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Antibacterial activity of the essential oils of myrtle leaves against *Erysipelothrix rhusiopathiae*

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PEER REVIEW

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Comments

The authors of this important research have proved that essential oils of *M. communis* are potential and promising antibacterial agents which could be used as antibiotic in the protection of domestic animals and humans against *E. rhusiopathiae*. This conclusion was the result of chemical composition and antibacterial activity investigation.
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ABSTRACT

Objective: To evaluate the antibacterial activity of the essential oil of *Myrtus communis* (*M. communis*) L. against *Erysipelothrix rhusiopathiae* (*E. rhusiopathiae*) *in vitro*.
Methods: Wild populations of *M. communis* collected from Khuzestan and Lorestan provinces, Southwest Iran, were examined for antibacterial activity and chemical variability in leaves. The *in vitro* antibacterial activity against *E. rhusiopathiae* was performed by agar disc diffusion and micro-dilution assays.
Results: The essential oils of *M. communis* have strong antibacterial against *E. rhusiopathiae* in both assays. The results showed that the major components of the oil were α-pinene (22.3%–55.2%), 1,8-cineole (8.7%–43.8%) and linalool (6.4%–14.5%). The inhibition zones and MIC values for bacteria which were sensitive to the essential oils of *M. communis* were in the range of 14.7–27.0 mm and 0.031–0.25 mg/mL, respectively.
Conclusions: This study demonstrates that products with valuable antibacterial activity can be produced from leaves of *M. communis* against *E. rhusiopathiae*.

KEYWORDS
Myrtus communis L., *Erysipelothrix rhusiopathiae*, Essential oil, 1,8-cineole, α-Pinene

1. Introduction

Erysipelas is an animal disease caused by Gram-positive bacteria *Erysipelothrix rhusiopathiae* (*E. rhusiopathiae*). Among the domestic animals, it suffers most frequently from the disease in human environment. This is a typical animal-borne disease observed mainly in occupational groups employed in agriculture, farming (of animals and birds), fishing and manufacturing industry. Erysipelas infection is a result of contact with infected animal, animal-borne contamination, animal-derived products or wastes. Infection in humans may have the following clinical course: mild form of skin infection diagnosed as erythema (erysipeloid), disseminated form of skin infection and the most serious form of infection of systemic course (endocarditis and sepsis)[1].

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Myrtus communis L. (*M. communis*) (myrtle) (Myrtaceae) is an evergreen shrub which grows mainly in Mediterranean climates and has long been used by locals for its culinary and medicinal properties[2]. In Iran, the species commonly known as ''Mord or Mort'' is abundant in the Zagros mountainous range of the country[3]. *M. communis* is an important medicinal and aromatic plant, because of the high essential oil content in its leaf, flower and fruit glands. Leaves and berries are sources of essential oil that have medicinal properties including antimicrobial[4-7], antioxidant and antimutagenic[6,8,9], astringent, antiseptic, anti-hyperglycemic[6,7,10], antinociceptive and anti-inflammatory[11], insecticide[12,13], nematicidal activity[14,15]. In addition, myrtle berries and leaves are mostly employed for the industrial formulation of sweet liquors with digestive properties[16]. *M. communis* has been used since ancient times for medicinal, food, and spice purposes. In Iranian folk medicine, *M. communis* has been used as an infusion for various purposes such as for the skin discords, anti-septic (smoking), women diseases, wound (antimicrobial), digestive discords, astringent, good hair condition, bronchodilator, activities *etc*[17,18].

