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Zingiber Officinale : Chemical and phytochemical screening and evaluation of its antimicrobial activities

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ABSTRACT

Zingiber Officinale is a common condiment for various foods and beverages and a long history of important Traditional Medicine herb for the treatment of stomach disorders. The constituents present in ginger have potent antioxidant and anti-inflammatory activities. The study deals with antimicrobial activity of Zingiber Officinale (ZO) extract and their phytochemical composition. Phytochemical screening revealed the presence of alkaloids, saponins, tannins, flavonoids, terpenoid and phlobotannins in both the extracts. The GO extracts were obtained by sokhlet apparatus and their chemical profile was determined through GC and GC-MS analysis resulted in the identification of 40 compounds in methanolic and 32 compounds in ethanolic extract. Their antimicrobial activity was tested against nine microorganisms that cause various diseases in human. Zingiber extracts showed selective antimicrobial activities.

INTRODUCTION

Herbal medicines are also in great demand in the developed world for primary health care because of their efficacy, safety and lesser side effects. India despite its rich traditional knowledge, heritage of herbal medicines and large biodiversity has a dismal share of the world market due to export of crude extracts and drugs.

Ginger, the rhizome of *Zingiber officinale*, is one of the most widely used species of the ginger family (Zingiberaceae) and is a common condiment for various foods and beverages. It has a long history of medicinal use dating back 2,500 years in China and India for conditions such as headaches, nausea, rheumatism, and colds. Ginger is native to Southern Asia, but it is now extensively cultivated in Jamaica, Nigeria, China, India, Fiji, Sierra Leone and Australia.

The anti-inflammatory properties of ginger have been known and valued for centuries. The original discovery of ginger's inhibitory effects on prostaglandin biosynthesis in the early 1970s has been repeatedly confirmed. This discovery identified ginger as an herbal medicinal product that shares pharmacological properties with non-steroidal anti-inflammatory drugs. Ginger is a strong anti-oxidant substance and may either mitigate or prevent generation of free radicals. It is considered a safe herbal medicine with only few and insignificant adverse/side effects.

The approved modern therapeutic applications for ginger are supportable based on its history of use in well established systems of traditional and conventional medicine, extensive phytochemical investigations, pharmacological studies in animals, and human clinical studies.

The aim of the present study is to evaluate the phytochemical characterization of the methanol and ethanol extract of *Zingiber officinale* with GC-MS and check the antimicrobial activity against various human pathogens.

MATERIALS AND METHODS

Plant Material

Ginger rhizomes were collected in March 2011 from the farm near of Jaipur Rajasthan. Collection was performed by pulling plants out of the soil and transferring them to sealable plastic bags.

Sample Preparation

The rhizomes were washed to remove soil from the field, peeled and washed again in clean water. After washing the rhizomes dried, powdered and submitted to successive extraction by sokslet apparatus with 100% ethanol and methanol at room temperature. All the extract was fitered through membrane filter and then the extract dried in room temperature. The dried extract further diluted in the ethanol and methanol respectively .The extracts was further sterilized by filtration (0.22µm), for GC-MS study.

Gas Chromatography and Mass Spectroscopy Analysis

The qualitative and quantitative compositions of the alcoholic / ethanolic fractions were studied by GC-MS on a GCM spectrometer(shimadzu) consisting of an GC-2010 gas chromatograph and an GC-MS QP2010 plus GC mass spectrometer in Jawaharlal Nehru University, New Delhi. Components were separated on Rtx -5MS quartz capillary column (60m x 0.25mm) with crossbond R 5% diphenyl/95% dimethyl polysiloxane stationary phase. In the temperature program :80 d C for 1 min. then increased to 180^0 C at a rate of 10^0 C /min and kept for 4 minutes, then with 15d C / min. to 300d C and kept for 17 minutes. Sample injection volume was 0.3 uL with a split ratio 1:20, run time 35 minutes and pressure at the column inlet 163.3 kPa with helium carrier gas at 1.21 ml/min.flowrate. Compounds were identified by comparison of mass spectra with those in the Wiley and NIST Libraries.

Phytochemical screening of extract

The method described [4, 6, 7] with slight modification were used for screening of alkaloid, steroids, phlobotannins, flavanoids, glycosides, saponins, tannin and terpenoids

Alkaloids test

5g each of the ginger extracts and 5ml of honey was stirred with 5ml of 1% aqueous hydrochloric acid on a steam bath. 1ml of the filtrate was treated with few drops of Draggendoff's reagent. Blue black turbidity serves as preliminary evidence of alkaloids.

