

Original Research

Phytochemical composition and antimicrobial activity of essential oil and extracts from the leaves of *Peganum harmala* L. in southern Algeria.

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Abstract

Medicinal plants have a wide use in traditional medicine. The aim of our study is to valorize medicinal and aromatic plants of the Algerian flora. For this reason, we determined the phytochemical composition and antimicrobial activity of the essential oil of *Peganum harmala* in two different southern regions. Phytochemical screening reveals the presence of flavonoids, alkaloids, saponins, tannins, glycosides, terpenoids and steroids and the absence of anthraquinones in extracts. The essential oil of *P. harmala* leaves collected from two different regions (Bougat and Mechria) was analyzed by GC-MS. The results revealed the presence of 55 and 47 compounds with a yield of 0.217 % and 0.211 %. These essential oils do not contain the same major components for Bougat region: Carvone 21.15%, Phytol 17.41%, Carbamic acid 3.52%, Lauric acid 2.10%, Thymol 1.77% and Tetrapentacontane 1.32%. For the region of Mechria, Phytol 10.24%, Carvone 5.92%, Tetrapentacontane 2.80%, Phytone 2.18%, Butylated Hydroxytoluene 1.48%, Lauric acid 1.39%. The essential oils which were tested showed variable degrees of antimicrobial activity. In general, Gram-positive bacteria and fungi were found to be more sensitive to the essential oils than Gram-negative bacteria.

Keywords

Peganum harmala, Leaves, Essential oil and extracts, GC-MS, Phytochemical screening, Antimicrobial activity, southern Algeria.

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Fitokemična sestava in protimikrobna aktivnost eteričnega olja in izvlečkov iz listov *Peganum harmala* L. v južni Alžiriji.

Izvleček

Zdravilne rastline imajo široko uporabo v tradicionalni medicini. Namen naše raziskave je valorizirati zdravilne in aromatične rastline alžirske flore, zato smo določili fitokemično sestavo in protimikrobno delovanje eteričnega olja *Peganum harmala* v dveh različnih južnih regijah. Fitokemijski pregled razkrije prisotnost flavonoidov, alkaloidov, saponinov, taninov, glikozidov, terpenoidov in steroidov ter odsotnost antrakinonov v izvlečkih. Eterično olje listov *P. harmala*, zbranih iz dveh različnih regij (Bougatob in Mechria), je bilo analizirano z GC-MS. Rezultati so pokazali prisotnost 55 in 47 spojin z izkoristkom 0,217 % in 0,211 %. Ta eterična olja ne vsebujejo istih glavnih sestavin za regijo Bougatob Carvone (21,15 %), fitol (17,41 %), karbamsko kislino (3,52 %), lavrinsko kislino (2,10 %), timol (1,77 %) in tetrapentakontan (1,32 %). Za regijo Mechria, fitol (10,24 %), karvon (5,92 %), tetrapentakontan (2,80 %), fiton (2,18 %), butil hidroksitoluen (1,48 %), lavrinska kislina (1,39 %). Eterična olja, ki so bila testirana, so pokazala različne stopnje protimikrobnega delovanja. Na splošno je bilo ugotovljeno, da so po Gramu pozitivne bakterije in glive bolj občutljive na eterična olja kot po Gramu negativne bakterije.

Ključne besede

Peganum harmala, listi, eterično olje in izvlečki, GC-MS, fitokemični pregled, protimikrobna aktivnost, južna Alžirija.

Introduction

Since the highest antiquity, Men have used plants found in nature in several fields, such as perfumery, pharmacology, agri-food, and disease treatments. (Roosta et al., 2017)

More than 20,000 aromatic and medicinal plants are used in traditional medicine (Pandey et al., 2013). These plants continue to provide valuable therapeutic agents for both modern and traditional medicine (Bouzouita et al., 2008).

Aromatic plants are characterized by the biosynthesis of odorous molecules, which constitute the essential oils known for their antiseptic and therapeutic activity in popular medicine for a long time (Samarth et al., 2017). Essential oils are complex natural substances with well-defined physicochemical characteristics and meet quality criteria that must be known to avoid any risk of toxicity, which may prove dangerous to health because some essential oils and medicinal plants are very dangerous (Haddouchi et al., 2008) in except to those of some medicinal plants. In addition, these products have a great variability in their chemical constituents, giving them many medicinal and biological properties that should be known and valued (Rashid et al., 2010).

The great use of antibiotics in medicine to fight pathogenic micro-organisms is not completely harmless to the body and can cause many undesirable effects (Rudramurthy et al., 2016). It clearly drives the evolution of resistance (CDCPOIDA, 2013). Epidemiological studies have demonstrated a direct relationship between antibiotic consumption and the emergence and dissemination of resistant bacteria strains. In bacteria, genes can be inherited from relatives or can be acquired from nonrelatives on mobile genetic elements such as plasmids. This horizontal gene transfer (HGT) can allow antibiotic resistance to be transferred among different species of bacteria. Resistance can also occur spontaneously through mutation. Antibiotics remove drug-sensitive competitors, leaving resistant bacteria behind to reproduce as a result of natural selection (Read et al., 2014). Despite warnings regarding overuse, antibiotics are overprescribed worldwide (The Antibiotic Alarm, 2013). Therefore, it is necessary to search for new natural antimicrobial molecules to inhibit various pathogenic microorganisms (Rudramurthy et al., 2016).

Algeria, by its geographical location, offers rich and diverse vegetation. A large number of aromatic plants grow there spontaneously. Interest in these plants has continued to grow in recent years. The Algerian flora includes 3300

species (Quezel & Santa, 1963). *Peganum harmala* L., commonly known as Syrian rue or Pègano, is a herbaceous plant belonging to the Zygophyllaceae family. The primary origin of *Peganum harmala* is central Asia, but nowadays, it grows in Australia, north of Africa and southwest of Amecira (Bahmani et al., 2012). It is an herbaceous plant, glabrous, 30 to 60 cm in height with a lignified base, much branched with a winged stem at the top and alternate leaves. It possesses narrow leaves arranged alternately on fleshy, bright green stiff stems (Zargari et al., 1992). The flowers of the plant are isolated in the axils of the leaves, 1-2 cm, whitish-green in colour and round seed capsules containing more than 50 seeds. It has a strong, unpleasant odour (Zargari et al., 1992; Marwat et al., 2011).

