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E: ISSN NO.: 2455-0817 *Remarking An Analisation*

Antimicrobial Activity and Chemical Composition of Ginger Essential Oil collected from Chamoli District of Uttarakhand State, India

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Abstract

The chemical composition of essential oils obtained by steam distillation of ginger from Chamoli District of Uttarakhand was investigated. The yield of the essential oils was 3.2%. The main components of the essential oil obtained by steam distillation was Cuparene (8.21%), Isocaryophillene (6.26%), α-Geudesmal (5.95%), 1-Heptatriacontanol (5.57%), γ-Muurolene (5.14%), β-Cedrene (4.43%), α-Bunebrene (4.43%), Camphane (4.16%), α-Elemol (3.85%), Eucalyptol (3.43%), α-Phellandrene (3.08%), Nerolidol (2.93%) and 8-Epi-gama-eudesmol (2.32%).

The antimicrobial activity of essential oils from ginger was studied according to the agar diffusion method. The test microorganisms used were the Gram-positive bacteria Staphylococcus aureus, Bacillus subtilis, and Salmonella typhi; Gram-negative bacteria Escherichia coli, Pseudomonas aeruginosa and Klebsiella pneumoniae and the fungi Candida albicans, Aspergillus niger, Aspergillus fumigatus and Tricoderma. The essential oil had a weak effect on the Gram-positive and Gram-negative bacteria and had a substantial fungicidal effect on the fungi under study.

Keywords: *Zingiber officinalis*, Essential Oil Composition, Antimicrobial Activity.

Introduction

Ginger (*Zingiber officinalis* Roscoe) is cultivated in a number of tropical countries around the world. Its roots are used both in the traditional medicine and as flavorings in foods and beverages[1]. The constituents of the essential oil have been subject to a number of studies, which have shown a difference in its constituents depending on the region of cultivation. It has been found[2] that the oil from Nigeria contains zingeberene + geranial (29.0 %) and β-phellandrene (8.86 %); from Taiwan; β-sesquiphellandrene + ar-curcumene + geranyl acetate (12.32 %) and β-phellandrene (6.79 %); from Japan; geranial and β- sesquiphellandrene; from Ethiopia; zingeberen (35.56 %), β-sesquiphellandrene (12.3 %) and β-bisabolene (12.15 %); from Australia; caphene (13.9 %) and ar-curcumene, from India; ar-curcumene (18.9 %) and β-sesquiphellandrene (11.6 %); from Sri Lanka; β- bisabolene (20.1 - 60.6 %), ar-curcumene (5.7 - 27.1 %), geranial (1.8 - 15.3 %) and 1.8 cineole (2.1 - 12, %); from China; zingeberene (38.12 %) and ar-curcumene (17.06 %).

The investigations conducted by McLeon and Pieris[3] have shown that the main ingredient of the oil from Sri Lanka is ar-curcumene. According to Smith and Robinson[4], the oil from Fiji is richer in neral and geranial as compared with the oils from the rest of the regions in the world. It has been found that the rhizomes of ginger, as well as the essential oil, have a distinct antimicrobial activity[1, 5-7].

Aim of the Study

The aim of this work was to study the chemical composition of essential oil obtained from the roots grown in the hilly region of Uttarakhand, India and to investigate its antimicrobial effect.

Experimental

Plant Material

The rhizome of *Zingiber officinale* was purchased from local market of Uttarakhand State from Gopeshwar (Chamuli District), India which are situated in high altitude areas of Himalayan region of Uttarakhand State. Fresh rhizome of Ginger was washed and used for analysis. An initial quality evaluation of the plant material was carried out as per the guidelines on herbal quality control (WHO,1998)[8] and a voucher specimen (C1/Chem/DAV/12) has been deposited in the Department of Chemistry, Dayanand Anglo Vaidic (DAV) College, Kanpur, Uttar Pradesh, India for further reference. Oil was extracted from rhizome by using a Clevenger apparatus. Extracts were prepared according to Oke & Mhamburger[9].

Extraction of Essential Oil

The rhizome of *Zingiber officinale* was washed and use in steam and water distillation. Using the Clevenger apparatus, a perforated grid or plate is fashioned so that the plant material is raised above the water. This reduces the capacity of the fl ask but affords a better quality of oil. Thereby ensuring that the water, which is being used as the steam source, will never run out and condenser always flow with cool water.

