

## Chemical composition and antibacterial activity of the essential oils of Algerian *Myrtus communis* L.

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### Abstract

*Myrtus communis* L. leaf essential oils from Algeria were analyzed by gas chromatography (GC) and gas chromatography/mass spectrometry (GC/MS). A total of thirty-four components were identified accounting for 95% of the oil. The main compounds were limonene (23.4%), linalool (15.4%), geranyl acetate (10.9%),  $\alpha$ -pinene (10.7%), linalyl acetate (8.2%) and 1,8-cineole (6.6%). The antimicrobial evaluation showed that myrtle oil exhibited good antibacterial activity against *Staphylococcus aureus*, *Proteus mirabilis* and *Klebsiella pneumonia*, but differed according to the strains. Conversely, it was not active against *Pseudomonas aeruginosa*.

### Keywords

*Myrtus communis* L., essential oil, chemical composition, antibacterial activity

### 1. Introduction

The genus *Myrtus* comprises about fifty species native to the Mediterranean basin (1). In Algeria, *Myrtus communis* is known as 'rayhan' and is present in Bissa, W. Chlef (Tell) and on coastal areas from east to west (2). The desert species, *Myrtus nivellei*, is commonly found in Hoggar and Tassili. Its leaves are highly appreciated by the Touareg as a herbal medicine (3). Several studies have investigated the chemical composition of myrtle oil from leaves (4, 5). Nevertheless, only a small number of studies have investigated the pharmacological effects of the plant or its specific ingredients (6). The chemical composition of myrtle oils from various origins has been reported in the literature. A classification of *M. communis* essential oil was proposed by Bradesi et al. (7): myrtle oil can be categorized into two chemotypes on the basis of myrtenyl acetate content. Each chemotype can be further divided into two subgroups, according to the relative ratio of  $\alpha$ -pinene to myrtenyl acetate or  $\alpha$ -pinene to cineole. Myrtle contains unique oligomeric, non-prenylated acylphloroglucinols such as myrtucommulone (MC) and semimyrtucommulone (S-MC) (8). MC contained in the leaves of *M. communis* has been reported to suppress the biosynthesis of eicosanoids by inhibition of 5-lipoxygenase and cyclooxygenase-1 *in vitro* and to inhibit the release of

elastase and the formation of reactive oxygen species in activated polymorphonuclear leukocytes (6). MC acts as antioxidant and shows antibacterial activity against clinically relevant bacteria (8). As regards *M. communis* essential oil, Yadegarinia et al. (9) reported the antimicrobial activity against *Escherichia coli*, *Staphylococcus aureus* and *Candida albicans*.

The aim of the present study was to determine the chemical composition of essential oil extracted from myrtle leaves collected in the region of Chlef (North West of Algeria). To the authors knowledge, the myrtle essential oil of this region was not studied before and its antibacterial properties are not known. This study will contribute to the valorization of medicinal and aromatic plants of the Algerian flora.

## 2. Experimental

### 2.1 Plant materials and microorganisms

Fresh leaves of *M. communis* L. were collected in forest of Bissa (longitude 1°21'36"E and latitude 36°08'94"N) (Chlef, Algeria), in June 2010. A voucher specimen has been deposited in the Herbarium of the Plant Ecology of University d'Es-Senia, under voucher number 1856. Samples were air-dried in shade for seven days. The bacterial species and strains used in this study were: *Staphylococcus aureus* ATCC 25923; *Proteus mirabilis* ATCC 43862; *Proteus mirabilis*; *Pseudomonas aeruginosa* ATCC 27853; and *Klebsiella pneumoniae* ATCC 35657. The strains were revived from frozen (−70°C) stocks and subcultured for purity. Bacteria were grown on nutrient agar and incubated at 37°C for 24 hours.

### 2.2 Essential oil extraction

The leaves of *M. communis* L. were dried at room temperature (20–25°C). The essential oils were extracted by hydrodistillation using a Clevenger-type apparatus. The essential oil from myrtle leaves used for the antibacterial tests were extracted as follows: 70 g of grinded leaves + 700 mL of distilled water for 3 hours 30 minutes. The extracted oils were dried and stored at 4°C until use.

### 2.3 Gas chromatography (GC-FID)

The analysis of the oil was carried out on a HP GC 6890A gas chromatograph equipped with flame ionization detector (FID) and using a capillary column coated with 5% phenyl methyl siloxane (30 m × 0.25 mm × 0.25 µm film thickness); column temperature 40°C (1 minute) to 200°C at 6°C/minute, 200–280°C at 30°C/minute, 280°C (2 minutes). Injector (split/splitless) temperature 280°C; detector temperature 300°C, injection mode, splitless; volume injected, 1 µL of diluted oil (10 mg of oil/5 mL diethyl ether). The carrier gas was helium with constant flow of 1 mL/minute.

