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Research article

COMPARATIVE CHEMICAL CONSTITUENTS AND ANTIMICROBIAL ACTIVITY OF NORMAL AND ORGANIC GINGER OILS (*ZINGIBER OFFICINALE* ROSCOE)

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ABSTRACT: Essential oils obtained from the rhizomes of normal and organic ginger plants (*Zingiber officinale* Roscoe) were characterized by GC and GC-MS. Zingiberene was the chief compound in both ginger oils. Organic ginger oil contained geranial (10.5%) as the second main compound and had more oxygenated compounds (35.1%) compared to normal ginger oil (31.9%). The organic ginger oil also contained β-bisabolene (6.1%), ar-curcumene (5.8%), sesquiphellandrene (2.6%) and δ-cadinene (2.2%). Antimicrobial activity of both the extracted oils was assessed against *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Stephylococcus aureus, E. coli, Klebsiella pneumonia, Shigella flexneri, Candida albicans, Fusarium oxysporum, Aspergillus niger, Penicillium sp* by disc diffusion method and obtained results are comparable with the reference compounds. The MIC values of the oils ranged from 20µg/mL to 1 µg/mL which is very significant. The study shows a wide application of oil extracted from organic ginger in the treatment of many bacterial and fungal diseases.

Keywords: Organic ginger oil, zingiberene, geranial, antimicrobial activity, minimum inhibitory concentration.

INTRODUCTION

The biological properties, antimicrobial and antioxidant properties of essential oils of different aromatic and medicinal plants have been known from ancient times. Due to the increasing in the applications of essential oils in pharmaceutical products, a systematic study on these plant extracts have become very important. From ancient times spices were well known for various purposes like flavouring, controlling the pests etc. Ginger is a rhizomatous plant which is used as a spice and medicine in various countries. Ginger is widely used in ayurvedic medicines and since long time, ginger has been used to treat dyspepsia, gastritis, blood circulation disturbance and inflammatory diseases in many countries (Wang et al., 2009). Also, it show potential antipyretic, antiallergenic, analgesic, antitussive (Gurdip et al., 2008) and chemopreventive activities (Sabulal et al., 2007). This potential activity was believed to be attributed to the major compounds in oils zingiberene, and their activity could be multiple (Ali et al., 2005; Singh et al., 2008; Anwer et al., 2009). Reports are available on the chemical composition of fresh ginger oil and the naturally occurring flavouring compounds (Nishimura O, 1995; WS et al., 2001; RK et al., 2002). Ginger contains 1-2 % oil, which imparts the unique flavour to the spice which has been studied by different workers (S et al., 1997; PS et al., 1997; BM, 2000). Various reports are available on the antimicrobial property of the volatile oil extracted from the rhizomes of ginger (AP et al., 2001; M. Habsah et al., 2000; S. Guptha and S. Ravishankar, 2005; S. Nanasembat and R. Lohasupthawee, 2005; G. S. et al., 2010) and the essential oil from ginger was studied for antimicrobial activity against *Aspergillus niger, Saccharomyces cerevisiae, Trichoderma sp., Lactobacillus acidophilus* and *Bacillus cereus* by paper agar diffusion method (S. et al., 2005). C. Ficker et al. (2003) reported 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 and the gingerol content of the African land race was at least 3 times higher than that of typical commercial cultivars of ginger The compounds were active against 13 human pathogens at concentrations of <1 mg/mL. Many oils exhibit antimicrobial properties due to the presence of components such as thymol, eugenol, 1,8- ineole, α- and β- pinenes, linalool, α- terpineol etc (A. Srivastava et al., 2000). These compounds and their relative concentration vary from oil to oil and from different oils which accounts for a varied antimicrobial activity. A survey of the literature reveals that there are no reports on the antimicrobial properties of the organic ginger oil on the microorganisms. Therefore, the aim of this study was to characterize the chemical constituents, and antimicrobial activity of essential oils obtained from normal and organic ginger.

MATERIAL AND METHODS

Plant material

The fresh 500gm of both normal and organic ginger rhizome were collected from the pots where normal and organic ginger plants were cultivated.