Isolation of Essential Oil

The rhizome of ginger cultivars were separately scrubbed and washed to remove the sand and other foreign particles and dried to remove moisture content. Rhizomes were carefully cut to small mesh size particles and hydro- distilled for 5 hrs in a Clevenger-type apparatus to get the oils. After drying over anhydrous sodium sulphate the oils were analyzed by GC and GC-MS. Analysis was carried out in triplicate.

GC-MS analysis was recorded on an Agilent 7890 GC with 5977A Network Mass Selective Detector. Column: HP-5MS (30m x 0.25mm id x 0.25µm). Oven temperature kept initially at 50^0 C for 2 minutes and then increased at 10^0 C/minute to 280^0C and maintained for 5 minutes. The carrier gas used was helium at 1.2 ml/minute flow rate. Injector and detector were 250 ^{0}C and 280 ^{0}C, respectively. The area percentage data were obtained on a Mass hunter integrator. The constituents were identified by comparing their GC retention times and their identity confirmed by a computer matching of their mass spectral pattern with that of known compounds in computer library software coupled with GC-MS. Fragmentation pattern studies with those were reported in literature were also made. The oil constituents were identified by comparison of their mass spectra and the retention indices with those published and presented in a library developed by ourselves[10]

Antimicrobial Effect

Test microorganisms used in this study were the Gram-posi- tive bacteria Staphylococcus aureus, Bacillus subtilis and Salmonella typhi the Gram-negative bacteria Escherichia coli, Pseudomonas aeruginosa and Klebsiella pneumoniae and the fungi Candida albicans, Aspergillus niger, Aspergillus fumigatus and Tricoderma.

The microbiological samples Staphylococcus aureus, Bacillus subtilis, Salmonella typhi, Escherichia coli, Pseudomonas aeruginosa and Klebsiella pneumonia were collected from Microbiology division of Shivam Dental Hospital, Kanpur, Uttar Pradesh, India and the fungi Candida albicans, Aspergillus niger, Aspergillus fumigatus and Tricoderma were taken from the plant pathology laboratory, Dayanand Anglo Vaidic College, Kanpur, Uttar Pradesh, India. . The isolates were identified according to published guidelines of Burneti *et al.,* (1994)[12]. The bacterial and fungal strains were maintained on Mueller–Hinton agar (MHA) and Potato dextrose agar (PDA) plates respectively at 4 °C.

S.No.	RT	Area %	Compound Name
1	5.38	1.31	α -Pinene
2	5.72	4.16	Camphane
2	6.39	0.37	Pseudolimonene
3	6.72	0.74	α -Myrcene
4	7.71	3.08	α -Phellandrene
5	7.77	3.43	Eucalyptol
6	14.76	0.82	2-Camphanyl acetate
7	18.29	1.05	Caryophyllene
8	19.33	0.75	Alloaromadendrene
9	19.83	8.21	(+)-Cuparene
10	20.14	4.43	α -Bunebrene
11	20.24	5.14	γ-Muurolene
12	20.46	6.26	Isocaryophillene
13	20.58	0.84	α -Copaene
14	20.84	4.43	β-Cedrene
15	21.24	0.92	α -Ledene oxide-(II)
16	21.51	3.85	α -Elemol
17	21.67	1.08	Acoradiene
18	21.80	2.93	(e)-Nerolidol
19	22.57	1.84	Trans-sesquisabiene hydrate
20	22.63	1.23	α -Acorenol
21	23.02	2.33	cis-Sesquisabinene hydrate
22	23.19	2.32	8-Epi-gama-eudesmol
23	23.35	1.03	Ledene oxide – (I)
24	23.42	2.16	Cubenol
25	23.89	5.95	α -Geudesmal
26	33.04	4.14	1,3,5-Cycloheptatriene 2,4-dihexyl-7,7 dimethyl
27	35.49	0.88	6-Paradol
28	36.41	0.92	1-Heptatriacontanol
29	36.45	1.77	1-Heptatriacontanol
30	39.72	1.14	Rhodopin
31	40.25	5.57	1-Heptatriacontanol
32	41.02	0.59	Dimethoxylycopene

