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Determination of the Antimicrobial, Antioxidant Activities and Fatty Acid Composition of *Peganum harmala* Seed

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HIGHLIGHTS

- *S. aureus, E. coli, Y. enterocolitica* bacteria showed the highest antimicrobial effect.
- The antioxidant activities were investigated four different extracts prepared with solvents of different polarities.
- Fatty acid content was analyzed by GC-FID.
- Linoleic acid was the most abundant fatty acid detected.

Abstract: The purpose of this study was to investigate the antioxidant and antimicrobial activity of *Peganum harmala* seed extract, as well as to determine its fatty acid profile. The highest zone diameter of *P. harmala* seed extract formed on *S. aureus*, *E. coli*, and *Y. enterocolitica* was determined to be 30 mm, and the MIC value on these bacteria was 31.2 µg mL^{-1}. It has a high level of antimicrobial effect. Among the four different solvent extracts, the highest absorbance value in the CUPRAC method was observed in water extract (0.714), the highest inhibition value in the DPPH method methanol extract (74.06%) and in the ABTS method (72.06%) ethanol extract. A total of 17 fatty acid components were detected in *P. harmala* seeds, with the highest proportion of linoleic acid with 61.46%. The *P. harmala* seed can be a good alternative to drugs in the prevention of microbial resistance.

Keywords: Antimicrobial; Antioxidant; MIC; GC-FID; *Peganum harmala* seed.

INTRODUCTION

Infections caused by multidrug-resistant bacteria are increasingly being one of the most difficult problems in modern medicine. New antibacterial components must be generated in order to handle this issue. Plants have been used in the treatment of diseases since ancient times. Plants are a popular source of treatment in the primary health care system, particularly in impoverished communities, because they are both cheaper and more accessible than medicines [1]. However, in developed countries, the use of chemical and synthetic

medications has increased significantly, resulting in the emergence of pathogenic multidrug-resistant microorganisms that cause considerable medical problems [2].

Peganum harmala (wild rue) is widely found in the Mediterranean region, Asia, North Africa and America [3]. This herbaceous plant is a perennial, shrub, flowering, and wild plant that can range in size of 30-100 cm. It is a member of the Zygophylaceae family and is a significant medicinal plant [4]. It is used for the treatment of various human ailments such as rheumatism, amnesia, asthma, diabetes, hypertension, apoplexy, jaundice, arthralgia, hemiplegia, lumbago, enteritis, malaria dysentery, and some skin diseases. Its seeds are known to have hallucinogenic and hypothermic properties. Research has shown that *P. harmala* has antispasmodic, antihelmitic, emetic antipyretic and abortive properties. It is also a drug used with traditional methods as a central nervous system stimulant [5]. Traditions in Turkey and Iran understand the smoke obtained by burning the seeds as a type of disinfectant, as well as a ward against the evil eye [6].

Plants exhibit medicinal properties as they contain secondary metabolites in their structure, and these secondary metabolites are responsible for different effects on the human body [7]. Various secondary metabolites are present in *P. harmala*. The list includes alkaloids, flavonoids, saponins, terpenoids, phenolic and tannins compounds [6]. These compounds have been found to exhibit various bioactivities such as antimalarial, antimicrobial, antitumoral, antioxidant and anticancer activities, analgesic, anti-inflammatory, cytotoxic and immunomodulatory [3].

Many herbs can have pharmacological activity since they contain active alkaloids. Alkaloids are nitrogen-containing heterocyclic compounds in a negative oxidation state that can form salts with acids that make them water-soluble [8]. *P. harmala* seeds contain 2-6% pharmacologically active alkaloids. These alkaloids are derived from β-carboline (bCs) and tetrahydro-β-carboline (TH bCs) and naturally occur in foods that are chemically concentrated between indolamine and aldehydes or α-keto acids. Most-carboline and quinazoline alkaloids are known as harmala alkaloids all together. Harmane, harmin, harmaline, harmol, pegan and harmalol are representational harmala alkaloids [5].

