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Research Article

LIPOXYGENASE INHIBITORY, ANTIOXIDANT, AND ANTIMICROBIAL ACTIVITIES OF SELECTED ESSENTIAL OILS

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ABSTRACT

Objective: To evaluate lipoxygenase inhibitory, antioxidant, and antimicrobial activities of selected essential oils.

Materials and Methods: Essential oils were obtained by hydro distillation using a Clevenger-type apparatus and oils composition was analyzed by gas chromatography-mass spectrometry. Lipoxygenase inhibitory activity of essential oils was evaluated by lipoxygenase inhibition assay and antioxidant activity by ferric reducing antioxidant power, 2,2-diphenyl-1-picrylhydrazyl and β-carotene-bleaching assay. Antimicrobial activity was determined by agar dilution, broth dilution, and poisoned food method.

Results: Lipoxygenase activity was inhibited significantly by *Cymbopogon winterianus* oil, and the inhibition was 96% at 12.5 µg/ml concentration. Concentration providing 50% inhibition (IC_{50}) value of *C. winterinus* oil for lipoxygenase inhibitory activity was 3.98 µg/ml. Lipoxygenase inhibition was 56, 58, 60 and 51% by *Chamomilla recutita*, *Mentha arvensis*, *Cymbopogon martini* and *Cymbopogon flexuosus* oil at 12.5 µg/ml concentration, respectively and IC_{50} values were in the range of 10-12.2 µg/ml. Among major terpenes components of the oils, lipoxygenase inhibitory activity was highest in the citronellal (IC_{50} value 1.66 µg/ml) followed by citronellol and citral. The essential oil of *Ocimum sanctum* exhibited significant reducing potential and antioxidant activity. Antibacterial activity was the highest in *C. flexuosus* oil while antifungal activity was highest in *C. flexuosus* and in *M. arvensis* oil as compared to other oils.

Conclusion: *C. winterianus* oil and its component citronellal with high antilipoxygenase activity are suitable for analgesic and anti-inflammatory purposes. *C. flexuosus* oil is suitable for checking of food spoilage as well as for dandruff due to high antimicrobial activity. *O. sanctum* oil can be used as a good source of antioxidants.

Keywords: Essential oils, Lipoxygenase inhibitory activity, Antioxidant activity, Antimicrobial activity.

INTRODUCTION

Aromatic plants are the source of secondary metabolites with biological activities [1]. Terpenoids are the main constituent of plant essential oils, but many of these oils are also composed of other chemicals like phenylpropanoids [2]. Essential oils are widely used in aromatherapy, cosmetics, and perfumery as well as flavoring agent and mosquito replants [3,4].

The present study was undertaken to examine the effect of essential oils of selected plants on lipoxygenase inhibition and on antioxidant and antimicrobial activities. Lipoxygenase inhibitory and antioxidant activity of aromatic grasses oils were examined as the effect has not been studied in detail. Three aromatic grasses (Java citronella, palmarosa, and lemongrass) were selected as lemongrass oil is used in pharmaceutical industries for controlling pathogens and for anti-inflammatory and analgesic purposes [5]. Lemongrass oil and citral inhibit cytokines production [6]. Java citronella oil is used in replants for mosquitoes and ticks [7]. Antifungal activity is reported in the palmarosa (*Cymbopogon martini*) against *Saccharomyces cerevisiae* [8]. Chamomile flowers and *Ocimum sanctum* leaves are used in herbal teas as relaxing infusion and in common cold [9]. Antioxidant and cyclooxygenase inhibitory activity is reported in eugenol and other compounds from holy-basil (*O. sanctum*) oil [10]. Antibacterial and antifungal activity is also reported from *O. sanctum* and *Ocimum kilimandscharium* oil against fungi causing bio-deterioration of food stuff during storage [11,12]. Vetiver oil is used in perfumes, scents, message blends for insomnia, compresses for acne, and as an ingredient in aftershave lotions. Geranium oil is good for skin as well as it is used as anti-depressant [13]. Japanese mint is the source of menthol, both the essential oil and menthol are used in medicine and in flavor and in cosmetic industries [14]. The essential oils of clove, basil, coriander, nutmeg, mint, black pepper, cinnamon, sage, fennel, etc., have been studied for antioxidant activity [15].

Lipoxygenases play an important role in the development of inflammation and other diseases [16,17]. Various essential oils such as thyme, clove, bitter orange, alove vera, bergamot, etc., were evaluated for lipoxygenase inhibitory activity [18,19]. Lipoxygenase inhibition activity is reported in other secondary metabolites such as curcumin and quercetin [20].