Many related works show that *Peganum harmala* essential oils have antibacterial, antiviral, antioxidant, anti-parasitic and anticancer activities, among other biological activities. The purpose of our research is to evaluate the chemical composition and antimicrobial activity of essential oils extracted from the leaves of *Peganum harmala*. This study aims to valorize medicinal and aromatic plants of the Algerian flora in order to find new bioactive natural products by the evaluation of the chemical composition and antimicrobial activity of essential oils which are extracted

from the leaves of *P. harmala* collected from two different regions (Bougtoob and Mechria).

Materials and Methods

Plant material

The leaves of *P. harmala* (Figure 1) were collected in April 2020 from the Bougtoob region (ElBayed-Algeria) and Mechria region (Naama-Algeria), situated in the South of Algeria. The plant was identified by a botanist from the Faculty of Science and Life at the University of Mascara. The leaves were cleaned, shaded, dried, and conserved in a closed and dark room.

Essential oil extraction

For the extraction of essential oils by hydrodistillation, Clevenger-type was used (Chouitah et al., 2017), and the dried plant 50g was submitted in a balloon of 500 ml for 3 hours. Sodium sulfate anhydrous (Na_2SO_4) (used to dry the extract) was added to the essential oil that was recovered and stored in the refrigerator at 4°C and in the dark until use.



a. Mechria region
a. regijo Mechria



b. Bougtoob region
b. regijo Bougtoob

Figure 1. *Peganum harmala* L. plant

Slika 1. Rastlina *Peganum harmala* L.

The yield of essential oil was determined by its contribution to the dry material.

$$R (\%) = (m / m_0) \times 100$$

Where m: essential oil mass (g), m_0 : fresh leaves mass (g), R: essential oil yield (%) (Boutekedjiret et al., 2003).

Preparation of extracts

Ten grams of each powder sample (leaves) were quenched separately in 100 ml of hydromethanolic solution (water/methanol 20:80 v/v) for 48 hours with stirring to obtain two extracts (Mechria and Bougtob region hydromethanolic extracts). The extracts were then filtered using a Whatman No. 1 filter paper. All filtrates were pressured to a boiling temperature of 50 °C, then they dried at 40 °C and stored at 4 °C until their analysis (Pharmacognosie, 1999).

Analysis of the essential oils

According to AFNOR NF ISO 280: 1999, essential oils must respond to analytical characteristics that are established by international committees of experts. Standard organoleptic tests and measuring of some physicochemical parameters were performed.

Organoleptic characters

It is a very important test in determining the quality of an essential oil. It is based on the colour, smell and appearance of the essential oil and is carried out according to AFNOR NF ISO 280 (1999).

Physical properties of the essential oil

Essential oil is said to be miscible with V volume and more ethanol of alkalimetric strength determined at a temperature of 20°C (AFNOR 75-101, 2000). When the mixture of 1 volume of the essential oil with V volumes of this ethanol is clear, and the remainder after gradual addition of ethanol of the same strength up to a total of 20 volumes.

Briefly, ethanol of suitable alcoholic strength is gradually added to a test portion of essential oil at a temperature of 20°C, and then their miscibility was evaluated by stirring vigorously during the addition of the solvent. When the obtained solution was perfectly clear, note that the volume (V) of dilution of ethanol was used with continuous addi-

tion of solvent in 0.5ml portions up to a total of 20ml, and stirring was performed after each addition. The miscibility of essential oils with ethanol of title t, at a temperature of 20°C, is expressed by One volume of essential oils in V volumes of ethanol with title t.

The acid index (NF T 75 103, 1982) expresses the number of milligrams of potassium hydroxide (KOH) required to neutralize the free acids contained in one gram of essential oil. 2 g of essential oil was added to 5 ml of ethanol 95% and five drops of phenolphthalein at 0.2%. The solution was neutralized by the solution of KOH (0.1 mol / l) until a pink colour. We denoted the volume of the KOH solution added. The calculation of AI is given by the formula: $AI = 5.61 \times V / M$; 5.61: Corresponds to 0.1 mol / L KOH; M: mass in grams of the essential oil; V: Volume in millilitres of ethanol solution of KOH (0.1 mol / l) used for titration.

The ester index value (NFT 75 104, 1982) is the number of milligrams of KOH needed to neutralize the free acids by hydrolysis of esters contained in one gram of essential oil. 2 g of essential oil was added to 25ml of ethanol solution of KOH (0.5 mol / l). It adopted the condenser, placed the ball on the heating mantle and allowed it to heat for one hour. Then, the solution was added to 20 ml of distilled water and five drops of 0.2% PP. The excess KOH solution was titrated with hydrochloric acid 0.5 mol / l. A blank test was carried out under the same conditions and with the same reagents. The calculation of EI is given by the formula: $EI = (28.05 \times (V_0 - V_i) / M) - IA$; 28.05 g / l: corresponding to 0.5 mol / L KOH; M: mass in grams of the test; V_0 : Volume in ml of the HCl solution (0.5 mol / l) used for the blank; V_i : volume in ml of the HCl solution (0.5 mol / l) used to determine the EI of the EO.

Physical properties of the essential oil

Relative density was performed using a pycnometer with a volume of 1 ml and temperature of 20 °C (NF T 75 111, 1982). Rotator power was obtained according to NF T 75 113 (1982) using a polarimeter-type VISTA C25. Light source (sodium-vapour lamp) for obtaining a light wavelength of $589.3 \text{ nm} \pm 0.3$ and a viewing tube $100 \pm 0.5 \text{ mm}$ in length. The refractive index was carried out using a refract meter to Bellingham type with a precision of ± 0.0002 , a direct reading of refractive index between 1.3000 and 1.7000 situated (NF T 75 112, 1977). The freezing point was determined according to AFNOR 75-111 (2000). The essential oil was put in test tubes inside a freezer, accompanied

by a thermometer, hence the observation of temperature variations accompanying the solidification of the oil, which was cooled slowly and gradually.