In Iran, myrtle grows wild in different bioclimatic zones extending from the upper semi-arid to the lower humid. Populations of *M. communis* grow at altitudes ranging from 900 to 1 700 m, under a rainfall ranging from 400 to 600 mm/year. Piras *et al.* showed a variation in anthocyanins, flavonols and α-tocopherol from alcoholic extracts of myrtle berries obtained from seven different sites[19]. In 2000, Moradi reported that essential oil of leaves of *M. communis* growing in Iran contains 1,8-cineole, α-pinene, limonene, linalool, α-terpineol, β-myrcene, *cis*-isoeugenol, α-terpinyl acetate and linalyl acetate as major components[20]. Population fragmentation and wild harvesting with no rational control were the major factors influencing genetic diversity, structuring and population dynamics. Population bioclimatic preferences and geographic distances separation play a major role in this differentiation. To our knowledge, no documented reports on antibacterial activity of the essential oils of *M. communis* against *E. rhusiopathiae* are available. The aim of this study was to evaluate the antibacterial activity of the essential oil of *M. communis* against *E. rhusiopathiae in vitro*.

2. Materials and methods

2.1. Plants material

The leaves (0.5 kg) of five wild populations of *M. communis* were collected from different localities of two provinces (Lorestan and Khuzestan) in Iran at the early flowering stage on 1-20 June 2012 (Figure 1). The samples of the plants were identified by regional floras and authors with floristic and taxonomic references[21], and voucher specimens were deposited at the herbarium of I.A.U, Shahrekord Branch (No. IAUSHK-231).

Figure 1. The leaves of wild of *M. communis* were collected from different localities in Iran at the early flowering stage.

2.2. Essential oil extraction

Harvested leaves of *M. communis* were dried at room temperature for 5 d. Dried leaves were grinded, and 100 g of tissue was distillated with 1 000 mL water for 3 h using a Clevenger-type apparatus according to the method recommended in British Pharmacopoeia[22]. The separated oil was dried over anhydrous sodium sulfate, and stored in dark glass bottles at (4±1) °C prior to use.

2.3. Identification of the oil components

The oils were analyzed by an Agilent Technologies 5975 mass system with Agilent Technologies 7890 GC. HP-5 MS column (30 m×0.25 mm i.d., film thicknesses 0.25 μm) was used with helium as the carrier gas at flow of 0.8 mL/min. Column temperature was from 60 °C to 280 °C. Programmed temperature increase was 4 °C /min. Split ratio was adjusted at 40:1. The injector temperature was set at 300 °C. The purity of helium gas was 99.999% and 0.1 μL samples were injected manually in the split mode. GC/MS analysis was performed on above mentioned Agilent Technologies 5975 mass system. Mass spectra were recorded at 70 eV. Mass range was from m/z 50-550. Retention indices were calculated for all components using a homologous series of *n*-alkanes (C_5-C_{24}) injected in conditions equal to samples ones. Identification of oil components was accomplished based on comparison of their retention times with those of authentic standards and by comparison of their mass spectral fragmentation patterns (Wiley/ChemStation data system)[23].

2.4. Antibacterial test

2.4.1. Antibacterial activity with disc diffusion assay

The strain of *E. rhusiopathiae* was isolated from patient chickens provided by the Microbiology Laboratory, Veterinary Medicine Faculty, (I.A.U.) Iran. Bacteria strain was identified using polymerase chain reaction-restriction fragment length polymorphism. The density of bacteria culture required for the test was adjusted to 5.0

McFarland standards, (1.0×10^{7} CFU/mL) measured using the spectrophotometer (Eppendorf, AG, Germany). These experiments were performed by the disc diffusion method with some modification[24,25]. Sterile paper discs (6 mm in diameter) were impregnated with 60 μL of dilutions of known essential oil concentrations (0.03–0.50 mg/mL) and incubated at 37 °C for 24 h. Bacterial growth inhibition was determined as the diameter of the inhibition zones around the discs (mm). The growth inhibition diameter was an average of three measurements, taken at three different directions.

2.4.2. Determination of minimum inhibitory concentration (MIC)

The MIC values were evaluated using the broth micro-dilution method according to standard methods[26]. Stock solutions of the essential oil and antimicrobial standards (penicillin and gentamicin) were prepared in 5.0% (v/v) dimethyl sulfoxide. After incubation at 37 °C for 24 h, the microorganism growth inhibition was evaluated by measuring absorbance at 630 nm, using a spectrophotometer. Experiments were performed in triplicate but at three different times.

