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Antibacterial and Antioxidant Potential of Essential Oil of *Eucalyptus camaldulensis* and Its Major Components

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Abstract: The present study evaluated the antibacterial and antioxidant potential of the essential oil (EO) extracted from the fresh foliage of *Eucalyptus camaldulensis* Dehnh and its major constituents. The chemical profiling of the EO revealed that it is a mixture of monoterpenes (58.85%) and sesquiterpenes (39.16%). The GC-MS analysis revealed the presence of 25 chemical compounds constituting ~98% of EO with 1,8-cineole, *β*-pinene, alloaromadendrene, and *β*-eudesmol as the main constituents. The antibacterial potential of EO and three major consituents–1,8-cineole, *α*-pinene, and *β*-pinene was assessed against four bacteria: *Escherichia coli* (MTCC 2961), *Staphylococcus aureus* (MTCC 3160), *Pseudomonas aeruginosa* (MTCC 424), and *Bacillus subtilis* (MTCC 441). The antibacterial screening revealed that all the treatments except *α-pinene* were bioactive against the studied bacterial species. EO and its major constituents exhibited free radical scavenging and antioxidant activity in a concentration-dependent manner. The highest DPPH radical scavenging was observed with *α*-pinene (~72%) and the minimum (~63%) was observed in EO at the highest concentration (800 µg ml^{-1}). EO exhibited the highest potential (~52%) for reducing ferric ion as compared to its major constituents. At the lowest concentration (100 µg ml^{-1}), the reducing activity was ~10% in 1,8-cineole, ~13% in *α*-pinene, ~15% in *β*-pinene, and ~43% in EO. The maximum $^{\cdot}OH$ scavenging activity was found in 1,8-cineole in contrast to the minimum activity in the case of *β*-pinene. TAA ranged from 10–75% at the concentration range of 100–800 µg ml^{-1} of EO or its major components. Maximum H_2O_2 scavenging activity was found in *α*-pinene (~72%), whereas the minimum activity was noticed with EO (~61%) at the highest concentration. The study concludes that EO from *E. camaldulensis* and its major constituents possess antibacterial and antioxidant activity that can be exploited for industrial and pharmaceutical applications.

Keywords: Essential oil, Oxygenated monoterpenes, Sesquiterpenes, Antibacterial activity, Antioxidant potential

The traditional system of medicine since time immemorial is based on the usage of natural products originating from plants (Chaves et al 2018). Higher plants are a treasure of variety of phytochemicals that can combat the major fatal diseases (Dhakad et al 2018, Gwinn 2018). With the rapid increase in number of diseases and the growing interest in natural remedies as a cure, the extracts from different parts of medicinally important flora are widely being researched for their various bioactivities including the anticancer and antioxidant properties (Ashraf et al 2015, Ashrafi et al 2020). The natural compounds including the oils find their usage extensively in diverse sectors such as food and fragrance industry, aromatherapy, cosmetics, medicines, and agrochemicals. (Bakkali et al 2008, Pavela and Benelli 2016, Isman 2020, Irshad et al 2020) and are in much demand worldwide owing to their importance in medicines (Blowman et al 2018, Gwin 2018). The plant family Myrtaceae is the ninth largest among the flowering plant families, and includes trees and shrubs found growing abundantly in wet tropics, especially South America, Australia, and Tropical Asia (Casceas et al 2015). *Eucalyptus* is one of the largest genera of family Myrtaceae, which includes about 900 species and subspecies (Brooker and Kleinig 2006). Among the different *Eucalyptus* species, *E. camaldulensis* Dehnh. (formerly *E. rostrata* Schl.) . *E. camaldulensis* possesses EO in its various parts like leaves, buds, and fruits. EOs have been traditionally used in everyday life owing to their antiseptic, anti-inflammatory, and antipyretic properties. Despite their valuable relevance for the human consumption, there is a paucity of information on their usage relating to the pharmacological arena. The current study aimed at evaluating the chemical composition and determining Antibacterial and Antioxidant potential of the leaf essential oil of *Eucalyptus camaldulensis* and its major components.