The selection of microorganism for antibacterial activity was based on as its role in skin infection such as *Staphylococcus aureus* and *Staphylococcus epidermidis* and bacteria of food spoilage, food poisoning, wound, and eyes infection such as *Bacillus cereus* and *Bacillus subtilis* [21,22]. *Malassezia furfur* is causative agent of dandruff and *Aspergillus parasiticus* is responsible for spoilage of food, fruit, and vegetables and produces mycotoxins are selected for antifungal activity [23-25].

In the present work, the antioxidant ability of the essential oils was determined by three different methods: Scavenging effect on 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical method, β-carotene-linoleic acid assay and ferric reducing antioxidant power (FRAP) method.

MATERIALS AND METHODS

Chemicals and reagents

Linoleic acid, DPPH, lipoxygenase, β-carotene, ascorbic acid, ferric chloride, butylated hydroxytoluene (BHT), anhydrous sodium sulphate,

menthol (≥99%), quercetin, methanol, ethanol, potassium ferricyanide, trichloroacetic acid (TCA), chloroform, mueller hinton agar, dimethyl sulfoxides (DMSO), and Tween-80 were purchased from Hi-media. citral (≥96%), geraniol (≥98%), linalool (≥97%), α-bisabolol (≥95%), citronellol (≥95%), and citronellal (≥95%) were purchased from Sigma-Aldrich.

Plant materials

The seeds/slips/plants of lemongrass (*Cymbopogon flexuosus* [Nees ex Stued]cv. Krishna), Java citronella (*Cymbopogon winterianus* [Jowitt.] cv. Bio-13), Palmarosa (*C. martinii* [Roxb. Wats. var. *motia* Burk.] cv. PRC-1), Vetiver (*Vetiveria zizanioides* [Nash]cv. KS-1), Japanese mint (*Mentha arvensis* cv. Shivalik), Geranium (*Pelargonium graveolens* [Herit. ex Ait.] cv. CIM-Pawan), *Chamomilla recutita* (Rausch.) cv. Vallary, and *O. sanctum* cv. CIM-angna was purchased from Central Institute of Medicinal and Aromatic Plants, Lucknow, India and cultivated into the herbal garden of faculty of Pharmacy Integral University, Kursi road, Lucknow, India.

Isolation of essential oils

The essential oils of *C. flexuosus*, *C. winterinus*, *C. martinii*, *P. graveolens*, *O. sanctum*, and *M. arvensis* were obtained by hydro-distillation for 1 hr using a Clevenger-type apparatus (Guenther) fresh leaves of these plants [26]. The essential oil of *C. recutita* was obtained from flowers and *V. zizanioides* oil was obtained from roots by hydro-distillation for 6 hr. The essential oils were dried over anhydrous sodium sulfate and stored at 4°C in air-tight vials. Oil content is expressed as a percentage.

Gas chromatography-mass spectrometry (GC-MS) analysis of essential oils

GC-MS analysis was performed on a Shimadzu GC-MS-QP2010 Plus system operating in electron ionization mode (70 eV) equipped with a split/splitless injector (270°C), split ratio 1/150, using an Omega wax capillary column (30 m × 0.25 mm (i.d.), film thickness: 0.25 µm). The column temperature was program from 70°C (1.0 minutes hold) to 180°C at a rate of 4°C/minutes (8.0 minutes hold) to 230°C (11.0 minutes hold) and ion source and interface temperature were 250°C and 280°C, respectively. Helium was used as a carrier gas. Injection volume of each sample was 0.2 µl. MS condition was: Start time: 2.00 minutes, end time: 55.82 minutes, ACQ Mode: Scan, start m/z: 40.00, end m/z: 850.

The percentage composition of the major component of the oils is reported as total peak area. The identification of the essential oil constituents is based on a comparison of their retention times using their mass spectra with data from WILEY8.LIB, NIS5.LIB, SZTERP.LIB.

Lipoxygenase inhibitory assay

Lipoxygenase inhibition was performed by the method developed by Tappel with slight modification [27]. Five µl of essential oils solution in DMSO (which provide 50, 25, 12.5, 6.2, 3.1, 1.55 µg essential oil/ml reaction mixture) was mixed with 1.74 ml borate buffer (0.2 M, pH 9.2) and mixed with 5 µl enzyme solution (50,000 unit/ml) in borate buffer (0.2 M, pH 9.2). The reaction was initiated by adding 250 µl linoleic acid solution (5.0 mg linoleic acid was mixed with 15 µl ethanol then 15 ml borate buffer (0.2 M, pH 9.2) was added with vigorous shaking). The increase in absorbance at 234 nm was recorded for 5 minutes in a spectrophotometer (Genesys 10S ultra violet-visible, Thermo Scientific). Quercetin at same concentration as essential oils was used as a positive control. The reaction mixture containing 5 µl DMSO in place essential oil solution was used as a negative control. The percent inhibition was calculated as follows:

Percent inhibition = $[(\Delta A_0 - \Delta A_1/\Delta A_0) \times 100]$

Where, ΔA_0 is the absorbance of control without essential oil, ΔA_1 is the absorbance of test solution. All the tests were carried out in triplicate; the sample concentration providing 50% inhibition (IC_{50}) was obtained by software Prism 5.0.

