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Phytochemical and antimicrobial properties of *Lavender angustifolia* and *Eucalyptus camaldulensis* essential oils

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ABSTRACT

This study was aimed to investigate chemical composition, antibacterial and antifungal activities of Lavender (*Lavandula angustifolia*) and Eucalyptus (*Eucalyptus camaldulensis*) essential oils. The essential oils which prepared via distillation by Clevenger unit analyzed by GC- MS. Antibacterial effect of essential oils were tested against *Listeria monocytogenes*, *Bacillus cereus*, *Salmonella thyphimurium,* and *Escherichia coli* using disc diffusion, agar well diffusion and broth microdilution methods and against *Candida* strains by broth micro dilution. The most inhibition zone of *Lavender* essential oil was related to *S. thyphimurium* and *L. monocytogenes* and *B. cereus* in disk diffusion and *B. cereus* in agar well diffusion method. The lowest minimum inhibitory concentration (MIC) for *Lavender* essential oil was 6 µl/ml, against *S. thyphimurium*. The MIC value of *Lavender* against *L. monocytogenes, E. coli* and *B. cereus* were 25, 12.5, 12.5 µl/ml respectively. The MIC value of *Eucalyptus* against *S. thiphymurium, L. monocytogenes, E. coli* and *B. cereus* were 25, 12.5, 12.5, 12.5 µl/ml respectively. The MIC value of *Eucalyptus* essential oil against *C. albicans*, *C. tropicalis*, *C. dubliniensis* and *C. parapsilosis* were 0.2%, 0.1%, 0.4% and 0.4%, respectively. The MIC value of *Lavender* essential oil against *C. albicans*, *C. tropicalis*, *C. dubliniensis* and *C. parapsilosis* were 0.4%, 0.4%, 1.6% and 1.6%, respectively.

1. Introduction

Expansion 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 (1). Scientific experiments since the late 19th century have documented the antimicrobial properties of some herbal components (2).

The *Myrtacea* family is composed of at least 3000 species in 130-150 genera, widely distributed in tropical and warm temperature regions of the world (3). The Eucalyptus belongs to *Myrtaceae* family and comprises of about 900 species (4). It is a tall tree native to Australia and Tasmania, successfully introduced worldwide, now extensively planted in many other countries including Iran (5). Essential oils of various plant species

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have been used for pharmaceutical (6) and cosmetic purposes (7). Essence of eucalyptus and its major component is widely used in manufacturing of softeners, pomades, antitussive syrups, toothpastes and also as flavor in many medicines. Also this component is used as fragrance in soaps, powders and other washing materials (8).

Eucalyptus trees are able to survive in between 45 degrees north and 45 degrees south latitude (9). This plant is a rich source of polyphenols and terpenoids (10). Leaves of this plant accumulate a very large amount of secondary metabolites which yield upon hydrodistillation and possess many biological effects (11, 12).

Lavender (*lavandula spp.*) belongs to labiatae (Lamiacae) family and has been used either dried or as an essential oil for centuries for a variety of therapeutic and cosmetic purposes (13). It is cultivated in France, Spain and Italy. Recently, this plant is cultivated in some areas of Iran. The most common species believed to have medicinal value are *L. dentata, L. angustifolia, L. latifolia, L. intermedia, L. stoechas* and *L. dhofarensis.* The essential oils derived from Lavender has significant properties such as antioxidant and hypolipidemic (14) and anti-inflammatory (15).

Ashour studied antimicrobial and cytotoxic activities of oil and extract of leaf, stem, and flower of eucalyptus sideroxylon and eucalyptus torquata grown in Egypt. An agar diffusion method was used to analyze antimicrobial activities of essential oil and extract of eucalyptus against medically important gram-positive and gram-negative bacteria. Gram-positive bacteria were highly susceptible to oils and extracts of both Eucalyptus species. With the exception of *Escherichia coli*, gram-negative bacteria were resistant to the extracts, but susceptible to the oils obtained from at least one organ of E. sideroxylon and E. torquata. Although *Aspergillus flavus* and *Aspergillus niger* were resistant to the extracts, essential oils of E. sideroxylon and E. torquata generally exhibited moderate to high antifungal activities against *Candida albicans*, *A. flavus* and *A.niger* (16).

