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Chemical composition and antimicrobial activity of the essential oil of *Ocimum basilicum* L. (sweet basil) from Western Ghats of North West Karnataka, India

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Abstract

Context:

Ocimum basilicum L. (Lamiaceae) commonly known as sweet basil, has been used as a traditional medicinal plant for the treatment of headaches, coughs, diarrhea, constipation, warts, worms, and kidney malfunctions.

Materials and Methods:

The essential oil of the flowering aerial parts of *O. basilicum* growing in the Western Ghats region of North West Karnataka, India, was obtained by hydro-distillation and analyzed by gas chromatography equipped with flame ionization detector and gas chromatography coupled to mass spectrometry (GC–MS). The oil was tested against six Gram-positive, eight Gram-negative bacteria, and three fungi by the tube-dilution method at a concentration range of 5.00-0.009 mg/mL.

Results:

Twenty-five constituents were identified in the essential oil of *O. basilicum*. The major constituents were identified as methyl eugenol (39.3%) and methyl chavicol (38.3%), accounting for 98.6% of the total oil. The oil was found to be active against Gram-positive, Gram-negative bacteria, and

fungi with minimal bactericidal concentration values in the range of 0.143 ± 0.031 to 0.572 ± 0.127 mg/mL, 0.781 ± 0.382 to 1.875 ± 0.684 mg/mL, and 0.312 ± 0.171 to 0.442 ± 0.207 mg/mL, respectively.

Conclusion:

The essential oil of *O. basilicum* of this region contains methyl eugenol/methyl chavicol chemotype and has bactericidal properties.

KEY WORDS: Bactericidal property, essential oil composition, gas chromatography-mass spectrometry, Lamiaceae, methyl chavicol, methyl eugenol, *Ocimum basilicum* L.

INTRODUCTION

Ocimum basilicum L. (sweet basil) belongs to the family Lamiaceae, which includes about 200 species occur in various botanic varieties and forms.[1] Traditionally, sweet basil has been used as a medicinal plant in the treatment of headaches, coughs, diarrhea, constipation, warts, worms, and kidney malfunctions.[2] *O. basilicum* is a popular culinary herb and a source of essential oils extracted by steam distillation from the leaves and the flowering tops which are used to flavor foods, in dental and oral products, and in fragrances.[3,4,5] The aromatic character of each type of basil is determined by genotype and depends on the major chemical compounds of essential oils primarily consisting of monoterpenes and phenylpropanoids.[6,7] The essential oil has antimicrobial, [8] antifungal, and insect-repelling,[9] anticonvulsant, hypnotic,[10] and antioxidant[11] activities. Various workers have reported chemical composition of the essential oil of *O. basilicum* from different parts of the world is summarized in Table 1. [12,13,14,15,16,17,18,19,20,21,22,23,24,25,26,27] In a study, on 270 sweet basil accessions, the major constituents were found to be linalool, methyl chavicol, or citral and 1,8-cineole, camphor, thymol, methyl cinnamate, eugenol, methyl eugenol, methyl isoeugenol, and elemicine.[28] According to Marotti *et al*.,[6] the European basil type has linalool and methyl chavicol as the major oil constituents. The reunion basils, another chemotype have methyl chavicol as a major constituent, whereas tropical chemotypes of basil have methyl cinnamate as the major constituent. Another basil chemotype grown in North Africa, Russia, Eastern Europe, and parts of Asia has eugenol as the major constituent.[6] Reports on the chemical composition of the essential oil of *O. basilicum* from Western Ghats region of Karnataka are very inadequate, and there is no report on the terpenoid composition of this plant from this region. Hence, this study was carried out to describe detailed chemical investigation and antimicrobial property of the essential oil of *O. basilicum* from Western Ghats region (one of the 34 global biodiversity hotspots[29]).

MATERIALS AND METHODS

Plant material

The flowering aerial parts of *O. basilicum* were collected in May 2011, at the height of 800 m from district Belgaum (N 15.88668; E 74.52353), Karnataka, India. The plant was identified by Dr. Harsha Hegde, Research Scientist, Regional Medical Research Centre (Indian Council of Medical Research), Belgaum (voucher specimen No. RMRC-532).

