruginosa* and *Escherichia coli*.

Several published studies have studied Essential oil from seeds for its antibacterial properties [16, 29]. Present the study's findings with the existing literature [60-62]. In their research, Selim et al. [28] found essential oil from the seeds of *Peganum harmala* L. to have the most potent antibacterial properties against four bacterial species: *Staphylococcus aureus*, *Salmonella indicia*, *Escherichia coli*, and *Bacillus cereus*.

Many studies show that plants are nature's greatest gift due to their therapeutic properties. Medicinally, all parts perform different functions. *Peganum harmala* L. seeds, for example, have hallucinogenic and hypothermic properties and the entire plant has been shown to have galactagogue, antispasmodic, anthelmintic, abortifacient, antipyretic, emmenagogue, and emetic properties. Nine components found in essential oil were shown to have

antioxidant and antibacterial activities in this investigation. Antioxidant activity increases as the concentration of essential oil increases. Seeds showed more powerful antioxidant activity at 100 $\mu\text{g/mL}$ with 88 % inhibition, while leaves revealed 71 % inhibition at 100 $\mu\text{g/mL}$. The unpaired t-test proved that the result of the antioxidant potential of essential oil is statistically significant because the probability was equal to 0.0074 ($p < 0.05$).

Based on the results of the investigation, it was determined that *Peganum harmala* L. volatile oil showed the highest zone of inhibition for antibacterial action against *Escherichia coli* and *Staphylococcus aureus*. i.e., 18.0 and 18.1 mm, respectively, after 24 hours of incubation, while zones of inhibitions, i.e., 12.1 mm, were obtained by both *Klebsiella pneumoniae* and *Pseudomonas aeruginosa*. It was surprising to see that the volatile oil of the leaves of *Peganum harmala* L. does not demonstrate any area of inhibition. The results of antibacterial potential proved to be statistically significant because they offered a probability equal to 0.0039 ($P \leq 0.05$). The average and standard deviation for ampicillin and *Peganum harmala* L. volatile oil were 15.075 ± 3.434 and 23.1 ± 0.8165 , respectively. Hence, not only have phytochemicals proved to have therapeutic effects, but each part also has some beneficial importance. It is impossible to say which essential oil constituents are responsible for antioxidant and antibacterial effects. Nonetheless, this data will prove valuable for further studies.

Conclusion

The volatile oil was extracted from the leaves and seeds of *Peganum harmala* L. using the hydro-distillation method. The oil was then characterized using gas chromatography and mass spectrometry techniques. Antioxidant and antibacterial screenings were conducted using the DDPH assay and the well diffusion method.

The studies have revealed that the volatile oil extracted from the seeds and leaves seeds of *Peganum harmala* L. possesses potential for application as natural ingredients in pharmaceuticals and food products. A comprehensive analysis revealed the presence of nine distinct components within the essential oil derived from *Peganum harmala* L. The volatile oil of the seeds demonstrated the highest antioxidant potential (88%) at a 100 µg/mL concentration. Similarly, notable antibacterial efficacy was noted against *Escherichia coli* and *Staphylococcus aureus*.

