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Original Research

Essential Oil Chemical Composition, Antioxidant and Antibacterial Activities of *Eucalyptus largiflorens* F. Muell

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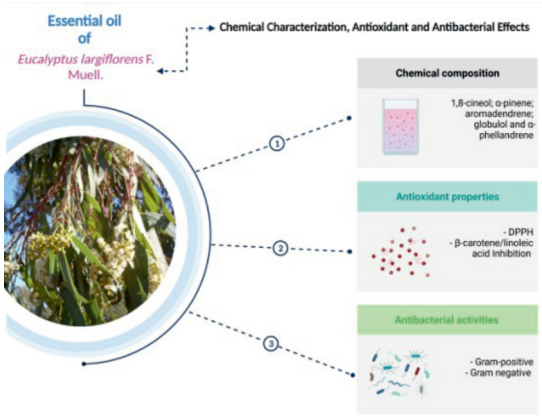
Abstract

In this study, we investigated the chemical composition, the antibacterial activity, and the antioxidant properties of extracts obtained from the leaves, flowers, and fruits of *Eucalyptus largiflorens* F. Muell. The antioxidant effects of the methanolic extract of aerial parts of the plant, obtained by Soxhlet apparatus, were estimated using 1,1-diphenyl-2-picrylhydrazyl (DPPH) and *β*-carotene/linoleic acid methods. The essential oils were chemically characterized by gas chromatography (GC) coupled with mass spectrometry (MS) and flame ionization detector (FID). Thirty compounds were identified, with 1,8-cineol, *α*-pinene, aromadendrene, globulol and *α*-phellandrene being the major components. The antibacterial activities of the essential oils were tested against several bacterial isolates, including *Staphylococcus aureus, Staphylococcus epidermidis* and *Escherichia Coli,* using the disc diffusion method. All the essential oils exhibited



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antibacterial activity. The maximum zone of inhibition was observed for *Escherichia coli*, with the fruit essential oil in the concentration (100 µL). There was no significant antioxidant activity in the essential oils, while the extracts exhibited considerable antioxidant effects in comparison to butylated hydroxytoluene (BHT). In this regard, the flower extract showed the highest antioxidant activity in both DPPH (IC_{50} = 21.5±0.2 µg/mL) and *β*-carotene/linoleic acid tests (Inhibition (%) = 84.3±0.7). Finally, the fruit essential oil exerts a significant antibacterial activity against several bacterial strains and exhibits a significant antioxidant effect in the *β*-carotene/linoleic acid tests, thus it may represent an innovative nutraceutical tool able to act on bacteria and host oxidative stress related to infections.

Keywords: eucalyptus, antioxidant, antibacterial, essential oils, GC/MS, extracts

Introduction

In recent years, vegetal products, including phytocomplexes and isolated compounds, have attracted increasing interest among cosmetic, pharmaceutical, and food industries [1-3]. In this sense, the scientific research has made important advances in the field of chemical composition and biological activities of plants such as, The border between Iran and Iraq [4-7] and their derivatives which often show antioxidant and antibacterial activities, potentially applicable in various fields of human health preservation. For example, the ability of many polyphenols to affect several targets involved in the onset and progression of chronic diseases such as atherosclerosis, may represent the starting point to set up nutraceuticals endowed with preventive effects towards cardiovascular pathologies [8, 9]. In addition, several aromatic and medicinal plants represent good sources of bioactive compounds that [10-14], due to their antimicrobial and antioxidant effects, may be used in many different fields, including the protection of body tissues, food, and pharmaceutical materials from oxidative damage [15-17].

The genus *Eucalyptus* (Myrtaceae family) consists of about 700 species of evergreen trees and shrubs native to Australia and Tasmania that have also been found to grow in many tropical and subtropical countries [18-20]. *Eucalyptus* oils are commonly acrid and bitter, and they have been used for their astringent, expectorant, insect repellant, rubefacient, antipyretic, antiseptic, antifungal, digestive, cardiotonic, and diuretic properties [21]. Some *Eucalyptus* species have also been investigated for their antioxidant activities [22].