MATERIAL AND METHODS

Plant material: The plant material, i.e. the leaves of *E. camaldulensis* Dehnh. were collected from the trees growing in the sector 14 campus of Panjab University, Chandigarh (30º45'34º N 76º45'59º E), India. The voucher specimen (PAN 21999) of the plant material was deposited in the Herbarium of the Botany department, Panjab University, Chandigarh, India.

Extraction of EOL: The freshly chopped leaves of *E. camaldulensis* were used for extraction of EO by the method of hydro-distillation using Clevenger's apparatus. About one

kg of plant leaves and 5L of distilled water were put in a round-bottom flask fitted with a condenser. After boiling the contents for about 4h, a pale-yellow aromatic, volatile oil was collected through the nozzle of the condenser. The oil obtained was dried over anhydrous sodium sulphate and refrigerated at 4ºC for its chemical profiling and the study of various bioactivities. The process of extraction of EO was repeated 3-4 times during the research work.

GC-MS: The chemical profiling of EO was determined through GC-MS (Gas chromatography-Mass spectrometry) analysis. GC was done on Shimadzu QP 2010 gas chromatograph equipped with a Flame Ionization Detector (FID) fitted with a ZB-5 column (30 m × 0.25 mm × 0.25 µm in length, diameter and thickness, respectively). Helium gas was used as a carrier gas with a flow rate of 1.05 ml min^{-1}. The split ratio was 1:10. The temperature of injector and ion source were fixed at 270°C. The ionization energy of mass spectra was 70 eV. The oven temperature was initially 100°C which was held isothermally for 2 min then increased to 200°C at the rate of 6 °C per min and finally held at 230°C for 19 min.

Chemical profiling of the oil: The relative percentage of different constituents was calculated automatically from the peak of total ion chromatograms. Constituents of the oil were identified by comparing their retention indices relative to a homologous *n*-alkane (C_9-C_{21}) series and matching their mass spectra with those of reference compounds in Wiley 275 and NBS 75 K libraries (Adams 2007).

Anti-bacterial activity: Antibacterial activity was assayed against four bacteria, *Escherichia coli* (MTCC 2961), *Staphylococcus aureus* (MTCC 3160), *Pseudomonas aeroginosa* (MTCC 424), and *Bacillus subtilis* (MTCC 441) using disk diffusion method. Each 1% nutrient agar plate was inoculated with 100 µl of 10^4 cfu ml^{-1} bacterial culture. To each sterile disk, 20 µl of sample was loaded. Antibiotic disk of norfloxacin of concentration 10 mcg $disc^{-1}$ was used as positive control. These plates were then incubated for 24 h at 37°C. After 24 h of incubation, the antibacterial activity of test samples was observed by measuring the zone of inhibition and noted to calculate the minimum inhibitory concentration (MIC) of the test samples. MIC was determined by sub-culture (as described above) as the lowest concentration resulting in the reduction in the number of organisms in inoculums. The tests were repeated at least three times and modal MIC values were selected.

Antioxidant and free radical scavenging activity: The antioxidant activity of EO was estimated by dissolving EO in methanol or acetone. Four different oil concentrations of EO were prepared, 100, 200, 400 and 800 µg ml^{-1}.

Total antioxidant activity (TAA): The evaluation of TAA of EO was done by using phosphomolybdenum method according to Prieto et al (1999).

Ferric ion reducing antioxidant power (FRAP): The FRAP activity of EO was evaluated following the method of Oyaizu (1986).

Hydrogen peroxide (H_2O_2) scavenging activity: Hydrogen peroxide scavenging activity of EO was evaluated as per the method of Ruch et al (1989).

Hydroxyl (OH) radical scavenging activity: The hydroxyl radical scavenging activity of EO was evaluated based on Fenton reaction as described by Yu *et al.* (2004) with slight modification.

DPPH (2, 2-diphenyl-l-picryl hydrazyl) radical scavenging activity: It was determined in terms of radical scavenging or hydrogen donating ability by measuring the scavenging activity against DPPH radical as per Blois et al (1958).

