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Chemical composition and biological activities of leaf and fruit essential oils from *Eucalyptus camaldulensis*

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Abstract: The chemical composition of the essential oils from the leaves and fruit of *Eucalyptus camaldulensis* grown in Mersin, Turkey was analyzed using gas chromatography (GC) and gas chromatography-mass spectrometry (GC-MS) techniques. The biological activities (antibacterial and antifungal) were examined using the agar well diffusion method. The main leaf oil constituents were *p*-cymene (42.1%), eucalyptol (1,8-cineole) (14.1%), α-pinene (12.7%) and α-terpinol (10.7%). The main constituents of the fruit oil were eucalyptol (1,8-cineole) (34.5%), *p*-cymene (30.0%), α-terpinol (15.1%) and α-pinene (9.0%). Our results showed that both types of oils are rich in terms of monoterpene hydrocarbons and oxygenated monoterpenes. The leaf and fruit essential oils of *E. camaldulensis* significantly inhibited the growth of Gram-positive (*Staphylococcus aureus* and *Bacillus subtilis*) and Gram-negative (*Escherichia coli* and *Streptococcus* sp.) bacteria ($p < 0.05$). The oils also showed fungicidal activity against *Candida tropicalis* and *C. globrata*. Leaf essential oils showed more activity than fruit essential oils, probably due to the higher p-cymene concentration in leaves.

Keywords: biological activities; essential oil; eucalyptol; *p*-cymene.

1 Introduction

Eucalyptus is one of the world's important and most widely planted genera belonging to the family Myrtaceae. This family includes 140 genera and about 3800 species grown in tropical and subtropical parts of the world [1, 2]. The Myrtaceae family represents an important source of essential oils with diverse biological activities including bacteriostatic, fungistatic and anti-inflammatory effects. Various Myrtaceae species possess strong antimicrobial potential and their volatile oils are used as antimicrobial and antifungal agents in creams, soaps and toothpastes [3, 4].

Eucalyptus is native to Australia, and the genus contains about 600 species [5]. A smaller number are also native to New Guinea, Indonesia and the Phillipines [6]. *Eucalyptus* is a fast-growing tree, and is suitable for use in paper production. There has been extensive overseas forest plantation of *Eucalyptus* trees. Leaves are a by-product of tree cutting, and the use of the excess leaves for biomass resources is considered to be an important research subject [5]. *Eucalyptus* is a monotypic genus in the flora of Turkey. Mainly an Australian genus, in Turkey *Eucalyptus camaldulensis* is grown commercially for timber and is naturalized, while several other species, including *E. robustus* Sm., *E. globolus* Lab., *E. tereticornis* Sm., are occasionally planted, mainly for ornamental reasons. *Eucalyptus camaldulensis* is a large evergreen tree that grows up to 15 m in Turkey where it is commercially grown, especially around Mersin and Adana. It is also extensively naturalized and invasive on roadsides, sand dunes, etc. [7].

Several species of *Eucalyptus* are used in folk medicine as an antiseptic and against infections of the upper respiratory tract, such as colds, influenza and sinus congestion. The antimicrobial, analgesic and anti-inflammatory properties of *E. citriodora*, *E. globulus*, *E. teretcorni* and *E. camaldulensis* have been reported from different parts of the world [8]. The essential oil of the *Eucalyptus* species shows a wide spectrum of antimicrobial [9, 10], antifungal [11, 12], anticandidal [13], antibacterial [14, 15], expectorant and cough stimulant activity [16]. Due to its disinfectant action, the essential oil is used externally and applied to cuts and skin infections but it has a deleterious effect on the body in high doses [17, 18]. Besides its antimicrobial uses, the essential oils and their constituents have also been used for their herbicidal [19, 20], insecticidal [21, 22], antihelmintic [23], anti-tumor [24] and anti-leeching [25] properties, as well as in integrated nonspecific disease skin infections [26] and mastitis [27, 28]. Rahimi-Nasrabadi et al. [29–31]

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have studied the chemical composition, antioxidant and antimicrobial activities of different *Eucalyptus* sp. essential oils and methanol extracts.