Antioxidant activity

FRAP

FRAP was determined according to Oyaizu [28]. Briefly, different amounts of essential oils (5, 10, 20, 40, 80 mg/ml) were dissolved in DMSO to make 1.0 ml solution and mixed with 2.5 ml of phosphate buffer (0.2 M, pH 6.6) and 1.0 ml of potassium ferricyanide (1%). The mixture was incubated at 50°C for 20 minutes. 2.5 ml of TCA (10%) was added to the mixture, which was then centrifuged at 6000 g for 5 minutes. Finally, 2.5 ml of supernatant was mixed with 2.5 ml of distilled water and 0.5 ml of ferric chloride (0.1%). Absorbance was measured at 700 nm. Same amount of DMSO was used in place of essential oil solution as a blank. All the tests were carried out in triplicate. Ascorbic acid and BHT were used as a positive control. Higher absorbance of the reaction mixture indicates greater reducing power.

DPPH radical scavenging assay

The DPPH radical scavenging activity of essential oils was determined by the method developed by Blois with slight modification [29]. The different concentrations of 100 µl essential oils solution in methanol was mixed with 0.9 ml (100 µM) DPPH solution. After a 30 minutes incubation period at 37°C in the dark, the absorbance was measured against blank at 517 nm. All the tests were carried out in triplicate. Same amount of methanol was used in place of essential oil solution as a blank. Ascorbic acid and BHT were used as a positive control. Percent radical scavenging activity was calculated as follows:

Percent radical scavenging activity = $[(A_0 - A_1/A_0) \times 100]$

Where, A_0 and A_1 are the absorbances of blank and sample, respectively. The sample IC_{50} was obtained by software Prism 5.0.

***β-carotene*-bleaching (BCB) method**

β-carotene-linoleic acid assay was performed by the method developed by Miller [30]. 2.0 mg of β-carotene was dissolved in 10 ml chloroform, 1.0 ml of this solution was pipetted into a round-bottom flask containing 20 mg of linoleic acid and 200 mg of Tween-80. Chloroform was completely evaporated using a vacuum evaporator, and 50 ml of aerated distilled water was added to the residue with vigorous shaking. 1.9 ml of this emulsion was mixed with 100 µl of essential oil dilution in DMSO. All the test tubes incubated in the water bath at 50°C for 2 hr, together with two blanks one containing BHT as a positive control and other with the same amount of DMSO in place of essential oils solution. The absorbance of the reaction mixture was measured at 470 nm. The percent inhibition was calculated as follows:

Percent inhibition = $[(\Delta A_0 - \Delta A_1/\Delta A_0) \times 100]$

Where, ΔA_0 is the absorbance of control without essential oil, ΔA_1 is the absorbance of test solution. All the tests were carried in triplicate, the sample IC_{50} value was obtained by software Prism 5.0.

Antimicrobial activity

Antibacterial activity of essential oils of *C. flexuosus*, *C. winterinus*, *C. martinii*, *C. recutita*, *M. arvensis*, *P. graveolens*, and *V. zizanioides* was performed by the "CLSI M7-A7" agar dilution assay [31], against *Klebsiella pneumoniae* (NCIM 2957), *B. cereus* (NCIM 2156), *B. subtilis* (NCIM 2920), *S. aureus* (NCIM 2079), and *S. epidermidis* (NCIM 2493). Mueller Hinton agar was used nutrient media. Minimum inhibitory concentration (MIC) were determined as the lowest concentration of the oils resulting no growth. All the tests were carried out in triplicate.

Antifungal activity was performed by "NCCL M27-A2" broth dilution assay against *M. furfur* (MTCC 1374) and poisoned food method [32,33], against *A. parasiticus* (NCIM 898). Sabouraud glucose broth was used for susceptibility testing of *M. furfur* and Potato dextrose agar was used for *A. parasiticus*. MIC_s were determined as the lowest concentration of the oils resulting no growth in broth dilution assay and ≥90% inhibition

in mycelial growth was recorded as MICs in poisoned food method. All the tests were carried out in triplicate.

Statistical analysis

Graph Pad Prism 5.0 software (Graph Pad software Inc. CA, USA) was used for all statistical analyses. One-way ANOVA followed by Turkey multiple comparison tests was used to calculate the significance. The level of significance was set at $p<0.01$. Statistical calculations and IC_{50} was analyzed by using non-linear regression.