Furthermore, in our research, methods based on electron transfer were used to test the antioxidant effect of the secondary metabolites reported in the seed. These are the methods that measure the oxidant reduction ability of the antioxidant by color change. It includes techniques such as ABTS/TEAC, CUPRAC and DPPH each of which employs a different chromogenic redox reagent with a different standard potential [9]. There has been a lot of study done on the antioxidant and antibacterial benefits of this plant's leaf extracts, which are used for therapeutic purposes today; however, there haven't been enough studies done on seeds with much more active components.

The study also aims to determine the antimicrobial activity of the extracts against some Gram positive and Gram negative bacteria and yeasts, and the antioxidant activity values of the extracts using CUPRAC, DPPH, and ABTS methods, and to reveal the fatty acid composition by using GC-FID analysis. We investigated the antibacterial, antioxidant, and fatty oil properties of *P. harmala* grown in the Kars region. There is no such study in the literature for *P. harmala* seeds grown in the Kars region.

MATERIAL AND METHODS

Reagents and standards

2,9-dimethyl-1,10-phenanthroline (Neocuproine), 2-azino-bis (3-ethylbenzthiazoline-6-sulfonic acid) (ABTS), butylated hydroxytoluene (BHT), Butylated hydroxyanisole (BHA), 1,1-diphenyl-2-picryl-hydrazyl (DPPH) and 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) were obtained from Sigma (Sigma-Aldrich GmbH, Sternheim, Germany). Mueller Hinton Agar and broth obtained from Merck. All other chemicals used were analytical grade and obtained from either Sigma-Aldrich or Merck.

Microorganisms

In order to determine the antimicrobial effects of extracts *Bacillus cereus* (ATCC 14579)*, Bacillus subtilis* (ATCC 6633)*, Escherichia coli* (ATCC 25922)*, Klebsiella pneumoniae* (ATCC 13883), *Pasteurella multocida* (ATCC 12945), *Pseudomonas aeruginosa* (ATCC 15442), *Staphylococcus aureus* (ATCC 25213), *Yersinia enterocolitica* (ATCC 9610) and *Candida albicans* (ATCC 10231) standard strains were used.

Preparation of seed extract

P. harmala seeds were purchased from a local market in the Kars province (Turkey) in September 2020, and dried in the shade. After the dried seeds were ground in the Warning blender until they turned into powder, they were diluted with a mixture of water and ethanol (water-ethanol 2:8 v/v) in a ratio of 1:5 (25

grams of seeds dissolved in 500 mL ethanol) and kept in a shaking water bath for 48 hours at room temperature [10]. After 48 hours, they were filtered through filter paper and then removed from the collected extracts by a rotary evaporator at low temperature and pressure. A total of 3.08 g of extract was obtained from this process and subsequently stored in a glass bottle at room temperature until used in antimicrobial analysis.

For the different solvent (water, ethanol, methanol and chloroform) extract from the powdered seed (1:20 ratio), 200 mL of solvents were added onto 10g of powder and stirred overnight in a magnetic stirrer [11]. Prepared extracts were filtered through Whatman No.1 filter paper. The same processes were repeated 3 times, then the solvents were removed from the collected extracts by using an evaporator at 40°C. The acquired extracts were stored at 4^{o}C to be used in analysis. For the water extract, the filtrates were frozen at -20^{o}C and lyophilized in the lyophilizator for 18 hours. Stock solutions were used for antioxidant analysis.

Preparation of Microorganism Cultures and Agar Well Diffusion Technique

The Agar well diffusion method was used to determine the antimicrobial activity [12]. As a medium, Mueller Hinton Agar (MHA) was used to determine the antimicrobial activity of bacteria and yeasts. After the bacteria and yeast suspension was adjusted according to the 0.5 McFarland standard, 100 µL was seeded in petri dishes which were left to dry at room temperature for 5-15 minutes. At the end of the period, 50 µL (50 µg) of the extracts were transferred to the wells with a diameter of 5 mm on the agar, and the zone diameters formed at 37°C after 24 hours. Antimicrobial activity experiments against all test microorganisms were carried out in triplicate.