Eucalyptus extracts are often used in oral hygiene products (mouth rinses, toothpastes, anti-plaque chewing gums) and surface cleaning wipes etc. Many screening reports, using disc diffusion and dilution techniques, have established the antimicrobial activity of *Eucalyptus* extracts from various species against a number of pathogens including *Haemophilus influenzae*, *Streptococcus pneumoniae*, *Staphylococcus aureus, Escherichia coli*, *Aspergillus niger*, *Porphyromonas gingivalis*, *Streptococcus sobrinus, Streptococcus mutans, Salmonella typhimurium*, *Pseudomonas aeruginosa* and *Staphylococcus epidermidis* [32–37].

In this study, we aimed to identify the chemical composition of the leaf and fruit essential oils of *E. camaldulensis*, to show their antimicrobial activities on some bacteria and fungi and also to compare them with the *Eucalyptus* genus patterns.

2 Experimental

2.1 Plant material

The leaves and fruit of *E. camaldulensis* were collected from natural habitats in Mersin, in July 2015. The leaves and fruit were dried in the shade at room temperature. The voucher specimen for *E. camaldulensis* (Dogan 2439) has been deposited in the Fırat University Herbarium (FUH).

2.2 Extraction of essential oil

The essential oil was extracted by hydrodistillation using a modified Clevenger apparatus coupled to a 2 L round-bottom flask. A total of 100 g of fresh plant material (aerial parts) and 1 L of water were used for the extraction. The chemical analyses were performed in the Plant Products and Biotechnology Research Lab at Firat University. The extraction was performed over a 3-h period. The oil was transferred to black-colored vials, wrapped in parafilm and aluminum foil and stored at 4 °C until analysis. The yields of the oils were calculated on the basis of the dry mass.

2.3 Gas chromatography (GC) analysis

The essential oil was analyzed using a HP 6890 GC equipped with a flame ionization detector (FID) and a HP-5ms (30 m × 0.25 mm i.d., film thickness 0.25 μm) capillary column was used. The column and analysis conditions were the same as in GC-MS, as expressed below. The percentage composition of the essential oils was computed from GC-FID peak areas without correction factors.

2.4 Gas chromatography/mass spectrometry (GC-MS) analysis

GC-MS analyses of the oils were performed on a Hewlett Packard Gas Chromatography HP 6890 interfaced with a Hewlett Packard 5973 mass spectrometer system equipped with a HP-5ms capillary column (30 m × 0.25 mm i.d., film thickness 0.25 μm) (J&X Scientific). The oven temperature was programed from 70 to 240 °C at the rate of 5 C/min. The ion source was set at 240 °C and the electron ionization at 70 eV. Helium was used as the carrier gas at a flow rate of 1 mL/min. The scanning range was 35–425 amu. Diluted oil in *n*-hexane (1.0 μL) (Merck) was injected into the GC-MS. The identification of constituents was performed on the basis of the retention index (RI) determined by co-injection with reference to a homologous series of *n*-alkanes (C8–C25) [38] under identical experimental conditions [39]. Further identification was performed by comparison of their mass spectra with those from NIST 98 Libraries (on ChemStation HP) and Wiley 7th Version. The relative amounts of individual components were calculated based on the GC (HP-5ms column) peak area (FID response) without using correction factors. The identified constituents of the essential oils are listed in Table 1.

Table 1: Phytochemical composition of leaf and fruit essential oils from *E. camaldulensis*.

Name of Compound	RI	Leaf (%)	Fruit (%)
α-pinene	1020	12.7	9.0
p-cymene	1090	42.1	30.0
Limonene	1094	5.5	–
Eucalyptol (1,8-cineole)	1096	14.1	34.5
γ-terpinene	1115	–	5.1
Borneol L	1198	5.5	5.3
α-terpineol	1214	10.7	15.1
Spathulenol	1493	3.2	–
Monoterpene hydrocarbons		60.3	44.1
Oxygenated monoterpenes		30.3	54.9
Oxygenated sesquiterpenes		3.2	–
Total identified		93.8	99.0

RI, Retention index relative to C8–C25 n-alkanes on HP-5 column.