The extracts from some *Eucalyptus* species have been used as food additives, such as ‘‘eucalyptus leaf extract’’ that has been reported in the list of food additives drawn up by the Japan Ministry of Health and Welfare [22-24] observed the antioxidant activities of some *Eucalyptus* species including *Eucalyptus citriodora* Hook. and *Eucalyptus staigeriana* F. Muell. ex F.M. Bailey. Also, an essential oil from the leaves of *Eucalyptus camaldulensis* Dehnh. from Thailand showed significant antioxidant activities in both 1,1-diphenyl-2-picrylhydrazyl (DPPH) and *β*-carotene tests [25]. In other studies, a considerable antioxidant activity was reported for leaf extracts of *Eucalyptus robusta* Sm. cultivated in Japan, and *E. globulus* from Spain and the Ivory Coast [26-28].

Eucalyptus largiflorens F. Muell. is an endemic tree of western Australia, but it is now widely cultivated in other areas, including Iran. This plant is susceptible to variations dependent on the growing environment features. More in detail, it has been demonstrated that several geographical and edaphological parameters, including sunlight exposure and weather, may strongly influence the tree morphology and the secondary metabolite composition, affecting consequently the biological activity of the plant extracts. Accordingly, substantial differences in the extracts from the same species grown in different conditions have been described. In this context, despite some reports have already characterized the chemical composition of *Eucalyptus* essential oils obtained from the tree leaves [29], further investigations on extracts from plants grown in specific environmental conditions may unravel novel biologic effects.

The main aim of the present study was to investigate and compare the chemical composition, the antibacterial effects, and the antioxidant activities of the essential oils obtained from the leaves, flowers, and fruits of *Eucalyptus largiflorens* F. Muell. In this study, the plant materials were harvested from the southern saline plains of Iran, characterized by a salinity of around 50 ds/m.

Materials and Methods

Plant Material Collection

The plant’s samples were collected in November from cultivated *Eucalyptus largiflorens* F. Muell. plants grown in Behbahan Agricultural Research Center (Behbahan Province in Iran). The leaves, flowers, and fruits were separated from the stem and air-dried. A voucher specimen (No. KRBG548) of the plant was deposited in the herbarium of the Faculty of Natural Resources, University of Kashan, Iran.

Essential Oils Extraction

Air-dried leaves, flowers and fruits were crushed separately in a grinder. Essential oils were obtained by hydrodistillation for 3.5 hours by using an all-glass Clevenger-type apparatus, as recommended by European Pharmacopoeia [30]. The essential oils were dried over anhydrous sodium sulfate and stored in the dark at a low temperature (4ºC) until analysis.

Preparation of Methanolic Extract

The plant materials were air dried at room temperature 10-14 days during the dry weather condition. The air-dried plant was ground, and 1000 g of the powder was extracted using 5000 mL of 70% methanol by continuous extraction in a Soxhlet apparatus. The extracts were concentrated using a rotary evaporator (Buchi, Flawil, Switzerland) at a maximum temperature of 45ºC and dried in a vacuum oven (45ºC overnight). Then, samples were stored in suitable closed containers in a refrigerator (4ºC) for further analysis. The yield of the dried extract from the leaves, flowers, and fruits were 54.3%, 45.6%, 53.7% (w/w), respectively.

Gas Chromatographic (GC) Analysis

The essential oils were analyzed using an Agilent HP-6890 gas chromatography (Agilent Technologies, Palo Alto, CA, USA) with HP-5MS 5% phenylmethylsiloxane capillary column (30 m × 0.25 mm i.d., 0.25 µm film thickness; Restek, Bellefonte, PA) equipped with an FID detector. The oven temperature was programmed from 60 to 270ºC at an increment rate of 3ºC/min. Injector and detector temperatures were set at 220 and 240ºC, respectively. Helium was used as carrier gas at a flow rate of 1 mL/min, and diluted samples (1/1000 in n-pentane, v/v) of 1.0 µL were injected manually and in the slitless mode. The proportion of each component of the essential oils was computed from its peak area percent relative to the respective spectrum total area (100%) [1].

