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Chemical Composition and Antimicrobial Activity of Fresh Rhizome Essential Oil of *Zingiber Officinale* Roscoe

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ABSTRACT

Background: Zingiber officinale Roscoe (Zingiberaceae) is a rhizomatous perennial herb found in tropical Asia. It is extensively used worldwide as a spice, flavoring agent and herbal remedy for cold, throat and chest infections and cough. The present study was carried out to analyse an essential oil from the fresh rhizomes of Z. officinale of Ghaziabad region and to evaluate its antimicrobial activity. **Materials and Methods:** The fresh rhizomes were hydrodistilled to get the essential oil which was analysed by GC and GC-MS techniques. The oil was evaluated for antimicrobial activity by disc diffusion method. **Results and Discussion:** The essential oil was characterized by high percentage of sesquiterpenes (66.66%), monoterpenes (17.28%) and aliphatic compounds (13.58%). The predominant sesquiterpene (3.09%) and selina-4(14),7(11)-diene (1.03%). The major monoterpenes were characterized as citronellyl *n*-butyrate (19.34%), β-phellandrene (3.70%), camphene (2.59%) and α -pinene (1.09%). The essential oil exhibited significant antimi-

crobial activity against *Bacillus subtilis, Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, Candida albicans* and *Aspergillus niger.* **Conclusion:** The essential oil mainly contained a large number of sesquiterpenes and monoterpenes and exhibited significant antimicrobial activity against pathogenic microorganisms.

Key words: Antimicrobial activity, Chemical composition, Essential oil, GC-MS analysis, *Zingiber officinale*.

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INTRODUCTION

Zingiber officinale Roscoe (Zingiberaceae) known as adrak or ginger, is an important plant with several ethnomedicinal and nutritional uses. It is a rhizomatous perennial herb reaching up to 90 cm in height. The rhizomes are aromatic, thick lobed, white to yellowish-brown, irregularly branched, annulated and compressed with smooth surface.^{1,2} It is originally found in tropical Asia and now cultivated as a commercial crop in India, China, Australia, America, Africa as well as South East Asia.³⁻⁵ Ginger is cultivated in tropical and subtropical regions. It is grown by vegetative means. Ginger is planted during April-May and harvested about 7-8 months (December-January) after planting when the leaves turn yellow and gradually wither. Harvesting is done by digging the rhizomes. They are washed properly and then dried to improve the colour.6 It is extensively utilized worldwide as a spice, flavoring agent and herbal remedy. In different traditional systems it is taken to cure a variety of diseases such as nausea, vomiting, asthma, palpitation, inflammation, dyspepsia, loss of appetite, constipation, digestion and pain.7,8

Ginger contains 1-2% of essential oil, which imparts the unique flavor to the spice and it has been studied by many workers.⁸⁻¹³ Many reports are available on the chemical composition of fresh ginger oil and the naturally occurring flavoring compounds.¹⁴⁻¹⁶ Various reports are available on the antimicrobial property of the essential oil from the rhizomes of ginger.¹⁷⁻²⁴ The essential oils are the reservoir of biologically active compounds and there has been increased interest in looking at their antimicrobial properties.²⁵ The major pungent compounds of fresh ginger are active gingerols which are a homologous of phenols. The most abundant is 6-gingerol. The pungency of dry ginger is mainly due to the presence of shogaols which are dehydrated forms of gingerols.²⁶ The ginger oil contains a mixture of constituents such as monoterpenes, namely phellandrene, camphene, cineole, linalool, limonene, citral, geraniol, citronellol, borneol and sesquiterpenes, namely α -zingiberene, *ar*-curcumene, β -bisabolene, β -sesquiphellandrene, zingiberol and zingiberenol along with some aliphatic aldehydes and alcohols.²⁷⁻⁴⁰ The composition of volatile oil is highly variable depending upon a variety of factors including their geographical origin, distillation procedures, post harvest treatment, processing, drying conditions and temperature.⁴¹⁻⁴³ In the present communication, we report essential oil composition of the rhizomes of *Z. officinale* from Ghaziabad region and its antimicrobial activity.