RESULTS AND DISCUSSION

GC-MS analysis of EO: GC-MS analysis revealed the presence of 25 chemical constituents, contributing ~98% of the total oil composition (Table 1). The main constituents identified in the oil were 1,8-cineole, *β*-pinene and *α*-pinene, analysis corresponding to their retention times (RT) of 7.88, 6.32, and 5.19 min, respectively The oil was a mixture of monoterpenes and sesquiterpenes, however, the relative percentage of monoterpenes was slightly higher. Among the monoterpenes, the fraction of hydrocarbons (36.38%) was more than the oxygenated ones (22.47%). The total percentage of the monoterpenes in the oil was 58.85% whereas sesquiterpenes constituted 39.16% of EO. As for the sesquiterpenes, its hydrocarbons accounted for 19.09% and the oxygenated sesquiterpenes were 20.07% (Fig. 1). Most abundant constituent of the oil was 1,8-cineole, an oxygenated monoterpene (17.14%) followed by *β*-pinene, a monoterpene hydrocarbon (16.48%), and aromadendrene, a sesquiterpene hydrocarbon (11.32%). In addition, significant quantities of *β*-eudesmol (10.85%), limonene (9.51%), and *α*-pinene (7.96%) were also present in the leaf oil of *E. camaldulensis.*

Antibacterial potential: Antibacterial screening of EO and its major components - 1,8-cineole, *α*-pinene, and *β*-pinene revealed that all the treatments except *α*-pinene were bioactive against the studied bacterial species (Table 2). MIC values of EO and its constituents varied from 9-27 mg ml^{-1} for each bacterial species. *S. aureus* and *P. aeruginosa* were most susceptible to 1,8-cineole and EO at MIC of 9.0 and 9.2 mg ml^{-1}, respectively . Similarly, *B. subtilis* was susceptible to 9.0 mg ml^{-1} of 1,8-cineole. One of the most resistant species was *E. coli* with MIC ranging from 17–27 mg ml^{-1} EO or its

major components. The inhibition zones of all the treatments were >10 mm, with the largest inhibition effect exhibited by 1,8-cineole for *P. aeruginosa* (Table 3). The smallest inhibition diameter was observed for *β*-pinene in *E. coli*. It was concluded that 1,8-cineole was the most effective antimicrobial agent found in EO.

Table 1. Chemical profiling of *E. camaldulensis* EO by GC-MS analysis

Constituent	RT[b]	RI_{cal}[c]	RI_{lit}[d]	Percentage
Monoterpene hydrocarbons (36.38%)				
α-Pinene	5.197	929.23	931	7.96
β-Pinene	6.325	973.21	975	16.48
β-Myrcene	6.541	980.73	978	0.96
p-Cymene	7.639	1018.20	1023	1.47
Limonene	7.798	1023.63	1025	9.51
Oxygenated monoterpenes				
1,8-Cineole	7.889	1026.68	1031	17.14
β-Citronellal	12.842	1170.71	1167	0.35
4-Terpineol	12.914	1172.60	1177	1.02
α-Terpineol	13.437	1186.03	1189	3.96
Sesquiterpene hydrocarbons				
α-Copaene	19.732	1364.10	1372	0.60
α-Gurjunene	20.807	1393.66	1402	0.61
trans-Caryophyllene	21.221	1405.72	1415	1.88
Calarene	21.59	1417.57	1423	0.49
Aromadendrene	21.865	1426.26	1436	11.32
Patchoulene	22.077	1432.89	1459	0.38
Alloaromadendrene	22.558	1447.70	1460	2.41
α-Amorphene	23.499	1475.78	1481	0.89
α-Muurolene	23.575	1478.00	1500	0.51
Oxygenated sesquiterpenes				
Isoaromadendrene epoxide	26.451	1568.77	1579	0.42
Globulol	26.59	1573.11	1575	4.71
Viridiflorol	26.851	1581.20	1584	1.12
α-Eudesmol	27.742	1609.67	1618	0.44
di-*epi*--1,10-Cubenol	27.97	1617.64	1623	3.37
β-Eudesmol	28.687	1642.29	1645	10.85
Epiglobulol	25.84	1549.43	1564	1.16
Total oil percentage		98.01%		

