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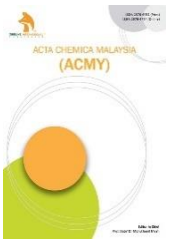

RESEARCH ARTICLE

IDENTIFICATION OF CHEMICAL COMPOSITION OF ESSENTIAL OIL OF HARMALA USING GC-MS ANALYSIS

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ABSTRACT

Peganum harmala belongs to the Zygophyllaceae family and has a history in traditional medicine for its wide range of therapeutic benefits. These include deterring intestinal parasites, relieving rheumatic symptoms, regulating bodily functions, combating parasites, promoting perspiration, exhibiting potential in anticancer treatment, and providing pain relief. The chemical composition of *Peganum harmala L.* generally comprises phytochemicals, essential oils, and fixed oils. Additionally, GC-MS analysis was conducted to characterize the extracted oil, revealing nine volatile constituents present in both the seeds and leaves of *Peganum harmala L.* In terms of antioxidant potential, it demonstrated an impressive 98.085% inhibition rate at a concentration of 100 µg/mL. The extracted essential oils can be explored for medicinal activities.

KEYWORDS

Medicinal plant; Essential oil; *Peganum harmala* L; Zygophyllaceae;

1. INTRODUCTION

Peganum harmala is classified within the Zygophyllaceae family, found in the order Zygophyllales, which encompasses approximately 22 genera and over 250 species Species of *Peganum* can be found throughout regions from North Africa and the Mediterranean to the Middle East, reaching into countries like Pakistan, India, and also in the southern areas of Iran. Additionally, these species have been introduced to North America and Australia (Asgarpanah et al., 2012). *Peganum harmala,* also known as Harmal, is a flowering plant that belongs to the Zygophyllaceae family. It is characterized by its herbaceous nature and typically reaches a height of 0.30 to 0.80 meters. The leaves of this plant are green and are arranged in a stiff twigs-like pattern. The roots of *Peganum harmala* can extend up to 5-6 meters, and it produces approximately 1000-2500 seeds annually. Hypothermic and hallucinogenic qualities are shown by the seeds (Kuhn and Winston, 2000; Lamchouri et al., 1999).

Peganum harmala is well-adapted to endure challenging environmental conditions, such as drought and high salinity. It is commonly found in alkaline and salty soils where the annual rainfall typically ranges from 100 to 250 millimeters (Hayet et al., 2010).

Since early history, *Peganum harmala*, generally referred to as Syrian rue, harmal, esphand, and other names, belongs to the Zygophyllaceae family. This herbaceous perennial is indigenous to regions surrounding the Mediterranean, the core of Sahara, sections of the Middle East, the Indian subcontinent, southern Australia, and the western U.S. (Mahmoudian et al., 2002; Chen et al., 2004; Bremner et al., 2009).

P. harmala extract is a rich source of bioactive substances , including large amounts of primary and secondary metabolites (Khlifi et al., 2013; Li et al., 2017; Li et al., 2018). The therapeutic and pharmacological effects of P. harmala can be linked to numerous active substances found in its seeds. These include β-carboline alkaloids like harmine, harmaline, and others in addition to steroids, anthraquinones, flavonoids, amino acids and quinazoline alkaloids such as vasicine (peganine) and related compounds (Astulla et al., 2008; Pulpati et al., 2008; Herraiz et al., 2010; Shao et al., 2013). The seeds of *Peganum harmala L.* contain alkaloids derived from β-carboline (βCs) and tetrahydo-β-carboline (THβCs). These alkaloids are naturally present in food due to chemical interactions between indoleamines and either aldehydes or α-keto acids. These interactions might take place during the stages of food production, processing, or even storage. Notably, traces of harman and norharman have been detected in properly cooked meat and fish, estimated at the ng/g level. Additionally, minor levels have been detected in multiple alcoholic drinks and other food products (Herraiz and Chromatogr, 2000).