Minimum Inhibition Concentration (MIC) and (Minimum Lethal Concentration) MLC

The MIC test was performed by the Broth dilution method [13]. 1 mL was completed with DMSO added on 1 mg extract so that an extract with a concentration of 1 mg mL^{-1} was obtained. Two-fold dilutions were made to achieve a concentration between 0.0039-1 mg mL^{-1}. The lowest concentration at which bacterial growth was visibly inhibited was determined as the MIC value. To determine the Minimum Lethal Concentration (MLC), non-turbid media were cultured on solid media. The lowest concentration that did not indicate media growth was determined to be the Minimum Lethal Concentration (MLC) value. Gram-positive and Gram-negative bacteria were treated with ampicilin as positive controls. A control sample for the fungal strain was flukanazole.

Cu (II) reduction antioxidant activity (CUPRAC) measurement method

Cupric ion (Cu $^{+2}$) reducing activities of water, ethanol, methanol and chloroform extracts of *P. harmala* seed performed using solvents with different polarities, were made according to the method developed by Apak and coauthors [14]. After adding 1x10^{-2} M $CuCl_2$ solution, 7.5x10^{-3} M neocuproin solution and 1M ammonium acetate buffer to the test tubes, the absorbance at 450 nm was recorded by adding water, ethanol, methanol and chloroform extracts prepared in different concentrations (5, 10, 20, 40, 80 µg mL^{-1}). BHT, BHA, Trolox were used as standard antioxidant substances. Experiments were done in triplicate.

DPPH free radical scavenging activity method

The free radical scavenging activity of water, ethanol, methanol and chloroform extracts of *P. harmala* seed was determined according to the method developed by Blois [15], after which 1 mL of DPPH radical solution (1 mM) was added to the amounts taken from the concentrations of the seed, water, ethanol, methanol and chloroform extracts (5, 10, 20, 40, 80 µg mL^{-1}). Absorbance measurements at 517 nm were recorded after waiting at room temperature for 30 minutes. BHT, BHA, Trolox were used as standard antioxidant substances. Experiments were done in triplicate.

ABTS radical scavenging activity method

ABTS radical scavenging activity of water, ethanol, methanol and chloroform extracts of seed was made according to the method developed by Re *et al.* [16]. ABTS solution prepared as 2 mM was mixed with 2 mM ratio $K_2S_2O_8$ solution and kept in magnetic stirrer in the dark to obtain ABTS $^{\bullet+}$ radical. The absorbance of this radical solution at 734 nm was adjusted to 0.700 ± 0.025 nm with a phosphate buffer. The required amounts of water, ethanol, methanol and chloroform extracts prepared in different concentrations (5, 10, 20, 40, 80 µg mL^{-1}) were taken and 1 mL of ABTS $^{\bullet+}$ solution was added to them. They were held for 30 minutes and the

absorbance at 734 nm was recorded. BHT, BHA, Trolox were used as standard antioxidant substances. Experiments were done in triplicate.

Determination of Oil Content and Fatty Acid Components

The oil content of the *P. harmala* seed was determined by the soxhlet device (Foss Soxtec 2055). Approximately 2-4 grams of the seed powder, which was homogeneously ground, was taken, placed in soxhlet cartridges, and then analyzed. The oil accumulated in the containers was dried for 30 minutes in the drying oven and held in the desiccator, and then weighed. Finally, the fat % was calculated from the weight difference.

Gas chromatography (GC-FID) analysis was conducted at the KSU USKIM Laboratory in Kahramanmaraş, Turkey. Fatty acid methyl esters were made with transmethylation [17]. Fatty acid content was analyzed by Shimadzu Gas Chromatography (Model 2025) with a flame ionizer detector (FID), 60 m x 0.25 µm x 0.20 mm TR-CN100 column. Supelco 37 Component Mix certified STD from Supelco was used in the analysis process. For fatty acid components, helium was used as a carrier gas and the flow rate was adjusted to 30 mL min^{-1}. The column temperature was held at 80°C for 2 minutes, then increased to 140°C at a rate of 5°C/min^{-1} (kept at 140°C for 2 minutes). Later it was increased again, this time by 3°C to 240°C. (kept at 240 °C for 5 minutes). Detector and injection temperatures were programmed as 250°C and 240°C, respectively. The gas flows used were determined as H_2 = 40 mL min^{-1} and dry air = 400 mL min^{-1}. Experiments were done in triplicate.