Isolation of essential oil

About 100 gm of rhizomes of each type 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.

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 ^{0}C-200^0 C, rate at 5^0 C /min, hold at 80^{0}C for 1 min, hold at 200^0 C for 20 min, FID temperature 300^0 C, injection temperature 250^0 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, 80^0 -200^0 C, rate at the rate of 5^0 C per min, hold at 80^0 C for 1 min, hold at 200^0 C for 25 min, column start temperature 80^0 C, injection temperature 250 ^{0}C, interface temperature 270^0 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. The components were identified based on the comparison of their GC retention times, interpretation of their mass spectra and confirmed by mass spectral library search using the National Institute of Standards and Technology (NIST) database (Massada, 1976; Adams, 2007).

Microorganisms

The microorganisms selected for the studies are Gram-positive bacteria *Bacillus subtilis* (MTCC 441); *Staphylococcus aureus* (MTCC 96). Gram-negative bacteria *Pseudomonas aeruginosa*; *E.coli* (MTCC 443); *Klebsiella pneumoniae* (MTCC 619) and *Shigella flexneri* (collected from RIMS) and fungal species *Candida albicans* (collected from DRL, Tezpur), *Fusarium oxysporum, Aspergillus niger* and *Penicillium* sp. The bacterial and fungal test organisms were maintained on freshly prepared nutrient agar and saboured's agar medium respectively.

Antimicrobial activity

For antimicrobial assay nutrient agar plates were inoculated with 0.2 ml of overnight grown culture of each test bacterial suspension. Similarly Sabouraud's agar plates were inoculated with 0.2 ml of cultured test fungi. The plates were evenly spread out with the help of a sterile cotton swab. A standard disc diffusion method by Baurer *et al* (1966) was used. Sterile discs (Whatman's No. 1; 6 mm in diameter) were impregnated with 10 µL of the essential oils and placed on the surface of the test plate. The plates were incubated at 37°C for 24h and zone of inhibition was measured and compared with the control. Replication was maintained for each case. The magnitude of antimicrobial action was assessed by the diameter of inhibition zones (mm) and compared with the co-assayed antibiotics tetracycline and ciprofloxacin as antibacterial and Fluconazole as antifungal agent.

Determination of minimum inhibitory concentration (MIC)

The minimal inhibitory concentration (MIC) values determined as described by Daw et al. (1994). The MIC was defined as the lowest concentration tested samples showing no visible bacterial growth after 24 h incubation period at 37ºC. The 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 20 µg/mL to 1 µg/mL. The least concentration of each oil showing a clear of inhibition was taken as the MIC.

Statistical analysis

All data are expressed as mean values ± standard deviation (S.D). Statistical analysis was performed using SPSS Software (version 10). Difference on statistical analysis of data were considered significant at $P<0.05$.

RESULTS AND DISCUSSION

The chemical compositions of both organic and normal ginger oils are given in the Table-I. In organic ginger oil ninety nine percent of the compounds were identified among which zingiberene was the major compound (29.8%), followed by geranial (10.5%), β-bisabolene (6.1%) and ar-curcumene (5.8%).

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Table 1: Chemical composition of organic and normal ginger oils

