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*Full Length Research Paper*

# Antibacterial activity and composition of the essential oils of *Eucalyptus camaldulensis* Dehn. and *Myrtus communis* L. growing in Northern Cyprus

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**Water-distilled essential oils from leaves of *Eucalyptus camaldulensis* Dehn. and *Myrtus communis* L., collected from Northern Cyprus, were analysed by GC-MS. The major constituents of the essential oil from *E. camaldulensis* Dehn. were ethanone (25.36%), eucalyptol (13.73%), β-caryophyllene (11.55%) and carvacrol (9.05%). Eucalyptol (50.13%) was identified as the main constituent of the essential oil of *Myrtus communis* L. The other important components were linalool (12.65%), α-terpineol (7.57%) and limonene (4.26%). *M. communis* showed some activity on Gram positive and Gram negative bacteria while *E. camaldulensis* was found to have a low activity. The higher efficacy of *M. communis* was confirmed by the agar dilution method.**

**Key words:** *Eucalyptus camaldulensis* Dehn., *Myrtus communis* L., Myrtaceae, essential oil composition, gas chromatography, antibacterial activity.

## INTRODUCTION

Development of microbial resistance to antibiotics is a global concern. Isolation of microbial agents less susceptible to regular antibiotics and recovery of increasing resistant isolates during antibacterial therapy is rising throughout the world which highlights the need for new principles. The use of essential oils as functional ingredients in foods, drinks, toiletries and cosmetics is gaining momentum, both for the growing interest of consumers in ingredients from natural sources and also because of increasing concern about potentially harmful synthetic additives (Reische et al., 1998). Within the wide range of the above-mentioned products, a common need is availability of natural extracts with a pleasant taste or fragrance combined with a preservative action, aimed at avoiding lipid deterioration, oxidation and spoilage by microorganisms. Until recently, essential oils have been studied mostly from their flavor and fragrance viewpoints only for flavoring foods, drinks and other goods. Actually, however, essential oils and their components are gaining increasing interest because of their relatively safe status, their wide acceptance by consumers and their exploitation for potential multi-purpose functional use (Ormancey et al., 2001).

Aromatic plants dominate in Mediterranean-type ecosystems. *Eucalyptus camaldulensis* Dehn. (Myrtaceae) is a mediterranean species. This species is a well-known aromatic and medicinal plant. *E. camaldulensis* is a perennial, single-stemmed, large-boled, medium-sized to tall tree to 30 m high (Bren and Gibbs, 1986), although some authors (e.g. Boland, 1984; Brooker et al., 2002) record trees to 45 m. Eucalyptus is one of the world's important and most widely planted genera. Among its main uses is the production of essential oils, which are used for medicinal and pharmaceutical purposes (Ghisalberti, 1996; Leung and Foster, 1996). The composition of the essential oils from *E. camaldulensis,* especially from the leaves, has been widely studied. Thus, the first two main components were spathulenol and *p*-cymene detected in trees from Morocco (Zrira and Benjilali, 1996), 1,8-cineole and *β*-pinene from Mozambique (Pagula et al., 2000), *p*-cymene and spathulenol from Jerusalem (Chalchat et al., 2001) and 1,8-cineole and limonene from Burundi (Dethier

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et al., 1994). There are reports on the composition of volatiles from the immature flowers (Giamakis et al., 2001) and the fruits (Tsiri et al., 2003).

*Myrtus communis* L. (Myrtaceae) is an annual plant that has been used since ancient times for medicinal, food and spices purposes. The leaves contain tannins, flavonoids such as quercetin, catechin and myricetin derivatives and volatile oils (Baytop, 1999; Romani et al., 1999). The fruits of this plant are mostly composed of volatile oils, tannins, sugars, flavonoids and organic acids such as citric and malic acids (Baytop, 1999; Martin et al., 1999). In past times, ripe fruits were used as food integrators because of their high vitamin contents. The fruit decoction was used to bath new-borns with reddened skin, while the decoction of leaves and fruits was useful for sore washing. The decoction of the leaves is still used for vaginal lavage, enemas and against respiratory diseases (Maccioni et al., 1994 - 1995; Marchini and Maccioni, 1998). The essential oil obtained from this species has been widely investigated. Its composition is quite variable (Lawrence, 1976 - 1977, 1979 - 1980, 1990, 1993a, 1993b, 1996). One of the main constituents of myrtle essential oil is 1,8-cineole (Bradesi et al., 1997). The essential oil obtained from the leaves by steam distillation is also important in perfumary (Baytop, 1999). The oil in leaves of *M. communis* growing in Turkey contains 1,8-cineole, linalool, myrtenyl acetate and myrtenol as major components (Ozek et al., 2000).

Northern Cyprus is rich in aromatic plants. The majority of the people living the countryside of the region use naturally grown plants for medicinal purposes. It is therefore, important to find out the medicinal value of those plants scientifically. In the present work, the composition of the essential oil from leaves of *E. camaldulensis* and *M. communis* grown in Northern Cyprus is studied.