RESULTS

Oil content and composition

Oil content in *M. arvensis* was 1.0% (Table 1) and main components were menthol (49%) and menthone (6.5%). Oil content of *C. recutita* was 0.2% and the major components were α-bisabolol oxide (44.6.0%), β-farnesene (12.2%), α-bisabolone oxide A (9.5%), α-bisabolol (7.4%) and chamazulene (1.5%). Oil content in *P. graveolens* was 0.25% and the major components were geraniol (20.1%), citronellol (17.3%), linalool (11.7%), citronellyl formate (8.4%) and γ-eudesmol (6.2%). *C. winterinus* leaves oil content was 1.1% and the major components were citronellal (22.2%), citronellol (6.3%), geraniol (21.0%). Oil content in *C. flexuosus* was 0.9% (major components were citral [47.9%], geraniol [14.1%], linalool [4.7%]). Oil content was 1.2% in *C. martinii*, and the major components were geraniol (54.8%). The essential oil yield in *V. zizanioides* was 2.4%, and main components were khusimol (23.9%), vetinol (8.7%), khusinol (5.8%), and khusimone (4.3%). Oil content in *O. sanctum* was 0.45% with a major component was eugenol (24.9%).

Lipoxygenase inhibition assay

The essential oil of *C. winterinus* exhibited the highest lipoxygenase inhibition activity than other oils and inhibition was 31%, 44%, 66%, and 96% at 1.55, 3.1, 6.2, and 12.5 µg/ml concentration (Table 2). IC_{50} value of *C. winterinus* oil for lipoxygenase inhibition was 3.98 µg/ml. The IC_{50} values of *C. recutita*, *M. arvensis*, *C. martinii*, and *C. flexuosus* oils were 10.04, 10.91, 10.27, and 12.23 µg/ml, respectively, while *O. sanctum* and *P. graveolens* oils have IC_{50} values 18.71, 24.25 µg/ml, respectively. Lipoxygenase inhibition was not observed in *V. zizanioides* oil up to 50 *µg*/ml test solution.

Citronellal exhibited highest lipoxygenase inhibitory activity with IC_{50} value 1.66 µg/ml. Citronellol and citral exhibited lipoxygenase inhibition and IC_{50} values were 3.6, 7.89 µg/ml, respectively. The IC_{50} values of geraniol and α-bisabolol for lipoxygenase inhibition were 18.53 and 14.98 µg/ml, respectively. The menthol and linalool exhibited weak lipoxygenase inhibition with IC_{50} values 28.84, 45.14 µg/ml, respectively. The IC_{50} value of positive control quercetin was 23.6 µg/ml.

Antioxidant activity

In FRAP method, ferric reducing power of essential increased with increasing concentration of oils (Table 3). The highest ferric reducing power was shown by *O. sanctum* oil, and it was equal to BHT but marginally lower than ascorbic acid. The reducing power of other oils was lower than BHT and ascorbic acid.

In the DPPH radical scavenging method, the highest activity was shown by *O. sanctum* with IC_{50} value 7.85 µg/ml (Table 2), followed by oils of *C. flexeosus*, *C. winterinus*, and *C. recutita* (IC_{50} values 660, 552.8, and 1669 µg/ml). DPPH scavenging activity of oil constituents was lower than the activity of essential oils. The IC_{50} value of citral was 45970 µg/ml. Geraniol and citronellal showed 27.2 and 26.7% activity at 100,000 µg/ml concentration, and activity was not observed in citronellol, linalool, α-bisabolol, and menthol at 100,000 µg/ml (data not presented).

Antioxidant activity as determined by BCB method and expressed as IC_{50} values is presented in Table 2. IC_{50} value of BHT was lower than the essential oils and their constituents. IC_{50} value of *O. sanctum* oil was the lowest (20.49 µg/ml) in comparison with other oils and constituents.

Antibacterial activity

The highest antibacterial activity was shown by *C. flexuosus* oil against all bacterial strains (Table 4) with MICs values in the range of 200-600 µg/ml. While, minimum activity was present in the oil of *C. martinii* (MICs values 700-950 µg/ml).

Antifungal activity

Substantial antifungal activity was found in oils of *M. arvensis* and *C. flexuosus* against *M. furfur* with MICs values 50 and 60 µg/ml by broth dilution assay (Table 5). Oil of *C. flexuosus* also showed maximum activity against *A. parasiticus* by poisoned food method than other essential oils.