Gas Chromatographic/Mass Spectrometric (GC/MS) Analysis

The GC/MS analysis of the plant essential oils was performed on an Agilent HP-6890 gas chromatograph (Agilent Technologies, Palo Alto, CA, USA) with an HP-5MS 5% phenylmethylsiloxane capillary column (30 m × 0.25 mm i.d, 0.25 µm film thickness; Restek, Bellefonte, PA) equipped with an Agilent HP-5973 mass selective detector (Agilent Technologies, Palo Alto, CA, USA) in the electron impact (Ionization energy: 70 eV) mode operating under the same condition as above. Retention indices were calculated for all components using a homologous series of *n*-alkanes injected in conditions equal to sample ones. Identification of components of the essential oils was carried out based on retention indices (RI) relative to *n*-alkanes and computer matching with the Wiley275.L and Wiley7n.L as well as comparisons of the fragmentation pattern of the mass spectra with data published in the literature [29].

Antioxidant Activity Evaluation

DPPH Method

DPPH (2,2-diphenyl-1-picrylhydrazyl) method usually involves hydrogen atom transfer (HAT) reactions, but based on kinetic data, an electron transfer (ET) mechanism has been suggested for this method [31]. Previously published DPPH radical scavenging activity assay method with minor modifications was used to determine radical scavenging activity (RSA) of the essential oils and extracts of *E. largiflorens* [29]. Briefly, stock solutions (10 mg/mL) of the essential oils, extracts, and synthetic standard antioxidant BHT were prepared in methanol and diluted to obtain concentrations ranging from 1 to 5×10^{-10} mg/mL. The diluted solutions (2 mL each) were mixed with 2 mL of a freshly prepared 80 µg/mL DPPH methanol solution and allowed to stand for 30 minutes in the dark at room temperature avoiding unwanted chemical reactions. Ultraviolet-visible (UV-Vis) absorption spectra of these solutions were recorded using a GBC Cintra 6 spectrometer (Australia) at 517 nm using a blank containing the same concentration of the essential oils, extracts or BHT without DPPH. Inhibition percentages (I%) of free radical DPPH were calculated in the following way: $I\% = [(A_{blank} - A_{sample})/A_{blank}] \times 100$ Where A_{blank} is the absorbance of the control reaction (containing all reagents except the test compound) and A_{sample} is the absorbance of the test compound. The sample concentration providing 50% inhibition (IC_{50}) was calculated by plotting inhibition percentages against concentrations of the samples. All tests were carried out in triplicate and IC_{50} values were reported as means±SD of triplicates.

β-Carotene/Linoleic Method

In this method, antioxidant activity was determined by measuring the inhibition of the formation of volatile organic compounds and conjugated diene hydro peroxides arising from linoleic acid oxidation. The method which was described by [32] was used with slight modifications. A stock solution of *β*-carotene and linoleic acid was prepared with 0.5 mg of *β*-carotene in 1 mL chloroform, 25 µL of linoleic acid, and 200 mg Tween 40. Chloroform was evaporated under vacuum and 100 mL of aerated distilled water was then added to the residue. The samples (2 mg/mL) were dissolved in DMSO and 350 µL of each sample solution was added to 2.5 mL of the above mixture in test tubes. The test tubes were incubated in a hot water bath at

50ºC for 2 h together with two blanks: one contained the antioxidant BHT as a positive control and the other one contained the same volume of DMSO instead of the samples (negative control). The test tube with BHT maintained its yellow color during the incubation period. The absorbencies were measured at 470 nm. Antioxidant activity (Inhibition percentage, I%) of the samples was calculated using the following equation: $I\% = (A_{\beta\text{-carotene after 2 h assay}}/A_{\text{initial}\beta\text{-carotene}}) \times 100$. Where $A_{\beta\text{-carotene}}$ after 2 h assay is the absorbance of β-carotene after 2 h assay remaining in the samples and $A_{\text{initial }\beta\text{-carotene}}$ is the absorbance of β-carotene at the beginning of the experiments. Tests were carried out in triplicate. Inhibition Percentages of the samples were compared with those of the positive and negative standards