Phytochemical screening

Phytochemical analysis of plant extracts is necessary to reveal the presence of certain chemical families. The detection of different phytochemical components of *P.harmala* extracts was performed by chemical detection tests based on phenomena of precipitation or colouration using the methods described in the literature. To test for phenols, we added to 2 ml of each extract solution a drop of 2 % FeCl_3 alcoholic solution. The positive reaction was expressed by the appearance of a green or blue-black colouration (Adou et al., 2016). For the test of flavonoids, we added 0.5 ml of each extract, ten drops of concentrated hydrochloric acid and a few milligrams of sunflower magnesium. Positive results were indicated by a pink-red or yellow colouration after three minutes of incubation (Haddouchi et al., 2016). To test for tannins, we took 3 ml of extract, placed it in a test tube and diluted with chloroform. 1 ml of acetic anhydride was added. Then, 1 ml of sulphuric acid was poured in carefully. A green colour was obtained, indicating the presence of tannins (Hossain et al., 2013). For the test for saponins, the dry powder was vigorously mixed with water. A positive result was indicated by the appearance of foam for more than 5 minutes (Joshi et al., 2013). For the test for coumarin glycosides, a few drops of FeCl_3 alcoholic solution were added to each extract. The presence of coumarins is indicated by the appearance of a green colouration after the addition of concentrated HNO_3 (Joshi et al., 2013). For the test for anthraquinones, approximately 0.5 g of the extract was stirred with 5 ml chloroform for ten minutes and filtered. The filtrate was shaken with 5 ml of ammonia solution. The presence of pink colour in the ammonia phase indicates the presence of anthraquinones. For a combined anthraquinones test, 1 g of powdered extract boiled with 5 ml of 10 % hydrochloric acid for five minutes. The cold filtrate was diluted with chloroform. The chloroform layer was then transferred to a test tube. An equal volume of 10 % ammonia was added to the chloroform extract. The presence of combined anthraquinones was indicated by a pink, red, or purple colour (Abodunrin et al., 2015). For the test for alkaloids, 0.5 g of the extract was mixed with 5 ml of 1 % aqueous hydrochloric acid in a water bath. A few drops of Dragendorff's reagent were added to 1 ml of the filtrate.

Turbidity or precipitation was considered to have a positive result (Ismail et al., 2016). For the test for steroids, 2 ml acetic anhydride and 2 ml H_2SO_4 were added to 5 ml of the extract. The colour change from violet to blue confirmed the presence of steroids (Kumar, 2015). For the test for proteins, 2 ml of NaOH (4 %) and a few drops of 3 % CuSO_4 solution were mixed with 2 ml of solution extract. The presence of protein is indicated by the formation of purple or pink colour (Maria et al., 2018). For the test of quinones, 3 ml of each extract was treated with 3 ml of chloroform, and then the chloroform layer was separated. A volume of potassium hydroxide (5 %) was then added to the chloroform layer. The appearance of red colour indicates the presence of quinones (Amezouar et al., 2013).

Chromatographic analysis: Gas chromatography/mass spectrometry analysis (GC-MS)

The volatile constituents of the essential oil were analyzed by "Shimadzu" GCMS-TQ8030 Gas chromatograph mass spectrometer with a Stabilwax silica capillary column (30 m X 0.32 mm, film thickness, 0.25 μm). Maximum Temp 350°C. The carrier gas was helium, Column Oven Temperature: 50°C, Injection Temperature: 220°C, rate change of 5°C/min, Equilibrium Time: 3min, vial capacity: 1.5 ml, and syringe capacity: 10 μl .

Microbial strains

The antimicrobial activity of the essential oil samples was tested against seven different microorganisms: The strains of the international ATCC collection (American Type Culture Collection).

Gram+: *Staphylococcus aureus* ATCC 2592, *Bacillus cereus* ATCC 11778.

Gram- : *Pseudomonas aeruginosa* ATCC 27853, *Klebsiella pneumoniae* ATCC 700603, *Escherichia coli* ATCC 25922.

Fungus: *Candida albicans* ATCC 30031, *Aspergillus brasiliensis* ATCC 16404.

Suspensions were adjusted to 1×10^7 CFU/ml (equivalent to 0.5 Mc Farland). These strains were maintained on slants of Nutrient Agar for bacteria and Sabouraud Agar for the fungus at 4°C. The tested microbial strains were provided by the Pasteur Institute (Algiers, Algeria).

Antimicrobial activity

The antimicrobial activity of essential oils from *P. harmala* leaves was evaluated using the paper disk diffusion method (Davis et al., 1994). Petri dishes (90 mm in diameter) were prepared with 20 ml of sterile Mueller-Hinton agar and dimethyl sulfoxide (DMSO) solution for bacteria and Sabouraud agar for fungi. Agar plates were stored at 4°C before use (Fauchère et al., 2002).

The antimicrobial activity test

Saturated with essential oil, the impregnated discs (with 3 µl of essential oil at a final concentration of 1-20 µg/ml) were then placed on the surface of the agar (Dean et al., 1987). The bacteria were spread over the discs. The spreading of the tested product over the entire disc determined the degree of concentration, the micro-organisms grew over the entire surface of the agar except where they encountered a sufficient concentration of the product, which would inhibit their growth after incubation 24h at 37°C (bacteria) and 48h at 25°C (fungi) around of the discs, we observed a clear circular zone formation. This is the zone of inhibition. In addition, this zone of diameter is large; the sensitivity of antibiotics induces more resistance to the bacterium. The diameter of the growth inhibition zone was measured with a calliper and expressed in mm (including the diameter of the 6 mm disc). (Billerbeck et al., 2002; NIST, 2002)

The minimum inhibition concentrations (MICs)

Were performed according to the Muller-Hinton broth microdilution method in 96 multiwall microtiter plates (Marino et al., 2001). The essential oils were dissolved in aqueous DMSO, and the initial concentration was 25 µg/ml. (Smânia et al., 1995). The initial concentration was serial dilution, which is a two-fold serial dilution. Each well contained 10⁷ CFU/ml of bacteria. After incubation for 24h at 37°C (bacteria) and 48h at 25°C (fungi), the CIM was determined as the lowest concentration at which no visible microorganism growth had occurred. All tests were performed in triplicate (Gilles et al., 2010).

Results and Discussion

The physicochemical analysis of the leaf extracts of *P. harmala* demonstrated the following results: an oily appearance, a yellowish colour, and a strong, unpleasant odour. The yield of essential oil obtained by hydro-distillation of dry plants was 0.217 ± 0.03% for the *P. harmala* Bougtob region and 0.211 ± 0.05% for the Mechria region sample. In a study by Ida et al. in the Bousaada region (South of Algeria), hydrodistillation yielded 0.005% (Ida et al., 2016).

