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Research Article

COMPARATIVE CHEMICAL COMPOSITION AND ANTIMICROBIAL ACTIVITY FRESH & DRY GINGER OILS (*ZINGIBER OFFICINALE ROSCOE*)

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ABSTRACT

The volatile oils from fresh and dried ginger rhizomes (Nedumangadu variety) (*Zingiber officinale* Roscoe) were analysed by GC and GC-MS. Zingiberene was the major compound in both ginger oils. Fresh ginger oil contained geranial (8.5%) as the second main compound and had more oxygenated compounds (29.2%) compared to dry ginger oil (14.4%). The dry ginger oil also contained ar-curcumene (11%), β-bisabolene (7.2%), sesquiphellandrene (6.6%) and δ-cadinene (3.5%). Antimicrobial activity of the oils against *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Candida albicans*, *Trichoderma* spp, *Aspergillus niger, Pencillium* spp. and *Saccharomyces cerevisiae*, was assessed by disc diffusion method and results obtained are comparable with the reference compounds. The MIC values of the oils ranged from 10μg/mL to 1 μg/mL which is very significant. The study shows a wide application of ginger oil in the treatment of many bacterial and fungal diseases.

Keywords: Fresh Ginger oil, Disc diffusion, Minimum inhibitory concentration, *Psuedomonas aeruginosa*, *Candida albicans*

INTRODUCTION

It has been known from ancient times that essential oils from aromatic and medicinal plants possess biological activity, antibacterial, antifungal and antioxidant properties. Due to the growing interest in the use of essential oils in both the food and the pharmaceutical industries, a systematic study on these plant extracts have become very important. Spices were used from ancient times for different purposes viz flavouring, keeping away the pests, and in perfumery.

Ginger is a rhizomatous plant grown throughout South-eastern Asia, China and in parts of Japan, Austria, Latin America, Jamaica and Africa. Ginger has been used as a spice and medicine in India and China since ancient times. Ginger plants were grown in pots and carried to abroad on sea long voyages to prevent scurvy. The spice was known in Germany and France in the ninth century and in England in 10th century for its medicinal properties. Many oils exhibit antimicrobial properties due to the presence of components such as thymol, eugenol, 1,8- cineole, α- and β- pinenes, linalool, α-terpineol etc [1,2]. Since these compounds and their relative concentration vary from oil to oil and from different oils which accounts for a varied antimicrobial activity [3].

Over three quarters of the world population still rely on plants and plant extracts for health care. Ginger is widely used in ayurvedic medicines and in folklore medicines [1]. About 8000 herbal remedies have been codified in ayurveda and are still used throughout India. Most of the ayurvedic preparations contain dry pepper and ginger.

Ginger contains 1-2 % oil, which imparts the unique flavour to the spice and it has been studied by many workers [4-9]. Many reports are available on the chemical composition of fresh ginger oil and the naturally occurring flavouring compounds [10-12].

Some reports are available on the antimicrobial property of the volatile oil from the rhizomes of ginger [13-20]. The essential oil from ginger, was studied for antimicrobial activity against Aspergillus niger, Saccharomyces cerevisiae, Mycoderma sp., Lactobacillus acidophilus and Bacillus cereus, as determined by paper agar diffusion method [20] .Another study [21] reports on the bioassay-guided isolation of antifungal compounds from an African land race of ginger, *Zingiber officinale* Roscoe, and the identification of 6, 8 and 10-gingerols and 6-gingerdiol as the main antifungal principles. The compounds were active against 13 human pathogens at concentrations of <1 mg/mL. The gingerol content of the African land race was at least 3 times higher than that of typical commercial cultivars of ginger [21].

A survey of the literature reveals that there are no reports on the antimicrobial properties of the fresh ginger oil on the selected microorganisms. So this study was carried out.

MATERIALS AND METHODS

Plant material

The fresh rhizomes (500g) of ginger plant were collected from the Agricultural college plantation (Vellayani, Trivandrum).Voucher specimen was deposited in the agriculture college herbarium. Dry ginger was obtained by drying chipped fresh ginger at a temperature of 50°C in air oven for 24 hrs.

Isolation of essential oil

The ground fresh ginger (200g) and dry ginger (50g) rhizomes were hydro distilled for 5 hrs in a Clevenger type apparatus. The oils were dried over anhydrous sodium sulphate and used for GC, GC-MS analyses and to make solutions for antimicrobial activity studies. The oil yields were 2.2±0.02% in fresh ginger and 1.5±0.01 % in dry ginger. Experiments were carried out in triplicate and average was taken.