Antibacterial Activity

60 clinical specimens (urine and wound) from patients of Shahid Beheshti Hospital in Kashan city were collected. From the 60 bacterial isolates, 33 were taken from women (55%) and 27 from males (45%). Clinical specimens were selected and cultured on microbiological environments with hot staining and biochemical tests using Klegler, Voges, Sulfite Indol Motility (SIM), Methyl Red (MR), Proskauer (VP), Iron Agar (KIA) and fermentation tests of various sugars were detected; the isolates were then transferred to the Muller Hinton agar plate for the evaluation of antibiotic susceptibility by the method of disk diffusion in agar. To carry out antibiograms, bacterial suspensions were prepared with a standard equivalent of half McFarland and cultured on a Muller Hinton medium agar culture. The antibiotics used in this study (produced by Padtanteb company, Iran) included Gentamicin (10 µg), Ampicillin (10 µg), Ceftazidime (30 µg), Penicillin (10 U), Ceftriaxone (30 µg), Ithromycin (15 µg) and Tetracycline (30 µg). After 24 hours of incubation at 37ºC, the diameter of the inhibition zones was measured and the strain susceptibility and resistance were determined and compared to the CLSI standard [33].

Essential oils of the plant were used for antibacterial studies. In addition to the essential oils, n-hexane was selected as a negative control by the diffusion method through wells and from tetracycline disk as a positive control and analyzed by the method of agar disk diffusion. The bacteria used in this study included Staphylococcus aureus, Staphylococcus epidermidis and Escherichia coli resistant to antibiotics and isolated from patients. Standard strains are *Staphylococcus aureus* (ATCC 25923) *Staphylococcus epidermidis* (12228 ATCC) and *Escherichia coli* (ATCC 12228). In this study, the antimicrobial activities of essential oil were performed by Agar well diffusion method. In this method, a suspension from each of the tested bacteria was prepared with an equivalent to the half-MacFarland standard, and after diffusion of the bacteria on the surface of the culture medium of the Muller Hinton Agar, the wells created on the above medium from the essential oil with 5 mm diameter, Then, 50 and 100 µL of essential oil was added in the well and n-hexane solvent used as the control, then the plates were placed in an incubator at 37ºC for 24 hours.

Statistical Analysis

All data were expressed as the mean±SD (standard deviation), tests were performed in triplicate and one-way ANOVA (analysis of variance) followed by the Dun-nett's test was used for statistical analysis using Excel software. Values of $p<0.05$ were considered statistically significant.

Results

Chemical Composition

The dried leaves, flowers and fruits of *Eucalyptus largiflorens* F. Muell. contained 1.45%, 0.68%, and 0.82% (v/w) of yellow-colored essential oils, respectively. The essential oils were analyzed both quantitatively and qualitatively using GC/FID and GC/MS systems. The components were identified by comparing the Kovats index (RI) and the mass spectrum of each component with those mentioned in the literature and stored in the Wiley 275.L and Wiley 7 n. L spectral libraries.

Antioxidant Activity

The free radical scavenging activity and lipid peroxidation inhibition of the essential oils and methanolic extracts from different parts of *Eucalyptus largiflorens* F. Muell. were evaluated by two different methods. The results are presented in Table 2.

The methanolic extracts showed a significant DPPH· scavenging activity and a strong antioxidant activity in the β-carotene/linoleic method. For example, the methanol flower extract reduced the stable DPPH free radicals to yellow-colored molecules of diphenylpicrylhydrazine with an IC_{50} value of 21.5 µg/mL. This value was highly comparable to the IC_{50} of the positive synthetic control Butylated hydroxytoluene (BHT) (IC_{50}= 18.5 µg/mL). As shown in Table 2, the antioxidant activity of the leaf and fruit extracts was about one-third of that of BHT. On the other hand, methanol extracts of the leaves, flowers, and fruits of *Eucalyptus largiflorens* F. Muell. showed similar results in the β-carotene/linoleic acid bleaching test: the inhibition percentages of these extracts were in a range between 84.3% and 88.2% (Table 2), comparable to that of the synthetic standard BHT. In contrast, essential oils did not exhibit considerable antioxidant activity in this experimental model.

