Indian Journal of Novel Drug Delivery

IJNDD

An Official Publication of Karnataka Education and Scientific Society

Research Article

Physicochemical and Biological Properties of Fixed and Essential Oils from Seeds and Leaves of *Peganum harmala*

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ARTICLE DETAILS

Article history:
Received on 22 September 2021
Modified on 11 October 2021
Accepted on 16 October 2021

Keywords:
Physicochemical Properties,
Fixed Oil,
Essential Oils,
Biological Activities,
Peganum harmala.

ABSTRACT

In this study, fixed and essential oils of seeds and leaves of *Peganum harmala* were screened for their physicochemical, phytochemical properties and antioxidant, antimicrobial activities. Leaves essential oil was extracted following hydrodistillation method, whereas seed fixed oil was isolated with hexane using Soxhlet apparatus. Furthermore, the reported organoleptic and physicochemical characterizations were in accordance with AFNOR norms. Moreover, the antioxidant activity was determined by DPPH free radical scavenging assay. Antibacterial effects were also estimated according to the agar well diffusion method. Besides, the determined quality indices generally meet the standards followed. A quantitative analysis of polyphenols and flavonoids was also performed. The IC_{50} values were 22.52 ±2.40 and 139±2.86 µg/ml, respectively, for leaves essential and seed fixed oils. Concerning the antibacterial activity, good inhibition was observed against *Escherichia coli* and *Pseudomonas aeruginosa*. Therefore, the results indicate that *Peganum harmala* is a rich source of oils with important activities.

INTRODUCTION

Because of their effectiveness, safety, and efficacy, several efforts to find alternative antioxidants and antibacterial agents from natural sources that have been intensified in recent years [1]. The antimicrobial and antioxidant properties of plants and the aromatic products derived from them are due to the different chemical substances in their composition – essential oils and glyceride oils, alkaloids, flavonoids, tannins, glycosides, and other compounds [2]. One of the most well-known plants used in popular medicine is *Peganum harmala* L, which belongs to the Zygophyllaceae family and grows abundantly in steppe areas and sandy soils throughout the Mediterranean region, North Africa, and the Middle East [3]. *Peganum harmala* is an important medicinal plant traditionally used as an analgesic, antihelmintic, antimicrobial and cancer-destroying agent [4].

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Different parts of this plant have been used in folk medicine for the treatment of human sicknesses such as asthma, colic, lumbago, jaundice and menstrual flow [5]. A lot of studies searched for the composition, antibacterial, antifungal, and antioxidant properties of different extracts coming from *Peganum harmala* [6, 7]. However, few reports on the extraction, characterization and biological activities of fixed and essential oils from this plant have been discovered. Thus, this research aimed at investigating the phytochemical and physicochemical proprieties of the essential and fixed oils extracted from seeds and leaves of *Peganum harmala* as well as to evaluate their antioxidant and antibacterial activities.

MATERIALS AND METHODS

Plant Material

The seeds of *Peganum harmala* were collected in July. Whereas the leaves were collected in April which is the maturing period in the region of Touama-Wilaya of Bordj Bou Arreridj- (North-East of Algeria) (Fig. 1).

Figure 1: Seeds (**a**) and Leaves (**b**) of *Peganum harmala*

Extraction

Extraction of Fixed Oil

The seeds fixed oil of *Peganum harmala* was prepared by using a Soxhlet apparatus fitted with a condenser. 50 g of ground seeds was transferred to a filter paper extraction thimble, then extracted with 250 ml hexane. After about 8 extraction cycles, the solvent loaded with the plant extract is recovered to be concentrated to dryness under vacuum using a rotary evaporator. Ultimately, the extracts were stored at 4°C until analysis.

Extraction of Volatile Oil

A fresh aerial part of the plant was hydrodistilled separately in a Clevenger. While extracted essential oils were dried over anhydrous sodium sulphate, filtered, weighed and stored in a dark sealed vial at 4°C.

Analysis of Bio-Oil

The sensory qualities of fixed and essential oils were examined and reported, including appearance, odor, and color. To evaluate the quality of the extracted oils, relative density, refractive index, hydrogen potential (pH), acid value, ester value, peroxide value, saponification value and miscibility in ethanol, were measured. These tests were carried out in accordance with AFNOR regulations [8].

Qualitative Phytochemical Screening of Fixed Oil

Total Phenolic Content

The total phenolic content of seeds fixed oil was evaluated according to the Folin-Ciocalteu method [9]. The sample solutions at different concentrations of oils (200µL) were mixed with Folin-Ciocalteu reagent (1mL) and incubated for 4 min. Subsequently, Na2CO3 solution (7.5%) was added. Furthermore, the mixture was left to react for two hours at the room temperature, the absorbance at 765 nm was measured. Thus, the total phenolic content was then expressed as µg/mg gallic acid equivalent (GAE).