Gas chromatography

Gas chromatographic analyses were carried out in a Hewlett-Packard Model 5890-II GC equipped with electronic integrators. Methyl silicone column was used (50m x 0.2mm, 0.17μ m) for the analyses. The conditions were as follows: temperature programming from 80 °C-200° C, rate at 5° C /min, hold at 80°C for 1 min, hold at 200° C for 20 min, FID temperature 300° C, injection temperature 250° C, carrier gas : nitrogen at a flow rate of 1mL/min, split ratio of 1:75. Quantitative analysis data were retained from electronic integration of area percentage without the use of response factors.

GC-MS analyses

GC-MS analyses were carried out in a Shimadzu GC-MS model GC-17A equipped with Mass spectrophotometer GC-MS QP 5050 A. A 30 M capillary silicon column was used for the analysis. Temperature programming conditions were as follows,80° -200° C, rate at the rate of 5° C per min, hold at 80° C for 1 min, hold at 200° C for 25 min, column start temperature 80° C, injection temperature 250 °C, interface temperature 270 ° C, carrier gas helium, flow rate of 1 ml/min, split ratio 1:50.

The percentage composition of the oil was calculated automatically from the FID peak areas without any correction. The retention indices of compounds were determined relative to the retention times of a series of n-alkanes with linear interpolation.

Table 1: Percentage chemical composition of ginger oils

Component	RI*	Fresh ginger oil (%)	Dry ginger oil (%)
Hexanal	770	0.1	T
Hexanol	858	0.0	T
o-xylene	884	<0.1	-
Amyl acetate	895	<0.1	-
α-pinene	943	0.1	0.3
Camphene	954	4.0	1.0
Heptanol	957	0.2	-
Sabinene	976	3.0	0.8
β-pinene	981	1.6	0.6
Myrcene	986	0.0	2.1
6-methyl-5-hepten-2-one	994	0.9	-
1,8 –cineole	1027	2.4	1.7
Limonene	1030	1.9	1.0
(E)-β-ocimene	1038	1.3	-
γ- terpinene	1057	0.8	-
Trans-linalool oxide(furanoid)	1092	0.1	-
Undecane	1100	0.4	0.2
Camphor	1136	0.2	-
Menthone	1143	0.2	-
Borneol	1157	1.2	0.5
Terpinen-4-ol	1170	0.2	0.1
Menthol	1171	<0.1	T
α-terpineol	1185	1.3	0.5
Decanal	1188	0.3	T
Nerol	1218	0.4	0.2
Neral	1227	1.8	T
Geraniol	1243	1.8	0.5
Geranial	1252	8.5	4.4
Trans-carvone oxide	1260	0.6	0.4
Bornyl acetate	1268	0.2	Tr
2-undecanone	1276	0.1	Tr
Undecanal	1284	0.2	-
β-cubebene	1359	-	2.40
α-copaene	1373	-	1.50
Geranyl acetate	1367	0.1	-
δ-elemene	1382	0.5	1.30
β-elemene	1403	0.4	1.00
β-caryophyllene	1418	-	1.40
α-bergamotene*	1436	1.3	1.90
β-farnesene	1448	0.1	1.50
Germacrene-D	1469	1.3	4.20
γ-muurolene	1477	1.2	3.40
ar-curcumene	1474	5.6	11.0
α-muurolene	1490	1.0-	2.20
Zingiberene	1487	28.6	30.3
β-bisabolene	1504	5.8	7.20
β-sesquiphellandrene	1516	2.5	6.60
δ-cadinene	1523	2.2	3.50
(Z)-nerolidol	1524	1.5	0.20
Elemol	1540	1.2	0.20
(E)-nerolidol	1553	1.4	1.20
Eudesma-3,7(11)diene	1542	-	0.20
Cubenol	1560	-	0.20
β-guiacol	1598	-	T
Epi-α-cedrenol	1609	-	T
Sesquisabinene hydrate*	1605	0.1	-
Zingiberenol*	1620	0.1	-
Zingerone	1625	0.6	-
α-murrolol	1630	0.2	T
β-eudesmol	1650	0.1	-
β-bisabolol	1659	0.3	0.30
γ-eudesmol	1660	0.5	-
Z-α-bergamotol	1692	0.0	0.10
(Z,Z)farnesol	1693	0.1	0.10
(Z,E)farnesol	1699	0.6	-
α-eudesmol	1701	1.4	-
(E,Z)-farnesol	1718	0.2	-
(E,E)-farnesol	1749	<0.1	0.10
(Z)-lanceol	1763	-	0.10
Total		92.2	92.3
Total oxygenated compounds		29.2	14.4
Total hydrocarbons		63	77.9