Component	RI*	Organic ginger oil (%)	Normal ginger oil (%)
Hexanal	770	0.1	0.1
Hexanol	855	0.0	0.0
o-xylene	885	<0.1	<0.1
Amyl acetate	895	<0.1	<0.1
α-pinene	945	0.1	0.1
Camphene	955	4.0	4.0
Heptanol	965	0.2	0.2
Sabinene	975	3.0	3.0
β-pinene	981	1.6	1.6
Myrcene	986	0.0	0.0
6-methyl-5-hepten-2-one	995	0.9	0.9
1, 8 –cineole	1025	3.5	2.9
Limonene	1030	1.9	1.9
(E)-β-ocimene	1040	1.3	1.3
γ- terpinene	1057	0.8	0.8
Trans-linalool oxide(furanoid)	1095	0.1	0.1
Undecane	1100	0.4	0.4
Camphor	1135	0.2	0.2
Menthone	1145	0.2	0.2
Borneol	1155	2.3	1.8
Terpinen-4-ol	1170	0.2	0.2
Menthol	1175	<0.1	<0.1
α-terpineol	1185	2.1	1.7
Decanal	1188	0.3	0.3
Nerol	1218	0.4	0.4
Neral	1227	2.7	2.3
Geraniol	1243	1.8	1.8
Geranial	1252	10.5	9.2
Trans-carvone oxide	1260	0.6	0.6
Bornyl acetate	1268	0.2	0.2
2-undecanone	1276	0.1	0.1
Undecanal	1284	0.2	0.2
β-cubebene	1359	2.40	-
α-copaene	1365	1.50	-
Geranyl acetate	1375	0.1	0.1
δ-elemene	1380	0.5	0.5
β-elemene	1403	0.4	0.4
β-caryophyllene	1418	1.40	-
α-bergamotene	1436	1.3	1.3
β-farnesene	1448	0.1	0.1
Germacrene-D	1469	1.3	1.3
γ-muurolene	1475	1.2	1.2

α-muurolene	1485	1.0	1.0
Zingiberene	1490	29.8	28.6
β-bisabolene	1505	6.1	5.8
β-sesquiphellandrene	1516	2.6	2.2
δ-cadinene	1525	2.2	2.2
(Z)-nerolidol	1524	1.5	1.2
Elemol	1540	1.2	1.2
(E)-nerolidol	1553	1.6	1.4
Eudesma-3, 7(11) diene	1542	0.20	-
Cubenol	1560	0.20	-
β-guiacol	1598	Trace	-
Epi-α-cedrenol	609	Trace	-
Sesquisabinene hydrate	1605	0.1	0.1
Zingiberenol	1620	0.1	0.1
Zingerone	1625	0.6	0.6
α-murrolol	1630	0.2	0.2
β-eudesmol	1650	0.1	0.1
β-bisabolol	1659	0.3	0.3
γ-eudesmol	1660	0.5	0.5
Z-α-bergamotol	1692	0.0	0.0
(Z, Z) farnesol	1693	0.1	0.1
(Z, E) farnesol	1699	0.6	0.6
α-eudesmol	1701	1.5	1.4
(E, Z)-farnesol	1718	0.2	0.2
(E, E)-farnesol	1749	<0.1	<0.1
(Z)-lanceol	1763	0.10	-
Total		99.0	94.3
Total oxygenated compounds		35.1	31.9
Total hydrocarbons		63.9	62.4
* RI – Retention Indices			

The monoterpene hydrocarbons present in the organic ginger oil were camphene (4%), sabinene (3%), neral (2.7%) and geraniol (1.8%). The total oxygenated compounds found present in the organic ginger oil was 35.1% and the hydrocarbon content was 63.9%. The major oxygenated compound was geranial (10.5%) followed by 1,8 cineole (3.5%), neral (2.7%), borneol (2.3%), α- terpineol (2.1%). Similarly main sesquiterpene compound was zingiberene and other sesquiterpene hydrocarbons present were ar-curcumene (5.8%), β-bisabolene (6.1%), β-sesquiphellandrene (2.6%), δ-cadinene (2.2%). Main sesquitepene alcohols present were (Z)-nerolidol (1.5%), (E)-nerolidol (1.4%), and α-eudesmol(1.4%). The naturally occurring component of ginger aroma zingerone was present in minute quantities (0.6%). Similarly, ninety four percent compounds of normal ginger were identified. As like as organic ginger oil, zingiberene was also the main compound (28.6%) of normal ginger oil which is followed by geranial (9.2%), β-bisabolene (5.8%) and ar-curcumene (5.6%). All together 31.9% oxygenated compounds and 62.4% hydrocarbon contents were recorded in normal ginger oil. The main oxygenated compound was geranial (9.2%). The level of geranial was decreases in normal ginger oil than organic ones. The level of monoterpene compound neral was decreased to 2.3% in normal ginger oil. The amount of total oxygenated compounds also decreased up to 31.9% from 35.1%.

