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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.
Details on Page S508

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