MATERIALS AND METHODS

Plant material

The fresh rhizomes of *Z. officinale* were purchased from the local market of Ghaziabad. The plant material was identified and authenticated by Dr. K C Bhatt, National Bureau of Plant Genetic Resources, Pusa Campus, New Delhi. A specimen sample was deposited in the herbarium division of our Institute.

Preparation of plant material

Fresh rhizomes of the plant *Z. officinale* were collected and washed thoroughly under running tap water to remove adhered soil and foreign matter. The rhizomes were cut into small pieces and pulverized to course powder using a mechanical grinder and the powder was preserved in air sealed polyethene bag.

Isolation of the essential oil

The fresh rhizomes (1 kg) of *Z. officinale* were hydrodistilled in Clevenger type glass apparatus for 4 h. The essential oil was collected, measured and dried over anhydrous sodium sulphate and stored at 4°C in the dark. This oil was used for GC, GC-MS and evaluation of antimicrobial activity. The yield of essential oil obtained from the fresh ginger was 1.26%.

GC Analysis

The gas chromatographic analysis of the essential oil was carried out on a GC-2010 (Shimadzu) equipped with a flame ionization detector (FID)

and ULBON HR-1 fused silica capillary column (60 m×0.25 mm×0.25 µm). The injector and detector (FID) temperatures were maintain at 250 and 270°C, respectively. The carrier gas used was nitrogen at a flow rate of 1.21 ml/min with column pressure of 155.1 kPa. The sample (0.2 µl) was injected into the column with a split ratio of 80:1. Component separation was achieved following a linear temperature programmed from 60 to 230°C at a rate of 3°C/min and then held at 230°C for 9 min, with a total run time of 55.14 min. Percentage of the constituents were calculated by electronic integration of FID peak areas.

GC-MS Analysis

The GC-MS analysis was carried out on a GC-MS-QP 2010 Plus (Shimadzu) fitted with a Column AB-Innowax (60 m×0.25 mm i.d., film thickness 0.25 µm), The carrier gas was nitrogen at a flow rate 1.21 ml/min. The oven column temperature was initially kept at 60°C for 10 min and increased up to 230°C at a rate of 4°C/min, then held at 230°C for 10 min and increased up to 260°C at a rate of 1°C/min and then held at 260°C for 10 min. The split flow was 101 ml/min. The split ratio was 1:80. The injector temperature was 240°C and detector temperature was 280°C. Injection volume was 0.3 µl. The ionization energy (voltage) was 70 eV and mass scan range (m/z) was 40-850 amu. The precentage composition of the oil was calculated automatically from the FID peak area without any correction.

Identification of compounds

The individual compounds were identified by comparing their Kovat's indices (KI) of the peaks on Innowax fused silica capillary column with literature values, matching against the standard library spectra, built up using pure substances and components of known essential oils. Further identification was carried out by comparison of fragmentation pattern of the mass spectra obtained by GC-MS analysis with those stored in the spectrometer database of NBS 54 K L, WILEY 8 libraries and published literature (Adams *et al.*, 2001; Ali 2001; Joulain and Konig 1998; McLafferty 1994). Relative amounts of identical components were based on peak areas obtained without FID response factor correction.

Antimicrobial activity Study design:

Test microorganisms or isolates

Microorganisms selected for the study were *Bacillus subtilis* (MTCC 441), *Staphylococcus aureus* (MTCC 737), *Escherichia coli* (MTCC 443), *Pseudomonas aeruginosa* (MTCC 424) and fungi species namely *Candida albicans* (MTCC 227), *Aspergillus niger* (MTCC 404). All the test strains were obtained from the Institute of Microbial Technology (IMTECH), Chandigarh, India. All strains were maintained at 4°C over nutrient agar slants throughout the experiment and used as stock cultures.

Media

Nutrient agar media was composed of beef extract (1.0 g), yeast extract (2.0 g), peptone (5.0 g), sodium chloride (5.0 g), agar (15.0 g) and distilled water (1.0 L).