### 2.4 Gas chromatography–mass spectrometry (GC-MS)

GC/MS was conducted using an Agilent 5973 GC/MS coupled to an Agilent 6890 gas chromatograph fitted with a split–splitless injector at 250°C (splitless mode). The analytical conditions were fixed as follows: Agilent HP-5MS capillary column (30 m × 0.25 mm, df=0.25 µm); temperature programme: from 40° to 250°C at 6°C/minute; mobile phase: He at 1 mL/minute. The mass spectra were recorded in electron impact (EI) mode at 70 eV (scanned mass range: 35–500 amu). Source and quadrupole temperatures were fixed at 230° and 150°C, respectively. The identification of the components was performed on the basis of chromatographic retention indices (RIs) and by comparison of the recorded spectra with computed data libraries (Wiley 275.L). For sesquiterpene hydrocarbons, further confirmations were obtained by comparing the mass spectra with data from the literature. RI values were measured on an HP-5MS column. For RI calculation, a mixture of homologues *n*-alkanes (C<sub>7</sub>–C<sub>30</sub>) was used, under the same chromatographic conditions.

## 2.5 Antibacterial tests

*In vitro* antibacterial activity of the essential oils of myrtle was evaluated by the disc diffusion method, minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) according to Motyl et al. (10). All tests were performed in Mueller–Hinton broth and oil extracts were weighed and dissolved in 3.2% dimethylsulfoxide (DMSO), 10 mg/mL, followed by sterilization using a 0.45- $\mu$ m membrane filter. Each microorganism was suspended in sterile saline (0.9%) solution and diluted to 10<sup>5</sup> CFU/mL. The sterilized Whatman filter paper No. 1 discs (6 mm diameter) were thoroughly moistened with 20  $\mu$ L of different concentrations of oils. The commercially available standard antibiotics (ciprofloxacin 5  $\mu$ g, trimethoprim/sulfamethoxazole 1.25/23.75  $\mu$ g and gentamicin 10  $\mu$ g) were also tested under similar conditions against all tested bacteria. The antibacterial activity of oils and antibiotics was demonstrated by a clear zone of inhibition around the disc. The zone of inhibition was measured after 24-hour incubation at 37°C.

## 3. Results and discussion

The essential oil yields obtained of *M. communis* leaves and physicochemical properties are given in Table 1. The myrtle oil yield was 0.6  $\pm$  0.1% ( $n=03$ ). This value is close to those reported in the literature, 0.3% for a north-center region *M. communis* (5) and 0.2–1.2% for the north-eastern region (11). Tuberoso et al. (12) reported a yield of 0.52% for Sardinian *M. communis*, and 0.61 and 0.40 for two *M. communis* varieties (*beatica* and *italica*) studied in Tunisia (13), and Bouzouita et al. (14) reported the yield of 0.5% for *M. communis*. The yields for leaves of Portuguese myrtle (15) and Moroccan myrtle (16) varied from 0.3% to 0.7% and from 0.3% to 0.4%, respectively. The results show a strong correlation between the extraction yield and the vegetative cycle of the plant, with a maximum synthesis of essential oil at the flower stage. Jamoussi et al. (17) showed that the yield of essential oil reached a peak at the beginning of the flowering stage (0.54%) and a minimum after the flowering period (0.27%).

**Table 1. Physicochemical characteristic of the essential oil of Algerian *Myrtus communis* L.**

The major chemical constituents of *M. communis* leaf oils and their relative amounts were determined by GC and GC/MS analysis. The relative contents (%) are shown in Table 2. Thirty-four components were identified for *M. communis*, representing 95% of the total oil. The main compounds were limonene (23.4%), linalool (15.4%), geranyl acetate (10.9%),  $\alpha$ -pinene (10.7%), linalyl acetate (8.2%) and 1,8-cineole (6.6%). Oxygenated monoterpenes was the predominant chemical group (50.5%) in *M. communis*, followed by the monoterpenes (40.5%), whereas the sesquiterpenes (1.9%) and oxygenated sesquiterpenes (2.1%) were low. The chemical composition of myrtle oils from various origins has been reported in the literature. Recent reports have been shown that Turkian myrtle oil contains 1,8-cineole (36.1%),  $\alpha$ -pinene (22.5%) and linalool (8.4%) (18), whereas  $\alpha$ -pinene and 1,8-cineole are the main components of Italian samples (12). According to our results, Algerian myrtle oil is characterized by the lack of myrtenyl acetate. The results also show that the oil is rich in limonene, linalool, geranyl acetate and  $\alpha$ -pinene.