The antimicrobial activity was evaluated by food poison technique which has been described by Nene and Thapliyal (1993)[11]. The essential oils of rhizome of *Zingiber Officinale,*

were used to prepare 100, 200 and 500 ppm concentrations of nutrient agar for antibacterial and PDA for antifungal assay. The control plates were poured with the respective medium without plant material. The 16 hrs culture of bacteria were diluted with sterile physiological saline solution (PS: 0.85% w/vsodium chloride) so that a concentration of inoculums approximately 108 cfu mL^{-l} could be achieved. The Whatman filter paper disc of 5 mm diameter was dipped in this bacterial growth and was placed in the centre of the above prepared nutrient agar plate. PDA petriplates containing different concentrations of plant material and were inoculated with a 5 mm mycelial disc of the fungal species from 7 day old culture grown on PDA. The inoculated plates were incubated at 25 $\pm 1^0$C till the fungus covered the control plates. The colony diameter was recorded and percent inhibition in each treatment was calculated. The zones < 8 mm were not considered significant.

Results and Discussion

The yield of the essential oil obtained by steam distillation was 3.2%. Total 32 components were identified from GC-MS of essential oil as shown in Table 1.

It can be seen from the data that the major constituents of the Chamuli essential oil were Cuparene (8.21%), Isocaryophillene (6.26%), α-Geudesmal (5.95%), 1-Heptatriacontanol (5.57%), γ-Muurolene (5.14%), β-Cedrene (4.43%), α-Bunebrene (4.43%), Camphane (4.16%), α-Elemol (3.85%), Eucalyptol (3.43%), α-Phellandrene (3.08%), Nerolidol (2.93%) and 8-Epi-gama-eudesmol (2.32%).

It can be seen from the data given below that both oils had a weak effect on the Gram-positive and the Gram-negative bacteria. However, they did not have any effect on the Gram-negative bacteria *Pseudomonas aeruginosa.* Among the yeast, *Saccharomyces cerevisiae* were considerably more sensi- tive to the oils, which had a weak effect on *Candida albicans.* The essential oils sup- pressed the growth of the must fungi. The fungicidal effect was strongest on *Penicillium sp.* followed by *Aspergillus niger, Botrytis cinerea* and *Rhizopus nigricans.*

Bacterial Pathogens

Table 1. Zone of inhibition (in mm) of *Zingiber Officinale* essential oil against six bacterial pathogens

S.No.	Bacterial Pathogens	Zone of inhibition (mm)
		Chamoli essential oil
1	*E coli*	17.6 ± 0.8
2	*P. aeruginosa*	20.0 ±0.5
3	*S aureus*	23.3 ± 0.8
4	*B subtilis*	20.9 ±0.4
5	*S typhi*	19.1 ± 0.2
6	*K. pneumoniae*	19.1± 0.4

Fungal Pathogens

Table 2. Zone of inhibition (in mm) of *Zingiber Officinale* essential oil against four fungal pathogens

S.No.	Pathogens	Zone of inhibition (mm)
		Chamoli essential oil
1	*C albicans*	12.1 ± 0.4
2	*A niger*	12.0 ± 0.2
3	*Tricoderma*	14.3 ± 0.6
4	*A fumigatus,*	13.3 ± 0.3

MIC Test

Bacterial pathogens

Table 3. MIC (in mg/ml) of *Zingiber Officinale* essential oil against six bacterial pathogens

S.No	Bacterial pathogens	Zone of inhibition (mg/ml)
		Chamoli essential oil
1	*E coli*	40.0
2	*P. aeruginosa*	43.4
3	*S aureus*	54.0
4	*B subtilis*	46.3
5	*S typhi*	65.2
6	*K. pneumoniae*	53.7

Fungal Pathogens

Table 4. MIC (in mg/ml) of *Zingiber Officinale* essential oil against fungal pathogens

S.No.	Fungal pathogens	Zone of inhibition (mg/ml)
		Chamoli essential oil
1	*C albicans*	43.0
2	*A niger*	39.6
3	*Tricoderma*	50.1
4	*A fumigatus,*	46.5