Sabouraud dextrose agar media was composed of dextrose (40.0 g), mycological peptone (10.0 g), agar (15.0 g) and distilled water (1.0 L).

Preparation of media

Nutrient agar medium (28 g) was accurately weighed, suspended in 1000 ml of distilled water in a conical flask and heated to boiling to dissolve the medium completely. The conical flask containing the nutrient agar medium was plugged with a non-absorbent cotton plug and covered properly with an aluminum foil. It was sterilized by autoclaving at 15-lbs/in² pressure (121°C) for 15 min. Sabouraud dextrose agar medium (65 g) was accurately weighed, suspended in 1000 ml of distilled water in

a conical flask and heated to boiling to dissolve the medium completely. The conical flask containing the sabouraud dextrose agar medium was plugged with a non-absorbent cotton plug and covered properly with an aluminum foil. It was sterilized by autoclaving at 15-lbs/in² pressure (121°C) for 15 min.

Preparation of organisms or inoculums

The test organisms were maintained on freshly prepared medium slants. The slants were incubated at 37°C for 24 h. The organisms from the medium slants were washed using 3 ml of saline solution and incubated for 24 h at 37 \pm 2°C. The developed organisms from the nutrient media were washed using 50 ml of distilled water. A dilution factor was determined which gave 25% light transmission at 530 nm. The amount of suspension to be added to each 100 ml nutrient broth was determined by use of test plates or test broth. The test organisms were stored under refrigeration.

McFarland turbidity standard

A McFarland standard No. 0.5 was used for quality control. It was prepared by adding 0.5 ml of a 1.175% (w/v) barium chloride dihydrate (BaCl,.2H,O) solution to 99.5 ml of 1% (v/v) sulphuric acid.

Antimicrobial standard

The stock solution of 100 μ g/ml of Tetracycline and Fluconazole were prepared by adding 10 mg of drug in 100 ml of dimethyl sulphoxide (DMSO). The further 10 μ g/ml of Tetracycline and Fluconazole were obtained by diluting 1 ml of stock solution upto 10 ml of dimethyl sulphoxide (DMSO).

Test procedure

Susceptibility testing by disc diffusion method:

Determination of Zone of inhibition (ZOI)

A standard disc diffusion method by Baurer *et al.* (1966)⁴⁴ was used. The antimicrobial activity of essential oil was investigated by disc diffusion method in triplicate using 24-48 h grown strains reseeded on nutrient media. The cultures were adjusted with saline water to obtain a suspension at concentration of 1×10^6 CFU/ml with a McFarland standard No. 0.5, then 100 µl of the suspension was spread on nutrient agar media plates to obtain uniform microbial growth. Sterile filter paper discs (Whatman's No. 5, 6 mm in diameter) were impregnated with 10 µl of the essential oil and placed on the surface of the agar test plate. Control discs were saturated with Tetracycline (10 µg/disc). Plates were subsequently incubated at 37°C for 24 h. The zones of inhibition were calculated by measuring the diameter in mm.

In the case of fungi, the test was performed in sterile petri dishes containing sabouraud dextrose agar (SDA). The oil was adsorbed on sterile paper disc and placed on the surface of the medium previously inoculated with a suspension of fungus. Control discs were saturated with Fluconazole (10 μ g/disc). All petri dishes were sealed with a sterile laboratory film to avoid evaporation of the test samples and incubated at 27°C for 48 h. The zone of inhibition was determined by measuring the diameter in mm of the clear zone around each disc.

Determination of minimum inhibitory concentration (MIC)

The minimal inhibitory concentration (MIC) was determined as described by Daw *et al.* (1994).⁴⁵ The MIC was defined as the lowest concentration of tested samples showing no visible bacterial growth after 24 h incubation period at 37°C. Disc diffusion method was used for determination of MIC in triplicate. Petri dishes contained 15 ml of nutrient agar for bacteria and sabouraud dextrose agar (SDA) for fungi, supplemented by test strains at a density of 1×10^6 CFU/ml. Four discs (6.0 mm in diameter) were made in each agar plate. The essential oil

was dissolved in dimethyl sulphoxide (DMSO) in two-fold serial dilutions were made in concentration range from 10 μ l/ml to 1 μ l/ml. DMSO blank was used as negative control. The plates of bacteria were incubated at 37°C for 24 h and plates of fungi were incubated at 27°C for 48 h. The least concentration of the essential oil showing a clear of inhibition was taken as the MIC.