Isolation of essential oil

The fresh plant material (500 g) was subjected to hydro - distillation using Clevenger type apparatus for 3 h. The oil was collected and dried over anhydrous sodium sulfate and stored in sealed vials at −4°C until analysis.[30] The oil yield was 0.21% v/w.

Gas chromatography

The gas chromatography (GC) analysis of the oil was carried out on Varian 450 gas chromatograph equipped with flame ionization detector (FID), using stationary phase CP Sil-8-CB (30 m × 0.25 mm i.d., 0.25 µm film thickness) column under the experimental conditions reported earlier.[31,32] Nitrogen was a carrier gas at 1.0 mL/min flow rate. Temperature was increased at the rate of 3°C/min between 60 and 220°C. Injector and detector temperatures were 230 and 250°C, respectively. The injection volume was 1.0 µL diluted in *n*-hexane; split ratio was 1:50.

Gas chromatography-mass spectrometry

The GC–mass spectrometry (MS) analysis of the oil was carried out on Thermo Scientific Trace Ultra GC interfaced with a Thermo Fisher Scientific SpA. Strada Rivoltana, 20090 Rodano-Milan, Italy. fitted with TG-5 (30 m × 0.25 mm i.d., 0.25 µm film thickness) column. The oven temperature was increased at the rate of 3°C/min between 60 and 220°C using helium as a carrier gas at 1.0 mL/min. The injector temperature was 230°C, injection size 0.1 µL prepared in *n*-hexane; split ratio 1:50. MS were taken at 70 eV with mass scan range of 40-450 amu.[33,34]

Identification of the components

Identification of constituents was done on the basis of retention index (RI) (determined with reference to the homologous series of *n*-alkanes C_8-C_{25}, under identical experimental condition), MS library search (NIST and WILEY), and by comparison with MS literature data.[35] The relative amounts of individual components were calculated based on GC peak area (FID response) without using the correction factor.

Antimicrobial strains

The microorganisms screened for antimicrobial activity were obtained from the National Collection of Industrial Microorganisms (NCIM), National Chemical Laboratory, Pune. The microorganisms were *Staphylococcus aureus* (NCIM 2079), *Staphylococcus epidermidis* (NCIM 2493), *Streptococcus faecalis* (NCIM 2080), *Micrococcus flavus* (NCIM 2379), *Micrococcus luteus* (NCIM

2103), *Bacillus subtilis* (NCIM 2063) (Gram-positive); *Escherichia coli* (NCIM 2574), *Enterobacter aerogenes* (NCIM 2694), *Klebsiella pneumoniae* (NCIM 2957), *Pseudomonas aeruginosa* (NCIM5029), *Proteus vulgaris* (NCIM 2813), *Proteus mirabilis* (NCIM 2241), *Serratia marcescens* (NCIM 2078), *Salmonella typhimurium* (NCIM 2501) (Gram-negative bacteria), *Aspergillus niger* (NCIM 620), *Aspergillus fumigatus* (NCIM 902), and *Penicillium chrysogenum* (NCIM 733) (fungi).

Preparation of test sample

The essential oil of the flowering aerial parts of *O. basilicum* was dissolved in 10% dimethylsulfoxide (DMSO), which is reported to be nontoxic to microorganisms at this concentration,[36,37] with Tween 80 (1% v/v for easy diffusion). Erythromycin (Alembic Ltd., Solan, Himachal Pradesh, India), amikacin (Iskon Remedies, Sirmour, Himachal Pradesh, India), and amphotericin B (Chandra Bhagat Pharma Pvt. Ltd., Ankleshwar, India) were used as a positive reference standard for Gram-positive, Gram-negative bacteria and fungi, respectively.

Preparation of inocula

The inocula of bacterial strains were prepared from 18 h old cultures using nutrient broth, and Sabourad's dextrose broth was used for fungi. The suspensions were adjusted to 0.5 of the McFarland standard turbidity -10^4 for bacteria and -10^3 for fungal colony forming units (CFU)/mL.[38]