The variations found in the yield of essential oil view of our samples compared to some previous studies may be due to some environmental factors (such as soil composi-

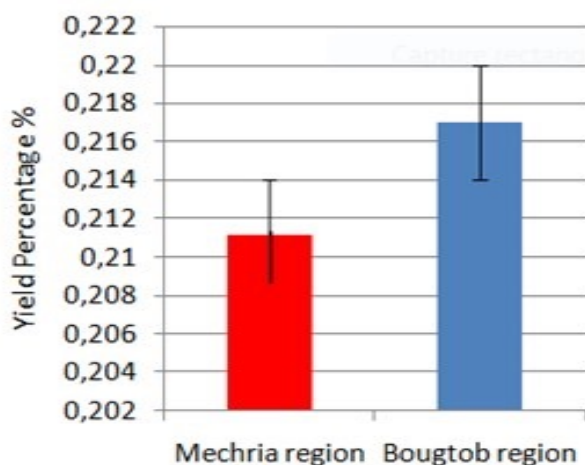


Figure 3. The yield of essential oil of *P. harmala* was obtained by hydro-distillation for different regions.

Slika 3. Donos eteričnega olja *P. harmala*, pridobljenega s hidrodestilacijo za različne regije.

tion, geographical location and climate conditions), the part of the plant used, the age of the plant and the period of the growing season or even to genetic factors (Hussain et al., 2009; Anwar et al., 2009).

Phytochemical screening

Phytochemical screening in the leaves of *P.harmala* showed the presence of flavonoids, alkaloids, saponins, tannins, glycosides, terpenoids and steroids and the absence of anthraquinones for two regions.

Phytochemical Compounds of Essential Oil

The chromatographic analysis of the essential oil of *P.harmala* from the Bougtob region revealed the presence of 55 volatile, constituting 66.59% of the total essential oil recovered from dry material (Table 4, 5). The chemical analysis showed that the characterized essential oil possessed many potentially bioactive substances, which were identified, and the following compounds are representing the major constituents: (1) Carvone 21.15%, (2) Phytol 17.41%, (3) Carbamic acid 3.52%, (4) Lauric acid 2.10%, (5) Thymol

Table 1. Physicochemical composition of *Peganum harmala*.

Tabela 1. Fizikalno-kemijska sestava *Peganum harmala*.

Specification	Peganum harmala (M)	Peganum harmala (B)
Density	0.905	0.907
Rotating power	+2°	+ 2°
Refractive index	+1.4693	+ 1.4695
Freezing point	-18	-18
Solubility in éthanol 90%	1 :3	1 :3
pH	6.02	6.06
Acid index	0.517	0.521
Ester index	22.7	23.2

(M): Mechria region, (B): Bougtob region.

Table 2. Phytochemical screening of *Peganum harmala* (leaves) hydromethanolic extracts (MM: hydromethanolic extract of Mechria region, MB: hydromethanolic extract of Bougtob region).

Tabela 2. Fitokemijski pregled hidrometanoličnih izvlečkov *Peganum harmala* (listi) (MM: hidrometanolni izvleček regije Mechria, MB: hidrometanolni izvleček regije Bougtob).

Extract constituent	Test	Observation	Extract MM	MB
Steroids	Liebermann–Burchard reaction	Purple to blue colour	+	+
Saponins		Foam on the surface	++	++
Tannins		Green color	+++	++
Quinones	Bortragers test		-	-
Phenols		Green-black	+	+
Coumarin glycosides		Green to yellow	++	++
Free anthraquinones		Red or pink colour	-	-
Combined anthraquinones		Pink, red or purple	-	-
Flavonoids		Pink-red or yellow	+	+
Alkaloids	Mayer	Colored precipitate	+	+
Cardiac glucosides		Brown ring	++	++
Proteins	Biuret test	Purple or pink colour	-	-

(-) absence, (+) presence, (+++) presence in high quantity.

1.77%, (6) Tetrapentacontane 1.32%, (7) Dihydroactinidiolide 1.21%, (8) 2-Aminobenzaldehyde 1.03%. In contrast, the chromatographic analysis of the essential oil of *P.harmala* from the Mechria region demonstrated the presence of 47 volatile, constituting 43.6% of the total essential oil recovered from dry material. Chemical analysis of essential oil from Mechria region showed that its major products are: (1) Phytol 10.24%, (2) Carvone 5.92%, (3) Tetrapentacontane 2.80%, (4) Phytone 2.18%, (5) Butylated Hydroxytoluene 1.48%, (6) Lauric acid 1.39%, (7) Hexatriacontane 1.27%, (8) 3,5-di-tert-Butyl-4-hydroxybenzaldehyde 1.14% .

In a study by Ida et al.(2016), the chromatographic analysis of the essential oil of *P.harmala* from the Bousaada region demonstrated the presence of 45 volatile constituting 89% of total essential oil showed that its major products are Eugenol 17.5%, Thymol 7%, Carvone 2.7%.

The variations found in the chemical composition from

the qualitative and quantitative point of view of our samples compared to the study by Ida et al.(2016) may be due to some environmental factors (such as soil composition, geographical location and climate conditions), the part of the plant used, the age of the plant and the period of the growing season or even to genetic factors (Hussain et al., 2009; Anwar at al., 2009). The methods that use water can induce hydrolysis of esters and also rearrangements, isomerizations, racemizations, and oxidations (Bruneton et al., 1993).

Khadhri et al.(2011) also say that the variation in the percentage of the chemical composition of essential oils can be explained by the differences in the geographic origins, climate, nature of the soil, solar radiation, age of the plant and part of plants.

All these factors involve the activation or inactivation of certain enzymatic groups, leading to the predominance of a particular biosynthetic pathway (Khadhir et al., 2011).