In the study of Benbelaid et al, unlike many other essential oils extracted from many medicinal plants which present activity only on gram positive bacteria, the essential oil of eucalyptus has shown an interesting activity on gram positive and gram negative bacteria. The most important activity was observed in species of the genus *Bacillus* and *Candida albicans*. The bacterial species with the smallest inhibition zones was *Klebsiella pneumonia* and *Pseudomonas aeruginosa* with a diameter of 13 and 7 mm respectively at 2 µl of essential oil (17).

In another study, the antimicrobial activity of lavender and lavandin was evaluated by the Kirby-Bauer method. Antimicrobial tests showed antimicrobial activity against *Shigella flexneri*, *Staphylococcus aureus*, *E.coli* and *Salmonella typhimurium*, while *Streptococcus pyogenes* was not sensitive to the action of the two essential oils. The study revealed that essential oils isolated and analyzed from lavender (*L. angustifolia*) and lavandin (*Lavandula x intermedia*) display significant bactericidal effects against microorganisms (18).

The main objective of this study is to determine the chemical components, antibacterial and antifungal activities of lavender and eucalyptus essential oils and their effects on significant food pathogens and candida species by disk and agar well diffusion and microdilution methods.

2. Material and Methods

2.1. Plant material and EOs Extraction

Lavender (*Lavandula angustifolia*) and eucalyptus (*Eucalyptus camaldulensis*) were purchased from a local grocery in Tehran (capital of Iran). The plants were verified in botanical department of Tehran Medicinal Herbs Research Centre of Tehran University of Medical Science. Essential oils of aerial parts of plants were extracted via hydrodistillation for 3 h using a Clevenger unit, according to the method recommended by the European Pharmacopia to produce oils (19). The essential oils were put in amber color glass containers and stored at 4°C after dehydration with dry sodium sulphate until further evaluations.

2.2. Gas Chromatography- Mass Spectrometry (GC-MS) analysis

Chemical composition of these essential oils were analyzed by GC-MS. The chromatograph (Agilent 6890 UK) was equipped with an HP-5MS capillary column (30 × 0.25 mm ID × 0.25 mm film thickness) and the data were taken under the following conditions: initial temperature 50°C, temperature ramp 5°C/min, 240°C/min to 300°C (holding for 3

min), and injector temperature at 290°C. The carrier gas was helium and the split ratio was 0.8 mL^{-1}/min. To confirm the analysis results, two essential oils were also analyzed by gas chromatography-mass spectrometry (Agilent 6890 gas chromatograph equipped with an Agilent 5973 mass-selective detector; Agilent UK) with the same capillary column and analytical conditions. The MS was run in electron-ionization mode with ionization energy of 70 ev (20).

2.3. Microbial strains

The antibacterial activity of essential oils were tested against two gram negative and two gram positive bacteria including *L. monocytogenes ATCC 19118*, *B. cereus ATCC 11178* , *S. thyphimurium ATCC 13311*, *and E. coli ATCC 43894*. This EOs also were tested against 4 Candidal strains including *C. albicans ATCC 10231, C. tropicalis ATCC 750, C. dubliniensis CD36 and C. parapsilosis ATCC 22019*. In this study, the selection of bacterial species was based on the importance of these species in food contamination and foodborne poisoning in humans. Also candida species were selected because these species are the most common cause of fungal infections in human especially in immunosuppressed patients.

2.4. Antimicrobial assay

The antimicrobial assay was performed by three methods: disc diffusion, agar well diffusion and broth microdilution.

2.5. Disc diffusion assay

Sterile paper discs (6 mm in diameter) were impregnated with different levels of essential oils (14, 7, 3.5 µl) and allowed to dry. 100 µl of bacterial suspension containing 10^8 cfu/ml were inoculated in nutrient agar (Merck, Germany). Then paper discs were impregnated in the inoculated agar. Kanamycin (30 µg/disc), Ofloxacin (5 µg/disc), Amoxicillin (25 µg/disc) (sigma) were used as positive reference standards to determine the sensitivity of one strain/isolate for each microbial species tested. The inoculated plates were incubated at 37°C for 24 h. Antimicrobial activity was evaluated by measuring the inhibition zones. Each assay in this experiment was repeated three times (21).