The seed is the primary therapeutic component of the plant. According to traditional medicine references, the plant offers a diverse range of pharmacological properties like aiding digestion, stimulating milk production, increasing urine flow, regulating menstrual periods, preventing blood clotting, and providing pain relief. Recent studies in herbal medicine have revealed that *P. harmala* has multiple potential health benefits, including effects on heart health, neurological systems, microbial defense, pest deterrence, cancer resistance, cell growth control, digestive health, and diabetes management (Nirounmand et al., 2015). *Peganum harmala* contains several phytochemical compounds, including alkaloids, flavonoids, and anthraquinones. The alkaloid content ranges from 2% to 5%. The extract of *Peganum harmala* predominantly consists of Harmaline, Harmalol, Harmine, tetrahydroharmine and harmol which are the main beta-alkaloids. Notably, alkaloids are not present in the flowers. Dry seeds of *Peganum harmala* contain approximately 4.3% to

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5.6% harmine and harmaline, while the roots contain about 2.0% to 1.4% harmol (Herraiz et al., 2010). Additionally, other compounds such as pegamine, isopeganine, dipeganine, and deoxypeganine have been identified in *Peganum harmala* (Virot et al., 2007).

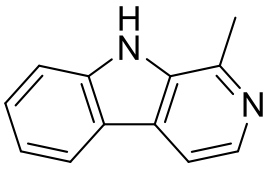


Figure 1: Harmane

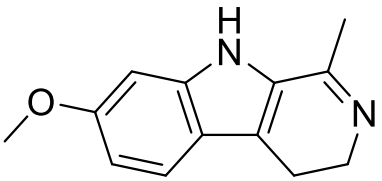


Figure 2: Harmaline

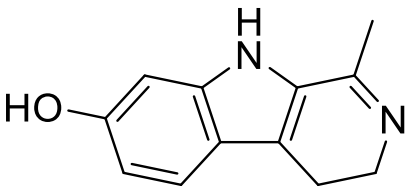


Figure 3: Harmalol

Harmaline, harmine, harmalol, and Harman are naturally occurring compounds which are present in both the seeds and roots of *Peganum harmala.* One can also find harmol and tetrahydroharmine in the seeds. The whole plant and its seeds contain various chemical derivatives of Quinazoline, including Deoxypeganine, Deoxyvasicinone, Vasicine, and peganol. Moreover, the plant has ingredients like 9,14-dihydroxyoctaecanoic acid, ash, calcium, copper, Dipegene, lipids, fibers, proteins, urine, and moisture. Studies have indicated that the plant's active components, notably harmine and harmaline, inhibit the mono-amino oxidase type A enzyme, leading to mental disorders including hallucinations (McKenna et al., 1984).

The amounts of harmaline in several components of the plant, such as seeds, fruits, and capsule walls, examined using Reverse Phase High Performance Liquid Chromatography (RP HPLC). The results revealed values of 56.0 mg/g, 4.55 mg/g, and 0.54 mg/g, respectively (Herraiz et al., 2010).

In Algeria, an analysis of *P. harmala* by the application of GC-MS disclosed various chemicals in its seed extract. Notably, this study revealed harmaline at 48.009%, harmine at 38.440%, tetrahydroharmine at 8.513%, tetrahydroharman at 0.061%, and 6-methoxytetrahydro-1-norharmanone at 0.057%. It is worth highlighting that there is currently no accessible research examining the active ingredients of the plant's essential oil utilizing the HS-SPME approach (Sassoui et al., 2015). In present research on the plant compounds, aromatic extracts and essential oils were derived from the dried seed powder of *Peganum harmala*. The chemical constituents of these extracts were subsequently analyzed using the GC-MS method.

The objective of this work lies in its dual focus. Firstly, it sought to ascertain the concentration of phytochemical components within different parts of the harmala plant. Secondly, it aimed to evaluate the inhibitory activity of these components against microorganisms. By addressing these aspects, the research contributes to a deeper understanding of the harmala plant's potential uses, offering valuable knowledge for both medicinal and scientific purposes.

2. Experimental Work

2.1 Extraction

In current research, plant samples were collected from the indigenous territories of the Dera Gazi Khan district in Punjab. Following this, the plant material was allowed to naturally dry in a shaded area. After a few days, the dried material underwent processing into a semi-coarse powder, which was then stored in airtight containers. The total dry mass of the entire plant amounted to approximately 12 kg. The research involved setting up a Soxhlet extractor and utilizing 4 kg of the dry substance for extraction. Hexane was chosen as the solvent, and the process was conducted at its boiling point. After the extraction, the concoction was filtered, and the hexane was allowed to dissipate over a span of three days. Ultimately, a dense and viscous extract was obtained, which represented the unrefined hexanoic derivative of the *peganum* plant.