Statistical Analysis

All analyses were performed triplicated and data obtained were analyzed by One-Way Analysis of Variance (One Way ANOVA/Duncan). The comparisons of means were made through the Duncan test at the 0.05 significance level. Statistical analyses were carried out with the SPSS program (standard version 20).

RESULTS

Antimicrobial Activity Results

The antimicrobial activity results of the *P. harmala* seed extract we used in the study are shown in Table 1.

Table.1. Inhibition zone diameters and Minimum Inhibition Concentration and Minimum Lethal Concentration of *P. harmala* seed extract on test microorganisms.

	İnhibition zone (mm)	**Minimum Inhibition Concentration (MIC µg mL^{-1})**	**Minimum Lethal Concentration (MLC µg mL^{-1})**	**Control Minimum Inhibition Concentration (MIC µg mL^{-1})**	**Control Minimum Lethal Concentration (MLC µg mL^{-1})**
Bacillus subtilis	28.00 ± 1.00 [b]	31.2	62.5	31.2	31.2
Bacillus cereus	26.40 ± 0.70 [b]	62.5	250	31.2	31.2
Pseudomonas aeruginosa	26.20 ± 1.00 [b]	125	125	31.2	62.5
Klebsiella pneumoniae	22.00 ± 0.30 [d]	62.5	125	31.2	31.2
Staphylococcus aureus	30.00 ± 0.06[a]	31.2	125	15,6	31.2
Escherichia coli	30.10 ± 0.30 [a]	31.2	125	15,6	15,6
Yersinia enterocolitica	30.00 ± 0.30 [a]	31.2	250	15,6	31.2
Pasteurella multocida	24.40 ± 1.00 [c]	62.5	125	31.2	62.5
Candida albicans	26.30 ± 0.70 [b]	31.2	125	15,6	31.2

Control: Ampicilin for bacteria; flukanazole for fungus
Each point shows the average value of three replicates ± SD
Difference between expressions with different letters in the same column $p<0.05$ is significant (according to one way ANOVA/Duncan).

When we examine Table.1, it was determined that the highest zone diameter was formed on *S. aureus, E. coli, Y. enterocolitica* (30 mm), and it had a high level of antimicrobial effect on all test microorganisms. The MIC values were expressed as µg mL^{-1}, which is the lowest concentration that inhibits the growth of microorganisms. It was observed that *P. harmala* seed extract had a high effect on microorganisms. The MIC value of *S. aureus, E. coli, Y. enterocolitica* with the highest zone diameter was determined as 31.2 µg mL^{-1}. Furthermore, MIC was observed at the same concentration for *C. albicans* and *B. subtilis* as well. It has an inhibitory effect on microorganisms even at low concentrations. It can be observed that the antibacterial activity of the *P. harmala* extract is present, although less so than the positive control. *P. harmala* extract shows a lower effect Minimum Lethal Concentration (MLC) than control antibiotics for all test microorganisms. Regarding MLC, the effect of *P. harmala* extract is remarkable.

Antioxidant Activity Results

P. harmala seed extracts of prepared with water, ethanol, methanol and chloroform solvents with standard antioxidants substances were determined antioxidant activities in different concentrations. The CUPRAC, DPPH and ABTS method results of the extracts obtained from *P. harmala* seeds are shown in Table 2.

CUPRAC is a method that has been used frequently in recent studies to determine the antioxidant activities of herbal extracts. Extracts with a high absorbance in the method have a high antioxidant capacity. Standard antioxidant substances and water, ethanol, methanol and chloroform extracts of *P. harmala* seed, the reduction activities of cupric ion (Cu^{+2}) at a concentration of 80 µg mL^{-1} were compared, according to the results (Table 2), respectively BHT>Trolox>BHA>Water extract>Ethanol extract>Methanol extract> Chloroform extract.

The effect of extracts obtained with different solvents on DPPH radical is shown in Table 2. The radical scavenging activity increased as the concentration of the extracts made with different solvents increased. The highest activity at all concentrations was detected in the methanol extract. In this case, the 80 µg mL^{-1} activities of the extracts and standard antioxidants were determined as BHA>Trolox>BHT> Methanol extract> Ethanol extract>Water extract >Chloroform extract, respectively.