Total Flavonoid Content

The total flavonoid content was estimated using the method described by Meziti et al [10]. A volume of 1 ml of the oil extracts or standard is added to 1 ml of the AlCl3 solution (2% in methanol). After 10 minutes of reaction, the absorbance at 430 nm was measured. Total flavonoid content was expressed as µg/mg of quercetin equivalent.

Antioxidant Activity of Fixed and Essential Oils

The antioxidant activity was evaluated using the 2, 2- dipheny1-1 picrylhydrazyl (DPPH) assay according to the method described by Mansouri et al [11]. A volume of 0.5 ml of the DPPH solution is mixed with 1.5 ml of oil extracts or standard antioxidants (Ascorbic acid). After 30 minutes of incubation in the dark at room temperature, the decreases in the absorbance values were measured at 517 nm.

Antibacterial Activity

The antibacterial capacity of fixed and essential oils was evaluated against the following bacterial strains*: Escherichia coli (ATCC* 25922); *Staphylococcus aureus (*ATCC 25923); *Pseudomonas aeruginosa (*ATCC 27853). The

inhibition zone diameter was assayed according to the agar well diffusion method. This technique involves making wells using sterile tips followed by the addition of 25 µL of oil to each well. The area where bacteria did not grow was measured [12].

Statistical Analysis

IC_{50} values were expressed as mean ± SD. The significance of the difference was tested by the one-way ANOVA using GraphPad Prism 5.01. Values of $P < 0.05$ were regarded as significant.

RESULTS AND DISCUSSION

Extraction Yield

The yield of fixed and essential oils was expressed as a percentage of the weight of the extract obtained relative to the weight of vegetable mass used. The result obtained is described in Table 1.

Table1: Yield of Peganum harmala fixed and essential oils

Leaves Essential Oil	Seeds Fixed Oil
3.77 %± 1.31	19.32± 12.74

According to our results, the yield obtained (3%) showed that the leaves of the harmel are very rich in essential oil. This higher yield could be explained by the right choice of the harvest period. According to Laëtitia [13], during this period, the plants produced their highest oil content, therefore harvesting the essence during the vegetative stage specific to each aromatic plant is required for optimal yield. The results of the fixed oil yield extracted from the seeds of *Peganum harmala* remain quantitatively different in comparison with those found by Lalla et El Hadeke [14] from seeds collected in Morocco. These variations in the content may be due to several factors, in particular the degree of maturity of the seeds, their interaction with the environment (type of climate, soil, etc.), as well as the time of harvest and the method of extraction [15].

Physicochemical Properties

The standards for defining the quality of oil have been determined by several well-known organizations around the world. In our work, we determined the indices following the AFNOR standard. The results obtained are presented in Table 2.

Table 2: Organoleptic and physicochemical characteristics of the fixed and essential oils from *Peganum harmala*

Characteristics	Seeds Fixed Oil	Leaves Essential Oil
Organoleptic Characteristics		
Color	Brownish yellow	Pale yellow
Odor	Pleasant	Strong, pleasant
Aspect	Low viscosity liquid	liquid
Physical and Chemical Characteristics		
Relative density at 20°C	0.908	1.02
Refractive index	1.460	1,441
Hydrogen potential (pH)	6.53	6.60
Acid value (mg KOH/g)	2.80	4.48
Ester value (mgKOHg^{-1} oil)	187.29	56 .1
Peroxide value (mEq O2/kg oil)	12.2	-
Saponification value (mgKoHg^{-1} oil)	190.1	-
Miscibility in ethanol	no	miscible

The hydrodistillation extraction of *Peganum harmala* leaf plant yielded essential oil having a pale yellow coloration with a strong and pleasant odor with a liquid aspect. However, the fixed oil extracted from the seeds showed a low viscosity liquid appearance and a very characteristic brownish yellow color with a pleasant odor. A liquid aspect which may be due to the presence of higher unsaturated fatty acids like oleic acid and linoleic acid and other unsaturated fatty acids [16].