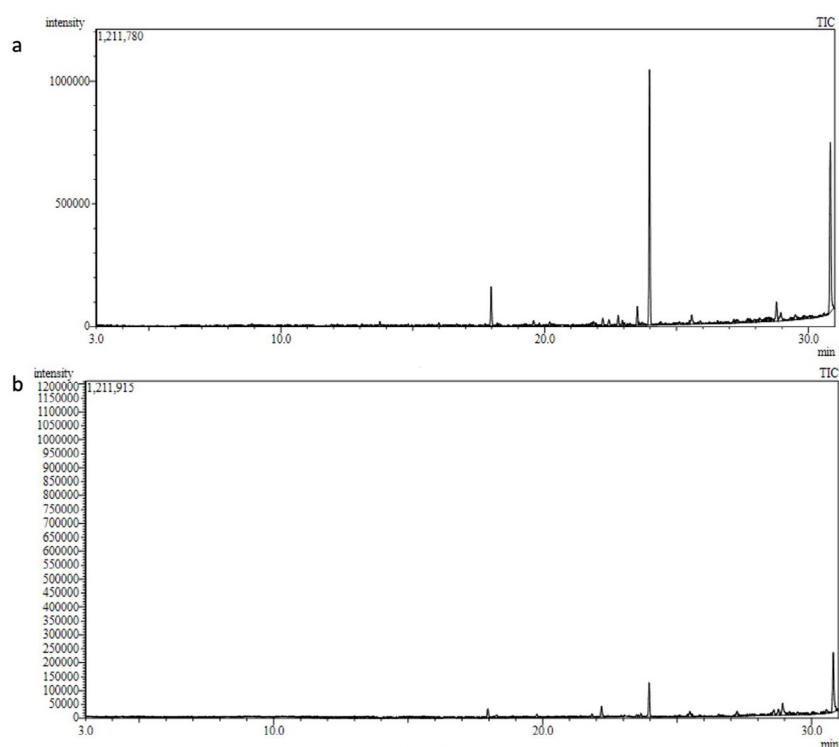


Figure 3. Gas chromatographic profiles the major constituents of *Peganum harmala* essential oils for two regions; a) Gas chromatographic profiles the major constituents of *Peganum harmala* essential oils for Bougtob region; b) Gas chromatographic profiles the major constituents of *Peganum harmala* essential oils for Mechria region.

Slika 3. Profili plinske kromatografije glavnih sestavin eteričnih olj *Peganum harmala* za 2 regiji; a) Profili plinske kromatografije glavnih sestavin eteričnih olj *Peganum harmala* za regijo Bougtob; b) Profili plinske kromatografije glavnih sestavin eteričnih olj *Peganum harmala* za regijo Mechria.

Table 3. Phytochemical components identified in *Peganum harmala* essential oil (Bougtoob region) by GC-MS.Tabela 3. Fitokemične sestavine, identificirane v eteričnem olju *Peganum harmala* (regija Bougtoob) z GC-MS.

Compound Name	Molecular Formula	RT (min)	Area (%)
Eicosane	C ₂₀ H ₄₂	13.393	0.30
D - carvone	C ₁₀ H ₁₄ O	13.758	0.53
Anisole	C ₁₀ H ₁₂ O	15.986	0.36
5,9-undecid-2-one, 6,10-dimethyl-, (e)-	C ₁₃ H ₂₂ O	16.655	0.31
Heptadecane, 8-methyl-	C ₁₈ H ₃₈	17.742	0.22
Carbamic acid	C ₁₇ H ₂₇ NO ₂	17.973	3.52
3,7,11,15-Tetramethyl-2-hexadecen-1-ol	C ₂₀ H ₄₀ O	18.295	0.24
Caryophyllene oxide	C ₁₅ H ₂₄ O	18.816	0.13
Ketone	C ₈ H ₁₂ O	19.287	0.18
Ethanone, 1-(1,3a,4,5,6,7-hexahydro-4-hydroxy-3,8-dimethyl-5-azulene)-	C ₁₄ H ₂₀ O ₂	19.584	0.56
Hexatriacontane	C ₃₆ H ₇₄	19.807	0.25
6,8-nonadien-2-one, 6-methyl-5-(1-methyl ethylidene)-	C ₁₃ H ₂₀ O	20.196	0.33
2-Benzyl-3-oxo propanoic acid, ethyl ester	C ₁₃ H ₁₆ O ₃	20.305	0.20
P-Cymen-7-ol	C ₁₀ H ₁₄ O	21.670	0.23
Tetrapentacontane	C ₅₄ H ₁₁₀	21.768	0.31
2,6-di(t-butyl)-4-hydroxy-4-methyl-2,5-cyclohexadiene-1-one	C ₁₅ H ₂₄ O ₂	21.847	0.43
Phytone	C ₁₈ H ₃₆ O	22.205	0.82
Δ-Cadinene	C ₁₅ H ₂₄	22.438	0.67
2-aminobenzaldehyde	C ₇ H ₇ NO	22.786	1.03
Eugenol	C ₁₀ H ₁₂ O ₂	22.944	0.46
Thymol	C ₁₀ H ₁₄ O	23.514	1.77
Hexacontane	C ₆₀ H ₁₂₂	23.695	0.32
D-(+)-carvone	C ₁₀ H ₁₄ O	23.978	21.15
Ar-tumerone	C ₁₅ H ₂₀ O	24.401	0.27
Octaborane	B ₈ H ₁₂	24.955	0.14
2-Nonen-1-ol, 2-methyl-	C ₁₀ H ₂₀ O	25.000	0.18
Decanoic acid	C ₁₀ H ₂₀ O ₂	25.110	0.32
Pentadecane, 8-hexyl-	C ₂₁ H ₄₄	25.495	0.31
Dihydroactinidiolide	C ₁₁ H ₁₆ O ₂	25.578	1.21
Quinoline, 6-butyl-	C ₁₃ H ₁₅ N	25.818	0.19
Phenol, 2,4-bis(1,1-dimethylethyl)-	C ₁₇ H ₃₀ OSi	25.901	0.36
Dihydroartemisinin, 6-dehydro-5-dehydroxy-3-deoxy-	C ₁₅ H ₂₂ O ₄	26.246	0.18
Diethyl phthalate	C ₁₂ H ₁₄ O ₄	26.552	0.26
Solasonine	C ₄₅ H ₇₃ NO ₁₆	26.671	0.19
2-propenoic acid, 3-(dimethylamino)-3-ethoxy-, ethyl ester	C ₇ H ₁₄ O ₃	26.954	0.19
P-vinyl phenol	C ₈ H ₈ O	27.174	0.39
Tetracosane	C ₂₄ H ₅₀	27.260	0.34
4-quinoline carboxaldehyde	C ₁₀ H ₇ NO	27.315	0.35
Formamide, N-phenyl-	C ₇ H ₇ NO	27.695	0.40
Indole	C ₈ H ₇ N	27.782	0.32
5.alpha.-cholestan-6.beta.-amine, n,n-dimethyl-	C ₂₉ H ₅₃ N	27.962	0.31
Methanone, diphenyl-	C ₁₃ H ₁₂ N	28.154	0.48
N-Hexadecanoic acid	C ₁₆ H ₃₂ O ₂	28.385	0.24