The level of sesquiterpene alcohols were also decreased in normal ginger oil in comparison to organic ginger oil. Several studies have shown that ginger oils are very complex mixtures of compounds and many variations have been found in the chemical composition (Singh et al., 2008; Felipe et al., 2008). For instance, Wohlmuth et al., 2006; Felipe et al., 2008 reported zingiberene and β-sesquiterpene as main components in ginger essential oil, ranged from 10 to 60%. Again, based on quantitative analysis, the amounts of some main compounds calculated in our present results are out the range generally identified in other reports. Singh et al. (2008) identified geranial (25.9%) as the major constituent in ginger oil, but it was detected in low amount (10.5%) in our study. Such variations in the chemical composition of distilled oils was recorded not only due to the existence of different subspecies but also might be attributed to the different agro-climatic condition of the regions, stage of maturity, adaptive metabolism of plants and distillation conditions etc. (Anwar et al., 2009; Abd El Baky and El Baroty, 2008; Singh et al., 2008;).

Antimicrobial activity

The antimicrobial activity of the essential oils of both organic and normal ginger as well as tetracycline, ciprofloxacin and fluconazole against selected microbial strains were assessed (Table 2 and 3). The results from the disc diffusion method revealed that both oils showed significant antimicrobial activity toward all tested organisms. The zone of inhibition was recorded 7-12.7 mm in case of organic ginger oil whereas for normal ginger oil it was 6.5-11.5 mm for tested bacterial strains. Similarly the zone of inhibition was recorded 7.7-13.02 mm in case of organic ginger oil whereas for normal ginger oil it was 7.3-12.6 mm for tested fungal strains. The result indicates that organic ginger oil showed significant antimicrobial activity in comparison to normal ginger oil. The antibacterial activity of organic ginger oils was recorded lower than the antifungal activity which was also higher than the fluconazole. Table-2 showed that organic ginger oil showed strong antibacterial activity against *Stephylococcus aureus* and *Pseudomonas aeruginosa* followed by *E. coli* which was on par with standard antibiotic and weaker than standards towards *Bacillus subtilis*, *Shigella flexneri* and *Klebsiella pneumonia*. Similarly organic ginger oil was more active towards all the tested fungal and on par with standard except *Penicillium sp*. The composition of organic ginger oil shows that it contains more oxygenated compounds (35.1%) compared to normal ginger oil (31.9%). The higher content of geranial and other oxygenated as well as hydrocarbon compounds may make the organic ginger oil more potent than normal ginger oil (GK Sinha and BC Gulati, 1990). Thus, the findings emphasize the main compounds were mainly contributor to the antibacterial property of oil. Our results also are comparable with the previous investigations (Baratta et al., 1998; Senhaji et al., 2007; Ali et al., 2005). They 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 main components of oil. The result indicates that there is a relationship between the chemical constituents of oils and its antimicrobial activity. As both the oils had a different chemical profiles, difference in antimicrobial activity could be expected. It has been reported that ginger (rich in sesquiterpenes) essential oils possessed a wide spectrum of antimicrobial activity (Baratta et al., 1998; Singh et al., 2008; Anwer, 2009). The obtained results corroborated with the earlier reported data of Daw et al., (1994) and Farag et al. (1989b). In general, the extract of antimicrobial mechanism of essential oils has not been completely elucidated. However, it has been proposed that the chemical structure of essential oils or their main could play an important role for the antimicrobial activity (Farag et al., 1989b; Daw et al., 1994), which may enable them to destroy the cellular structure leading to death. Therefore, the bioactivity of essential oils is dependent not only on the major compounds but also on the chemical structures of these compounds (Farag et al., 1989 b, c).