The organoleptic characteristics give only very limited information on these oils. Indeed, other more precise characterization techniques are more than necessary. Relative density is the

mass of a given volume of oil compared to the mass of an equal volume of distilled water at 20 °C. The relative densities ranged from 0.908 and 1.02 for seeds and leaves essential oils respectively. The refractive index was 1.460 for the fixed oil and 1.441 for the essential oil. This parameter depends on properties like molecular weight, fatty acid chain length, degree of instauration, and degree of conjugation [17]. The values obtained fall within the range of some common oils, with values between 1.3-1.5 [18]. According to AFNOR, the refractive index is used to check the purity of a product or of an essential oil. The pH value of 6.53 for fixed oil and 6.60 for essential oil is an important parameter, since it indicates the inhibitory range of several bacterial strains [19]. The acid value which measures the amount of free fatty acids resulting from reactions of hydrolytic triglyceride is a quality criterion for reporting the condition conservation of oil [20]. It is measured by the mass of potassium hydroxide (KOH) in milligrams that is required to neutralize the free fatty acid present in 1 g of fat. The low acid value obtained from seeds fixed and leaves essential oils (2.80 and 4.48) in this study are indicators of their ability to resist lipolytic hydrolysis and oxidative deterioration [21].

The ester value is the number of milligrams of potassium hydroxide (KOH) necessary for the neutralization of the acids released by the hydrolysis of the esters contained in 1 g of fatty substance. The peroxide value of seeds fixed oil of *Peganum harmala* was evaluated as 12.2 meq/O^2. It is a measure of the amount to which rancidity processes occurred during storage. Furthermore it can be used to determine the quality and stability of fats and oils [18]. The peroxide value was also found to increase with the storage time, temperature and contact with air of the oil samples.

The saponification index of a fatty substance tells us about the length of the carbon chain of its constituting acids. It increases as the carbon chain length of fatty acids shortens [22]. The fixed oil from the seeds of *Peganum harmala* has a saponification value of 190.1 mg KOH/g of the oil. The saponification value gives an indication of the molecular weight of the fatty acid and the purity status of the oil or whether the oil is adulterated. Higher saponification values suggest that the oil has little impurities and could be good for soap making.

Phytochemical Screening of Fixed Oil

The total phenolic content of *Peganum harmala* seed oil was calculated with a regression equation based on a standard curve using gallic acid and is shown in Table 3.

Table 3: Total phenolic and flavonoid contents of *Peganum harmala* seeds oil

	Total Phenolic Content µg / GAE / mg	Total Flavonoid Content µg/EQE /mg
Seeds Fixed Oil	57.22 ± 0.2	0.816± 0.31

Results showed that the total phenolic content of *Peganum harmala* seed oil was 57.22±0.2 µg gallic acid equivalents in mg of oil extract. The results obtained resemble those obtained by Khadhr et al [23] with a content of (53.4 µg /100 mg) in the seed hexanic extract.

The content of total flavonoids was also measured spectrophotometrically by using the Aluminum chloride colorimetric assay. It was expressed as quercetin equivalents in the µg /mg extract, and a calibration curve for quercetin was used. The result obtained showed a value of 0.816±0.31 µg quercetin equivalents in mg of oil extract. This value is significantly higher than the result found by Khadhr et al [23] with a content of (49.3 µg QE / 100 mg). This difference can be explained by geographical climatic factors or harvesting and extraction conditions.

Antioxidant Activity

DPPH assay is a rapid and sensitive way to identify the antioxidant of extracts [24, 25]. The effect of antioxidants DPPH radical scavenging is generally attributed to their ability to donate hydrogen [26].

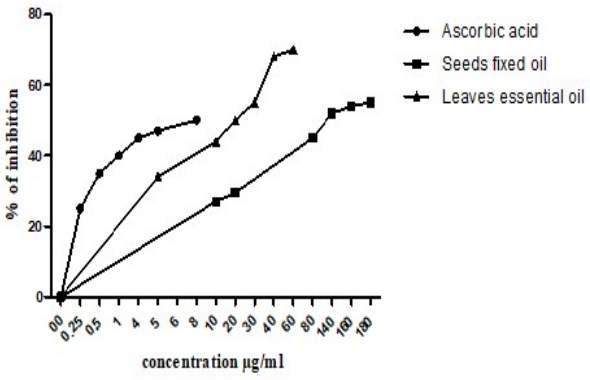

Figure 2: Inhibition of the DPPH free radical by ascorbic acid and oil extracts of *Peganum harmala* at different concentrations.

Fig. 2 shows that fixed and essential oil extracts have a significantly higher tendency to scavenge the DPPH radicals in a dose dependent. However, ascorbic acid showed a very potent inhibition against the free radical DPPH at lower concentrations.

The concentration of an antioxidant needed to decrease the initial DPPH concentration by 50% (IC_{50}) is a parameter widely used to measure antioxidant activity.

In Fig. 3, results showed that fixed and essential oils exert less significant ($p < 0.05$) antioxidant power in comparison with the antioxidant capacity of standard (IC_{50}= 4.24 µg/ml).