In the ABTS radical scavenging activity method, the activities of water, ethanol, methanol and chloroform extracts of the plant and standard antioxidant substances were compared at a concentration of 80 µg mL^{-1}, respectively as: Trolox>BHA>BHT> Ethanol extract>Methanol extract >Water extract >Chloroform extract. When the different solvent extracts of the plant were compared, it was determined that the highest ABTS radical scavenging activity (72.06%) was found in the ethanol extract. Out of all of the antioxidant activity determination methods, the activity of extracts obtained with different solvents showed lower radical scavenging activity than standard antioxidants.

Table 2. CUPRAC (Cu^{+2}) reduction, DPPH and ABTS radical scavenging activity results of extracts from *P. harmala* seed (80 µg mL^{-1})

Antioxidant Methods	Water	Ethanol	Methanol	Chloroform	BHT	BHA	Trolox
Cu^{+2} reducing power*	0.71±0.00 d	0.67±0.00 e	0.51±0.00 f	0.30±0.00 g	0.98±0.00 a	0.81±0.00 c	0.92±0.00 b
$DPPH^{\cdot}$ scavenging activity**	52.33±0.66 f	67.23±0.90 e	74.06±0.55 d	33.40±09 g	82.20±0.43 c	89.26±0.82 a	87.43±0.68 b
$ABTS^{\cdot+}$ scavenging activity**	58.81±0.24 f	72.06±0.80 d	64.83±0.56 e	25.73±0.54 g	76.23±0.70 c	82.16±0.25 b	90.10±0.20 a

Each point shows the average value of three replicates ± SD

*: Expressed as absorbance values.

**: Expressed as percent (%) inhibition/scavenging effect

BHT: Butylated hydroxytoluene, BHA: Butylated hydroxyanisole, DPPH: 2, 2-diphenyl-1- picrylhydrazyl radical, ABTS: 2,2-azinobis (3-ethylbenzo-thiazoline-6-sulfonic acid)

Difference between expressions with different letters in the same line $p<0.05$ is significant (according to one way ANOVA/Duncan).

Results of Fatty Acid Components

The oil content of *P. harmala* seed was determined as 13.19%, and a total of 17 fatty acid components were detected. These are shown in Table 3 and Figure 1.