Statistical analysis

Tests were carried out in triplicate and the results were calculated as standard means (\pm SD). Difference on statistical analysis of data were considered significant at P<0.05.

RESULTS AND DISCUSSION

The chemical constituents of the essential oil were identified by analysis of GC and GC-MS. The chemical composition of ginger oil is tabulated in Table 1 with their Kovat's indices and respective percentage. The essential oil was characterized by high percentage of sesquiterpenes (66.66%), monoterpenes (17.28%) and aliphatic compounds (13.58%). In the essential oil eighty compounds were identified among which zingiberene (46.71%) was the major component followed by citronellyl *n*-butyrate (19.34%), valencene (7.61%) and β -phellandrene (3.70%). Among the 52 sesquiterpenes (66.66%), the major ones were zingiberene (46.71%), valencene (7.61%), β-funebrene (3.09%) and selina-4(14), 7(11)-diene (1.03%). The other remaining sesquiterpenes were present in less than one percent. Among 14 monoterpenes, there were eight monoterpene hydrocarbons (9.87%), two monoterpene ketones (2.46%) and four monoterpene esters (4.93%). The predominant monoterpenes were citronellyl *n*-butyrate (19.34%), β-phellandrene (3.70%), camphene (2.59%) and α -pinene (1.09%). The other monoterpenes were detected in less than one per cent. A total 11 aliphatic constituents were characterized in the essential oil. Methyl eugenol was the only aromatic component present in the oil. The other components present in the essential oil included a-agarofuran and ledene oxide.

Various studies have shown that ginger oils are very complex mixtures of compounds and many variations have been found in the chemical composition, geranial (25.9%) as the major constituent in ginger oil.⁴⁶ Zingiberene and β -sesquiterpene were the main components in the range of 10 to 60 percent.47 The main constituents of Sikkim ginger oil were geranyl acetate (18.8%), zingiberene (16.3%) and geranial (8.2%).48 The main components of Nigerian ginger oil were zingiberene (29.5%) and sesquiphellandrene (18.4%).49 Ar-curcumene (11%), β -bisabolene (7.2%), sesquiphellandrene (6.6%) and δ -cadinene (3.5%) were the prominent constituents of dry ginger oil of Nedumangadu variety from Trivendrum (Kerala).37 a-Zingiberene (15.92%), geraniol (11.75), bisabolene (11.12%), neral (9.25%), β -phellandrene (7.73%) and β -sesquiphellandrene (5.26%) were the main components of the rhizome oil of Zingiber officinale from Pantnagar (Uttarakhand).⁵⁰ Such variations in the chemical composition of distilled oils were recorded not only due to the existence of different species but also might be attributed to the different agro-climatic conditions like climatic conditions, regions, stage of maturity, harvesting time of plants and distillation conditions.46,51

The antimicrobial activity of volatile oil was tested against *Bacillus subtilis, Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, Candida albicans* and *Aspergillus niger*. As shown in Table 2, the volatile oil showed significant antimicrobial activities in comparison to the standards, Tetracycline and Fluconazole. The zones of inhibition were in the range of 10.4 to 12.5 mm and 8.3 to 12.5 mm for bacterial and fungal strains, respectively. The standard, Tetracycline had a 10.0 to 15.0 mm zone of inhibition in case of bacterial strains. The standard, Fluconazole had a 8.2 to 12.2 mm zone of inhibition in case of fungal strains. A strong