* **RI** – Retention Indices

Identification of the oil components was done by comparison of their mass spectra with the Wiley GC-MS library as well as by comparing them with those reported in literature. The identification of each compound was confirmed by comparison of its retention index either with those of authentic compounds or from literature [22-26]

Microorganisms

The microorganisms selected for the study are *Bacillus subtilis* (gram positive bacteria), *Pseudomonas aeruginosa* (gram negative), three fungi species namely *Candida albicans*, *Aspergillus niger, Penicillium* spp, *Saccharomyces cerevisiae* (yeast) and one dermatophyte which is a *Trichoderma* spp . All the organisms are collected from IMT, Chandigarh, India.

Antimicrobial activity

A standard disc diffusion method by Baurer *et al* [27] was used. In each experiment, microorganisms were cultured at 37 °C for 4 h and prepared to turbidity equivalent to McFarland standard No. 0.5 (National Committee of Clinical Laboratory Standards, 2000). Then 100 µL of the suspension was spread on the test plate (Nutrient Agar). Sterile discs (6 mm diameter) were impregnated with 10 µL of the essential oils and placed on the surface of the test plate. Control discs were saturated with water or tetracyclin (10 µg/disc). Plates were subsequently incubated at the appropriate temp for 24 hrs and zones of inhibition were calculated by measuring the diameter in mm.

In the case of fungi, dermatophyte and yeast, the test was performed in sterile Petri dishes containing saboraud dextrose agar. The oils were adsorbed on sterile paper disc and placed on the surface of the medium previously inoculated with a suspension of fungus, yeast and dermatophyte. All Petri dishes were sealed with a sterile laboratory film to avoid evaporation of the test samples and incubated at 28^{0}C.The zone of inhibition was determined by measuring the diameter of the clear zone around each disc. The standard antibiotics Griseofulvin was used for dermatophyte and Nyastatin used for fungi.

Determination of minimum inhibitory concentration (MIC)

The minimum inhibition concentration (MIC) values were determined by micro broth dilution method. The inoculated bacteria as prepared from 24 h nutrient broth cultures and suspensions were adjusted to 0.5 McFarland turbidity standard. Essential oils dissolved in DMSO were first diluted to the highest concentration (1 mg/mL) to be tested, and then serial two-fold dilutions were made in a concentration range from 10 µg/mL to 1 µg/mL. The least concentration of each oils showing a clear of inhibition was taken as the MIC.

Statistical analysis

Data are expressed as means ±SD. Statistical analysis was performed with Microsoft excel 2007. Difference on statistical analysis of data were considered significant at $P<0.05$.

RESULTS AND DISCUSSION

The chemical compositions of the oils are given in the Table-I. Nintey two percent of the compounds were identified in fresh ginger oil. Zingiberene (28.6%) was the major compound, followed by geranial (8.5%), ar-curcumene (5.6%) and β-bisabolene(5.8%). Monoterpene hydrocarbons present were camphene(4%), sabinene(3%), neral(1.8%) and geraniol(1.8%).The content of oxygenated compounds in the fresh ginger oil was 29.2% and the hydrocarbon content was 63%.The main oxygenated compound was geranial (8%) followed by 1,8 cineole(2.4%) neral (1.8%), borneol (1.2%), α-terpineol (1.3%) and other compounds. Main sesquiterpene compound was zingiberene and other sesquiterpene hydrocarbons present were ar-curcumene (5.6%), β-bisabolene (5.8%), β-sesquiphellandrene (2.5%), δ-cadinene (2.2%). Main sesquitepene alcohols present were (Z)-nerolidol (1.5%), (E)-nerolidol (1.4%), and α-eudesmol(1.4%). Zingerone, naturally occurring component of ginger aroma was present in lesser quantities (0.6%).