Antioxidant potential: EO obtained from the leaves of *E. camaldulensis* was assessed for its radical scavenging and antioxidant potential along with its major components -1,8-cineole, *α*-pinene, and *β*-pinene against 1,1-diphenyl-2-picrylhydrazyl (DPPH) and hydroxyl radicals, hydrogen peroxide, and for total antioxidant activity and ferric ion reducing activity

DPPH scavenging potential: Gradual increase in DPPH scavenging activity with increase in the concentration of EO and its major components (Fig. 2). At the lowest concentration (100 µg ml^{-1}), the scavenging activity of EO and its constituents ranged between 20.5–48.1%. Maximum % scavenging activity (~72%) was noticed in case of *α*-pinene at highest concentration of 800 µg ml^{-1} which was significantly higher than that of EO (~63%) at the same concentration. The scavenging activities of *β*-pinene and 1,8-cineole were comparable with *α*-pinene, however, were significantly more than EO at 800 µg ml^{-1} (Fig. 2). In general, the DPPH scavenging activity was in the order - *α*-pinene > *β*-pinene > 1,8-cineole > EO. Different alphabets over the bars represent significant difference among the various treatments (EO and its constituents) at a particular concentration applying post hoc Tukey's test ($p \leq 0.05$).

Ferric ion reducing antioxidant power (FRAP) activity: EO and its components showed an increasing trend in the FRAP activity with increase in concentration of EO or its major components. In general, EO exhibited the highest (significant at $p \leq 0.05$) potential (~52%) for reducing ferric ion as compared to its other components (Fig. 3). On the other hand, at the lowest concentration (100 µg ml^{-1}), the reducing activity was around 10% in 1,8-cineole, 13% in *α*-pinene, 15% in *β*-pinene, and 43% in EO. The activity of EO was significantly more ($p \leq 0.05$) than its other constituents.

Values presented as means ± standard error. Different alphabets over the bars represent significant difference among the various treatments (EO and its constituents) at a particular concentration applying *post hoc* Tukey's test ($p \leq 0.05$).

Total antioxidant activity (TAA): TAA significantly increased with the increase in EO concentration or its major

Table 2. Minimum inhibitory concentration (MIC) of *E. camaldulensis* EO and its major constituents on the selected bacterial strains

Bacteria	EO (mg ml^{-1})	1,8-cineole (mg ml^{-1})	*α*-Pinene (mg ml^{-1})	*β-Pinene* (mg ml^{-1})
Staphylococcus aureus	$9.2 \times 10^{-3} \pm 0.00021$	$9.0 \times 10^{-3} \pm 0.0012$	–	$1.78 \times 10^{-2} \pm 0.00074$
Bacillus subtilis	$18.4 \times 10^{-3} \pm 0.00021$	$9.0 \times 10^{-3} \pm 0.0018$	–	$1.75 \times 10^{-2} \pm 0.00028$
Escherichia coli	$1.7 \times 10^{-2} \pm 0.00169$	$1.8 \times 10^{-2} \pm 0.0016$	–	$2.7 \times 10^{-2} \pm 0.00163$
Pseudomonas aeruginosa	$9.2 \times 10^{-3} \pm 0.00024$	$9.0 \times 10^{-3} \pm 0.0009$	–	$1.74 \times 10^{-2} \pm 0.00020$

components (100–800 µg ml^{-1}) and ranged from 10–75% (Fig. 3). The maximum antioxidant potential was observed in 1,8-cineole (~75%) at the highest concentration of 800 µg ml^{-1}, which was significantly ($p \leq 0.05$) more than the other constituents and EO. This was followed by EO with ~72% activity, *α*-pinene with ~63% activity, and *β*-pinene showing ~60% activity. At the minimum concentration (100 µg ml^{-1}), TAA was around 10% in EO followed by 26% in 1,8-cineole, 36% in *α*-pinene, and 38% in *β*-pinene.Different alphabets over the bars represent significant difference among the various treatments (EO and its constituents) at a particular concentration applying *post hoc* Tukey's test ($p \leq 0.05$).