References

1. Edziri H, Mastouri M, Mahjoub MA, Patrich G, Matieu M, Ammar S, Ali SM, Laurent G, Zine M, Aouni M. Antibacterial, antiviral and antioxidant activities of aerial part extracts of *Peganum harmala* L. grown in Tunisia Toxicological & Environmental Chemistry. 2010 Aug 1;92(7):1283-1292.
2. Jones FA. Herbs—useful plants. Their role in history and today: European journal of gastroenterology & hepatology. 1996 Dec 1;8(12):1227-1231.
3. Li JJ. Laughing gas, Viagra, and Lipitor: the human stories behind the drugs we use: Oxford University Press. 2006.
4. Barrett B. Medicinal properties of Echinacea: a critical review. Phyto-medicine. 2003 Jan 1;10(1):66-86.
5. Goodman J, Walsh V. The story of taxol: nature and politics in the pursuit of an anti-cancer drug. Cambridge University Press; 2001.
6. Bukhari IA, Khan RA, Gilani AH, Ahmed S, Saeed SA. Analgesic, anti-inflammatory and anti-platelet activities of the methanolic extract of *Acacia modesta* leaves. Inflammo pharmacology. 2010 Aug;18: 187-196.
7. Park GH, Park JH, Song HM, Eo HJ Kim MK, Lee JW, Lee MH, Cho KH, Lee JR, Cho HJ, Jeong JB. Anti-cancer activity of Ginger (*Zingiber officinale*) leaf through the expression of activating transcription factor 3 in human colorectal cancer cells. BMC complementary and alternative medicine. 2014;14: 408.
8. Spencer KA. Host specialization in the world Agromyzidae (Diptera). Springer Science & Business Media; 2012.
9. Asgarpanah J, Ramezanloo F. Chemistry, pharmacology and medicinal properties of *Peganum harmala* L. African Journal of pharmacy and pharmacology. 2012 Jun 15;6(22):1573-1580.
10. Niroumand MC, Farzaei MH, Amin G. Medicinal properties of *Peganum harmala* L. in traditional Iranian medicine and modern phytotherapy: a review. Journal of Traditional Chinese Medicine. 2015 Feb 15;35(1):104-109.
11. Zargari, A. Medicinal plants. Tehran university press, Iran.1988; (2): 618-619.
12. Piacentini MP, Piatti E, Fraternali D, Ricci D, Albertini MC, Accorsi A. Phospholipase C-dependent phosphoinositide breakdown induced by ELF-EMF in *Peganum harmala* calli. Biochimie. 2004;86(4-5):343-349.
13. Darabpour E, Bavi AP, Motamedi H, Nejad SM. Antibacterial activity of different parts of *Peganum harmala* L. growing in Iran against multi-drug resistant bacteria. EXCLI journal. 2011;10: 252.
14. Abbas M, Shahid M, Iqbal M, Anjum F, Sharif S, Ahmed S, Pirzada T. Anti-termite activity and phytochemical analysis of fifteen medicinal plant seeds. Journal of Medicinal Plants Research. 2013; 7(22):1608-1617.
15. Benbott A, Bahri L, Boubendir A, Yahia A. Study of the chemical components of *Peganum harmala* and evaluation of acute toxicity of alkaloids extracted in the Wistar albino mice.

- Journal of Material and Environmental Science. 2013; 4:558-565.
16. Apostolico I, Aliberti L, Caputo L, De Feo V, Fratianni F, Nazzaro F, Souza LF, Khadhr M. Chemical composition, antibacterial and phytotoxic activities of *Peganum harmala* seed essential oils from five different localities in Northern Africa. *Molecules*. 2016 Sep 15;21(9):1-13.
 17. El Gendy MA, El-Kadi AO. *Peganum harmala* L. differentially modulates cytochrome P450 gene expression in human hepatoma HepG2 cells. *Drug Metabolism Letters*. 2009 Dec 1;3(4):212-216.
 18. Fortunato JJ, Réus GZ, Kirsch TR, Stringari RB, Stertz L, Kapczinski F, Pinto JP, Hallak JE, Zuardi AW, Crippa JA, Quevedo J. Acute harmine administration induces antidepressive-like effects and increases BDNF levels in the rat hippocampus. *Progress in Neuro-Psychopharmacology and Biological Psychiatry*. 2009 Nov 13;33(8):1425-1430.
 19. Schwarz MJ, Houghton PJ, Rose S, Jenner P, Lees AD. Activities of extract and constituents of *Banisteriopsis caapi* relevant to parkinsonism. *Pharmacology Biochemistry and Behavior*. 2003 Jun 1;75(3):627-633.
 20. Moloudizargari M, Mikaili P, Aghajanshakeri S, Asghari MH, Shayegh J. Pharmacological and therapeutic effects of *Peganum harmala* and its main alkaloids. *Pharmacognosy reviews*. 2013 Jul;7(14):199.
 21. Samoylenko V, Rahman MM, Tekwani BL, Tripathi LM, Wang YH, Khan SI, Khan IA, Miller LS, Joshi VC, Muhammad I. *Banisteriopsis caapi*, a unique combination of MAO inhibitory and antioxidative constituents for the activities relevant to neurodegenerative disorders and Parkinson's disease. *Journal of ethnopharmacology*. 2010 Feb 3;127(2):357-367.
 22. Flattery DS, Schwartz M. *Haoma and harmaline: the botanical identity of the Indo-Iranian sacred hallucinogen "soma" and its legacy in religion, language, and Middle Eastern folklore*. Univ of California Press. 1989;21.
 23. Farzin D, Mansouri N. Antidepressant-like effect of harmine and other β -carbolines in the mouse forced swim test. *European neuropsychopharmacology*. 2006 Jul 1;16(5):324-328.
 24. Astulla A, Zaima K, Matsuno Y, Hirasawa Y, Ekasari W, Widyawaruyanti A, Zaini NC, Morita H. Alkaloids from the seeds of *Peganum harmala* showing antiplasmodial and vasorelaxant activities. *Journal of Natural Medicines*. 2008 Oct; 62:470-472.
 25. Hayet E, Maha M, Mata M, Mighri Z, Laurent G, Mahjoub A. Biological activities of *Peganum harmala* leaves. *African Journal of Biotechnology*. 2010;9(48):8199-8205.
 26. Shapira Z, Terkel J, Egozi Y, Nyska A, Friedman J. Abortifacient potential for the epigeal parts of *Peganum harmala*. *Journal of ethnopharmacology*. 1989 Dec 1;27(3):319-325.
 27. Tahrouch, S.; Raplor, S.; Belashen, Y.; Bessiere, J. M.; Andary, C. (1998). Volatile constituents of *Peganum harmala* (*Zygophyllaceae*). *Acta Botanica gallica*, 145(2), 121-124.
 28. Faridi P, Ghasemi Y, Mohagheghzadeh A. Chemical composition of *Peganum harmala* smoke and volatile oil. *Journal of essential oil-bearing plants*. 2013 Jul 4;16(4):850-854.
 29. Selim SA, Aziz MH, Mashait MS, Warrad MF. Antibacterial activities, chemical constituents and acute toxicity of Egyptian *Origanum majorana* L., *Peganum harmala* L. and *Salvia officinalis* L. essential oils. *Afr. J. Pharm. Pharmacol*. 2013;7(13):725-735.
 30. Gupta AK. *Handbook on Unani medicine with formulae, processes,*