Table 1. Chemical composition of the essential oils of *Eucalyptus largiflorens* F. Muell.

Component[a]	RI[b]	RI[c]	Composition (%)		
			Fruit	Flower	Leaf
α-Pinene	927	939	12.4	22.3	25.4
β-Pinene	964	979	-	1.3	-
α-Phellandrene	996	1003	2.5	9.9	6.5
P-Cymene	1013	1025	2.0	-	1.7
1,8-Cineole	1027	1031	14.9	46.0	32.9
γ-Terpinene	1052	1060	-	2.3	0.6
Terpinolene	1080	1089	-	0.4	-
trans-Pinocarveol	1129	1139	1.0	0.5	4.4
Pinocarvone	1152	1165	-	-	1.2
Terpinen-4-ol	1172	1177	-	3.2	0.4
α-Terpineol	1181	1189	-	1.2	0.7
Piperitone	1345	1343	-	0.4	-
α-Gurjunene	1397	1410	3.1	-	-
Longifolene	1409	1408	-	-	1.4
β-Gurjunene	1417	1434	0.7	-	0.3
Aromadendrene	1426	1441	25.7	0.5	9.0
Alloaromadendrene	1445	1460	4.6	-	1.4
β-Selinene	1471	1490	0.6	-	0.8
β-Guaiene	1473	1493	0.7	-	-
γ-Gurjunene	1480	1477	3.3	-	0.5
γ-Cadinene	1497	1514	1.1	-	-
δ-Cadinene	1508	1523	1.6	-	-
Epiglobulol	1548	1564	3.4	0.9	1.7
Selina-3,7(11)-diene	1554	1547	0.7	0.4	0.5
Spathulenol	1566	1578	0.6	-	0.2
Ledol	1567	1569	-	-	3.6
Globulol	1578	1585	13.4	6.2	7.1
Viridiflorol	1583	1593	2.4	1.9	1.6
Widdrol	1591	1599	1.4	0.5	0.7
epi-*α*-Cadinol	1632	1640	1.0	0.5	-
Total			96.5	98.3	94.2

[a] Compounds listed in order of elution from HP-5MS column.
[b] Relative retention indices to C_8-C_{24} *n*-alkanes on HP-5MS column.
[c] Literature retention indices.

Antibacterial Activity

In vitro antibacterial activity of the extracts against bacterial isolates was evaluated. Bacteria were isolated and purified from urine and wound samples collected from patients. The isolated bacterial species *Staphylococcus aureus, Staphylococcus epidermidis, and Escherichia Coli* were tested for antibiotic susceptibility or resistance. The results are shown in Table 3. For several bacterial isolates, resistance to some

Table 2. Antioxidant activity of the essential oils and methanol extracts of *Eucalyptus largiflorens* F. Muell. By DPPH and *β*-carotene methods.

Sample	DPPH IC_{50} (μg/mL)	*β*-carotene/linoleic acid Inhibition (%)
Leaf extract	57.0 ± 0.1	86.3 ± 0.6
Flower extract	21.5 ± 0.2	84.3 ± 0.7
Fruit extract	64.2 ± 0.4	88.2 ± 0.5
Leaf essential oil	ND[a]	11.5± 0.7
Flower essential oil	ND[a]	21.2± 0.3
Fruit essential oil	ND[a]	5.2 ± 0.2
BHT	18.50 ± 0.6	87.6 ± 0.8
Negative control	NA	2.8± 0.4

[a] Less than 32% inhibition in the essential oils concentrations of up to 10 mg/mL, ND = Not, Determined, NA = Not Applicable.

antibiotics was observed. Penicillin and erythromycin showed the lowest antibiotic effect (Table 3).