## MATERIALS AND METHODS

### Plant materials

Leaves of *E. camaldulensis* and *M. communis* were collected from the Famagusta region of Northern Cyprus, in October 2000.

### Preparation of essential oil

The plant samples were separately water distilled in a Clevenger-type apparatus for 3 h.

### Gas chromatography

GC-MS analyses of volatile components were carried out on a MSD 5973 mass spectrometer,which was connected to a Agilent GC-6890 gas chromatography, equipped with a carbowax (30m x 320um id x 0.25). oven temperature was held at 50ºC for 3.3 min and then programmed from 50 to 240ºC at 3 /min. The carrier gas was helium with 1.2 ml/min flow rate for volatile constituents. Split ratio was 20:1. Identification of the constituents was based on comparison of the retention times (relative *n*-hydrocarbons and computer matching of the mass spectra with the National Bureau of Standards mass spectral library, as well as by comparison of the fragmentation patterns of the mass spectra with those reported in the literature (Jennings and Shibamoto, 1980; Adams, 1995).

### Organisms and media

Test organisms used in this study were as follows: *Staphylococcus aureus* ATCC 6538P, *Listeria monocytogenes* ATCC 7644, *Enterococcus durans* wild type, *Salmonella typhi* clinical isolate, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 9027 and *Bacillus subtilis* ATCC 6633. For the antimicrobial tests, Muller Hinton Agar and Muller Hinton Broth (Oxoid) was used.

### Disc diffusion test

Overnight broth cultures, adjusted to yield approximately 5.0 x $10^7$ cfu $ml^{-1}$ were streaked with a calibrate loop on plates containing solid medium. Filter paper discs (6 mm diameter) (Oxoid) were placed on the inoculated agar surfaces and impregnated with 10 µl of essential oil. The plates were observed after 18 h at 37°C. All tests were performed in duplicate and the antibacterial activity was expressed inhibition diameters (mm) produced by essential oils. Methicillin disc (5 µg), norfloxacin disc (10 µg) and gentamicin disc (10 µg) were used as positive control.

### Minimum inhibitory concentration (MIC)

The minimum inhibitory concentration of essential oils was determined by an agar dilution method according to the National Committee for Clinical Laboratory Standard NCCLS guidelines using a multipoint replicator and delivering 0.3 µl of standardized microbial suspension. The final concentration of essential oils in the medium ranging from 1% to 0.0625% v/v. The plates were incubated at 37 for 18 - 24 h and the MIC was defined as the lowest concentration of essential oil inhibiting the visible growth. All determinations were performed in duplicate and growth control consisting of MH medium was included.

## RESULTS

### Chemical composition of the essential oils

The results obtained by GC–MS analysis of the essential oils of *E. camaldulensis* and *M. communis* are presented in Tables 1. Twenty two constituents, which represented 82.09% of the total essential oil of *E.camaldulensis* leaves and sixteen constituents which represented 87.86% of the total essential oil of *M. communis* leaves were identified. As a result of GC–MS analyses, *E. camaldulensis* contained ethanone (25.36%), eucalyptol (13.73%), β-caryophyllene (11.55%) and carvacrol (9.05%) as the major compounds and pulegone (0.14%), thujone (0.16%), γ-terpinene (0.17%), nerolidol (0.19%) as the minor components. Eucalyptol was the predominant component (50.13%), linalool (12.65%), α-terpineol (7.57%) and limonene (4.26%). were the major compounds and nerol (0.24%), terpinene 4-ol (0.37%), 8-hydroxycarvatanacetone (0.47%) of *M. communis* oil.

**Table 1.** Compositions of the essential oils obtained from leaves of *E. camaldulensis* and *M. communis* collected in Northern Cyprus.

| Constituents | *E. camaldulensis* (%) | *M. communis* (%) |
|---|---|---|
| Camphene | 0.23 | - |
| β-Pinene | 0.24 | 0.92 |
| γ-Terpinene | 0.17 | _ |
| Eucalyptol | 13.73 | 50.13 |
| p-Cymene | 4.80 | 0.97 |
| Thujone | 0.16 | - |
| Linalooloxide | 0.21 | 2.48 |
| Camphor | 1.18 | - |
| Linalool | 1.01 | 12.65 |
| Terpinene-4-ol | 3.32 | 0.37 |
| Valencene | 1.95 | - |
| β-Caryophyllene | 11.55 | - |
| Pulegone | 0.14 | - |
| α-Terpinene | 4.57 | - |
| Guaiyl acetate | 0.20 | - |
| Caryophyllene oxide | 0.22 | - |
| Nerolidol | 0.19 | - |
| Ledol | 0.63 | - |
| Bisabolene | 0.40 | - |
| Thymol | 1.19 | 1.10 |
| Ethanone | 25.36 | 0.62 |
| Carvacrol | 9.05 | - |
| Isobutyl isobutyrate | - | 0.50 |
| Limonene | - | 4.26 |
| Exo-2-hydroxycineole acetate | - | 1.05 |
| α-Terpineol | - | 7.57 |
| Neryl acetate | - | 3.88 |
| Nerol | - | 0.24 |
| Geraniol | - | 0.65 |
| 8-hydroxycarvotanacetone | - | 0.47 |