- uses and analysis. Asia Pacific Business Press Inc. Delhi: India. 2003.
31. Hassan I. Some folk uses of *Peganum harmala* in India and Pakistan. *Economic Botany*. 1967 Jul;21(3):284.
 32. Moussa TA, Almaghrabi OA. Fatty acid constituents of *Peganum harmala* plant using Gas Chromatography–Mass Spectroscopy. *Saudi journal of biological sciences*. 2016 May 1;23(3):397-403.
 33. Fazal H, Ahmad N, Khan MA. Physicochemical, phytochemical evaluation and DPPH-scavenging antioxidant potential in medicinal plants used for herbal formulation in Pakistan. *Pak J Bot*. 2011 Dec 1;43(SI):63-67.
 34. Pennacchio M, Jefferson L, Havens K. Uses and abuses of plant-derived smoke: Its ethnobotany as hallucinogen, perfume, incense, and medicine. Oxford University Press; 2010 Jul 23.
 35. Mahdeb N, Mayouf S, Boukhari F, Souilah S, Bouzidi A. Hemolytic effect of total alkaloids from the seeds of *Peganum harmala* in vitro on erythrocytes of ruminants: Sheep, cattle and goats. *Asian J Plant Sci Res*. 2013;3(6):53-59.
 36. Abbasipour H, Mahmoudvand M, Rastegar F, Basij M. Insecticidal activity of *Peganum harmala* seed extract against the diamondback moth, *Plutella xylostella*. *Bulletin of Insectology*. 2010 Dec 1;63(2):259-263.
 37. Dastagir G, Husaain F, Rehman I U. Essential oil of some plants of the family *Zygophyllaceae* and *Euphorbiaceae*. *Pakistan Journal of Botany*. 2014; 46(6):2043-2049.
 38. Ismahane L, Sghir MM, Ibrahim M, Khadija B, Laid OM. Ovicidal, larvicidal and adulticidal activities of essential oils from *Peganum harmala* L. (*Zygophyllaceae*) against date moth *Ectomyelois ceratoniae* Zeller (Lepidoptera: Pyralidae). *Int. J. Biosci*. 2016; 8:146-152.
 39. Atta-ur-Rhman Choudhary MI, Thomsen WJ. Bioassay technique for drug development Harwood academic publishers.14-24.
 40. Dubey N K. *Plants are a source of natural antioxidants*. Centre for Agriculture and Bioscience International. 2014.
 41. Shahidi F, editor. Natural antioxidants: chemistry, health effects, and applications. The American Oil Chemists Society; 1997.
 42. Weber SU, Lodge JK, Saliou C, Packer L. Antioxidants. *Handbook of cosmetic science and technology*. CRC Press. 2001; 315-326.
 43. Pokorny J, Yanishlieva N, Gordon MH, editors. Antioxidants in food: practical applications. CRC press; 2001.
 44. Jayaprakasha GK, Rao LJ. Phenolic constituents from the lichen *Parmotrema stippeum* (Nyl.) Hale and their antioxidant activity. *Zeitschrift für Naturforschung C*. 2000; 55(11-12):1018-1022.
 45. Miliauskas G, Venskutonis PR, Van Beek TA. Screening of radical scavenging activity of some medicinal and aromatic plant extracts. *Food chemistry*. 2004 Apr 1;85(2):231-237.
 46. Espin JC, Soler-Rivas C, Wichers HJ. Characterization of the total free radical scavenger capacity of vegetable oils and oil fractions using 2, 2-diphenyl-1-picrylhydrazyl radical. *Journal of Agricultural and Food chemistry*. 2000;48(3):648-656.
 47. Hassan A, & Amjid I. Gas chromatography-mass spectrometric studies of stems essential oil of *Pinus roxburghii* and their antibacterial and antifungal activities. *Journal of Medicinal Plants Research*. 2009;3(9): 670-673.
 48. Mukherjee PK, Balasubramanian R, Saha K, Saha BP, Pal M. Antibacterial efficiency of *Nelumbo nucifera*