Hydroxyl radical (OH) scavenging activity: In general, OH scavenging activity ranged from ~23% to ~73% at concentrations ranging from 100 to 800 µg ml^{-1} (Fig. 4). With increase in the concentration of EO and its constituents, the activity also showed an increasing trend. The maximum OH scavenging activity was measured in 1,8-cineole and noticed

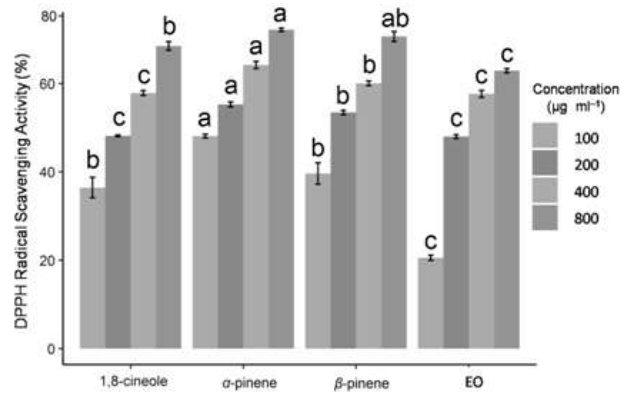

Fig. 1. DPPH radical scavenging activity of *E. camaldulensis* EO and its major constituents(mean ± standard error)

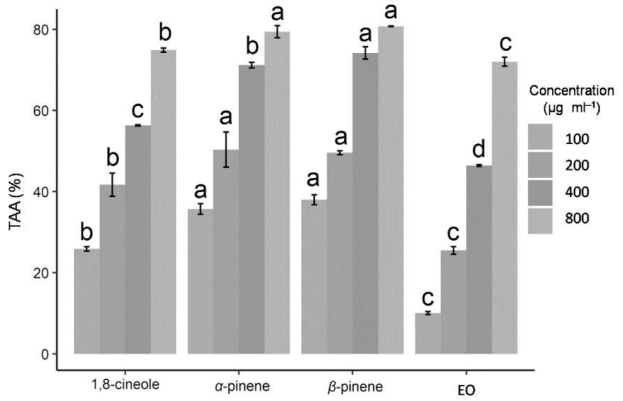

Fig. 3. Total antioxidant activity (TAA) of *E. camaldulensis* EO and its major constituents. (Mean ± standard error)

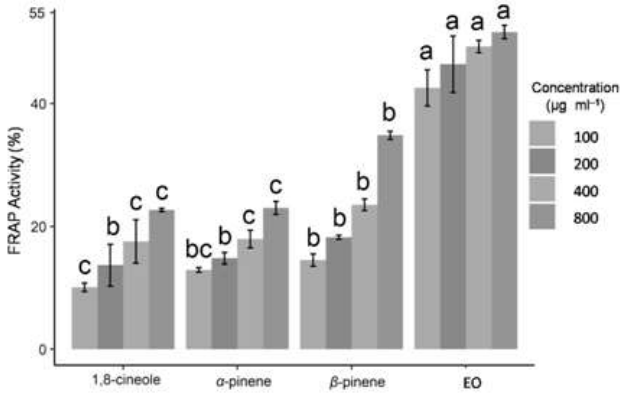

Fig. 2. Ferric ion reducing antioxidant power (FRAP) activity of *E. camaldulensis* EO and its major constituents. Data presented as mean ± standard error

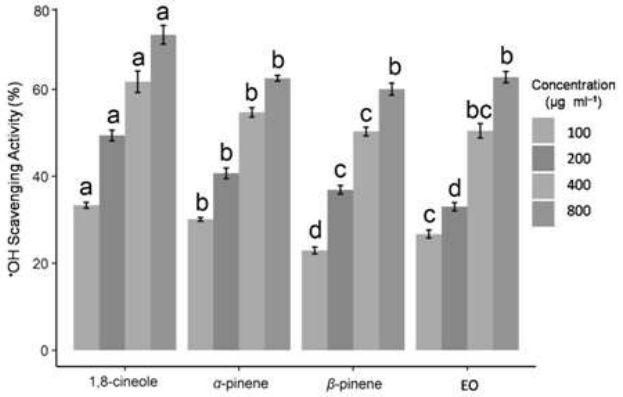

Fig. 4. Hydroxyl radical (OH) scavenging activity of *E. camaldulensis* EO and its major constituents (Mean ± standard error)