2.6. Agar well diffusion method

Suspensions 0.5 McF units (approximately 10^8 CFU mL/1) of each tested microorganism were spread on the surface of nutrient agar by a sterile cotton swab. For agar well diffusion method, four wells in each plate was prepared with the sterilized standard device (0.6 cm). 200 µl of lavender and eucalyptus essential oils were dissolved in 1 ml DMSO 10%. Then serially two fold dilutions of essential oils were prepared (100 µl/ml, 50 µl/ml and 25 µl/ml). 42 µl of serial dilutions of essential oils was introduced into relevant well. The plates were incubated for 24 h at 37 °C. Then zone of inhibition was measured (22).

2.7. Broth microdilution method

A broth microdilution method was used to determine the minimum inhibitory concentration (MIC) according to Gulluce et al method (23). To determine the MIC, bacterial strain suspensions from 24 h culture were prepared. The 96-well plates were prepared by dispensing into each well 95 µl of nutrient broth and 5 µl of the inoculum. A 100 µl aliquot from the stock solutions of essential oils initially prepared at the concentration of 100 µl/ml and was added into the first wells. Then, 100 µl from their serial dilutions was transferred into four consecutive wells. The last well containing 195 µl of nutrient broth without compound and 5 µl of the inoculum on each strip was used as the negative control. The final volume in each well was 200 µl. The MIC was defined as the lowest concentration of the compounds to inhibit the growth of microorganisms. The plate was incubated 37°C for 24 h. Essential oils tested in this study were screened two times against each microorganism.

2.8. Antifungal assay

For determination of MICs, microdilution broth method was used based on the Clinical Laboratory Standards Institute (CLSI, M27-A2). Stocks and dilutions of essential oils were prepared in DMSO 10%. Final concentrations in the microdilution plates ranged from 12.5-0.02% (v/v) for each essential oil. The microdilution plates were prepared using the RPMI 1640 broth medium (Sigma) with l-glutamine and without sodium bicarbonate and buffered at pH 7.0 with 0.165 mol/l of morpholine propan sulfonic acid (MOPS) (Sigma). Yeast suspensions were prepared after vortexing and adjusting to a 0.5 Mc Farland standard transmittance at a wave length of 530 nm. The final inoculum yielded

was of 0.5 ×10 ^{3} - 2.5 × 10 ^{3} cells/ml. Two wells served as the growth control and sterility check. MICs were visually determined at 24 h of incubation at 37 °C, and were observed for the presence or absence of growth. The growth in each well was compared with that of the growth control EO free well (24).

3. Results

3.1. Chemical compositions of EOs

The results obtained by GC-MS analysis of essential oils of eucalyptus and lavender are presented in Table 1 and 2. Twenty one components, which represented 83.63% of eucalyptus and 21 constituents which represents 95.24% of lavender essential oils were identified. The eucalyptus essential oil was characterized by high amounts of isopentyl isovalerate (19.12%), α-pinene (12.6%), trans-pinocarveol (11.4%) and β-pinene (8.81%). The lavender essential oil was characterized by high amounts of cis-3-Hexen-1-ol (18%), β-Bisabolene (17.17%), and α-farnesene (12.31%).

3.2. Disc and agar well diffusion assay

The eucalyptus and lavender essential oils were tested for antibacterial activity against foodborne pathogenic bacteria. The results of the antibacterial activities of essential oils by disc diffusion and agar well diffusion are shown in Table 3 and 4. According to the disc diffusion method gram positive bacteria were more sensitive to EOs. Results of disk diffusion assay showed high activity of eucalyptus essential oil against *B. cereus* and *L. monocytogenes* and lavender essential oil against *S. thyphimurium*, *L. monocytogenes*, *B. cereus*. The most susceptible organisms were *B. cereus* in agar well diffusion method.