2.2 Extraction and Analysis

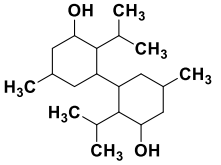
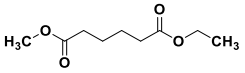
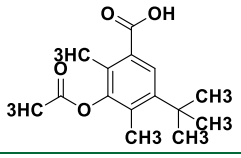
In the investigation, the raw plant extract underwent solvent extraction, a procedure relying on polarity differences. Carbon tetrachloride (CCl_4) was employed to extract the hexanoic component in a separatory funnel, resulting in a purified fraction of the hexane extract. Sample analysis was performed using GC-MS. The device specifications were established as follows: An Agilent 6890N gas chromatograph was utilized, which was linked to an Agilent 5973 mass detector. The chosen column was an HP-5, measuring 30 m in length, with an internal diameter of 0.25 mm and a stationary phase thickness of 0.25 µm. The injection method employed was a split/no-gap, with the initial column temperature set at 50°C (0.00 min hold time, no change rate).

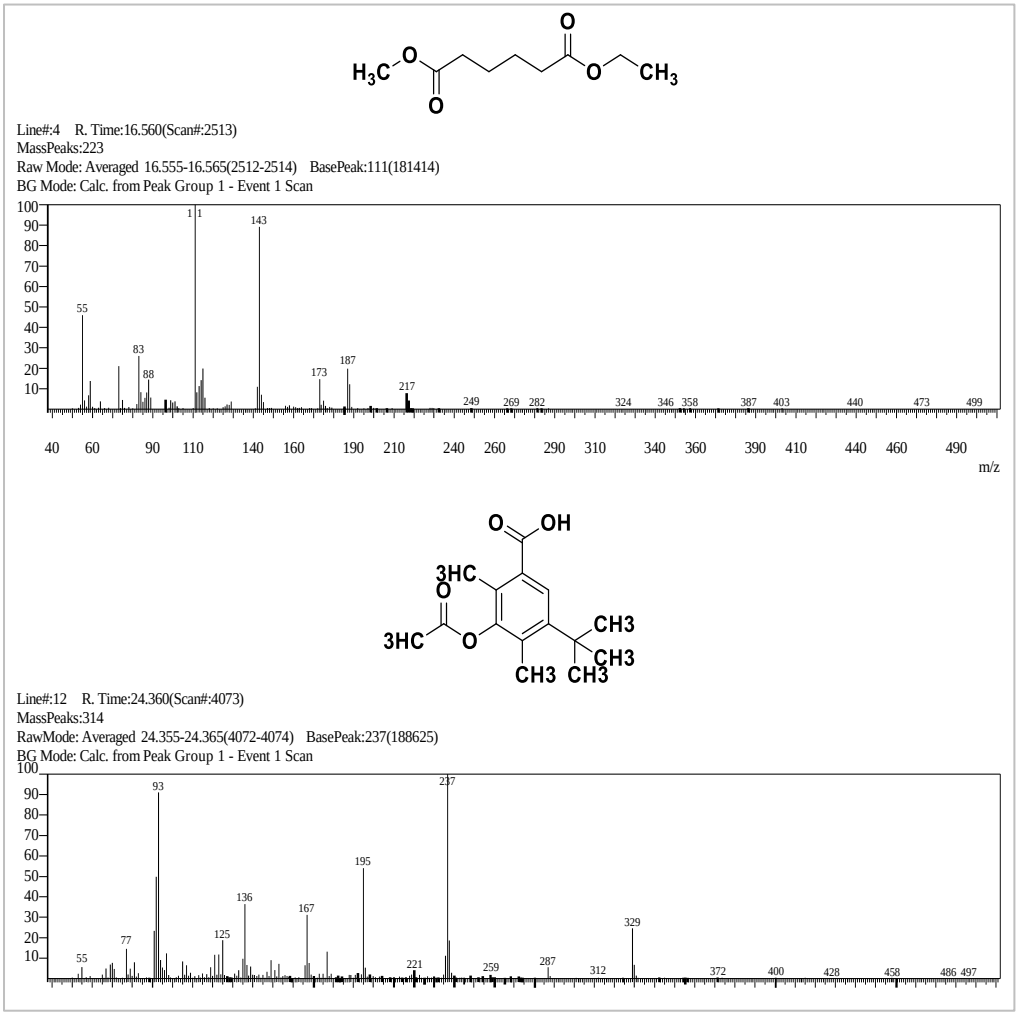
Subsequently, the temperature settings for the column were adjusted to 200°C (0.00 min hold time, increasing at a rate of 5°C/min), followed by 240°C (0.00 min hold time, increasing at a rate of 10°C/min). Helium, with a purity of 99.999%, was selected as the carrier gas. The injection method did not have a gap, and the library utilized was Willey 7n. The injector's temperature was maintained at 250°C with a flow rate of 0.9 ml/minute. The research employed a Hollow Stir Bar Sorptive Extraction (HSSPME) mode, utilizing SMPE fibers with a PDMS thickness of 100 micrometers, which were sourced from SUPELCO. The sample had a weight of 0.5 g. For extraction, the conditions involved a temperature of 60°C for 20 minutes, with an ultrasound duration of 10 minutes, utilizing a Euronda ultrasound device from Italy. Subsequently, the sample was kept in the GC-MS injector port for a period of 3 minutes.