In addition , leaves essential oil showed a significant ($p < 0.05$) free radical scavenging activity with a low IC_{50} value of (22.52±2.40 µg/ml) compared to the fixed seed oil which had a higher IC_{50} value (139±2.86 µg/ml) with low free radical scavenging activity.

The antioxidant potential of essential oils might be due to the presence of phytoconstituents chiefly the mono- and oxygenated terpenes [27]. The study of Dastagir et al [28] described the oxygenated monoterpenes as the major components of the plants of family Zygophyllaceae. In addition, it has been established in numerous studies that the antioxidant activity of essential oils is not only linked to the high percentage of the majority compounds, but also to the presence of other minority constituents and synergistic effects between them.

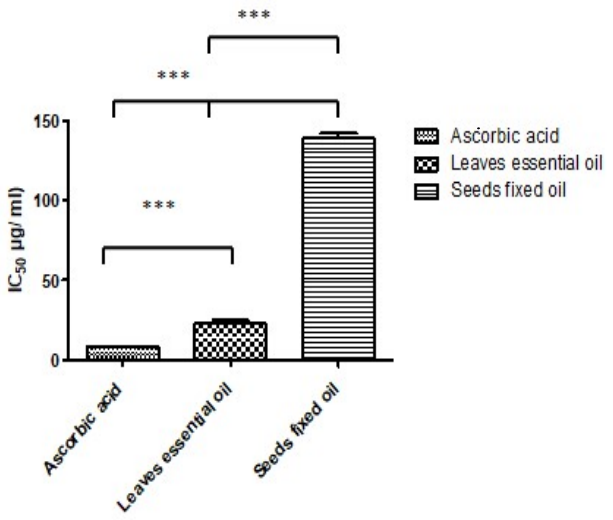

Figure 3: DPPH radical scavenging (IC_{50}) effect of oil extracts of *Peganum harmala* and standard (Acid ascorbic). Values were expressed as the mean ±SD of triplicate. *** $p < 0.05$.

Furthermore, the seed oil was studied as a potential inhibitor of DPPH free radical formation. The inhibition effect might be due to the presence of phytochemicals such as polyphenols. As has recently been demonstrated that the major polyphenols responsible for the antioxidant activity of seed oil are isomers of vitamin E (α- and γ-T) [1]. Also, the antioxidant activity of the seed oil may be due to its richness in polyunsaturated fatty acid (mainly linoleic acid).

Antibacterial Activity

The average diameters of the growth inhibition zones are shown in Table 4.

Table 4: Zone of inhibition of seeds fixed and leaves essential oils of *Peganum harmala* against different bacterial strains.

	Zone of inhibition (mm) of essential oils			**Zone of inhibition (mm) of fixed oil**			**Zone of inhibition (mm) of standard antibiotics**		
Test organisms	**1%**	**5%**	**10%**	**1%**	**5%**	**10%**	GET	CEZ	VAN
Escherichia coli	15.5	20	25	00	12	25	23	32	17
Pseudomonas aeruginosa	10	13	17	14	17	21	06	00	00
Staphylococcus aureus	00	00	00	00	00	00	16.5	20	24

GET: Gentamicin; CEZ: Cefazolin; VAN: Vancomycin

The results showed that the oils exert a considerable antibacterial effect on *Escherichia coli* and *Pseudomonas aeruginosa* with zones of inhibition increasing with an increase in the concentrations of the oils. The *Staphylococcus aureus* strain did not show any sensitivity towards the different concentrations of the oils tested. Moreover, the essential oil exhibits potent antibacterial activity against *Escherichia coli,* especially in low concentrations (1%, 5%) which was comparable to seed fixed oil. However, the fixed oil showed greater inhibitory activity against *Pseudomonas aeruginosa* when compared to an essential oil.

Thus, we found that leaves essential oil demonstrated activity against *Escherichia coli* and *Pseudomonas aeruginosa*. This activity may be related to the presence of oxygenated monoterpènes and phenolic compounds (e.g., carvacrol, thymol, and eugenol) are thought to be primarily responsible for their biological properties [29].

For the fixed oils, much of the responsibility attributed to the antibacterial potential is believed to be associated with fatty acids present in their compositions [30].

CONCLUSION

This study revealed the richness of leaves and seeds of *Peganum harmala* in oils. Furthermore, the determination of physicochemical characteristics is essential not only for the recognition of existing compounds and the purity of oils, but also to evaluate their biological properties. On the other hand, the results indicated that the essential and fixed oils tested exhibited higher antioxidant and antibacterial activities. Therefore, this ability is due to the presence of different phytochemicals.

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