Table 1. Chemical compositions of eucalyptus (Eucalyptus camaldulensis) essential oil

Number	Component	percent	RI[1]
1	α-pinene	12.6	935
2	Sabinene	0.20	947
3	Comphene	0.78	950
4	β-pinene	8.81	976
5	α-phellandrene	4.24	1003
6	ρ-cymene	5.60	1024
7	1,8-cineole	0.16	1027
8	isopentyl	19.12	1105
9	trans-pinocarveol	11.40	1136
10	cis-verbenol	0.74	1140
11	Pinocarvone	3.84	1161
12	Terpinen-4-ol	0.85	1175
13	α-terpineol	0.63	1194
14	Myrtenal	1.85	1225
15	α-guaiene	0.61	1435
16	trans-calamenene	0.74	1538
17	Spathulenol	2.52	1572
18	Viridiflorol	4.74	1585
19	Longiborneol	1.56	1598
20	l-epi-cubenol	0.43	1628
21	β-Eudesmol	1.25	1650
22	α-eudesmol	0.88	1653
	Total	83.63	

Table 2. Compositions of Lavender (Lavandula angustifolia) essential oil chemical

Number	component	percent	RI
1	α-thujene	0.21	926
2	ρ-cymene	0.12	1024
3	4,4-dimethyl 1-2-	0.15	1130
4	α-terpineol	0.26	1186
5	cis-hex-3-en-1-ol	18	1386
6	Linalool	1.12	1551
7	β-elemene	0.32	1588
8	β-caryophyllene	7.61	1596
9	Carvacrol methyl	6.18	1604
10	γ-elemen	0.31	1636
11	β-farnese	0.28	1667
12	Neroli	0.32	1692
13	Germacrene D	2.17	1712
14	β-Bisabolene	17.17	1732
15	(E,E) α-farnesene	12.31	1750
16	γ-cadinene	1.64	1760
17	Germacrene B	2.82	1832
18	ρ-cymen-8-ol	0.32	1852
19	α-calacorene	1.42	2108
20	$C_{15}H_4O$	0.67	2188
21	Carvacrol	21.84	2221
	Total	95.24	

3.3. Broth microdilution method

The findings clearly indicated that the essential oils of lavender and eucalyptus had antibacterial activity against a number of bacteria. The lowest MIC value for lavender was 6 µl/ml, against *S. thyphimurium*. The MIC value of lavender against *L. monocytogenes, E. coli* and *B. cereus* were 25, 12.5, and 12.5 µl/ml, respectively. MIC of eucalyptus against *S. thiphymurium, L. monocytogenes, E. coli* and *B. cereus* were 25, 12.5, 12.5, and 12.5 µl/ml, respectively.

3.4. Antifungal assay

Table 5 illustrates the inhibitory activity of essential oils on the growth of different Candida species including C. albicans, C. parapsilosis, C. tropicalis, C. dubliniensis. As shown in Table 5, MICs of eucalyptus essential oil against C. albicans, C. tropicalis, C. dubliniensis and C. parapsilosis were 0.2%, 0.1%, 0.4% and 0.4%, respectively. MICs of lavender essential oil against C. albicans, C. tropicalis, C. dubliniensis and C. parapsilosis were 0.4%, 0.4%, 1.6% and 1.6%, respectively.

4. Discussion

Twenty two components, which represents 83.63% of eucalyptus essential oil and 21 constituents which represents 95.24% of lavender essential oils were identified. The eucalyptus essential oil was characterized by

Table 3. Comparison of inhibition zone of lavender (Lavandula angustifolia) and eucalyptus (Eucalyptus camaldulensis) essential oils with inhibition zone (mm) of standard antibiotics in disk diffusion method

Bacterial strain	Eucalyptus EO(µl)			Lavender EO(µl)			Standard antibiotics		
	14	7	3.5	14	7	3.5	K	Ofx	Am
S. thyphimurium	14	10	7	14	10	9	14	26	18
L.monocytogenes	12	10	-	14	10	-	16	30	10
B. cereus	12	-	-	11	8	-	18	28	16
E. coli	14	10	8	12	8	8	14	34	10

EO: essential oil, -: no inhibition, K: Kanamycin, Ofx: Ofloxacin, Am: Amoxicillin

Table 4. Inhibition zone of different concentrations of lavender (Lavandula angustifolia) and eucalyptus (Eucalyptus camaldulensis) essential oils in agar well diffusion method

Bacterial strains	Lavender EO (µl/ml)				Eucalyptus EO (µl/ml)			
	200	100	50	25	200	100	50	25
S. thyphimurium	11	9	-	-	15	11	10	-
B. cereus	17	15	12	10	20	17	13	9
E. coli	11	10	-	-	19	17	9	-
L.monocytogenes	13	12	10	8	19	16	12	8