Saponins test

5g each of the extracts and 5ml of honey was shaken with distilled water in a test tube. Frothing which persists on warning was taken as preliminary evidence of the presence of saponins.

Tannins

5g each of the extracts and 5ml of honey was stirred with 100ml distilled water and filtered. Ferric chloride reagent was added to the filtrate. A blue-black or blue green precipitate determines the presence of Tannins [2].

Phlobotannins test

Disposition of red precipitate when an aqueous extract of the test samples was boiled with 1% hydrochloric acid determines the presence of phlobotannins [2].

Flavonoids test

5ml of diluted ammonia solution was added to aqueous filtrate of the test samples followed by the addition of concentrated H_2SO_4. A yellow coloration observation determines the presence of flavonoids.

Cardiac glycosides (keller-killiani test)
5g of each of the extracts and 5ml of honey was dissolved in 2ml glacial acetic acid containing a drop of ferric chloride solution. This was underplayed with 1ml concentrated H_2SO_4. A brown ring of the interface indicates a deoxy-sugar characteristic of cardenolides. A violet ring may appear below the brown ring, while in the acetic acid layer, a green ring may form just gradually spread throughout this layer [2].

Steriods
2 ml of acitic anhydride was added to 0.5 g of extract and 2 ml of sulphuric acid was added by the sides of the test tube and observed the colour change from violet or blue-green.

Terpenoids (Salkowski test)
To 0.5 g of the extract, 2 ml of chloroform was added: Conc. H_2SO_4 (3 ml) was carefully added to form a layer. A reddish brown coloration of the interface indicates the presence of terpenoid.

Antimicrobial assay
The antimicrobial activity of Ginger Methanolic and ethanolic extract against various human pathogens was determined by broth dilution method [5] . Ginger extracts concentration was measured by spectrophotometer. Serial double dilutions were made in Brain Heart Infusion (BHI) broth. A sample (100µL) of each concentration was pipetted into the corresponding well of a sterile microdilution tray. Bacterial suspensions from an overnight culture were standardized to 0.5 McFarland (1.5 X 108 CFU mL-1) using an API turbidometer. A 1:20 dilution was made to give a bacterial suspension of approximately 6 X 106 CFU mL-1. A sample (10µL) of the bacterial suspension was added to each well giving a final suspension of 6 x 106 CFU mL-1. Tray was incubated at 37^0C for overnight. Next day these serial diluted samples were inoculated on Nutrient Agar and Mac Conkey Agar plate. Plates were further incubated at 37 0 C for overnight. All experiments were run in duplicate. The minimum inhibitory concentration (MIC) was determined as the lowest concentration of extract that showed no visible growth in broth microdilution tray and showed mild growth when sub cultured on a suitable solid medium.

RESULTS AND DISCUSSION

Identification of Components
Interpretation on mass spectrum GC-MS was conducted using the database of National Institute Standard and Technology (NIST) having more than 62,000 patterns. The spectrum of the unknown component was compared with the spectrum of the known components stored in the NIST library. The Retention time, Name, Molecular weight and Structure of the components of the test materials were ascertained.

The GC-MS study of *Zingiber officinale* have shown many phytochemicals (Table 1) with peaks (Figure 1) which contributes to the medicinal activity of the plant .

Analysis of the chemical composition of the extract by GC-MS facilitated the identification of components in Ethanol Extract (Table 1) The major compounds identified in Ginger Ethanol Extract were α Gingiberene(20.57%) and β Seiquphellandrene (12.71%), α Curcumen (11,27%),Cyclo Hexane(10.61%), α Fernesene(9.77%) The other compounds were Cis-6-Shagole (7.45%), Gingerol (4.46%), Gingerol (1.98%) and many more.

The GC-MS study of Methanol extract was performed for the identification of the compounds in the extract. (Table 2) The major compounds identified in extract was α Gingiberene (15.32%), β Seiquphellandrene(11.80%),2,6,10 Dodecatrien-1-ol (8.29%), α Fernesene(8.22%), α Curcumen(8.11%), ϒ Cadinene(2.13%) and many more.

Comparable results were obtained but from petroleum ether extract [9, 3].