Ethyl ether	$C_4H_{10}O$	28.501	0.48
Phosphorochloridothioic acid, O,O-dimethyl ester	$C_2H_6ClO_2PS$	28.570	0.19
3,5-di-tert-Butyl-4-hydroxybenzaldehyde	$C_{15}H_{22}O_2$	28.615	0.29
Lauric acid	$C_{12}H_{24}O_2$	28.791	2.10
Tetrapentacontane	$C_{54}H_{110}$	28.950	1.32
3-methylheptadecane	$C_{18}H_{38}$	29.328	0.30
1,2-Benzenedicarboxylic acid, butyl 8-methylnonyl ester	$C_{22}H_{34}O_4$	29.500	0.75
21.xi-methyl-17-isocholest-16-en-3.Beta.-ol	$C_{27}H_{46}O$	29.848	0.35
Triethylene glycol mono dodecyl ether	$C_{18}H_{38}O_4$	30.058	0.44
Cyclohexanepropanol, .alpha.,2,2,6-tetramethyl-	$C_{13}H_{26}O$	30.144	0.25
Tetracosane	$C_{24}H_{50}$	30.595	0.33
Phytol	$C_{20}H_{40}O$	30.833	17.41

Table 4. Phytochemical components were identified in the *Peganum harmala* essential oil Mechria region by GC-MS.Tabela 4. Fitokemične sestavine, identificirane v eteričnem olju *Peganum harmala* Mechria regija z GC-MS.

Compound Name	Molecular Formula	RT (min)	Area (%)
Thiirane, octyl-	$C_{10}H_{20}OS$	14.355	0.38
Phosphoric acid	$C_{15}H_{41}O_6PSi_4$	14.962	0.36
Pentacosane	$C_{25}H_{52}$	15.602	0.33
Methyl 2,6-anhydro-.alpha.-d-altroside	$C_7H_{12}O_5$	15.853	0.52
Butanedioic acid, decyl phenylmethyl ester	$C_{21}H_{32}O_4$	17.162	0.38
2-Methyl-decahydro-4-quinolone	$C_{10}H_9NO$	17.268	0.29
Butylated Hydroxytoluene	$C_{15}H_{24}O$	17.960	1.48
β -Ionone	$C_{13}H_{20}O$	18.182	0.38
2-Butenoic acid	$C_8H_{17}NO_5$	18.288	0.69
2-Nonadecene	$C_{19}H_{38}$	19.147	0.38
Cyclobutaneacetamide, N-(4-methoxyphenyl)	$C_{10}H_{13}NO_2$	19.288	0.34
Citronellyl valerate	$C_{15}H_{28}O_2$	19.427	0.43
Eicosane	$C_{20}H_{42}$	19.780	0.63
o-Cymene	$C_{10}H_{14}$	20.255	0.24
Hexane, 2,3,4-trimethyl	C_9H_{20}	20.529	0.23
Phenanthrene, 3-methyl-	$C_{15}H_{12}$	20.785	0.57
Propanoic acid, ethyl ester	$C_5H_{10}O_2$	21.060	0.21
Pentanoic acid, 5-hydroxy-2-propyl	$C_{14}H_{32}O_3Si_2$	21.206	0.23
Perhydro-htx-2-one, 2-depenty-, acetate ester	$C_{16}H_{27}NO_3$	21.411	0.23
13-Methyltrtriacontane	$C_{34}H_{70}$	21.735	0.25
2,6-Di(t-butyl)-4-hydroxy-4-methyl-2,5-cyclohexadien-1-one	$C_{15}H_{24}O_2$	21.833	0.62
Phytone	$C_{18}H_{36}O$	22.189	2.18
Carvacrol	$C_{10}H_{14}O$	23.499	0.46
Phytol acetate	$C_{22}H_{42}O_2$	23.567	0.37
Tetrapentacontane	$C_{54}H_{110}$	23.657	0.71
Carvone	$C_{10}H_{16}O$	23.960	5.92
Octadecanoic acid	$C_{18}H_{36}O_2$	25.090	0.33
Hexatriacontane	$C_{36}H_{74}$	25.472	1.27
2(4H)-Benzofuranone, 5,6,7,7a-tetrahydro-4,4,7a-trimethyl-, (R)-	$C_{11}H_{16}O_2$	25.562	0.37
Quinoline, 6-butyl-	$C_{13}H_{15}N$	25.828	0.32

α -Pyrrolidone, 5-[2-butyrylethyl]-	$C_{10}H_{17}NO_2$	26.016	0.42
Malonic acid, 6-heptynyl	$C_{15}H_{28}O_4$	26.285	0.39
1,2-Benzenedicarboxylic acid, diethyl ester	$C_{12}H_{14}O_4$	26.541	0.63
Dotriacontane	$C_{32}H_{66}$	27.233	1.13
Spiro[cyclopropane-1,2'-(1'h)-phenanthrene]-1',4'-(3'h)-dione, 8'-[(formyloxy)methyl]-4'b,5',6',7',8',8'a,9',	$C_{21}H_{28}O_7$	27.281	0.34
1,3,2-Dioxaphosphorinane, 2-(benzyloxy)-2-oxo-	$C_{10}H_{13}O_4P$	27.811	0.57
2-(2-Bromo-ethyl)-3-methyl-oxirane	C_5H_9BrO	28.377	0.23
3-Quinuclidinol	$C_7H_{13}NO$	28.508	0.47
3,5-di-tert-Butyl-4-hydroxybenzaldehyde	$C_{15}H_{22}O_2$	28.600	1.14
Lauric acid	$C_{12}H_{24}O_2$	28.773	1.39
Benzenamine, 2,4-dinitro-N-phenyl-	$C_{12}H_9N_3O_4$	28.840	0.36
Tetrapentacontane	$C_{54}H_{110}$	28.924	2.80
Phosphonic acid, bis(1-methylethyl) ester	$C_6H_{15}O_3P$	29.059	0.40
Pentadecane, 2,6,10-trimethyl-	$C_{18}H_{38}$	29.181	0.55
1,2-Benzenedicarboxylic acid, butyl 8-methylnonyl ester	$C_{22}H_{34}O_4$	29.477	0.68
Tetrapentacontane	$C_{54}H_{110}$	30.554	0.76
Phytol	$C_{20}H_{40}O$	30.808	10.24

Antibacterial activity

The essential oil of *P.harmala* leaves of two regions has good inhibitory activity in vitro against the microorganisms tested. However, the microorganisms did not show the same sensitivity to the essential oil.