 Table 1: Chemical composition of the essential oil of the fresh rhizomes

 of Zingiber officinale from Ghaziabad region

Components	Kovat's Index	% Area
a-Pinene	937	1.09
Camphene	943	2.53
2-Methyl nonane	964	0.29
Myrcene	975	0.45
α-Phellandrene	987	0.20
3-Octen-2-one	1006	0.63
(+)-α-Limonene	1011	0.82
β-Phellandrene	1015	3.70
a-Terpinolene	1067	0.16
n-Nonenal	1130	0.14
2-Methyl undecane	1164	0.15
3-Methyl butanol	1205	0.54
n-Nonan-2-one	1280	0.11
α-Nagniatene	1317	0.18
α-Cubebene	1348	0.16
δ-Elemene	1345	0.53
p-Menth-1-en-8-ol acetate	1348	0.11
Cyclosativene	1357	0.55
a-Copaene	1375	0.95
n-Undecanol	1381	0.37
Geranyl acetate	1383	0.32
Camphor isomer	1391	0.10
Methyl eugenol	1404	0.68
Bergamotene	1417	0.95
α-Santalene	1419	0.54
Geranyl propionate	1430	0.25
(Z,E)-α-Farnesene	1433	0.10
y-Elemene	1437	0.41
n-Decanyl acetate	1439	0.39
Neryl acetone	1441	0.79
Allo-Aromadendrene	1455	0.32
Germacrene D	1458	0.82
β-Funebrene	1473	3.09
Zingiberene	1490	46.71
Valencene	1492	7.61
Selina-4(14), 7(11)-diene	1495	1.03
Citronellyl n-butyrate	1501	19.34
a-Muurolene	1503	0.10
Cuparene	1505	0.38
a-Bisabolene	1507	0.05
β-Bisabolene	1513	0.15
γ-Cadinene	1515	0.06
β-Curcumene	1516	0.04

Components	Kovat's Index	% Area
δ-Cadinene	1518	0.10
Myristicin	1519	0.14
a-Cubebene	1522	0.43
β-Sesquiphellandrene	1525	0.06
trans-Calamenene	1529	0.04
cis-Cadina-1, 4-diene	1535	0.31
Selina-3,7-diene	1542	0.10
α-Agarofuran	1546	0.41
Sesquisabinene hydrate	1548	0.12
Epiglobulol	1551	0.76
2-Pentadecen-4-yne	1555	0.24
4-(2,6,6 -Trimethyl-2-cyclohexen-1-ylidene)	1557	0.11
-2- butanone		
(E)-Nerolidol	1558	0.06
Caryophyllene alcohol	1560	0.03
Globulol	1584	0.34
10-Epi-γ-Eudesmol	1597	0.33
Zingiberenol	1601	0.86
(-) Globulol	1604	0.19
1,5,5,8-Tetramethyl -3,7-Cycloundecadien -1-ol	1607	0.06
Agaruspirol	1631	0.03
γ-Eudesmol	1635	0.29
Muurolol	1641	0.02
β-Eudesmol	1649	0.05
a-Eudesmol	1652	0.15
Eudesm-4(14) - en-11-ol	1654	0.19
Ledene oxide	1668	0.03
8-Cedren-13-ol	1671	0.05
2,2,5,5-Tetramethyl tricyclo [6.3.0.0.(3,7)]-undec-1(8)-en-3-ol	1683	0.01
3,7,11-Trimethyl-2,10-docecadien-1-ol	1688	0.03
α-Bisabolol pentanoate	1695	0.02
(E)-Nuciferol	1705	0.01
Longifolene aldehyde	1715	0.50
Caryophyllic acid	1731	0.03
β-Bisabolene-12-ol	1739	0.02
1,8-Dimethyl-8,9-epoxy-4-isopropyl spiro (4.5)decan-7-one	1755	0.07
Heptadecanoic acid	1761	0.05
a-Atlantone	1773	0.00

antibacterial activity against *S. aureus, P. aeruginosa* followed by *E. coli* which was on par with standard (positive control) and weaker than standard towards *B. subtilis.* The essential oil also showed strong antifungal activity against *C. albicans* and *A. niger* which was also on par with standard (positive control).