2.4.3. Determination of minimum bactericidal concentration (MBC)

The MBCs of essential oils were determined according to the MIC values. Five microliter from MIC tubes were transferred to agar plates and incubated at 37 °C for 24 h. The MBC was referred to the minimum concentration of essential oils with no viable bacteria.

2.5. Statistically analysis

Means and standard deviation of the samples were calculated. Each treatment was carried out with three replicates. Mean differences were determined by using Duncan's multiple range test at 5% level of significance. All statistical analyses were performed using SPSS version 19.0.

3. Results

The main chemical compositions of essential oils of various population of *M. communis* identified by GC-MS are presented in Table 1. Three main constituents of the essential oils were α-pinene (22.3%–52.2%), 1,8-cineole (8.7%–43.8%) and linalool (6.4%–14.5%).

Table 1

Main compositions of the essential oil of *M. communis* leaves collected from various regions.

Components	RI[a]	Percentage (%)[b]				
		Population-I	Population-II	Population-III	Population-IV	Population-V
α-pinene[c]	940	26.3	28.9	38.8	22.3	52.2
Limonene	1029	21.4	tr[d]	tr	tr	6.3
1,8-cineole	1034	11.4	27.9	32.3	43.8	8.7
Linalool	1087	14.5	8.6	8.4	12.7	6.4
α-terpineol	1189	5.2	7.9	5.2	5.9	7.9
Linalyl acetate	1252	6.3	2.8	2.4	4.5	1.7

[a]RI: Retention index determined on HP-5MS capillary column; [b]Calculated from TIC data; [c]Values of major compounds are given as means; [d]trace (<0.01%).

The *in vitro* antibacterial activity of the essential oil of wild populations of *M. communis* was assessed by the disc diffusion and micro-dilution methods against *E. rhusiopathiae*. Antibacterial activity was expressed as diameter of the inhibition zones, MIC and MBC values (Tables 2 and 3). The essential oils of *M. communis* exhibited varying levels of antibacterial activity against the investigated bacteria. The diameter of the inhibition zones values of different concentrations were between 14.7–27.0 mm. In general, a total of *M. communis* essential oil showed relatively high inhibitory activities against the bacteria tested (Table 2). The MICs of the essential oils were within concentration ranges 0.031–0.25 mg/mL, and the respective MBCs were 0.125–0.25 mg/mL (Table 3). The results showed that essential oils of various populations had high inhibitor activity against *E. rhusiopathiae*. The essential oil obtained from population-IV had the highest inhibitor activity against *E. rhusiopathiae* (Tables 2 and 3).

Table 2

Antibacterial activity of the essential oils of *M. communis* against *E. rhusiopathiae* by disc diffusion assay.

Bacterial	Concentration (μg/mL)	Growth inhabitation (mm)					ANOVA
		Population-I	Population-II	Population-III	Population-IV	Population-V	
E. rhusiopathiae	500	19.00±1.73	20.00±0.00	22.00±0.00	18.67±2.08	15.67±2.87	$P\leq0.01$
	250	18.00±5.21	18.00±0.00	17.00±0.00	20.00±1.73	18.00±0.00	$P\leq0.05$
	125	14.67±4.04	15.33±5.79	24.00±0.00	27.00±0.00	24.33±2.89	$P\leq0.05$
	62	21.67±0.57	20.00±0.00	24.00±0.00	25.00±1.73	17.00±5.19	$P\leq0.05$
	31	19.67±2.89	20.00±0.00	16.00±0.00	25.00±0.00	24.00±0.00	$P\leq0.05$
	Mean	18.60±4.13[b]	18.67±2.89[b]	20.61±5.18[b]	23.13±3.85[a]	19.80±5.51[b]	$P\leq0.05$

Values are expressed as mean±SD.

Table 3

MICs and MBCs of the essential oils of *M. communis* against *E. rhusiopathiae*.