2.5 Antimicrobial activity

2.5.1 Microbial strains and culture media

All media were supplied by LAB Ltd (UK). All microbial strains were obtained from the Microbiology Laboratory, Department of Biology, Science Faculty of Firat University. Stock cultures of Gram-positive *S. aureus* (ATCC 6538P) and *Bacillus subtilis* (ATCC 6633), Gram-negative *E. coli* (ATTC 25922), *Streptococcus* sp. (ATCC 8059) and the yeasts *Candida tropicalis* (ATCC 13803) and *C. globrata* (ATCC 66032) were subcultured and maintained in nutrient broth at 37 °C for 24 h. Briefly, 100 µL of test bacteria/fungi were grown in 10 mL of fresh media until they reached a count of approximately 10^8 cells/mL for bacteria or 10^5 cells/mL for fungi. Then, 100 µL of microbial suspension was spread onto agar plates corresponding to the broth in which they were maintained.

2.5.2 Antimicrobial screening

The agar well diffusion method was employed for the determination of the antimicrobial activities of the essential oils and standard antibiotics [40]. The results of the essential oils were discussed with standard antibiotics as the positive controls [amikacin (30 µg) AK30, gentamicin (10 µg) CN10, tetracycline (30 µg) TE30 and netilmycin (30 µg) NET30]. All tests were performed in triplicate.

2.5.3 Agar well diffusion method

A suspension of the test microorganism, 0.1 mL of 10^8 cells/mL, was spread on the Muller Hinton Agar (MHA). The wells (6 mm diameter) were cut from the agar and different concentrations (10, 20 and 30 µL) of essential oils were delivered into them. After incubation for 24 h at 37 °C, all plates were examined for each zones of growth inhibition, and the diameters of these zones were measured in millimeters.

3 Results and discussion

3.1 Chemical composition of essential oil

This study evaluated the chemical composition and antimicrobial activities of the essential oil from the leaves and fruit of *E. camaldulensis* in Mersin, Turkey. The yields of the leaf essential oils were 1.2% and 1.0% (v/w) in fruit essential oils. A total of seven and six compounds were identified representing 93.8% and 99.0% of the total oils, respectively. The major constituents of the leaves were *p*-cymene (42.1%), eucalyptol (1,8-cineole) (14.1%), α-pinene (12.7%) and α-terpinol (10.7%), and in the fruit were eucalyptol (1,8 cineole) (34.5%), *p*-cymene (30.0%), α-terpinol (15.1%) and α-pinene (9.0%). The results of the GC-MS analysis of the oils are presented in Table 1.

It was previously reported that the main constituents of oil in *E. camaldulensis* were 1,8-cineole (21.75%), β-pinene (20.51%), α-pinene (15.6%) and terpineol (9.41%); spathulenol (37.46%), *p*-cymene (17.20%) and crypton (8.88%) in *E. gomphocephala*; spathulenol (18.37%), *p*-cymene (19.38%) and crypton (16.91%) in *E. camaldulensis* var. *obtusa* [41].

In the study by Elaissi et al., the main components in the essential oil of 15 *Eucalyptus* species were reported to be 1,8-cineole, followed by spathulenol [42]. Traore et al. also found 1,8-cineole, *p*-cymene, α-pinene, limonene, γ-terpinene and trans-pinocarveol to be the main compounds in the essential oils of *E. camaldulensis* from Mali [43]. The chemical composition of the hydrodistilled essential oils of leaves from three species of *Eucalyptus*, *E. spathulata, E. microtheca* and *E. torquata*, was analyzed and the predominant and common components were 1,8-cineole, α-pinene, terpine-4-ol, α-terpineol, aromadendrene and viridiflorol [44]. In the Rahimi-Nasrabadi et al. [30] study, the chemical composition of the essential oil from the leaves of *E. procera* cultivated in central Iran was analyzed. Forty-five constituents representing 99.6% of the oil were identified. The main constituents of the oil were found to be 1,8-cineole (35.9%), α-pinene (25.6%) and viridiflorol (7.7%). In a similar study, on the essential oil from the aerial parts of *E. loxophleba*, 39 compounds were identified representing around 98.0% of the total oil. The major constituents of the oil were found to be 1,8-cineole (39.4%), methyl amyl acetate (19.8%), aromadendrene (10.0%), viridiflorol (6.0%) and α-pinene (5.4%) [31]. It is possible to say that our results have shown congruency with the other *Eucalyptus* essential oil studies especially on major compounds.