- (Nymphaeaceae) rhizomes extract. Indian drugs. 1995;32(6):274-276.
49. Zhu W, Asghari G, Lockwood GB. Factors affecting volatile terpene and non-terpene biotransformation products in plant cell cultures. *Fitoterapia*. 2000 Sep 1;71(5):501-506.
50. Viuda-Martos M, Ruiz Navajas Y, Sánchez Zapata E, Fernández-López J, Pérez-Álvarez JA. Antioxidant activity of essential oils of five spice plants widely used in a Mediterranean diet. *Flavour and Fragrance journal*. 2010 Jan;25(1):13-19.
51. Fang YZ, Yang S, Wu G. Free radicals, antioxidants, and nutrition. *Nutrition*. 2002 Oct 1;18(10):872-879.
52. Baiano A, Del Nobile MA. Antioxidant compounds from vegetable matrices: Biosynthesis, occurrence, and extraction systems. *Critical reviews in food science and nutrition*. 2016 Sep 9;56(12):2053-2068.
53. Abolhasani L, Salehi EA, Kenari RE. Study of antioxidant capacity and stability of phenolic compounds from the seeds of *Peganum harmala*. *J Appl Environ Biol Sci*. 2015;4(11):218-222.
54. Benarous K, Bombarda I, Iriepa I, Moraleda I, Gaetan H, Linani A, Tahri D, Sebaa M, Yousfi M. Harmaline and hispidin from *Peganum harmala* and *Inonotus hispidus* with binding affinity to *Candida rugosa* lipase: In silico and in vitro studies. *Bioorganic chemistry*. 2015 Oct 1; 62:1-7.
55. Mazandarani M, Sineh Sepehr K, Baradaran B, Khuri V. Autecology, phytochemical and antioxidant activity of *Peganum harmala* L. seed extract in North of Iran (Tash Mountains). *Journal of Medicinal Plants and By-products*. 2012 Sep 1;1(2):151-6.
56. Khadhr M, Bousta D, El Mansouri L, Boukhira S, Lachkar M, Jamoussi B, Boukhchina S. HPLC and GC-MS analysis of Tunisian *Peganum harmala* seeds oil and evaluation of some biological activities. *American Journal of Therapeutics*. 2017 Nov 1;24(6):706-712.
57. Singh VK, Mishra V, Tiwari S, Khaliq T, Barthwal MK, Pandey HP, Palit G, Narender T. Anti-secretory and cytoprotective effects of peganine hydrochloride isolated from the seeds of *Peganum harmala* on gastric ulcers. *Phytomedicine*. 2013 Oct 15;20(13):1180-1185.
58. Kaskoos, RA. Physico-chemical parameters, phytochemical screening and antioxidant activity of seeds of *Peganum harmala* collected from Iraq. 2014; 4(28):20-24.
59. Kanwal GZ, Hafeez A, Haq IU, Rehman TU, Muhammad SA, Shahzadi I, Fatima N, Rehman NU. Antioxidant, Antimicrobial and Antileishmanial Study of Different Parts of *Peganum harmala*. *International Journal of Biosciences*, 2016;9: 45-58.
60. Arshad N, Zitterl-Eglseer K, Hasnain S, Hess M. Effect of *Peganum harmala* or its β -carboline alkaloids on certain antibiotic resistant strains of bacteria and protozoa from poultry. *Phytotherapy Research: An International Journal Devoted to Pharmacological and Toxicological Evaluation of Natural Product Derivatives*. 2008 Nov;22(11):1533-1538.
61. Shahverdi A, Ostad S, Khodae S, Bitarafan L, Monsef-Esfahani H, Jamalifar H, Nikavar B, Mohseni M. Antimicrobial and cytotoxicity potential of *Peganum harmala* smoke. *Pharmacognosy magazine*. 2008 Jul 1;4(15):236-240.
62. Gaviraj EN, Babu GR, Murthy UD. An antibacterial activity guided the isolation of harmine from *Peganum harmala* seeds by bioautography. *Indian Drugs*. 1998; 35(8): 471-474.