-: no inhibition, EO: essential oil

Table 5. The minimum inhibitory concentration **(**MIC) values of fungal strains

Fungal strains	Lavender EO	Eucalyptus EO
C. albicans	0.4%	0.2%
C. dubliniensis	1.6%	0.4%
C. parapsilosis	1.6%	0.4%
C. tropicalis	0.4%	0.1%

EO= Essential Oil

high amounts of isopentyl isovalerate (19.12%), α-pinene (12.6%), trans-pinocarveol (11.4%) and β-pinene (8.81%). Akin et al reported ethanone, eucalyptol, caryophyllene and carvacrol as main components of E. camuldensis (25). In other studies, the major components of eucalyptus essential oils were 1,8 cineole, pinene and spathulenol (6, 26). The lavender essential oil was characterized by high amounts of Carvacrol (21.84%), cis-hex-3-en-1-ol (18%), β-Bisabolene (17.17%), α-farnesene (12.31%). Xiaolan et al reported that 1,5-Dimethyl-1-vinyl-4-hexenylbutyrate was the main constituent of lavender essential oil (43.73%), followed by 1,3,7-Octatriene, 3,7-dimethyl-(25.1%), Eucalyptol (7.32%), and Camphor (3.79%) (27). The observed differences in the constituents of essential oils may be due to different environmental and genetic factors, different chemotypes and the nutritional status of the plants.

Results of this study revealed that the essential oils of lavender and eucalyptus have antibacterial activity with varying magnitudes. The zone of inhibition above 7 mm in diameter was taken as positive result. Generally most of the tested organisms were sensitive to essential oils. The antimicrobial assay showed that different microorganisms tested had different susceptibility to the essential oils. In general, B. cereus as the gram positive bacteria was more sensitive to the EOs compared to other microorganisms. Ashour et al (16) and Benblaid et al. (17) showed that eucalyptus EO has potential antibacterial effect on gram positive bacteria especially on Bacillus cereus. According to similar research, the inhibition zone of essential oils increased by the increase of essential oil levels in all the microorganisms tested (28- 30).

The MIC results are in agreement with the results of agar methods, the highest MIC was observed in S. thyphimurium and minimum zone of inhibition was measured in both agar methods for this bacteria. Comparison of the inhibition zone of essential oils with standard antibiotics showed that inhibition zone of the higher amount of essential oils is similar to Kanamycin and Amoxicillin. These essential oils are potential antibacterial agents and can be used to prevent antibiotic resistant.

In our study, the lowest MIC value of Lavender essential oil was related to C. albicans (0.4%) and C. tropicalis (0.4%). The minimum MIC value of Eucalyptus EO was related to C. tropicalis (0.1%). the essential oil of Eucalyptus was more effective against Candidal species compared to Lavender essential oil. Ashour studied antifungal effect of extracts and essential oils of Eucalyptus sideroxylon and Eucalyptus torquata on some fungal strains. Aspergillus flavus and Aspergillus niger were resistant to the extracts. Essential oils exhibited moderate to high antifungal activities against Candida albicans, A flavus and A niger (16). Benbelaid et al reported high activity of eucalyptus essential oil against Candida albicans (17). Aligiannis et al (31) proposed classification for plant materials, based on MIC results as follows: strong inhibitors-MIC up to 0.5 mg/ml; moderate inhibitors- MIC between 0.6 and 1.5 mg/ml and weak inhibitors-MIC above 1.6 mg/ml. Based on the above classification, the eucalyptus essential oil has

strong activity against all fungal strains. Lavender essential oil antifungal activity against C. albicans and C. tropicalis was strong and against C. dubliniensis and C. parapsilosis was nearer to weak. This result shows that eucalyptus essential oil is more effective on Candida strains compared to lavender essential oil.

5. Conclusion

The study demonstrates that the eucalyptus essential oil has strong activity against all tested fungal strains. The antifungal activity of Lavender essential oil against C. albicans and C. tropicalis was strong and it was nearer to weak against C. dubliniensis and C. parapsilosis. This result demonstrates that eucalyptus essential oil is more effective on Candidal strains compared to lavender essential oil. Results of our study suggest a wider use of these essential oils in pharmaceutical and food preparations.

Conflict of Interests

Authors have no conflict of interest.

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