3.2 Antibacterial and antifungal activities of essential oils

The antibacterial and antifungal activity results are summarized in Tables 2 and 3. The results indicate that the inhibition zones (IZs) resulting from the antibacterial activities ranged between 9 and 25 mm at 10, 20 and

Table 2: Antibacterial activity of *E. camaldulensis* leaf and fruit essential oils against Gram-positive and Gram-negative bacterial strains.

Bacterial strains	Essential oils						Antibiotic			
	Leaf oil (µg/mL)			Fruit oil (µg/mL)						
	10	20	30	10	20	30	NET	AK	CN	TE
S. aureus IZ (mm)	14±1.9	15±1.5	17±1.0	15±1.5	18±1.7	21±2.1	18±0.0	22±0.0	20±0.0	15±0.0
B. subtilis IZ (mm)	18±1.4	20±1.2	21±1.8	11±1.3	12±1.6	14±1.2	20±0.0	19±0.0	19±0.0	16±0.0
E. coli IZ (mm)	22±1.7	23±2.2	25±1.9	9±2.5	11±1.8	12±1.9	17±0.0	15±0.0	16±0.0	18±0.0
Streptoccus sp. IZ (mm)	18±1.3	19±1.8	22±2.5	10±1.2	18±1.6	18±1.1	16±0.0	17±0.0	15±0.0	19±0.0

IZ, Diameter of inhibition zone (mean ± SD) ($p < 0.05$); NET, netilmycin; AK, amikasin; CN, gentamycin; TE, tetracyclin.

Table 3: Antifungal activity of *E. camaldulensis* leaf and fruit essential oils against fungal strains.

Fungal strains	Essential oils						Antibiotic			
	Leaf oil (µg/mL)			Fruit oil (µg/mL)						
	10	20	30	10	20	30	NET	AK	CN	TE
C. tropicalis IZ (mm)	18±1.6	20±1.7	22±1.1	12±1.7	15±2.1	18±1.5	NA	NA	NA	NA
C. globrata IZ (mm)	19±1.5	20±1.0	23±1.7	13±1.9	18±1.6	20±1.3	NA	NA	NA	NA

IZ, Diameter of inhibition zone (mean ± SD) ($p < 0.05$); NA, not analyzed.

30 µg/mL oil concentrations ($p < 0.05$). Both essential oils showed antibacterial activity against the studied bacterial strains. The inhibition zones resulting from the antifungal activities ranged between 12 and 23 mm at 10, 20 and 30 µg/mL oil concentrations ($p < 0.05$).

Eucalyptus camaldulensis essential oil significantly inhibited the growth of Gram-positive (*S. aureus* and *B. subtilis*) and Gram-negative (*E. coli* and *Streptococcus* sp.) bacteria strains. Fruit essential oil showed strong antibacterial activity against the *S. aureus* (21±2.1), *Streptococcus* sp. (18±1.1), *B. subtilis* (14±1.2) and *E. coli* (12±1.9), respectively ($p < 0.05$) (Table 2). The fruit oil showed very effective fungicidal activity against *C. tropicalis* (18±1.5) and *C. globrata* (20±1.3) fungi ($p < 0.05$). The leaf essential oil showed strong antibacterial activity against *E. coli* (25±1.9), *B. subtilis* (21±1.8), *Streptococcus* sp. (22±2.5) and *S. aureus* (17±1.05), respectively ($p < 0.05$). The leaf oil showed efficacious antifungal activity against the *C. tropicalis* (22±1.1) and *C. globrata* (23±1.7) ($p < 0.05$). These essential oils contain major compounds 1,8-cineole and *p*-cymene excessively which are suggested to show very rich antimicrobial activities against many significant pathogens.