-: Absent

## Antimicrobial activity

Antimicrobial activity was assayed against seven pathogen bacteria (*S. aureus*, *L. monocytogenes*, *E. durans*, *S. typhi*, *E. coli*, *Ps. aeruginosa* and *B. subtilis*). These microorganisms have different growth properties. The results of disc diffusion test and minimum inhibitory concentration of essential oils are listed in Tables 2 - 3. *M. communis* showed some activity on Gram positive and Gram negative bacteria while *E. camaldulensis* was found to have a low activity. The higher efficacy of *M. communis* was confirmed by the agar dilution method.

## DISCUSSION

The major components of the essential oil of *E. camuldensis* were ethanone (25.36%), eucalyptol (13.73%), β-caryophyllene (11.55%) and carvacrol (9.05%). However, the major components were 1,8-cineole, β-pinene and spathulenol (Tsiri et al., 2003; Zrira and Benjilali, 1996; Pagula et al., 2000). The chemical composition depends on the plant origin. In the essential oil of *M. communis* species, eucalyptol was the predominant component (50.13%). The other important components were linalool (12.65%), α-terpineol (7.57%) and limonene (4.26%). The major components were α-pinene, limonene and 1,8-cineole of the essential oil of leaves of *M. communis* (Flamini et al., 2004; Bouzouita et al., 2003, 1998; Tuberoso et al., 2006). We did not find α-pinene and 1,8-cineole. Scora (1973) reported that environmental factors such as geography, temperature, day length, nutrients, etc, were considered to play a key role in the chemical composition of myrtle oil. These factors influence the plant's biosynthetic pathways and consequently the relative proportion of the main characteristic compounds. This leads to the existence of different

**Table 2.** Disc diffusion test.

| Essential oil | Inhibition zone (∅ mm) | | |
|---|---|---|---|
| | ***S. aureus*** | ***E. coli*** | ***Ps. aeruginosa*** |
| *Eucalyptus camaldulensis* | 14 | - | - |
| *Myrtus communis* | 15 | 15 | 7 |
| Meticillin 5µg | 31 | | |
| Norfloxacin 10 µg | - | 30 | - |
| Gentamicin 10 µg | - | - | 21 |

-: No inhibition zone.

**Table 3.** Minimum inhibitory concentration (MIC).

| Essential oils | MIC (%, v/v) | | | | | | |
|---|---|---|---|---|---|---|---|
| | ***S. aureus*** | ***B. subtilis*** | ***E. durans*** | ***L. monocytogenes*** | ***E. coli*** | ***P. aeruginosa*** | ***S. typhi*** |
| *E. camaldulensis* | 0.5 | >1 | >1 | >1 | >1 | >1 | nd |
| *M. communis* | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 | >1 | 0.5 |

nd: Not detected.

origins, as well as seasonal variation throughout the plant's vegetative cycle (Flamini et al., 2004).

The results presented in this study (Tables 2 and 3) are in line with the small number of published papers on the effects on bacterial growth of *M. communis* and *E. camaldulensis*. Ghalem and Mohamed (2008) reported the major activity of essential oils from leaves of *E. camaldulensis* against *S. aureus* than that against *E. coli*. With regard to the *M. communis,* Salvagnini et al. (2008) have reported the antimicrobial activity of essential oil of leaves against *S. aureus, S. epidermidis, E. coli, B. subtilis* and *Serratia marcescens.* Yadegarinia et al., (2006) have demonstrated the activity of *M. communis* L. essential oil against *E. coli, S. aureus* and *Candida albicans.* However, it is difficult to compare the data with the literature because several variables influence the results, such as the different chemical composition due to the environmental factors (such as geography, temperature, day length, nutrients, etc) of the plant. However, the high proportions of eucalyptol (13.73%), ß-caryophyllene (11.55%), ethanone (25.36%) and carvacrol (9.05%) in *E. camaldulensis* and the large amounts of eucalyptol (50.13%), linalool (12.65%) and α-terpineol (7.57%) in *M. communis* could be responsible for the activity observed. Several authors have been pointed to the antimicrobial activity of carvacrol (Curtis et al., 1996; Kim et al., 1995); it interacts with the cell membrane, disrupting it (Thompson, 1996; Hollman, 2001). Linalool has previously been reported (Carson and Riley, 1995) as having antimicrobial activity.

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