Table 1: The essential oils composition (%) by GC-MS

Name of plants	Oil content % (v/w)	Main components of essential oil (%)
M. arvensis	0.80±0.28	Menthol (49.4%), menthone (6.5%).
C. recutita	0.20±0.03	α-Bisabolol oxide (44.6.0%), β-farnesene (12.2%), α-bisabolone oxide A (9.5%), α-bisabolol (7.4%), chamazulene (1.5%)
P. graveolens	0.25±0.03	Geraniol (20.1%), citronellol (17.3%), Linalool (11.7%), citronellyl formate (8.4%), γ-eudesmol (6.2%)
C. flexuosus	1.00±0.04	Citral (47.9%), geraniol (14.1%), linalool (4.7%)
C. winterianus	1.10±0.09	Citronellal (22.2%), citronellol (6.3%), geraniol (21.0%), citronellyl acetate (8.8%), geranyl acetate (7.8%), limonene (3.1%)
C. martini	1.10±0.07	Geraniol (54.8%)
V. zizanioides	2.36±0.03	Khusimol (23.9%), khusinol (5.8%)
O. sanctum	0.45±0.04	Eugenol (24.9%)

M. arvensis: Mentha arvensis, C. recutita: Chamomilla recutita, P. graveolens: Pelargonium graveolens, C. flexuosus: Cymbopogon flexuosus, C. winterianus: Cymbopogon winterianus, C. martini: Cymbopogon martini, V. zizanioides: Vetiveria zizanioides, O. sanctum: Ocimum sanctum, GC-MS: Gas chromatography-mass spectrometry

Table 2: Lipoxygenase inhibitory, DPPH radical scavenging and inhibition of β-carotene-bleaching of essential oils/components

Essential oil/ component	Lipoxygenase inhibition IC_{50}(µg/ml)	DPPH radical scavenging IC_{50} (µg/ml)	β-carotene bleaching IC_{50} (µg/ml)
M. arvensis	10.91±2.21	33130±748.90	142.40±25.20
C. recutita	10.04±0.99	1669±125.60	45.93±6.05
P. graveolens	24.25±6.56	16430±1156.0	734.00±74.66
C. flexuosus	12.23±0.65	660.2±11.60	125.50±19.28
C. winterianus	3.98±0.11	552.8±8.90	86.37±8.11
C. martini	10.27±1.66	37330±613.10	275.71±38.22
V. zizanioides	Nd*	3874±302.00	307.00±37.72
O. sanctum	18.71±3.13	7.85±0.72	20.49±2.15
Citral	7.89±1.55	45970±698.2	361.70±40.91
Geraniol	18.53±3.85	-	375.00±20.73
Linalool	45.14±3.08	-	315.80±92.71
α-Bisabolol	14.98±1.16	-	95.18±12.14
Menthol	28.84±3.47	-	592.1±184.10
Citronellol	3.60±0.72	-	370.20±26.06
Citronellal	1.66±0.11	-	253.60±35.39
Quercetin	23.63±8.57	-	-
BHT	-	5.4±0.68	0.64±0.24
Ascorbic acid	-	4.4±0.39	-

*Nd=not detected, DPPH: 2,2-diphenyl-1-picrylhydrazyl, IC_{50}: Concentration providing 50% inhibition, BHT: Butylated hydroxytoulene, *M. arvensis: Mentha arvensis, C. recutita: Chamomilla recutita, P. graveolens: Pelargonium graveolens, C. flexuosus: Cymbopogon flexuosus, C. winterianus: Cymbopogon winterianus, C. martini: Cymbopogon martini, V. zizanioides: Vetiveria zizanioides, O. sanctum: Ocimum sanctum*, DPPH: 2,2-diphenyl-1-picrylhydrazyl

Table 3: Antioxidant activity of essential oil/oil constituent at different concentrations measured by the FRAP method

Essential oil/oil constituent	5 mg/ml	10 mg/ml	20 mg/ml	40 mg/ml	80 mg/ml
M. arvensis	0.002±0.001	0.009±0.001	0.016±0.003	0.030±0.003	0.052±0.00
C. recutita	0.372±0.001	0.571±0.002	0.792±0.002	0.909±0.003	1.016±0.003
P. graveolens	0.071±0.002	0.090±0.003	0.113±0.001	0.131±0.002	0.148±0.002
C. flexuosus	0.548±0.003	0.680±0.017	0.851±0.002	0.985±0.004	1.366±0.005
C. winterianus	0.250±0.003	0.386±0.004	0.583±0.003	0.791±0.016	0.993±0.004
C. martini	0.128±0.004	0.227±0.003	0.347±0.004	0.480±0.001	0.594±0.002
V. zizanioides	0.142±0.004	0.300±0.003	0.514±0.001	0.738±0.007	0.842±0.004
O. sanctum	2.960±0.012	3.292±0.006	3.448±0.010	3.615±0.007	-
Citral	0.468±0.002	0.581±0.006	0.747±0.004	0.877±0.005	0.939±0.004
Geraniol	0.010±0.003	0.017±0.001	0.026±0.004	0.039±0.005	0.047±0.004
Citronellol	0.015±0.004	0.039±0.005	0.049±0.002	0.091±0.004	0.111±0.009
Citronellal	0.187±0.007	0.193±0.004	0.210±0.001	0.620±0.003	0.710±0.003
BHT	2.143±0.004	2.311±0.005	2.383±0.006	2.394±0.004	2.495±0.007
Ascorbic acid	3.940±0.007	4.293±0.009	-	-	-