Ninety two percent of the compounds were identified in dry ginger oil. The main compound was zingiberene (30.3%), followed by ar-curcumene (11%), β-bisabolene (7.2%),β-sesquiphellandrene (6.6%) and germacrene-D (4.2%).. Geranial content came down to 4.4% from 8% due to drying of ginger. The content of monoterpene compounds decreased in dry ginger oil. Except myrcene, the content of all the other monoterpene compounds decreased .The total content of oxygenated compounds decreased to 14.4% from 29.2%. The content of sesquiterpene alcohols also decreased in dry ginger oil.

Antimicrobial activity

The activity studies (Table-II) show that fresh ginger oil (FG) was on par with standard antibiotic against *Aspergillus niger, candida* and *Pseudomonas aeruginosa*, weaker towards *Saccharomyces cerevisiae* and inactive against *Bacillus subtilis*, *Pencillium spp* and *Trichoderma spp*. Dry ginger oil (DG) was more active towards *Pseudomonas aeruginosa* , on par with standard towards *Candida,* weaker than standard against *Bacillus subtilis, Aspergillus niger, Pencillium spp, Saccharomyces cereviseae*. The composition of fresh ginger oil shows that it contains more of oxygenated compounds (29%) compared to dry ginger oil (14%).The higher content of geranial and other oxygenated compounds makes fresh ginger oil more potent than dry ginger oil. The content of hydrocarbon compounds are more in dry ginger oil compared to fresh ginger oil. Earlier studies [1-2] have reported that monoterpene compounds are more active than sesquiterpene compounds. Dry ginger oil had higher content of sesquiterpene hydrocarbons. Hydrocarbon compounds are reported to have less activity compared to oxygenated compounds [1-2].

Table 2: Antimicrobial activity of ginger oils by disc diffusion method

Sample	Zone of inhibition (mm)						
	Bacillus subtilis	***Pseudomonas aeruginosa***	***Aspergillus niger***	***Penicillium spp***	***Candida albicans***	***Saccharomyces cerevisiae***	***Trichoderma***
FGO*	6.05±0.05	7.11±0.06	8.23±0.04	-	12.12±0.03	6.03±0.06	-
DGO*	5.12±0.05	9.06±0.11	5.41±0.03	4.16±0.11	12.23±0.02	7.09±0.04	-
Reference compound	7.20±0.06	8.10±0.08	8.25±0.03	8.08±0.12	12.09±0.11	12.14±0.04	8.06±0.05

*FGO-Fresh ginger oil, DGO-Dry ginger oil, Reference compounds: Tetracycline for bacteria, Nyastatin for fungi and Griseofulvin for dermatophyte.

Minimum inhibitory concentration

MIC values are given in Table 3. From the table it can be seen that dry ginger oil had greater activity towards *Pseudomonas aeruginosa*, *Penicillium spp.*and *Candida albicans*. Fresh Ginger Oil had higher activity towards *Aspergillus niger*, *Candida albicans* and lesser activity towards *Bacillus subtilis*. The Fresh ginger oil contains more oxygenated compounds compared to Dry ginger oil. The electronegativity assosciated with the oxygenated compounds present in the fresh ginger oil have more influence in controlling the microorganisms *Aspergillus niger* and *Candida albicans*. The Dry Ginger Oil contains more sesquiterpene hydrocarbons and they are more active compared to monoterpenes against certain microorganisms, since they contain more double bonds. Dry ginger oil's higher activity against *Pseudomonas aeruginosa, Pencillium* spp and *Candida albicans* is thus explained.

Table 3: Minimum inhibitory concentration (μg/mL) of fresh and dry Ginger oils

Organism	FGO	DGO
Bacillus subtilis	10	10
Pseudomonas aeruginosa	5	<1
Aspergillus niger	<1	5
Penicillium spp	>10	<1
Candida albicans	<1	<1
Saccharomyces cerevisiae	5	5

CONCLUSION

Both fresh and dry ginger oils contained zingiberene as the major compound but in different ratios. The GC-MS analysis showed that fresh ginger was more abundant in oxygenated compounds. The significant antimicrobial activity of the fresh ginger oil can be attributed to this. Fresh ginger oil had an MIC value of <1 μg/mL against *Aspergillus niger* and *Candida albicans* and. dry ginger oil had an MIC value of less than 1 μg/mL against *Pseudomonas aeruginosa* , *Pencillium* spp and *Candida albicans* and The study elucidates that the dry ginger oil and fresh ginger oil can be used against these organisms as ecofriendly alternative to synthetic chemicals. Further studies on detailed mechanism of action these two oils are in progress.

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