The antibacterial properties of the essential oils extracted from *Eucalyptus largiflorens* F. Muell. cultivated in Iran were evaluated using the agar disk diffusion method. The results are summarized in Table 4. All the essential oils exhibited antimicrobial activity against both Gram-positive and Gram-negative bacteria. The maximum zone of inhibition was shown by all three essential oils against *Escherichia Coli* and the activity increased along with the increasing concentration (50 μL, 100 μL). The comparison between the antimicrobial effects of essential oils and tetracycline is shown in Table 4. *n*-Hexane as negative control did not show any inhibitory effect on the studied bacteria. These results indicate the *in vitro* antibacterial effects of the essential oils at relative low concentrations.

The *in vitro* antimicrobial activities of *Eucalyptus largiflorens* F. Muell. fruits essential oil and extracts were further assessed against a panel of eight

Table 3. Pattern profile of the bacterial strains.

Antibiotics	Bacteria (number and percentage)			
	The type of reaction	*Staphylococcus epidermidis*	*Staphylococcus aureus*	*Escherichia coli*
Ceftriaxone	Sensitive	15(75)	8(40)	11(55)
	Resistant	5(25)	4(20)	7(35)
	Intermediate	0(0)	8(40)	2(10)
Ceftazidime	Sensitive	5(25)	10(50)	15(75)
	Resistant	5(25)	7(35)	3(15)
	Intermediate	10(50)	3(15)	2(10)
Erythromycin	Sensitive	9(45)	2(10)	0(0)
	Resistant	8(40)	17(85)	4(20)
	Intermediate	3(15)	1(5)	16(80)
Tetracycline	Sensitive	15(75)	4(20)	3(15)
	Resistant	5(25)	6(30)	16(80)
	Intermediate	0(0)	10(50)	1(5)
Penicillin	Sensitive	20(100)	0(0)	0(0)
	Resistant	0(0)	18(90)	18(90)
	Intermediate	15(75)	2(10)	2(10)
Ampicillin	Sensitive	3(15)	2(10)	3(15)
	Resistant	15(75)	14(70)	17(85)
	Intermediate	2(10)	4(20)	0(0)
Gentamicin	Sensitive	13(65)	15(75)	8(40)
	Resistant	9(35)	4(20)	9(45)
	Intermediate	0(0)	1(5)	3(15)

Table 4. Inhibition zone at various volumes of *E. largiflorens* essential oils on microorganisms isolated from patients (mm±SD).

Tetracycline	n-Hexane	Fruit		Flower		Leaf		Isolates
30 µL	-	50 µL	100 µL	50 µL	100 µL	50 µL	100 µL	
25±0.4	0	10.63±0.4	13.4±0.5	9.7±0.02	13.5±0.18	6.5±0.01	11.8±0.31	*Staphylococcus aureus*
19±0.3	0	12±0.82	14.67±0.93	8.6±0.18	11.6±0.7	7.3±0.2	9.5±0.3	*Staphylococcus epidermidis*
22±2	0	13.65±0.3	16±0.68	10.5±0.3	13.5±0.28	8.5±0.3	9.2±0.27	*Escherichia coli*

microorganisms using the inhibition zones, zone diameters, MIC and MBC values. According to the results presented in Table 5, the fruits essential oils of *Eucalyptus largiflorens* F. Muell. showed antimicrobial activity against the tested bacteria, except for *P. aeruginosa*. In comparison to the methanol extracts, the essential oil exhibited a stronger and broader activity. The highest inhibition zone (34.3 mm±2.1) and the lowest MIC value (6.7 µg/mL) was achieved for E. coli, showing that this microorganism is the most sensitive to this essential oil. Other sensitive microorganisms sesnsitive to the essential oils were *S. typhimurium* and *K. pneumoniae*, with MIC value of 14.5 µg/mL. Neither the essential oils nor the methanolic extract could inhibit the growth of *Pseudomonas aeruginosa*. As shown in Table 5, the flowers methanol extract did not exhibit antimicrobial activity, while the fruits methanol extract showed modest antibacterial activities against *S. aureus*, *S. epidermidis*, *B. subtilis*, and *B. cereus*