Table 2: Zone of Inhibition (mm) and Minimum inhibitory concentration (µl/ml)

	ZOI (mm) ± SD (n=3)			MIC (μl/ml) ± SD (n=3)
Microorganisms	Ginger Oil	DMSO (Negative Control)	Standard (Positive Control)	Ginger Oil
Gram Positive Bacteria				
Bacillus subtilis	12.5 ± 0.7	No Zone	15.0 ± 0.5	10 ± 0.8
Staphylococcus aureus	12.0 ± 0.2	No Zone	11.5 ± 0.2	8 ± 0.7
Gram Negative Bacteria				
Escherichia coli	10.6 ± 0.3	No Zone	10.5 ± 0.2	6 ± 0.1
Pseudomonas aeruginosa	10.4 ± 0.8	No Zone	10.0 ± 0.1	5 ± 0.2
Fungi				
Candida albicans	12.5 ± 0.4	No Zone	12.2 ± 0.1	1 ± 0.1
Aspergillus niger	8.3 ± 0.3	No Zone	8.2 ± 0.1	1 ± 0.3

ZOI (Zone of inhibition): Ginger oil concentration-10 μ /disc, Negative control -Dimethylsufoxide (DMSO), Standard (Positive control)-Tetracycline-10 μ g/disc for bacteria and Fluconazolae-10 μ g/disc for fungi.

MIC (Minimum inhibitory concentration): Oil concentration range from 1 $\mu l/ml$ to 10 $\mu l/ml.$

Previous investigations also reported that ginger essential oils exhibited an inhibitory effect against a wide range of pathogenic bacteria and fungi and their effect was probably due to their major components of oil. The result indicates that there is a relationship between the chemical constituents of oils and its antimicrobial activity. It has also been reported that ginger (rich in sesquiterpenes) essential oils possessed a wide spectrum of antimicrobial activity.^{46,51,52} However, antimicrobial activity (bioactivity) of essential oils was dependent not only on the major components but also on the chemical structures of these components.^{45,53}

The antimicrobial activity of oil was assessed by determination of minimum inhibitory concentration. Ginger oil exhibited significant inhibitory effect against all tested organisms with MIC values ranged from 1 to 10 μ l/ml. As indicated in Table 2, the ginger oil had greater activity towards *C. albicans* and *A. niger*. The MIC value was higher in case of *B. subtilis* and weaker in *C. albicans* and *A. niger*.

CONCLUSION

The major components of the essential oil of ginger from Ghaziabad region were zingiberene (46.71%), citronellyl n-butyrate (19.34%), valencene (7.61%) β -phellandrene (3.70%), β -funebrene (3.09%), camphene (2.59%), α -pinene (1.09%) and selina-4(14),7(11)-diene (1.03%). The essential oil mainly contained a large number of sesquiterpenes (66.66%) and monoterpenes (17.28%). The essential oil showed significant antimicrobial activity against pathogenic microorganisms. The presence of zingiberene in large amount in the essential oil makes it medicinally valuable.

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CONFLICT OF INTEREST

The author declare no conflict of interest.

ABBREVIATION USED

GC: Gas Chromatography, GC-MS: Gas Chromatography-Mass Spectroscopy, FID: Flame Ionization Detector, KI: Kovat's Index, MTCC: Microbial Type Culture Collection, SDA: Sabouraud Dextrose Agar, DMSO: Dimethyl Sulphoxide, CFU: Colony Forming Unit, ZOI: Zone of Inhibition, MIC: Minimum Inhibitory Concentration.

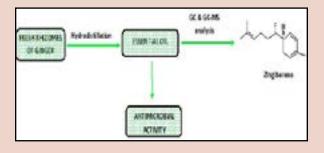
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PICTORIAL ABSTRACT



SUMMARY

- The essential oil was characterized by high percentage of sesquiterpenes (66.66%), monoterpenes (17.28%).
- The major component of the essential oil of ginger from Ghaziabad region was zingiberene (46.71%).
- Zingiberene is responsible for flavour and antimicrobial activity.
- The essential oil showed significant antimicrobial activity against pathogenic microorganisms.



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