Antimicrobial assay

The tube-dilution method was used to determine the minimum inhibitory concentration (MIC) of the essential oil of *O. basilicum* against the microorganisms under study. The oil was dissolved in 10% DMSO with Tween 80 (1% v/v for easy diffusion). The final concentration of the oil was 5.00 mg/mL. Serial two-fold dilutions were prepared from the stock solution to give concentrations ranging from 5.00 to 0.009 mg/mL of the essential oil for bacteria and fungi.[39] Erythromycin, amikacin, and amphotericin B were dissolved in sterile distilled water, and two-fold dilutions were prepared (1.0-0.002 mg/mL). About 1 mL of each concentration was mixed with 1.0 mL of sterile nutrient broth for bacteria at 10^4 CFU/mL concentrations, while Sabourad's dextrose broth for fungi at 10^3 CFU/mL concentrations obtained from McFarland turbidity (standard no. 0.5). Negative control was prepared with DMSO (10%) and Tween 80 (1% v/v), and blank control from virgin media. Tubes were incubated for 24 and 48 h at 37°C for bacteria and fungi, respectively. MIC was determined as the lowest concentration that inhibited the visible microbial growth.[40,41] The minimal bactericidal concentration (MBC) determination, 0.1 mL of the culture in each tube of MIC without visible growth was spread on nutrient agar plate and incubated for 24 and 48 h at 37°C for bacteria and fungi, respectively. The highest dilution at which 99.9% of the bacteria and fungi inoculum were killed was considered the MBC. The assays were replicated, and the mean value of six experiments was recorded (n = 6) with a standard error of the mean. The statistical analysis was performed using Graph Pad InStat software San Diego, California, USA.

RESULTS AND DISCUSSION

Twenty-five compounds were characterized and identified by GC–MS, comprising 98.6% of the total oil. The identified compounds are listed in Table 2 in elution order from the TG-5 column [Figure 1], along with the percentage composition of each component and its RI. The major constituents were methyl eugenol (39.3%) [Figure 2], and methyl chavicol (38.3%) [Figure 3]. Other minor constituents were terpinolene (7.7%), eugenol (4.5%), and cubenol (1.9%). On the basis of >200 analyses of essential oils isolated from *O. basilicum* classified four major essential oil chemotypes of basil: (1) Methyl chavicol-rich, (2) linalool-rich, (3) methyl eugenol-rich, (4) methyl cinnamate-rich, and also numerous subtypes.[42] The presence of essential oils and their composition determines the specific aroma of plants and the flavor of the condiment. Not only the type of cultivar but also the agronomical practices and environmental conditions affect the composition of sensory important compounds.[43,44] The presence of methyl eugenol and methyl chavicol has been reported in the high percentage in this region is contrary from the northern and rest of the southern part of India, suggested the methyl eugenol/methyl chavicol chemotype essential oil of *O. basilicum* was found from this region. This quantitative and qualitative divergence may be due to the geographical, climatic, and soil conditions in the southern part of India, which in turn may affect the composition and other secondary metabolites of the plant.

The antimicrobial activity expressed as mg/mL, of the essential oil of *O. basilicum* against various strains of bacteria and fungi is summarized in Table 3. The organisms *S. aureus*, *B. subtilis*, *A. fumigatus*, *S. faecalis*, *S. epidermidis*, *P. chrysogenum*, and *A. niger* were found to be more susceptible to the oil with MBC values of 0.143 ± 0.031, 0.260 ± 0.080, 0.312 ± 0.171, 0.364 ± 0.127, 0.416 ± 0.415, 0.416 ± 0.161, and 0.442 ± 0.207 mg/mL, respectively. The organisms *M. flavus*, *M. luteus*, *P. mirabilis*, *P. vulgaris*, and *P. aeruginosa* were found moderately susceptible with the MBC values of 0.520 ± 0.161, 0.572 ± 0.127, 0.781 ± 0.382, 0.833 ± 0.322 and 0.937 ± 0.342, respectively. The microorganisms *E. aerogenes*, *S. marcescens*, *S. typhimurium*, *E. coli*, and *K. pneumoniae* were less susceptible and showed higher MBC values (MBC > 1.0 mg/mL). The observation of MBC assay suggested that the oil has bactericidal property. According to Wan *et al*.,[45] the majority of the essential oils assayed for their antibacterial properties showed a more pronounced effect against the Gram-positive bacteria. The resistance of Gram-negative bacteria to essential oil has been ascribed to their hydrophilic outer membrane which can block the penetration of hydrophobic compounds into target cell membrane.[46] The presence of phenolic components in the essential oil could be contributing for antimicrobial activity by causing leakage of intracellular ATP and potassium ions leading to cell death.[47,48]

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Footnotes

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Conflict of Interest: None declared.