All the values are mean±SD of three experiments. The value of significance is set at $p<0.01$ and all the values are significant. BHT: Butylated hydroxytoulene, *M. arvensis: Mentha arvensis, C. recutita: Chamomilla recutita, P. graveolens: Pelargonium graveolens, C. flexuosus: Cymbopogon flexuosus, C. winterianus: Cymbopogon winterianus, C. martini: Cymbopogon martini, V. zizanioides: Vetiveria zizanioides, O. sanctum: Ocimum sanctum*, SD: Standard deviation

Table 4: MIC (µg/ml) of studied essential oils citral tested against Gram-negative and Gram-positive bacteria

Essential oil	Bacterial strain				
	K. pneumonia	***B. cereus***	***B. subtilis***	***S. epidermidis***	***S. aureus***
C. flexuosus oil	300	300	600	400	200
C. winterianus oil	750	700	900	600	700
C. martini oil	950	850	950	700	900
C. recutita oil	600	800	700	750	750
M. arvensis oil	550	500	600	500	600
Pelargonium graveolens oil	600	600	700	650	800
V. zizanioides oil	800	700	700	650	800
Citral	250	350	650	300	250
Ciprofloxacin	0.16	0.13	0.12	0.14	0.12

Ciprofloxacin was used as a positive control. *C. flexuosus: Cymbopogon flexuosus, C. winterianus: Cymbopogon winterianus, C. martini: Cymbopogon martini, C. recutita: Chamomilla recutita, M. arvensis: Mentha arvensis, P. graveolens: Pelargonium graveolens, V. zizanioides: Vetiveria zizanioides, K. pneumonia: Klebsiella pneumonia, B. cereus: Bacillus cereus, B. subtilis: Bacillus subtilis, S. epidermidis: Staphylococcus epidermidis, S. aureus: Staphylococcus aureus*, MIC: Minimum inhibitory concentration

Table 5: MIC (µg/ml) of studied essential oils tested against

Essential oil	Fungal strain	
	Malassezia furfur	**Aspergillus parasiticus**
C. flexuosus oil	60	750
C. winterianus oil	120	1200
C. martini oil	100	1200
C. recutita oil	400	1200
M. arvensis oil	50	900
P. graveolens oil	300	1000
Vetiveria zizanioides oil	140	950

C. flexuosus: Cymbopogon flexuosus, C. winterianus: Cymbopogon winterianus, C. martini: Cymbopogon martini, C. recutita: Chamomilla recutita, M. arvensis: Mentha arvensis, P. graveolens: Pelargonium graveolens, V. zizanioides: Vetiveria zizanioides, MIC: Minimum inhibitory concentration

DISCUSSION

Various studies have demonstrated the lipoxygenase inhibitory activity of essential oils [18]. The lipoxygenase inhibitory activity of *C. recutita* essential oil is due to chamazulene and α-bisabolol [34,35]. The IC_{50} value obtained in our study for α-bisabolol is comparable with other worker [36]. The mode of lipoxygenase inhibition is due to the reduction of the active ferric species of enzyme to its inactive ferrous form [20]. Another mode of lipoxygenase inhibition is the reduction of hydro peroxides that are essential activators of lipoxygenase [37]. But in our study *C. winterinus* oil and its main component citronellal showed the highest lipoxygenase inhibition, while their reducing power was low. Therefore, the mode of lipoxygenase inhibition by essential oils is unclear.

Ferric reducing capacity was evaluated by many workers [38]. Essential oils containing phenolics showed high activity [39]. In our study, *O. sanctum* oil showed high activity, and it may be due to the presence of 26% eugenol in the essential oil.

Many workers reported the antioxidant activity of essential by DPPH assay. Essential oils in which phenolic compounds were not present showed weak activity and oils containing phenolics showed good antioxidant activity by DPPH assay [40,41]. In our study, the essential oil of *O. sanctum* showed the highest scavenging activity compared to other essential oils. Antioxidant activity of tea and herbal infusions was examined by other workers, among them herbal teas, green tea, white tea, and black tea were characterized by the highest, whereas chamomile and peppermint tea by the lowest antioxidant activity [9]. This may be due to the presence of eugenol. All the essential oils showed high DPPH scavenging activity than the studied standards (main components) of the oil. This may be due to the synergistic effect of the oil constituents [42].