3. Result and Discussion

The hexane fraction of *Peganum harmala* was undergone through GC-MS analysis. The mass spectra were obtained in neutral mode. The spectra were interrupted to reach the structure of compounds. Structural interpretation is done by using website Metlin.peaks of fragments retention time, molecular formula and weight all are listed in the Table 1.

Table 1: GC-MS Data of Hexane Fraction

Sr. No.	R_t(min)	Molecular weight	Molecular formula	Tentative identification	other fragments ions (m/z)	Structure
1	11.44	236	$C_{18}H_{24}O_2$	3,3'-bimenthol	221, 166, 149	
2	16.56	223	$C_9H_{16}O_4$	Hexane dioic acid, ethyl methyl ester	187, 173, 143	
3	24.36	314	$C_{15}H_{19}O_4$	Benzoic acid, 3-acetyloxy-, tert. -butyl dimethyl	287, 259, 227, 195	



Graph 1: Spectrum of GC-MS analysis of compounds that are obtained from the hexane fraction.

The hexane fraction of *Peganum harmala* leaves was found to contain an array of compounds including alkaloids, phenols, steroids, and terpenoids. GC-MS analysis validated the phytochemical composition of this plant, with alkaloids being the predominant constituent and steroids present in smaller amounts.

4. Conclusion

In the recent study, the leaves of the *Peganum harmala* plant were examined. The hexane fraction was analyzed through Soxhlet extraction, followed by GC-MS analysis. The analysis revealed the presence of alkaloids, terpenoids, steroids, and phenols, all of which are biologically neutral. Notably, the plant exhibited inhibitory properties related to harmine. The structures of these compounds were determined using Metlin during the GC-MS analysis.

Therefore, it's evident that *Peganum harmala* offers valuable phytochemicals with potential medicinal benefits, displaying significant antibacterial potential. It's recommended for future studies to focus on isolating the bioactive compounds from the *Peganum harmala* extract and understanding their modes of action, paving the way for advancements in scientific research.

References

Asgarpanah, J., Ramezanloo, F., Chemistry, 2012. Pharmacology and medicinal properties of Peganum harmala L. J Afr Pharma Pharmacol 2012;6:1573-1580.

Kuhn, M.A., Winston, D., 2000. Herbal therapy and supplements, a scientific and traditional approach. New York: Lippincott. Pp. 350-374.

Lamchouri, F., Settaf, A., Cherrah, Y., Zemzami, M., Lyoussi, B., Zaid, A., Atif, N., and Hassar, M., 1999. Antitumour principles from Peganum harmala seeds. Thérapie, 54: Pp. 753-758.

Hayet, E., Maha, M., Mata, M., Mighri, Z., Laurent, G., and Mahjoub, A. (2010). Biological activities of Peganum harmala leaves. African Journal of Biotechnology, 9(48), 8199-8205.

Mahmoudian, M., Jalilpour, H., and Salehian, P., 2002. Toxicity of Peganum harmala: Review and a case report. Iranian J. Pharmacol. Therap., 1: Pp. 1-4.

Chen, Q., Chao, R., Chen, H., Hou, X., Yan, H., Zhou, S., Peng, W., and Xu, A., 2004. Antitumor and neurotoxic effects of novel harmine derivatives and structure-activity relationship analysis. Int. J. Cancer, 114: Pp. 675-682.