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Figures and Tables

Table 1

Major constituents of the essential oil of *Ocimum basilicum* from different countries

Country	Major constituents
Bangladesh	Methyl cinnamate, linalool, tau-cadinol, α-bergamotene, γ-muurolene, sulfone-methyl styryl, and methyl chavicol[12]
Brazil	Linalool, geraniol, and 1.8-cineole[13]
Czech Republic	Linalool, eugenol, 1,8-cineole, and bergamotene[14]
Guinea	Linalool, eugenol, α-bergamotene, and thymol[15]
Malaysia	Methyl chavicol[16]
Mississippi	Linalool, camphor, α-humulene, eucalyptol, eugenol, bornyl acetate, methyl chavicol, *trans*-caryophyllene, α-*trans*-bergamotene, and cadinol[17]
Northeast India	Camphor, limonene, and β-selinene[18]
Oman	Linalool, geraniol, 1,8-cineole, α-bergamotene, and geranyl acetate[19]
Poland	Linalool, 1,8-cineol, germacrene D, and β-elemene[20]
Romania	Linalool, elemene, farnesene, and guaiene in one sample, while *epi*-bicyclo sesquiphellandrene, farnesene, β-elemene, and γ-cadinene in another sample[21]
Southern, India	Methyl cinnamate, linalool, β-elemene, and camphor[22]
Thailand	Methyl chavicol[23]
Turkey	Menthone, estragole, isoneomenthol, menthol, pulegone, and linalool[24]; methyl chavicol, linalool, α-cadinol, germacrene D, and 1,8-cineole from Iran[25]; methyl eugenol, α-cubebene, nerol, and ε-muurolene[26]
USA	Linalool, estragole, methyl cinnamate, eugenol, and 1,8-cineole[27]

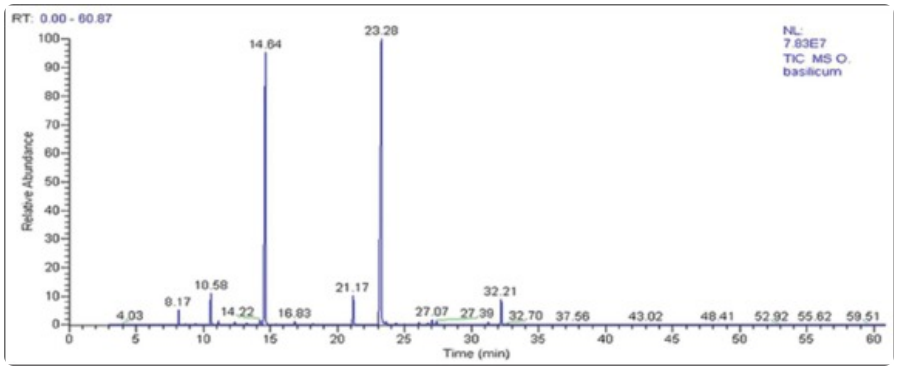
Table 2

Chemical composition of the essential oil of *Ocimum basilicum*

Compounds	RI	Area %	Identification
Sabinene	975	t	RI, MS
β-Pinene	976	0.1	RI, MS
Myrcene	990	0.3	RI, MS
Limonene	1029	0.2	RI, MS
Cineol-1,8	1031	2.1	RI, MS
(*E*)-β-Ocimene	1050	0.2	RI, MS
Terpinolene	1088	7.7	RI, MS
Camphor	1146	0.6	RI, MS
Borneol	1169	0.5	RI, MS
Terpin-4-ol	1177	0.1	RI, MS
α-Terpineol	1188	1.0	RI, MS
Methyl chavicol	1196	38.3	RI, MS
Isocarveol-dehydro	1214	0.2	RI, MS
Chavicol	1250	0.6	RI, MS
Eugenol	1356	4.5	RI, MS
α-Copaene	1376	t	RI, MS
β-Cubenene	1388	t	RI, MS
Methyl eugenol	1403	39.3	RI, MS
β-Copaene	1432	0.1	RI, MS
cis-Muurola-3,5-diene	1450	0.1	RI, MS
cis-Muurola-4 (14), 5-diene	1466	0.2	RI, MS
γ-Muurolene	1478	0.1	RI, MS
Methyl isoeugenol	1492	0.2	RI, MS
δ-Amorphene	1512	0.3	RI, MS
Cubenol	1646	1.9	RI, MS
Total identified		98.6	