This essential oil has strong antibacterial activity against Gram-positive bacteria, *Staphylococcus aureus* (inhibition zone 36 mm MIC: 10.5 µg/ml and 33 mm MIC: 11.5 µg/ml) and *Bacillus cereus*, (inhibition zone 21.7 mm MIC: 15 µg/ml and 18 mm MIC: 17.5 µg/ml) and very strong antifungal, *Candida albicans* (inhibition zone 25 mm MIC: 09.0 µg/ml and 22.5 mm MIC: 11.5 µg/ml) and *Aspargillus brasiliensis* (inhibition zone 18 mm MIC: 7.5 µg/ml and 17.5 mm MIC: 10.5 µg/ml). The essential oils have moderate antibacterial activity against Gram-negative bacteria, *Pseudomonas aeruginosa* (inhibition zone 10.5 mm MIC: 19.5 µg/ml and 8.5 mm MIC: 21 µg/ml), *Klebsiella pneumonia* (inhibition zone 13.5 mm MIC: 17.5 µg/ml and 12 mm MIC: 18.5 µg/ml), *Escherichia coli* (inhibition zone 17 mm MIC: 14.5 µg/ml and 15.5 mm MIC: 12 µg/ml) due to the presence of lipopolysaccharide barrier (LPS), which has a role in protecting bacteria against antibacterial agents.

Gram-negative bacteria are more resistant to essential oils than Gram-positive bacteria due to the structure of their outer membrane. Thus, the outer membrane of Gram-negative is richer in lipo-polysaccharides and proteins (Toure et al., 2015).

Conclusions

In this study, we determined the chemical composition and the antimicrobial activity of essential oil from the leaves of *Peganum harmala* grown in the region of Bougtob and Mechria. Fifty-five compounds have been identified in the essential oil of the sample of the first region, Carvone 21.15%, Phytol 17.41%, Carbamic acid 3.52%, Lauric acid 2.10%, and Thymol 1.77%, Tetrapentacontane 1.32% were the major compounds. In the sample of the region of Mechria, 47 compounds were identified when Phytol 10.24%, Carvone 5.92%, Tetrapentacontane 2.80%, Phytone 2.18%, Butylated Hydroxytoluene 1.48%, and Lauric acid 1.39% were the dominant. This difference in composition is due to several factors, such as soil composition, geographical location and climate conditions.

Phytochemical screening in the leaves of *P.harmala* showed the presence of flavonoids, alkaloids, saponins, tannins, glycosides, terpenoids and steroids and the absence of anthraquinones.

The antimicrobial effectiveness of the essential oil of *P.harmala* from southern Algeria has been demonstrated against five bacteria and fungi. This essence showed strong antimicrobial activity against all the tested strains. In general, Gram-positive bacteria and fungi were found to be more sensitive to the essential oils than Gram-negative bacteria. This great power is attributed mainly to their high

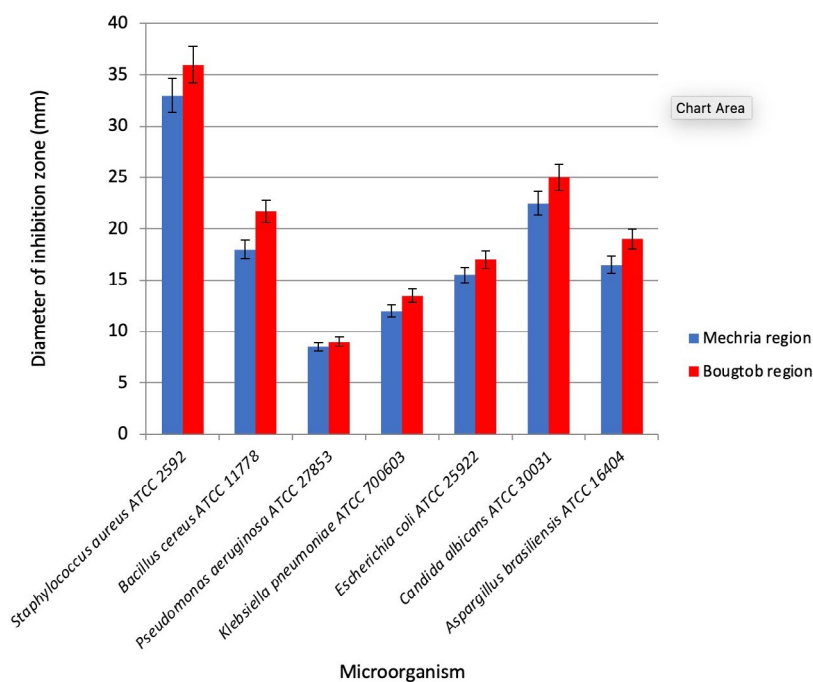


Figure 4. Zones of growth inhibition (mm) are used using a direct contact technique in an agar medium for the antimicrobial activity of *P.harmala* essential oils.

Slika 4. Območja zaviranja rasti (mm) so s tehniko neposrednega kontakta v agarškem mediju protimikrobna aktivnost eteričnih olj *P.harmala*.

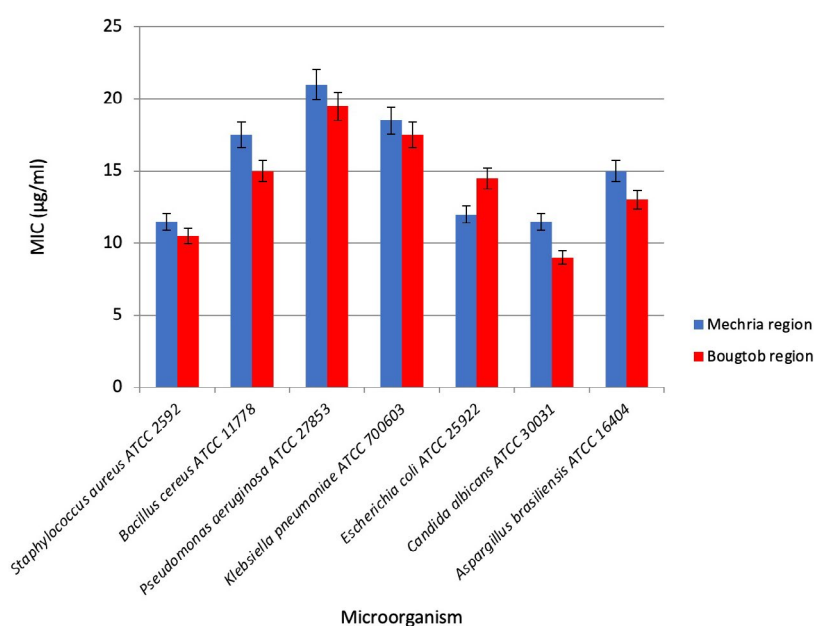


Figure 5. MIC ($\mu\text{g/ml}$) using microdilution method in 96 multiwell microliter plates showing antimicrobial activity of *P.harmala* essential oils.