Various workers reported the antioxidant activity of many essential oils by BCB assay [38]. The oils containing phenolic and sesquiterpene showed high antioxidant activity [43,44]. In our study, the essential oil of *O. sanctum* showed the highest activity. It may be due to the presence of eugenol a phenolic compound. High activity of essential oil of *C. recutita* may be due to the presence of sesquiterpene such as α-bisabolol and β-farnesene.

Strong antifungal activity was present in *C. flexuosus* oil against *M. furfur*. This is perhaps due to the use of sensitive broth dilution method. High antibacterial and antifungal activity in *C. flexuosus* oil is related with strong activity in citral which is main monoterpene in the oil.

CONCLUSION

C. winterianus oil and its component citronellal with high antilipoxygenase activity are suitable for analgesic and anti-inflammatory purposes. *C. flexuosus* oil is suitable for checking of food spoilage as well as for dandruff due to high antimicrobial activity. *O. sanctum* oil can be used as a good source of antioxidants.

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REFERENCES

1. Sunder K, Rani TP, Amma BV. GC-MS analysis and antibacterial activity of *Myristica fragrans* seed extracts against lower respiratory tract pathogen *Acinetobacter baumanii*. Asian J Pharm Clin Res 2014;7(3):126-9.
2. Sangwan NS, Farooqi AH, Shabih F, Sangwan RS. Regulation of essential oil production in plants. Plant Growth Regul 2001;34(1):3-21.
3. Nerio LS, Olivero-Verbel J, Stashenko E. Repellent activity of essential oils: A review. Bioresour Technol 2010;101(1):372-8.
4. Franz CM. Essential oil research: Past, present and future. Flavour Fragr J 2010;25(3):112-3.
5. Katsukawa M, Nakata R, Takizawa Y, Hori K, Takahashi S, Inoue H. Citral, a component of lemongrass oil, activates PPARα and γ and suppresses COX-2 expression. Biochim Biophys Acta 2010;1801(11):1214-20.
6. Bachiega TF, Sforcin JM. Lemongrass and citral effect on cytokines production by murine macrophages. J Ethnopharmacol 2011;137(1):909-13.
7. Sherwood K, Sherwood F. Repellent composition containing natural oils of citronella, cedar and wintergreen and use thereof. US Patent, 1992; No.5, 106, 622.
8. Prashar A, Hili P, Veness RG, Evans CS. Antimicrobial action of palmarosa oil (*Cymbopogon martinii*) on *Saccharomyces cerevisiae*. Phytochemistry 2003;63(5):569-75.
9. Rusaczonek A, Franciszek S, Wazkicwicz-Robak B. Antioixidant properties of tea and herbal infusions-a short report. Pol J Food Nutr Sci 2010;60(1):33-5.
10. Kelm MA, Nair MG, Strasburg GM, DeWitt DL. Antioxidant and cyclooxygenase inhibitory phenolic compounds from *Ocimum sanctum* Linn. Phytomedicine 2000;7(1):7-13.
11. Aggarwal SG, Goyal S. Comparative analysis of antimicrobial activity of essential oil of *Ocimum kilimandscharium*. Asian J Pharm Clin Res 2012;5(1):53-5.
12. Kumar A, Shukla R, Singh P, Dubey NK. Chemical composition, antifungal and antiaflatoxigenic activities of *Ocimum sanctum* L. essential oil and its safety assessment as plant based antimicrobial. Food Chem Toxicol 2010;48(2):539-43.
13. Maxwell-Hudson C. Aromatherapy Massage Book. London: Dorling Kindersley Limited; 1994.
14. Farooqi AH, Sharma S. Effect of growth retardants on growth and essential oil content in Japanese mint. Plant Growth Regul 1988;7(1):39-45.
15. Politeo O, Jukic M, Milos M. Chemical composition and antioxidant activity of essential oils of twelve spice plants. Croat Chem Acta 2006;79(4):545-52.
16. Duroudier NP, Tulah AS, Sayers I. Leukotriene pathway genetics and pharmacogenetics in allergy. Allergy 2009;64(6):823-39.
17. Mochizuki N, Kwon YG 15-lipoxygenase-1 in the vasculature: expanding roles in angiogenesis. Circ Res 2008;102(2):143-5.
18. Wei A, Shibamoto T. Antioxidant/lipoxygenase inhibitory activities and chemical compositions of selected essential oils. J Agric Food Chem 2010;58(12):7218-25.
19. Albano SM, Lima AS, Miguel MG, Pedro LG, Barroso JG, Figueiredo AC. Antioxidant, anti-5-lipoxygenase and antiacetylcholinesterase activities of essential oils and decoction waters of some aromatic plants. Rec Nat Prod 2012;6(1):35-48.