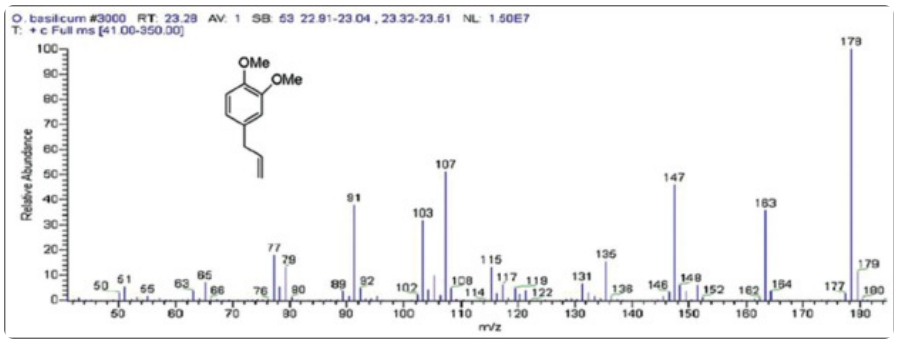
RI: Retention index relative to C_8-C_{25} *n*-alkanes on TG-5 column, MS=(GC/MS), t: Trace (<0.1%), MS: Mass spectrometry

Figure 1



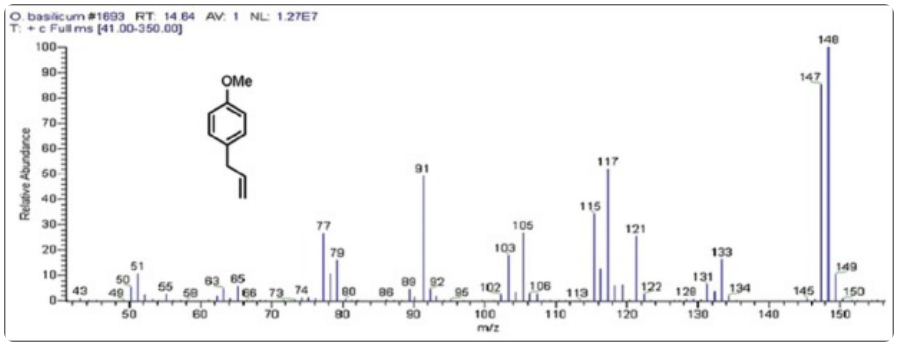
Gas chromatography-total ion current of the essential oil of *Ocimum basilicum*

Figure 2



Mass spectra and structure of methyl eugenol

Figure 3



Mass spectra and structure of methyl chavicol

Table 3

MBC values (mg/mL) of the essential oil of *Ocimum basilicum*

Microbial strains	MBC mean±SEM of essential oil	MBC mean±SEM of RA
Gram-positive		
Staphylococcus aureus	0.143±0.031	0.002±0.001
Staphylococcus epidermidis	0.416±0.161	0.002±0.001
Streptococcus faecalis	0.364±0.127	0.002±0.001
Micrococcus flavus	0.520±0.161	0.002±0.001
Micrococcus luteus	0.572±0.127	0.001±0.001
Bacillus subtilis	0.260±0.080	0.001±0.001
Gram-negative		
Escherichia coli	1.666±0.645	0.009±0.004
Enterobacter aerogenes	1.041±0.322	0.009±0.004
Klebsiella pneumoniae	1.875±0.684	0.005±0.002
Pseudomonas aeruginosa	0.937±0.342	0.004±0.003
Proteus vulgaris	0.833±0.322	0.005±0.003
Proteus mirabilis	0.781±0.382	0.002±0.001
Serratia marcescens	1.250±0.684	0.005±0.003
Salmonella typhimurium	1.562±0.765	0.012±0.002
Fungi		
Aspergillus niger	0.442±0.207	0.001±0.001
Aspergillus fumigatus	0.312±0.171	0.001±0.001
Penicillium chrysogenum	0.416±0.415	0.001±0.001

Values are mean±SEM of six experiments in replicate. RA: Reference antibiotics erythromycin for Gram-positive bacteria; amikacin for Gram-negative bacteria and amphotericin B for fungi, SEM: Standard error of the mean, MBC: Minimal bactericidal concentration