Slika 5. MIC ($\mu\text{g/ml}$) z metodo mikrorazredčevanja v 96 večstenskih mikrolitrskih ploščah, ki prikazuje protimikrobno delovanje eteričnih olj *P.harmala*.

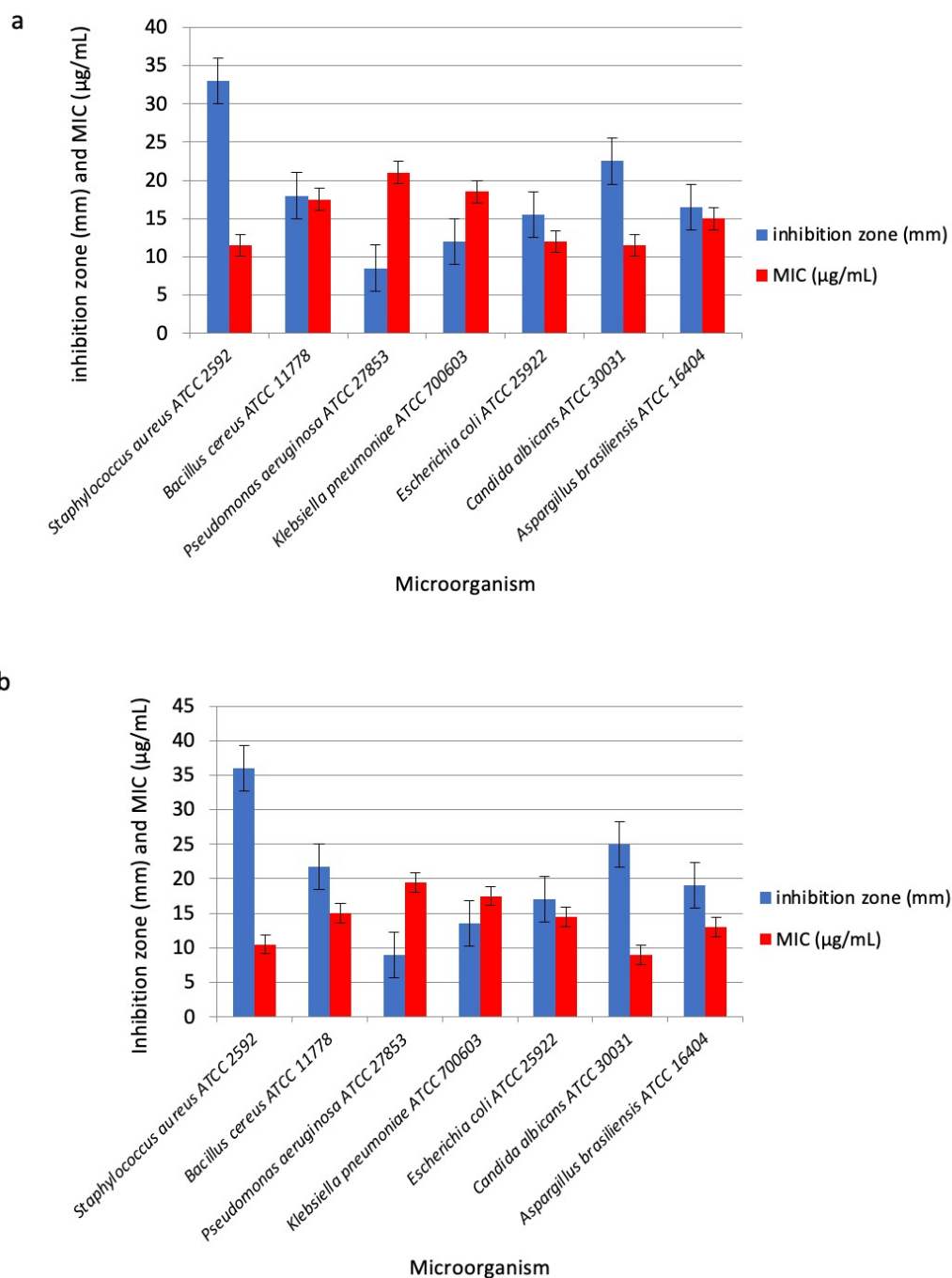


Figure 6. Comparison of Diameter of inhibition zone and MIC for the individual bacteria/fungi for two regions; a. Comparison of Diameter of inhibition zone and MIC for the individual bacteria/fungi for Mechria region; b. Comparison of the Diameter of the inhibition zone and MIC for the individual bacteria/fungi for the Bougtob region

Slika 6. Primerjava premera inhibicijskega območja in MIC za posamezne bakterije/glive za 2 regiji; a. Primerjava premera inhibicijskega območja in MIC za posamezne bakterije/glive za regijo Mechria; b. Primerjava premera inhibicijskega območja in MIC za posamezne bakterije/glive za regijo Bougtob

contents in oxygenated terpenes (Carvone, Phytol and Lauric acid). However, the antimicrobial activity registered confirms some of the traditional uses of the plant in the treatment of some diseases.

As a final remark, it is also vital to mention that the essential oil of *Peganum harmala* is a domain where much remains to be studied to exploit its properties.

Author Contributions

Conceptualization, G.A. and C.O.; methodology, G.A. and C.O.; software, G.A.; validation, G.A., C.O., K.F. and F.Z.; formal analysis, F.Z.; investigation, C.O. and K.F.; data curation, G.A., C.O., K.F., F.Z., and D.Z.; writing original draft preparation, G.A., C.O., K.F., F.Z., and D.Z.; writing review and editing, G.A., C.O., K.F., F.Z., and D.Z.; visualization, C.O.; supervision, C.O.; project administration, C.O. All authors have read and agreed to the published version of the manuscript.

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Data Availability

Data are available upon request to the corresponding author.

Conflicts of Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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