Various “in vitro” studies have been carried out to evaluate the effectiveness of essential oil obtained from ginger against bacteria and fungi as shown in table 1-4. It is well-known that the antimicrobial activity of ginger essential oil mainly depends on their chemical composition,[15,17] the extraction solvent,[18,19] the methodology used to obtain it,[13,14] and the process to which the ginger was submitted.[16] However, according to different authors, phenolic compounds (euge- nol, shogaols, zingerone, gingerdiols, gingerols, etc.) and their synergistic relationship with other compounds such as β-sesquiphellandrene, cis-caryophillene, zingiberene, α- farnesene, α- and β-bisabolene, are mainly responsible for the antimicrobial activity found in ginger essential oil.13,20

Ginger essential oil showed a high fungicide and antibacterial effect due to its high eugenol concentration.[21,22] Singh et al.[13] pointed out that the main compounds with antimicrobial activity present in

essential oil from fresh ginger are geranial, α-zingiberene, (E, E)- α-farnesene, neral, ar-curcumene, and β-sesquiphellandrene.

Ginger antimicrobial effect is also related to the applied treatment as reported by some authors. For example, essential oil obtained from fresh ginger contained a higher concentration of oxygenated compounds (29.2%), such as geranial, 1,8-cineole, neral, borneol, α-terpineol, than essential obtained from dried ginger, which has a low concentration of volatile compounds (14.4%)[16] and consequently low antibacterial and antifungal activities.[23]

Antibacterial activity

Table 2 shows the minimum inhibitory concentration (MIC) of ginger essential oil on three gram positive and three gram negative bacteria. Debbarma et al.[24] evaluated the effect of ginger essential oil on three gram-positive and nine gram-negative bacteria. As expected, the inhibition increased by increasing the concentration of ginger essential oil. Moreover, all gram-positive strains were more sensitive (MIC = 3.9–15.6 µL/mL) compared to gram-negative bacteria (MIC = 31.3–62.5 µL/mL). In our study more or less similar results are obtained i.e. gram positive bacteria were less sensitive (MIC = 40.0 - 53.7 mg/mL) compared to gram-negative bacteria (MIC = 46. 3 - 65. 2 mg/mL).

This result suggests that gram-negative bacteria are more resistant due to the composition and structure of their cell wall.[25] In this regard, gram- negative cells present a lipid bilayer, which provides extra protection against antimicrobial compounds.[26] Moreover, Sharma et al.27 pointed out that the antimicrobial activity of essential oil depends on its chemical composition and the concentration of its compounds.

E. coli (MIC = 40.0 mg/mL) was the most sensitive microorganism, while S. typhi (MIC = 65.2 mg/mL) was the most resistant to ginger essential oil followed by P. aeruginosa, B. subtilis, k. pneumonia and S. aureus.

Antifungal Activity

The MIC of ginger essential oil is reported in Table 3 and 4. In general, the studies indicated a high antifungal activity of ginger compounds. Moreover, selected works have highlighted that fungi are more sensitive to ginger compounds than bacteria. For example, Sharma et al.28 pointed out that the MIC value of ginger essential oil for both Candida albicans and Aspergillus niger was 1 µL/mL, while those for different bacteria ranged from 5 to 10 µL/mL. C. albicans inhibition has been widely studied using essential oil and extracts of ginger, with results indicating that essential oil has a higher effect than aqueous and organic extracts.[16,27] The action mechanism of essential oil has not been clarified, but Singh et al.[35] highlighted that essential oil is rich in zingiberene show high antifungal action. It can be seen from the data that essential oil has a adequate effect on the Gram-positive and the Gram-negative bacteria. Maximum Zone of inhibition found in Tricoderma followed by A. fumigates, C. albicans and A. niger. The fungicidal effect is strongest on Tricoderma and weakest on A. niger.

Based on the conducted studies it can be concluded that ginger oil has adequate effect on Gram-positive and Gram-negative bacteria and has a substantial fungicidal effect on the fungi under study, which did not contradict the data in the literature [1, 5 - 7.]

The MIC value of ginger essential oil for A. niger is 39.6 mg/ml followed by C. albicans 43.0, A. fumigates 46.5 and Tricoderma50.1mg/ml respectively.

Similar results were reported by other authors when methanol,[30] chloroform,[30] and water[31] were used as solvents. However, these results are strongly affected by the plant composition, the solvent used for the extraction of the anti-microbial compounds, the tested microorganisms and the used techniques.[32]

Endnotes

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