Bremner, P., Rivera, D., Calzado, M.A., Obon, C., Inocencio, C., Beckwith, C., Fiebich, B.L., Muñoz, F., and Heinrich, M., 2009. Assessing medicinal plants from South-Eastern Spain for potential anti-inflammatory effects targeting nuclear factor-Kappa B and other pro-inflammatory mediators. J. Ethnopharmacol., 124: Pp. 295-305.

Khlifi, D., Sghaier, R. M., Amouri, S., et al., 2013. Composition and anti-oxidant, anti-cancer and anti-inflammatory activities of Artemisia herba-alba, Ruta chalpensis L. and Peganum harmala L, Food and Chemical Toxicology, vol. 55, Pp. 202–208, 2013.

Li, S., Cheng, X., and Wang, C., 2017. A review on traditional uses, phytochemistry, pharmacology, pharmacokinetics and toxicology of the genus Peganum, Journal of Ethnopharmacology, vol. 203, Pp. 127–162.

Li, Y., et al., 2018. Study of Methanol Extracts from Different Parts of Peganum harmala L. Using 1H NMR Plant Metabolomics J. Anal. Methods Chem.

Astulla, A., Zaima, K., Matsuno, Y., Hirasawa, Y., Ekasari, W., Widyawaruyanti, A., Cholies, Z. N., and Morita, H., 2008. Alkaloids from the seeds of Peganum harmala showing antiplasmodial and vasorelaxant activities. J. Nat. Med., 62: Pp. 470-472.

Pulpati, H., Biradar, Y.S., and Rajani, M., 2008. Highperformance thin-layer chromatography densitometric method for the quantification of

harmine, harmaline, vasicine, and vasicinone in Peganum harmala. J. AOAC Int., 91: Pp. 1179-1185.

Herraiz, T., González, D., Ancín-Azpilicueta, C., Arán, V.J., and Guillén, H., 2010. β-Carboline alkaloids in Peganum harmala and inhibition of human monoamine oxidase (MAO). Food Chem. Toxicol., 48: Pp. 839-845.

Shao, H., Huang, X., Zhang, Y., Zhang, C., 2013. Main alkaloids of Peganum harmala L. and their different effects on dicot and monocot crops. Molecules, 18(3), Pp. 2623-2634.

Herraiz, T., Chromatogr, J., 2000. A 881 Pp. 483-499.

Niroumand, M. C., Farzaei, M. H., Amin, G., 2015. Medicinal properties of Peganum harmala L. in traditional Iranian medicine and modern phytotherapy: a review. Journal of Traditional Chinese Medicine, 35(1), Pp. 104-109.

Virot, M., Tomao, V., Colnagui, G., Visinoni, F., Chemat, F., 2007. New microwave-integrated Soxhlet extraction: an advantageous tool for the extraction of lipids from food products. Journal of chromatography A, 1174(1-2), Pp. 138-144.

McKenna, D. J., Towers, G. N., Abbott, F., 1984. Monoamine oxidase inhibitors in South American hallucinogenic plants: tryptamine and β-carboline constituents of ayahuasca. Journal of Ethnopharmacology, 10(2), Pp. 195-223

Sassoui, D., Seridi, R., Azin, K., Usai, M., 2015. Evaluation of phytochemical constituents by GC-MS and antidepressant activity of Peganum harmala L. seeds extract. Asian Pacific Journal of Tropical Disease, 5(12), Pp. 971-974.

Hashemi, P., Yarahmadi, A., Shamizadeh, M., and Khademi, K., 2012. Comparison of headspace solvent microextraction, hydrodistillation solvent microextraction, and solid-phase microextraction for the study of volatile components of Kelussia odoratissima Mozaff. by GC-MS. Acta Chromatographica, 24(1),Pp. 97-109.

Bahmani, M., Taherikalani, M., Khaksarian, M., Soroush, S., Ashrafi, B., Heydari, R., 2019. Phytochemical profiles and antibacterial activities of hydroalcoholic extracts of Origanum vulgare and Hypericum perforatum and Carvacrol and Hypericin as a Promising Anti-Staphylococcus aureus. Mini reviews in medicinal chemistry, 19(11), Pp. 923-932.