Nikbakht et al. [44] tested the antifungal activities of essential oils of five *Eucalyptus* species (*E. largiflorens*, *E. microtheca*, *E. oleosa*, *E. spathulata* and *E. torquata*) using the minimum inhibitory concentration and disc diffusion methods against *Aspergillus flavus*, *A. parasiticus*, *A. niger*, *Penicillium chryzogenum* and *P. citrinum*. A high antifungal activity was found in the leaf oil of *E. largiflorens*. They also reported the chemical composition, antioxidant and antimicrobial activities of essential oils and methanolic extracts of *E. largiflorens* and *E. oleosa* from Iran. The major constituents of the oil of *E. largiflorens* were 1,8-cineole, cryptone, terpinen-4-ol, 4-allyloxyimino-2-carene, α-pinene, α-terpinyl acetate, cuminic aldehyde and p-cymen-7-ol. The essential oil showed strong antibacterial activity against *E. coli*, *Salmonella typhimurium*, *Klebsiella pneumoniae*, *S. aureus, S. epidermidis*, *Bacillus cereus* and *B. subtilis*. Also, the main components of essential oil of *E. oleosa* were 1,8-cineole, α-pinene, α-terpineol and trans-pinocarveol. The essential oil showed strong antibacterial activity against the above mentioned microorganisms [44].

In the study of the antibacterial, antifungal and anticancer activities of volatile oils and methanolic extracts of the stems, leaves and flowers of *E. sideroxylon* and *E. torquata* species, it was found that the flower oil of *E. torquata* exhibited potent antifungal activity against *C. albicans* and showed a moderate antifungal activity against *C. albicans, A. flavus, and A. niger* [45].

Salem et al. showed that the antibacterial activities of the essential oils of three *Eucalyptus* species, *E. camaldulensis*, *E. camaldulensis* var. *obtusa* and *E. gomphocephala*, resulted in IZs between 10 and 18 mm at 2000 µL/mL oil concentration. All essential oils demonstrated good

antibacterial activity against the studied bacterial strains [41].

The antimicrobial activity of *E. citriodora* essential oil against pathogenic fungi, bacteria and drug-resistant mutants of *C. albicans*, *E. coli* and *Mycobacterium smegmatis* was evaluated following agar disc diffusion and broth dilution assay methods. Its effectiveness against *Trichophyton rubrum* followed by *Histoplasma capsulatum*, *C. albicans* and *Cryptococcus neoformans* was also assessed. It is reported that it is more active against Gram-positive than it is against Gram-negative bacteria and also showed more activity on drug-resistant mutants of *C. albicans* and *E. coli* [8]. Ghalem and Mohamed [46], demonstrated that leaf essential oils of *E. globolus* and *E. camaldulensis* exhibited inhibitory effects on *S. aureus* more than *E. coli*. Some correlation betweeen the amount of 1,8-cineole, *p*-cymene, α-pinene, of cryptone and the antibacterial activity were observed [47].

3.3 The relationship between antimicrobial activity and oil constituent

It is well demonstrated that the antimicrobial activity of an essential oil is linked to its chemical composition. The functional groups of compounds found in essential oils are associated with their antimicrobial characteristics [48]. It was reported that the antibacterial activity of *Eucalyptus* essential oils is generally due to components such as 1,8-cineole (eucalyptol), citronellal, citronellol, citronellyl acetate, *p*-cymene, eucamalol, limonene, linalool, β-pinene, γ-terpinene, α-terpineol, alloocimene and aromadendrene. For example several reports have shown that 1,8-cineole has strong antimicrobial activity against many important pathogens and spoilage organisms, including *S. aureus* and *Fusarium solani* [49]; *E. coli* and *B. subtilis* [50].

As a result of these findings, the antimicrobial activities of *E. camaldulensis* leaf and fruit oils could be attributed to eucalyptol (1,8-cineole), *p*-cymene, α-terpineol and α-pinene. The antimicrobial activities of these components have also been reported [32, 47, 51–53].

4 Conclusion

Our results showed that both oils are rich in terms of monoterpene hydrocarbons and oxygenated monoterpenes. This study demonstrates the occurrence of *p*-cymene/eucalyptol (1,8-cineole) and α-terpineol chemotype of *E. camaldulensis* in Turkey. This study clearly indicated that essential oils from both fruits and leaves of *E. camaldulensis* have antibacterial and antifungal effects on some pathogen microorganisms. These essential oils showed antimicrobial activity on both bacteria and fungi similar to the antibiotics used as positive controls. In some conditions the essential oils were determined to be more effective than the antibiotics. The major components of the essential oils, eucalyptol and *p*-cymene, could be responsible for these effects. Consequently, leaf essential oil appeared to be more effective than the fruit essential oil and this effect might be because of its rich *p*-cymene content.

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