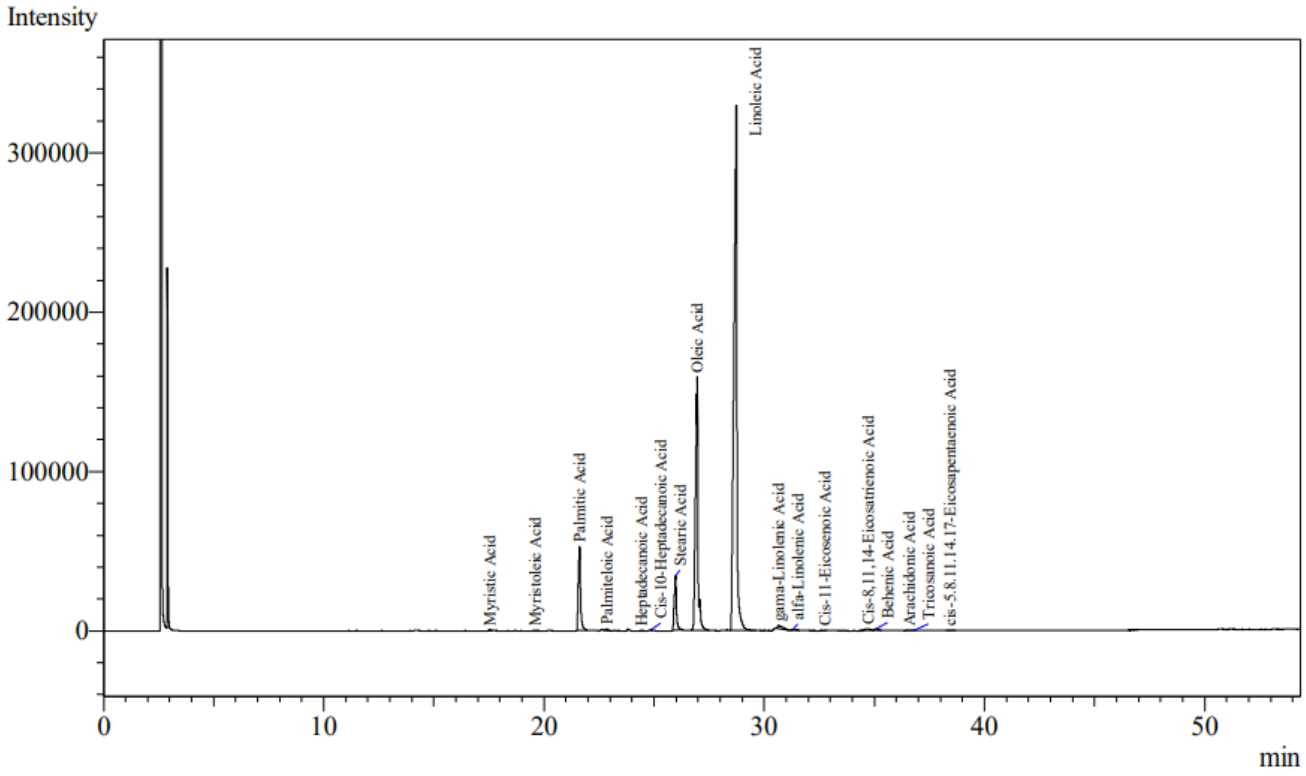

Figure 1. GC-FID choramotogram of *P. harmala* seed fatty acid analysis

Table.3. Fatty acid compositions (%) of *P. harmala* Seeds

Number of Carbon Atoms		Fatty acids	Fatty acid percentages (%)
1	C14:0	Myristic Acid	0.12 ± 0.01
2	C14:1	Myristoleic Acid	0.03 ± 0.01
3	**C16:0**	**Palmitic Acid**	**7.28 ± 0.03**
4	C16:1	Palmiteloic Acid	0.14 ± 0.01
5	C17:0	Heptadecanoic Acid	0.04 ± 0.00
6	C17:1	Cis-10-Heptadecanoic Acid	0.07 ± 0.01
7	**C18:0**	**Stearic Acid**	**4.66 ± 0.04**
8	**C18:1**	**Oleic Acid**	**24.76 ± 0.05**
9	**C18:2**	**Linoleic Acid**	**61.46 ± 0.04**
10	C18:3	Gama-Linolenic Acid	0.63 ± 0.01
11	C18:3	Alfa-Linolenic Acid	0.16 ± 0.01
12	C20:1	Cis-11 -Eicosenoic Acid	0.01 ± 0.00
13	C20:3	Cis-8,11,14-Eicosatrienoic Acid	0.36 ± 0.01
14	C22:0	Behenic Acid	0.20 ± 0.01
15	C20:4	Arachidonic Acid	0.02 ± 0.02
16	C23:0	Tricosanoic Acid	0.01 ± 0.00
17	C20:5	Cis-5.8.11.14.17-Eicosapentaenoic Acid (EPA)	0.05 ± 0.01
		Total	100.00

Each point shows the average value of three replicates ± SD

Of those 17 fatty acids determined by GC-FID analysis in *P. harmala* seed oil, 11 were unsaturated fatty acids and 6 were saturated fatty acids (Table 3, Figure 1). Unsaturated fat content (87.69%) was found higher than saturated fat (12.31%). Linoleic acid (61.46%) derived from omega-6, which is the unsaturated fatty acid required for the nutritional needs of the human body, is the highest fatty acid. In addition, omega-9 derivative

oleic acid (24.76%), which is a naturally occurring fatty acid in various vegetable and animal oils and fats, is one of the most common fatty acids. Linoleic acid and oleic acid, which are unsaturated fatty acids, compose 86.22% of the total fatty acids. Palmitic acid (7.28%), which is one of the saturated fatty acids, was found to be the third most abundant fatty acid.

DISCUSSION

Different reactive oxygen species and oxygen-containing free radicals are produced throughout physiological and metabolic activities in the human body. Compounds that remove or destroy reactive oxygen species in biological systems exist naturally in plants and fruits, as well as in metabolism. Phenolic compounds generally present in plants are compounds with antioxidant activity. Hence, medicinal herbs are known to be a potential source of pharmaceutical natural products. Therefore, this study sought to determine the bioactive contents of *P. harmala* such as antimicrobial, antioxidant activities and fatty acid contents.