**Table 2. Composition of the leaves essential oil of Algerian *Myrtus communis* (mean of triplicates).**

Variable zones of microbial growth inhibition by various dilutions of *M. communis* L. essential oil were noted. The MBCs and MICs of *M. communis* leaf oils against bacteria are presented in Table 3. The essential oil of *M. communis* has an inhibitory effect on the growth of all pathogenic bacteria tested except *P. aeruginosa*, showing resistance at all doses applied. The two strains of *Proteus mirabilis* are the most susceptible, with an MIC of 0.12 μg/mL and an MBC of 16 μg/mL for the reference strain. The application of a dose of 64 μg/mL proved moderately active against *S. aureus* and *K. pneumonia*, with inhibition zones of 12 and 10 mm, and an MIC of 4 μg/mL. This result reflects what is generally reported in literature, which showed that Gram-negative bacteria are always more resistant to the treatment than Gram-positive strains. The results of Yadegarinia et al. (9) from the disc diffusion method and determination of MICs and minimum lethal concentrations (MLCs), indicate that *S. aureus* and *E. coli* were almost equally affected by Iranian myrtle oil, with a mean inhibition zone of 10–13 mm., and MIC and MLC values of 4 and 8 μg/mL, respectively. Several authors reported no inhibitory effect of Corsican (19) and Iranian myrtle oil (4) on *P. aeruginosa*. The important chemical constituents of Algerian *M. communis* oil are limonene, linalool, α-pinene, geranyl acetate, linalyl acetate and 1, 8-cineole. The high monoterpenes hydrocarbons such as α-pinene and limonene seem to contribute to the strong antimicrobial activity of *M. communis* (4).

**Table 3. Inhibition of bacterial growth by essential oils (mean of triplicates).**

The available data suggest that essential oils may disrupt the permeability barrier of cell membranes. The investigations of Sikkema et al. (20) have shown that the site of action of cyclic hydrocarbons, including terpene hydrocarbons, is at the cell membrane. Terpene hydrocarbons like α-pinene, β-pinene, γ-terpinene and limonene are found to affect the structural and functional properties of artificial membranes. These compounds were shown to permeate the membranes, making them swell. This inhibited respiratory enzymes, which led to a partial dissipation of the pH gradient and electrical potential, each being crucial to the energy system in a cell. It was suggested that the free OH group possessed by the alcohols may be a key to their activity (21). In bacteria, the permeabilization of the membranes is associated with loss of ions and reduction of membrane potential, collapse of the proton pump and depletion of the ATP pool (22). Knobloch et al. (23) have found that essential oils cause a deterioration of the cytoplasmic membrane. Analyses of the lipid profiles by GC and of the cell envelope structure by scanning electron microscopy of several bacteria treated by some essential oil constituents showed a strong decrease in unsaturated and an increase in saturated fatty acids, as well as alterations of the cell envelopes (24). Damage to the cell wall and membrane can lead to the leakage of macromolecules and to lysis. A review of Bakkali et al. (25) has indicated that, in results from the literature on polyphenolic and phenolic compounds, it can be inferred that essential oils can act mainly as pro-oxidants by intermediate of their volatile constituents like phenolic constituents, terpenes or terpenoids, which turn themselves into pro-oxidants.

The major constituents of Algerian myrtle oil extracted in June were as follows: limonene, linalool, α-pinene, geranyl acetate, linalyl acetate and 1,8-cineole. These findings suggest that the investigated essential oils have strong antibacterial activity against the human pathogenic bacteria tested and need exploitation as an alternative source of natural antibacterial agents.

## Notes

tr, traces (<0.1%); RI<sup>a</sup>, retention indices (26); RI<sup>b</sup>, retention indices relative to C<sub>7</sub>–C<sub>30</sub> on the HP-5MS capillary column. Monoterpenes: 40.5; oxygenated monoterpenes: 50.5; sesquiterpenes: 1.9; oxygenated sesquiterpenes: 2.1; identified compounds: 95.0.

The zone of inhibition (ZI) < 7 mm: inactive oil. ZI (7–14 mm): moderately active oil; ZI > 14 mm: highly active oil. MIC, minimum inhibitory concentration (µg/mL); MBC, minimum bactericidal concentration (µg/mL); 1: ciprofloxacin (5 µg); 2: trimethoprim/sulfamethoxazole (1.25/23.75 µg); 3: gentamicin (10 µg).

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