Phytochemical screening
Phytochemical screening of Ethanolic and Methanolic plant extract showed presence of alkaloid,phlobotannins, flavanoids, glycosides, saponins, tannin and terpenoids and absence of steroids(Table 3).Similar results were obtained from ethanolic extract of *Zingiber officinale* [8].

Table 1 : Quantitative biochemical analysis of 100% ethanolic extract of *Zingiber officinate* (Ginger roots).

S. No.	RT	Area%	Name	Molecular weight	Molecular Formula
1	7.434	1.06	Cineole	$C_{10}H_{18}O$	154
2	9.408	0.68	Camphol	$C_{10}H_{18}O$	154
3	12.364	0.23	Cycloisosativene	$C_{15}H_{24}$	204
4	12.435	0.48	Tricyclo [4.4.0.0.2,7] dec 3 ene	$C_{15}H_{24}$	204
5	13.434	0.35	Beta Farnesene	$C_{15}H_{24}$	204
6	13.634	0.27	2,6,10 Dodecatrien-1-ol	$C_{15}H_{26}O$	222
7	13.812	1.84	Spiro [4,5] dec-7ene	$C_{15}H_{24}$	204
8	14.042	11.27	α Curcumen	$C_{15}H_{22}$	202
9	14.274	20.57	α Gingiberene	$C_{15}H_{24}$	204
10	14.352	9.77	α Fernesene	$C_{15}H_{24}$	204
11	14.531	10.61	Cyclo Hexane	$C_{15}H_{24}$	204
12	14.650	3.41	ϒ Cadinene	$C_{15}H_{24}$	204
13	14.872	12.71	β Seiquphellandrene	$C_{15}H_{24}$	204
14	15.050	1.15	α Panosinsen	$C_{15}H_{24}$	204
15	15.509	1.09	Nerolidol B	$C_{15}H_{26}O$	222
16	16.932	0.74	Guaiol	$C_{15}H_{26}O$	222
17	17.050	0.27	Naphthalene	$C_{15}H_{24}$	204
18	17.403	0.79	Rosifaliol	$C_{15}H_{24}O$	220
19	17.482	1.24	β-bisabolol	$C_{15}H_{26}O$	222
20	17.904	0.73	Farnesol 3	$C_{15}H_{26}O$	222
21	19.743	0.33	Widdrol	$C_{15}H_{26}O$	222
22	20.786	0.24	Sobivol	$C_{10}H_{16}O$	152
23	20.914	0.21	2 Heptene, 2-methyl-6-p-tolyl	$C_{15}H_{22}$	202
24	21.146	0.93	Farnnesene epoxide	$C_{15}H_{24}O$	220
25	23.229	1.64	2,5 Dibutyl Furane	$C_{12}H_{20}O$	180
26	23.465	0.35	Nerolidol acetate	$C_{17}H_{28}O_2$	264
27	23.693	7.45	Cis-6-Shagole	$C_{17}H_{24}O_3$	276
28	24.207	4.46	Gingerol	$C_{17}H_{26}O_4$	294
29	25.196	1.98	Gingerol	$C_{17}H_{26}O_4$	294
30	25.640	0.62	Capsaicin	$C_{17}H_{27}NO_3$	293
31	26.830	1.87	Trans-10-Shagole	$C_{21}H_{32}O_3$	332
32	27.343	0.67	δ Tocopherol	$C_{27}H_{46}O_2$	402

Table 2: Quantitative biochemical analysis of 100% methanolic extract of *Zingiber officinale* (Ginger roots)