It has been reported that *P. harmala* seeds contain β-carboline alkaloids and show antimicrobial activities against the tested bacteria and fungi species; moreover, research has shown that the chemical-derived harmala alkaloids exhibit moderate antimicrobial effects against *S. aureus, E. coli and P. vulgaris* and the tested microorganisms [18]. In our study, *P. harmala* seeds were found to have a strong antimicrobial effect. Darabpour and coauthors reported that the seed and root extract of *P. harmala* had the most active phytochemicals and showed a broad spectrum and strong antibacterial activity against clinical bacterial pathogens [19]. These results are similar to our study.

In Abderrahim and coauthors study, Gram negative bacteria *E. coli* were suppressed at 1 mg mL^{-1} but Gram positive bacteria *P. aeruginosa* were inhibited at 6 mg mL^{-1} [20]. In addition, *P. harmala* seed oil has the highest antibacterial activity against *B. cereus* and *S. aureus* with a concentration of 75 µg mL^{-1}. Concentration of 250 µg mL^{-1} is not effective on *S. indica* [21]. Alcoholic extract of *P. harmala* shows fungicidal activity on opportunistic yeasts, notably *Candida species* (*C. albicans* MFC: 2.5 mg mL^{-1}) [22]. In this study, on the other hand, the MIC value for *C. albicans*, *B. subtilis, S. aureus, E. coli* and *Y. enterocolitica* was determined as 31.2 µg mL^{-1}. These results indicate that *P. harmala* seed has the effect of inhibiting the growth of microorganisms even at low concentrations. The antimicrobial activity findings of *P. harmala* seed on Gram positive and Gram negative microorganisms are consistent with the findings of other researchers.

The *P. harmala* seed contains a wide variety of phenolic and flavonoid compounds that act as antioxidants and inhibit the production of oxidative species in the body. In many studies, antioxidant activity was determined by DPPH and ABTS radical scavenging method. Iqbal and coauthors studied the antioxidant activity of *P. harmala* seeds with DPPH method in different solvents and determined that methanol extract showed the highest antioxidant activity at a concentration of 50 µg mL^{-1} with a rate of 72% [6]. In our study, the highest activity was found in methanol extract with a rate of 74.06% at a concentration of 80 µg mL^{-1}. This result is similar to Iqbal and coauthors [6]. Khadhr and coauthors [23] reported at a concentration of 20 mg mL^{-1} 62.50 % antioxidant activity in the methanol extract of Tunisian *P. harmala* seed using the DPPH method, and Abderrahim and coauthors [20] reported strong activity in the methanol extract. When compared to the literature, *P. harmala* seed from Kars province exhibits higher antioxidant activity even at lower concentrations. The presence of phytochemicals such as polyphenols may account for *P. harmala* seeds' significant inhibition of DPPH free radical formation.

The ABTS radical cation ($ABTS^{\cdot+}$) is reactive to most antioxidants and soluble in both aqueous and organic solvents. Therefore, it can be used to identify both hydrophilic and lipophilic antioxidants in a variety of matrices. In the study conducted on the antioxidant activity of the methanol and ethanol extracts of *P. harmala* with ABTS and DPHH method, it was found that the high activity of methanol extract in DPPH method and ethanol extract in ABTS method. According to Khlifi and coauthors [24], *P. harmala* showed high antioxidant activity with ABTS and DPPH method. High antioxidant activities have also been reported in water, ethanol and water-ethanol mixture of *P. harmala* seed [25]. Consequently, the extraction of antioxidant compounds from plant material depends on the solubility of these compounds in various solvents. The polarity of the solvent used plays an important role in increasing their solubility. Differences in the polarity of antioxidative components may explain why the antioxidant activity of the extracts is different. The solubility of phenolic compounds is determined by the type of solvents used. In the extracts prepared with different solvents in our study, high activities were observed in the DPPH, ABTS and CUPRAC methods in methanol, ethanol and water extracts, respectively. It has also been reported that the changes in antioxidant activity observed in the extracts may be mostly dependent on the presence of phenolic chemicals that varies based on where they are collected [26]. Furthermore, as the concentration of herbal extracts increased, the radical scavenging activity and cupric ion (Cu^{+2}) reduction activities also increased. Studies on the reduction capacity

of *P. harmala* seeds with the CUPRAC method are quite limited in the literature. In our study, a high activity was obtained with the CUPRAC method, and these results could very well shed light in future research.