20. Jankun ES, Wynimko JC, Selman SH, Jankun J. Lipoxygenases - A challenging problem in enzyme inhibition and drug development. Curr Enzym Inhib 2007;3(2):119-32.
21. Kloos WE, Lambe DW. Staphylococcus. In: Balowa A, Hausler WJ, Herrmann KL, Isenberg HD, Shadomy HJ, editors. Manual of Clinical Microbiology. 5th ed. Washington, DC: American Society for Microbiology; 1991. p. 222-37.
22. Drobniewski FA. *Bacillus cereus* and related species. Clin Microbiol Rev 1993;6(4):324-38.
23. Selvakumar P, Naveena E, Prakash BD. Studies on the antidandruff activity of essential oil of *Coleus amboinicus* and *Eucalyptus globulus*. Asian Pac J Trop Biomed 2012;2(2):715-9.
24. Doster MA, Michailides TJ, Morgan DP. *Aspergillus* species and mycotoxins in figs from California orchards. Plant Dis 1996;80(5):484-9.
25. Calvo AM, Bok J, Brooks W, Keller NP. veA is required for toxin and sclerotial production in *Aspergillus parasiticus*. Appl Environ Microbiol 2004;70(8):4733-9.
26. Guenther E. The Essential Oils 1. New York: Van Nostrand; 1955. p. 316-9.
27. Tappel AL. Lipoxidase. Methods Enzymol 1962;5:539-42.
28. Oyaizu M. Studies on products of browning reactions: Antioxidative activities of browning reaction prepared from glucosamine. Jpn J Nutr 1986;44:307-15.
29. Blois MS. Antioxidant determinations by the use of a stable free radical. Nature 1958;18:1199-200.
30. Miller HE. A simplified method for the evaluation of antioxidants. J Am Oil Chem Soc 1971;48(2):91-7.
31. Clinical and Laboratory Standards Institute. Reference Method for Dilution Antimicrobial Susceptibility Tests for Bacteria that Grow Arobically. Approved Standard. 7th ed. Wayne, PA: Clinical and Laboratory Standards Institute Document M7-A7; 2006.
32. Clinical and Laboratory Standards Institute. Reference Method for Broth Dilution Antifungal Susceptibility Testing of Yeast. Approved Standard. 2nd ed. NCCL Document, M27-A2. Wayne, PA: Clinical and Laboratory Standards Institute; 2002
33. Mishra M, Tiwari SN. Toxicity of *Polyalthia longifolia* against fungal pathogen of rice. Indian Phytopathol 1992;45(1):56-61.
34. Berry M. The chamomiles. Pharm J 1995;254:191-3.
35. Safayhi H, Sabieraj J, Sailer ER, Ammon HP. Chamazulene: An antioxidant-type inhibitor of leukotriene B4 formation. Planta Med 1994;60(5):410-3.
36. Baylac S, Racine P. Inhibition of 5-lipoxygenase by essential oils and other natural fragrant extracts. Int J Aromatherapy 2003;13(2-3):138-42.
37. Schewe T, Sadik C, Klotz LO, Yoshimoto T, Kühn H, Sies H. Polyphenols of cocoa: Inhibition of mammalian 15-lipoxygenase. Biol Chem 2001;382(12):1687-96.
38. Miguel MG. Antioxidant and anti-inflammatory activities of essential oils: A short review. Molecules 2010;15(12):9252-87.
39. Yang Y, Yue Y, Runwei Y, Guolin Z. Cytotoxic, apoptotic and antioxidant activity of the essential oil of *Amomum tsao-ko*. Bioresour Technol 2010;101(11):4205-11.
40. Mothana RA, Al-Rehaily AJ, Schultze W. Chemical analysis and biological activity of the essential oils of two endemic *Soqotri Commiphora* species. Molecules 2010;15(2):689-98.
41. Mechergui K, Coelho JA, Serra MC, Lamine SB, Boukhchina S, Khouja ML. Essential oils of *Origanum vulgare* L. subsp. glandulosum (Desf.) Ietswaart from Tunisia: Chemical composition and antioxidant activity. J Sci Food Agric 2010;90(10):1745-9.
42. De Martino L, De Feo V, Fratianni F, Nazzaro F. Chemistry, antioxidant, antibacterial and antifungal activities of volatile oils and their components. Nat Prod Commun 2009;4(12):1741-50.
43. Aidi Wannes W, Mhamdi B, Sriti J, Ben Jemia M, Ouchikh O, Hamdaoui G, *et al*. Antioxidant activities of the essential oils and methanol extracts from myrtle (*Myrtus communis* var. italica L.) leaf, stem and flower. Food Chem Toxicol 2010;48(5):1362-70.
44. Joshi SC, Verma AR, Mathela CS. Antioxidant and antibacterial activities of the leaf essential oils of Himalayan *Lauraceae* species. Food Chem Toxicol 2010;48(1):37-40.