Table 3. Zone of inhibition (in mm) at minimum inhibitory concentration of *E. camaldulensis* EO and its major constituents on the selected bacterial strains

Bacteria	Zone of inhibition (mm)*				
	Norfloxacin (10 mcg/disc)	1,8-cineole	EO	*α*-Pinene	*β*-P*inene*
Bacillus subtilis	26	11.3 ± 1.24	10.6 ± 0.94	0	12.6 ± 2.05
Staphylococcus aureus	23	9.3 ± 0.94	9.0 ± 1.41	0	0
Escherichia coli	21	18.6 ± 0.94	15.3 ± 1.24	0	10.0 ± 1.63
Pseudomonas aeruginosa	22	19.3 ± 1.88	14.0 ± 1.63	0	14.0 ± 0.81

* values presented as mean ± standard error

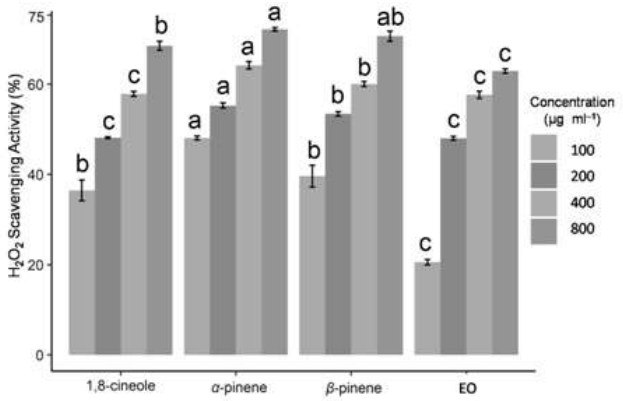

Fig. 5. Hydrogen peroxide (H_2O_2) scavenging activity of *E. camaldulensis* EO and its major constituents (Mean ± standard error)

to be ~73%, which was significantly ($p \leq 0.05$) more than EO and other constituents . At the minimum concentration (100 µg ml^{-1}), the OH scavenging activity was found to be lowest in *β*-pinene (~23%) followed by EO (~27%), *α*-pinene (~30%), and 1,8-cineole (~33%). Different alphabets over the bars represent significant difference among the various treatments (EO and its constituents) at a particular concentration applying *post hoc* Tukey's test ($p \leq 0.05$).

Hydrogen peroxide (H_2O_2) scavenging activity: Hydrogen peroxide scavenging activity of EO and its major components - 1,8-cineole, *α*-pinene, and *β*-pinene increased in a concentration-dependent manner (Fig. 5). The activity ranged from ~21 to ~48% at the minimum concentration (100 µg ml^{-1}) and ~61% to ~72% at the highest concentration, i.e. 800 µg ml^{-1}. The maximum scavenging activity was in *α*-pinene (~72%) at the highest concentration of 800 µg ml^{-1}. This was significantly more ($p \leq 0.05$)) . Different alphabets over the bars represent significant difference among the various treatments (EO and its constituents) at a particular concentration applying *post hoc* Tukey's test ($p \leq 0.05$).

CONCLUSION

Based on GC-MS, a total of 25 components were identified in EO which constituted ~98% of the oil. On the whole, the major compounds identified in EO were 1,8-cineole, *β*-pinene, alloaromadendrene, *β*-eudesmol, and *α*-pinene. Antibacterial efficacy of the oil and its constituents was also explored, and it was found to be maximum in 1,8-cineole. Further, the inhibition was most pronounced in the gram-negative bacteria, *Pseudomonas aeruginosa.* EO and its major constituents also exhibited free radical scavenging as well as antioxidant activity. *α*-Pinene showed maximum scavenging activity for DPPH radical, whereas EO showed the minimum activity. EO exhibited highest potential for reducing ferric ion compared to its major constituents. In case of OH radical**,** the scavenging activity was observed to be maximum in 1,8-cineole, whereas it was least in *β*-pinene.

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