Table 2: Antibacterial activity of organic and normal ginger oils by disc diffusion method

Treatment	Zone of inhibition (in mm)					
	Bacillus subtilis	*Pseudomonas aeruginosa*	*Stephylococcus aureus*	*E. coli*	*Klebsiella pneumonia*	*Shigella flexneri*
Organic ginger oil	7 ±0.2	9.1 ±0.1	12.7 ±0.2	8.6 ±0.4	8.7 ±0.2	7.5 ±0.2
Normal ginger oil	6.5 ±0.2	8 ±0.1	11.5 ±0.1	8.2 ±0.2	8.1 ±0.01	6.9 ±0.2
Tetracycline	7.5 ±0.5	8.5 ±0.2	11.5 ±0.2	8.3 ±0.2	8.8 ±0.2	8.4 ±0.01
Ciprofloxacin	7.3 ±0.3	8.2 ±0.2	12.2 ±0.2	8.5 ±0.4	9.2 ±0.3	9.1 ±0.01

Data are the mean of three replicates, ± S.D

Table 3: Antifungal activity of organic and normal ginger oils by disc diffusion method

Treatment	Zone of inhibition (in mm)			
	Candida albicans	*Fusarium oxysporum*	*Aspergillus niger*	*Penicillium sp*
Organic ginger oil	13.02 ±0.01	8.5 ±0.2	8.32 ±0.01	7.7 ±0.2
Normal ginger oil	12.6 ±0.2	6.3 ±0.2	8.2 ±0.2	7.3 ±0.2
Fluconazole	12.2 ±0.1	8.06 ±0.02	8.25 ±0.1	8.08 ±0.02

Data are the mean of three replicates, ± S.D

Table 4: Minimum inhibitory concentration (µg/mL) of organic and normal ginger oils

Organism	Organic ginger oil	Normal ginger oil
Bacillus subtilis	12 ±0.2	15 ±0.3
Pseudomonas aeruginosa	2 ±0.1	5 ±0.2
Stephylococcus aureus	<2 ±0.01	8 ±0.01
E. coli	3 ±0.1	7 ±0.2
Klebsiella pneumonia	8 ±0.1	13 ±0.4
Shigella flexneri	20 ±2	20 ±0.03
Candida albicans	<2 ±0.01	3 ±0.01
Fusarium oxysporum	3 ±0.02	9 ±0.3
Aspergillus niger	4 ±0.2	9 ±0.01
Penicillium sp	13 ±0.2	15 ±0.4

Data are the mean of three replicates, ± S.D.

Minimum inhibitory concentration (MIC)

The antimicrobial activity of both oils was assessed by determination of minimum inhibitory concentration. Organic ginger oil exhibited strong inhibitory action against all tested organisms with MIC values ranged from 2 to 12 µg/ml, followed by normal ginger oil (MIC 3 to 15 µg/ml). MIC values are given in Table 4. From the table it can be seen that both the ginger oil had significant activity towards all the tested organisms. The organic ginger oil showed greater activity towards *Stephylococcus aureus, Pseudomonas aeruginosa, E. coli* and all the fungal strains except Penicillium sp. in comparison to normal ginger oil. The MIC value was recorded maximum in case of *Shigella* flexneri, *Bacillus subtilis* and *Penicillium* sp. whereas it was lowest in *Stephylococcus aureus* and *Candida albicans*. The organic ginger oil contains more oxygenated compounds compared to normal ginger oil. The electronegativity associated with the oxygenated compounds of the organic ginger oil may have more influence in controlling the microorganisms. The organic ginger oil also contains more sesquiterpene hydrocarbons and they are more active compared to monoterpenes against certain microorganisms. Thus the increase of oxygenated and hydrocarbon compounds in the organic ginger oil makes it more active against tested organisms than normal ginger oil.

CONCLUSION

Both normal and organic ginger oils contained zingiberene as the major compound but their ratios are different. The GC-MS analysis showed that organic ginger was more abundant in oxygenated compounds as well as in hydrocarbon compounds. The significant antimicrobial activity of the organic ginger oil can be attributed to this. Organic ginger oil had an MIC value of <2 µg/mL against *Stephylococcus aureus* and *Candida albicans.* The study elucidates that the organic ginger oil can be used against these pathogenic organisms as natural ecofriendly alternative to antagonistic chemicals. This study also indicates the potential use of organic ginger essential oils in ethno-medicine as a preventer of cellular damage and as preserver of foodstuffs in food industries against spoilage bacteria and fungi besides its traditional uses.

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