Pathogen	Population-I (μg/mL)		Population-II (μg/mL)		Population-III (μg/mL)		Population-IV (μg/mL)		Population-V (μg/mL)		Pe[a] (μg/mL)	Ge[b] (μg/mL)
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MIC
E. rhusiopathiae	31.2	62.5	250	62.5	125	31.2	250	62.5	125	62.5	250	62.5

[a]Pe: penicillin, [b]Ge: gentamicin.

4. Discussion

The results of the present study indicated the essential oil components of various populations of *M. communis* can be varied with genetic (landrace), environmental conditions and geographic origin[27]. The essential oils of *M. communis* were characterized by high levels of oxygenated monoterpenes (24.7%–66.9%) including 1,8-cineole, linalool and α-terpineol, followed by monoterpene hydrocarbon (22.3%–58.5%) including α-pinene and limonene. These monoterpenes are widespread components of the essential oils and used as fragrances and flavours in the cosmetic, perfume, drug and food industries. Comparison of our results with literature data allows our samples to be assigned to the chemotype α-pinene/1,8-cineole because of the high content of these two compounds[7]. Other studies showed that among the constituents of the essential oil of leaves and fruits of *M. communis*, the myrtenol, myrtenal and myrtenyl acetate presented[28-31]. The essential oil that we used for antimicrobial *in vitro* assay contained a high quantity of monoterpenes that according to literature do have antimicrobial activity. The antibacterial activity of *M. communis* essential oil may be attributed to the high level of α-pinene, a compound with known antimicrobial properties. There are published papers dealing with antimicrobial activity of essential oil principal components, such as α-pinene[32]. Regarding the mechanism of action of 1,8-cineole, once the phenolic compound crossed the microbial cellular membrane, interactions with membrane enzymes and proteins would cause an opposite flow of protons, affecting cellular activity[33,34]. The mechanisms by which essential oil can inhibit microorganisms vary. In some cases it may be due to the hydrophobicity of the chemical (oil) which penetrates into the lipid bilayer of the cell membrane and makes the cells more permeable, leading to leakage of vital cell contents[35,36]. This property could be resulted from the relatively high amount of monoterpenes (α-pinene and 1,8-cineole) in the essential oils of various populations especially population-V. In conclusion, this study demonstrates that products with valuable antibacterial activity can be produced from leaves of *M. communis* against *E. rhusiopathiae*. The essential oil of *M. communis* can be used as an alternative preservative instead of synthetic ones in veterinary pharmacy industry.

Conflict of interest statement

We declare that we have no conflict of interest.

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Comments

Background

Many infections or diseases can be transmitted directly or indirectly between domestic animals and humans, for instance by consuming contaminated foodstuffs or through contact with infected animals, especially erysipelas. The need for new antimicrobial agents in the treatment of this animal disease is evident.

Research frontiers

The current investigation evaluates the *in vitro* antibacterial activity of essential oils of the leaves of *M. communis* against *E. rhusiopathiae* and their chemical composition were determined.

Related reports

E. rhusiopathiae causes an animal disease, erysipelas a superficial infection of the skin. In Iranian folk medicine, *M. communis* has been used as an infusion for various purposes such as for the skin discords, anti-septic (smoking), women diseases, wound (antimicrobial), *etc*.

Innovations and breakthroughs

Myrtus oil is used with great benefit in generally problematic skin. In the present study, authors have demonstrated the *in vitro* antibacterial activity of leaves oils of *M. communis* against *E. rhusiopathiae*.

Applications

The present study support and suggest the use of the essential oil of *M. communis* as an alternative preservative instead of synthetic ones in veterinary pharmacy industry.

Peer review

The authors of this important research have proved that essential oils of *M. communis* are potential and promising antibacterial agents which could be used as antibiotic in the protection of domestic animals and humans against *E. rhusiopathiae*. This conclusion was the result of chemical composition and antibacterial activity investigation.

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