S. No	RT	Area%	Name	Molecular Formula	MW
1	13.425	0.21	Beta Farnesene	$C_{15}H_{24}$	204
2	13.807	0.81	Spiro [4,5] dec-7ene	$C_{15}H_{24}$	204
3	14.037	8.11	α Curcumen	$C_{15}H_{22}$	202
4	14.265	15.32	α Gingiberene	$C_{15}H_{24}$	204
5	14.349	8.22	α Fernesene	$C_{15}H_{24}$	204
6	14.525	8.29	2,6,10 Dodecatrien-1-ol	$C_{15}H_{26}O$	222
7	14.645	2.13	ϒ Cadinene	$C_{15}H_{24}$	204
8	14.868	11.80	β Seiquphellandrene	$C_{15}H_{24}$	204
9	15.498	1.52	Nerolidol B	$C_{15}H_{26}O$	222
10	16.838	0.90	Zingiberenol	$C_{15}H_{26}O$	222
11	16.925	0.99	Guaiol	$C_{15}H_{26}O$	222
12	16.992	0.64	Dimethyl-3,8 Nonadien-2-one	$C_{11}H_{18}O$	166
13	17.196	0.76	Sesquisabinene Hydrate	$C_{15}H_{26}O$	222
14	17.393	1.48	Rosifaliol	$C_{15}H_{24}O$	220
15	17.467	1.54	β-bisabolol	$C_{15}H_{26}O$	222
16	17.886	1.20	Farnesol 3	$C_{15}H_{26}O$	222
17	18.598	0.29	Germacron	$C_{15}H_{22}O$	218
18	19.385	0.70	2-Norbornanone	$C_{15}H_{24}O$	220
19	19.441	0.83	Thiiofenchone	$C_{10}H_{16}S$	168
20	19.645	0.31	Veridiflorol	$C_{15}H_{26}O$	222
21	20.112	0.39	Dlepi αCedrenepoxide	$C_{15}H_{24}O$	220
22	20.468	0.33	Methyl Icosanoate	$C_{21}H_{42}O_2$	326
23	20.783	0.24	Verbenol 3 Caren	$C_{23}H_{34}O_2$	342
24	20.907	0.32	Ar-Curcumene	$C_{15}H_{22}O$	218
25	21.145	0.95	Carveol	$C_{10}H_{16}O$	152
26	21.738	0.15	β-pinen, 3(acetylmethyl)	$C_{12}H_{20}O$	192

27	22.054	0.46	Methyl linoleate	$C_{19}H_{34}O_2$	294
28	22.373	0.50	2,5 dibutylfuran	$C_{12}H_{20}O$	180
29	22.639	0.42	Decalin, 1-methoxymethyl	$C_{12}H_{22}O$	182
30	23.075	0.20	Nerolidyl propionate	$C_{18}H_{30}O_2$	278
31	23.687	5.72	Cis -6-shagaol	$C_{17}H_{24}O_3$	276
32	23.917	0.98	-	-	-
33	24.018	13.78	Gingirol	$C_{17}H_{26}O_4$	294
34	24.225	0.18	Nerolidyl propionate	$C_{18}H_{30}O_2$	278
35	24.639	0.20	2- Formythexadecane	$C_{17}H_{34}O$	254
36	25.195	1.75	Laricіresinol	$C_{20}H_{24}O_6$	360
37	25.453	2.36	Gingirol	$C_{17}H_{26}O_4$	294
38	26.812	1.42	Tran- 10 - Shagaol	$C_{21}H_{32}O_3$	332
39	27.144	3.13	δ - Tocopherol	$C_{27}H_{46}O_2$	402
40	27.251	0.48	Matairesinol	$C_{20}H_{22}O_6$	358

Table 3 : Quantitative phytochemical analysis of crude extract of *Zingiber officinale* (Ginger roots)

Bioactive Principles	Methanol extracts of ginger	Ethanol extracts of ginger
Alkaloids	+++	+++
Tannins	++	++
Glycosides	++	++
Saponins	+++	+++
Steriods	-	-
Flavonoids	++	++
Terpenoids	+	+
Phlobotannins	+	+

Key = +++ abundantly present , + fairly present, ++ moderately present, – absent

Antimicrobial Assays

The findings of the present study revealed that *Zingiber officinale* contain potent antimicrobial property against tested microbes. The antimicrobial activity of the ginger extracts (Ethanol and methanol) was initially evaluated by broth micro dilution method using four strains of pathogenic bacteria *(Escherichia Coli ATCC 25922, Pseudomonas aeruginosa ATCC27853, Staphylococcus aureus ATCC 25923, Enterococcus faecalis ATCC 29212).* These extracts exhibited strong antimicrobial activity. The results obtained in the broth dilution assay regarding the MIC range of the tested microbes are shown in (Table 4). The MIC range determined by Ginger ethanolic and methanolic extract were concentration dependent for all strains. Earlier, identical results were reported in this plant but via. different methods as disk diffusion method [3] and well diffusion method [1].

Table 4 : Antimicrobial screening of *Zingiber officinale* extracts

Test microorganism	Extract	
	Ethanolic	Methanolic
	MIC range (mg/ml)	MIC range (mg/ml)
E. Coli	2.0±0.04	3.50±0.02
P. aeruginosa	2.0±0.01	1.75±0.09
S. aureus	2.0±0.02	1.75±0.08
E. faecalis	2.0±0.06	3.50±0.01

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