Fatty acids are classified as either saturated or unsaturated fatty acids depending on the bonds they contain. Saturated fatty acids in nutrition prevent the removal of small amounts of lipoprotein in the blood; and as a result, the accumulation in the vessels can cause atherosclerosis. Unsaturated fatty acids are called monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA). Monounsaturated fatty acids are commonly found in foods as oleic acid, and polyunsaturated fatty acids are linoleic acid [27]. Linoleic and linolenic acids from polyunsaturated fatty acids should be taken with food, as they cannot be synthesized in the human body. Omega-6 fatty acids take its source from linoleic acid. Linoleic acid (61.46%), which is an omega-6 derivative and one of the unsaturated fatty acids required for the human body, is the highest fatty acid in *P. harmala* seed. Linoleic acid is particularly abundant in oils obtained from plant seeds. Additionally, linoleic acid (omega-6 fatty acids) is an important compound for brain tissue and cell membranes. Previous research has also found that polyunsaturated fatty acids can protect against lipid peroxidation [28]. Additionally, it is thought that the seeds' antioxidant and antibacterial capabilities are related to their high content of polyunsaturated fatty acids (especially linoleic acid). According to Richard and coauthors [29] polyunsaturated fatty acids, specifically omega 3 fatty acids, exhibit indirect antioxidant capabilities in vascular endothelial cells, lowering inflammation, atherosclerosis, and cardiovascular disease risk. The second fatty acid in *P. harmala* seed is oleic acid with a rate of 24.76%. Khadhr and coauthors [23] found Linoleic acid (62.05%) and Oleic acid (24.48%) in their investigation, and their findings are compatible with ours**.** According to the same study, seed oil has strong antioxidant and anti-inflammatory effect due to the presence of bioactive chemicals, polyunsaturated fatty acids such as linoleic acid. Omega fatty acids, particularly omega 9 (oleic acid), omega 3 (alpha-linoleic acid), and omega 6 (linoleic acid) have effects such as catalyzing brain development, supporting the immune system, and preventing coronary heart diseases [30]. In the deficiencies of these fatty acids, learning difficulties are also observed in humans along with asthma, dry skin, growth retardation, arthritis, some types of cancer and sugar [31].

Certain saturated fatty acids are necessary for the body's essential signaling and stability functions. Saturated fatty acids required in these processes are lauric acid, myristic acid and palmitic acid [32]. In this study, palmitic acid within *P. harmala* seed oil was determined as 7.28% and myristic acid was 0.12% (Table 3). Moussa and Almaghrabi [33] found that the *P. harmala* plant is a good source of omega-3 essential oil (α-linolenic acid, 14.79%) and omega-6 oil (linoleic acid, 10.61%), and included palmitic acid (48.13%) and stearic acid (13.8%) which are fatty acids used in manufacturing (soap, cosmetics, etc.).

CONCLUSION

Kars is located at a height of 1768 m, and the nutritional value of plants is affected by altitude. This study is unique to the Kars region. This study showed a high presence of important oils such as linoleic acid, oleic acid and palmitic acid in the seed of *P. harmala*. These oils have strong antibacterial and antioxidant properties. *P. harmala* seed extracts have the potential to be used against bacteria resistant to antibiotics, and show high antioxidant activity due to the phenolic components contained in *P. harmala* species. Hence, it is suitable to be used as a natural antimicrobial and antioxidant agent. All of these findings support the traditional medical practice of using this herb to treat inflammatory disorders. Furthermore, given the fatty acid content, antioxidant capacity and antimicrobial activity of *P. harmala* seed, it was highlighted in this study that it may have medicinal potential. Further research to discover the compounds responsible for the biological actions of *P. harmala* seed would be beneficial.

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Conflicts of Interest: The authors declare no conflict of interest.

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