Discussion

Infectious diseases represent a world health problem that has not been solved due to the development of bacterial strains that became resistant to multiple antibiotics. Therefore, the discovery of new substances able to inhibit bacterial growth and resistant strains may represent a strategy aimed at contrasting this global burden [34]. In addition to synthetic compounds, molecules isolated from vegetal matrices as well as whole plants extracts and related fractions may be also investigated for this purpose. In Folk Medicine of several countries, such as, for example, traditional Chinese medicine, Unani medicine, Ayurvedic and African traditional medicine, plants represent the main ingredient for the treatment and prevention of many pathologies, including those induced by pathogens [35]. Several extracts and essential oils have been shown to exert significant antibacterial activities against both gram positive and gram negative bacteria through several molecular mechanisms [36, 37].

After bacterial infections, often inflammatory processes occur along with an increase in oxidative stress [38-40]. Therefore, the identification of substances able to exert an antibacterial activity and to de-crease oxidative stress may represent an efficient tool able to act according to an approach that we may define host and guest-targeting. In this paper, we investigated the chemical composition, the antibacterial activity against several bacteria and the antioxidant activity of *Eucalyptus largiflorens* F. Muell. extracts, using different approaches. The main molecule found in essential oils from the three parts of the plant is the mon-terpenic oxide 1,8-cineole, followed by the monoterpenes hydrocarbons α-pinene and α-phellandrene. These compounds were identified in high amounts also in other *Eucalyptus* species, such as *Eucalyptus accedens* W.Fitzg, *Eucalyptus bosistoana* F.Muell., *Eucalyptus cladocalyx* F.Muell., *Eucalyptus robusta* Sm., *Eucalyptus punctata* DC, *Eucalyptus wandoo* Blakely [29].

The antibacterial activity of *Eucalyptus largiflorens* F. Muell. essential oil was observed for both Gram - and Gram + bacteria. As the antimicrobial resistance for the tested species is still a big issue, responsible for billions death all over the world, futher investigations aimed at evaluating these effects in common multidrug-resistant organisms (MDROs) strains are desirable [33, 41, 42]. The antibacterial activities of essential oils may be, at least in part, due to the presence of molecules acting as antibiotic agents. In particular, 1,8-cineole (eucalyptol), is one of the main compounds of the essential oils, endowed with strong antimicrobial activities (REF).

In general, bacteria outer membrane seems to represent the main target of essential oil terpenes. Indeed, terpenes may work as antimicrobial molecules through an augment of membrane fluidity and permeability, an alteration of membrane-embedded proteins, reduction of respiration, and modifications of ion transport processes. In this context, 1,8-cineol, one of the main compounds found in *E. largiflorens*, may inhibit *E. coli* through several mechanisms, involving, among others, the alteration of membrane fluidity occur-ring through a modification of total fatty acids composition [43]. In addition, this compound was shown to induce the release of cellular materials from bacterial cells, in *E. coli* and *S. aureus* (REF). Interestingly, scanning electron microscope analyses seem to demonstrate that some terpenes, including α-Terpineol, determine the formation of pores on the outer membrane of *E. coli*. that may lead to cells collapse [44] .

The strong antimicrobial activity of *Eucalyptus largiflorens* F. Muell. is accompanied by a significant

Table 5. Antibacterial activity of fruit essential oil and methanol extracts of *E. largiflorens*.

	Essential oil			Methanol extracts						Gentamicin	
				Fruit			Flower				
Test bacteria	DD[a]	MIC[b]	MBC[c]	DD	MIC	MBC	DD	MIC	MBC	DD	MIC
S. aureus	19.2	30.2	61.4	12.3	124.0	124	—	—	—	16.0	9.0
S. epidermidis	18.5	30.2	30.2	14.5	61.5	61.5	—	—	—	19.0	4.0
E. coli	34.3	6.7	6.7	—	—	—	—	—	—	10.0	16.0
K. pneumoniae	27.5	14.5	30.2	—	—	—	—	—	—	7.0	13.0
B. subtilis	17.0	30.3	61.5	13.0	61.5	61.5	—	—	—	13.0	19.0
B. cereus	19.0	61.5	61.5	12.0	61.5	124.0	—	—	—	12.0	19.0
S. typhimurium	26.0	14.5	14.5	—	—	—	—	—	—	9.0	11.0
P. aeruginosa	d—	—	—	—	—	—	—	—	—	9.0	24.0

[a]DD: Diameter of inhibition zone including disc diameter of 6 (mm).
[b]MIC: Minimum inhibitory concentration (μg/mL).
[c]MBC: Minimum bactericidal concentration (μg/mL).
d—: no antibacterial activity.

antioxidant power. In particular, the methanolic extracts showed a potent antioxidant activity in both DPPH and β-carotene/linoleic acid tests, while the essential oils produced this effect only in *β*-carotene/linoleic acid test. This functional difference is probably due to the presence of higher concentrations of polyphenolic compounds in the extracts than in the essential oils. Moreover, the essential oils likely exhibited this effect for a better affinity to the matrix test. In this paper, we demonstrated the in vitro ability of *Eucalyptus largiflorens* F. Muell. essential oils to act both as an antibacterial and antioxidant agent. The latter effect may de-crease oxidative stress related mechanisms such as inflammation, occurring in tissues infected with many bacteria including *E. coli*, *Staphylococcus aureus* and *Staphylococcus epidermidis*. Therefore, the essential oil may produce health promoting effects in subjects with *E. Coli* infection, that results in an increase of proinflammatory and pro-oxidative markers [45-47]. The present study has revealed that the chemical composition and antioxidant and antimicrobial activity of essential oils of *Eucalyptus largiflorens* F. Muell. species, varied greatly depending upon the different parts of the plant extract. The antimicrobial activity of essential oils may be directly associated with their major constituents, likely acting in synergy with minor compounds. As a result of the present study, the methanolic extract and the essential oils were found to exert antioxidant and antimicrobial effects in different in vitro assays thus they may be considered for applications in both human health care and food and pharmaceutical industries. The extracts studied in this paper are interesting as they were obtained from plants grown in conditions allowing to express higher percentage of major compounds than those reported in previous works. This may be due to the habitat conditions of the studied plant (saline plain). Also, the gram-positive bacteria are more sensitive to the essential oils than gram-negative bacteria [46] while our results did not show any selective antimicrobial activity on the basis of the cell wall differences of bacteria. However, the components responsible for the antioxidant and antimicrobial activities of its extracts are currently unclear; further work should be performed on the isolation and identification of the components of the extracts.

Conclusions

The results here reported suggest, *Eucalyptus largiflorens,* contain a wide range of biologically active volatile compounds such as, 33 components, accounting for 94.2% to 98.3% of the total oils, were identified in the essential oils from different parts of the plant. The main constituents of the essential oils from leaves (L), flowers (FL) and fruits (F) were: 1,8-cineol (L: 32.9%, FL: 46.0%, F: 14.9%), *α*-pinene (L: 25.4%, FL: 22.3%, F: 12.4%), aromadendrene (L: 9.0%, FL: 0.5%, F: 25.7%), globulol (L: 7.1%, FL: 6.2%, F: 13.4%), and *α*-phellandrene (L: 0.0%, FL: 9.9%, F: 2.5%). Additionally, this work demonstrates that the essential oils of this plant parts are a good correlation of volatile oils to antioxidant and antibacterial activities. As well as, the methanolic extract showed have noteworthy antioxidant activity. Therefore, the essential oil may produce health promoting effects in subjects with *E. Coli* infection, that results in an increase of proinflammatory and pro-oxidative markers.

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Conflict of Interest

The authors declare that they have no conflict of interest.

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