Available online www.jocpr.com

Journal of Chemical and Pharmaceutical Research, 2013, 5(7):296-301

Research Article

ISSN : 0975-7384
CODEN(USA) : JCPRC5

Phytochemical screening, chemical composition and antimicrobial activity of *Zingiber officinale* essential oil of Adamaoua region (Cameroon)

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ABSTRACT

Ginger (Zingiber officinale) is used as a spice and as a medicinal plant. It is especially known for its antibacterial and antifungal activities. Many other pharmacological properties are recognized to this plant. This study focuses on the phytochemical and antimicrobial properties of essential oil of a sample of ginger collected from Meiganga / Adamaoua region. The essential oil was obtained by steam distillation using the conventional method. Its fractionation by the method of Flash chromatography with pentane/petroleum ether gradient allowed separation of the hydrocarbon fraction of the oxygenated fraction. The essential oil was then tested on Escherichia coli, Listeria monocytogenes, Entercoccus faecalis, Bacillus cereus, Staphylococcus aureus, Pseudomonas fluorescens, Bacillus subtilis, Candida lusitaniae, Candida tropicalis, Cryptococcus neoformans, Aspergillus flavus, Aspergillus parasiticus strains for its biological activities. Twenty monoterpene and sesquiterpene compounds were identified in the crude essential oil in the analysis by GC-FID method. Six major components corresponding to camphene, linalool, citronelal, geranial, α-terpineol and neral showed intense peaks.

Keywords: Ginger essential oils, antimicrobial testing, GC-FID

INTRODUCTION

Herbs and plants have been in use as a source of therapeutic compounds in traditional medicinal system since ancient time. Medicinal plants play an important role in traditional heath care systems as well as in international herbals and pharmaceutical markets. The medicinal value of these plants lies in some chemical substances that produce a definite physiological action on the human body. The most important of these bioactive constituents of plants are alkaloids, tannins, flavonoids and phenolic compounds [1, 2].

Officinale (Zingiberaceae) is an important plant with several ethnomedicinal and nutritional values therefore, used extensively worldwide as a spice, favouring agent and herbal remedy. Traditionally, *Z. Officinale* is used in Chinese, Arabian, Africans, India and many other traditional systems to cure a variety of diseases viz, nausea, vomiting, asthma, palpitation, inflammation, dyspepsia, loss of appetite, constipation, digestion and pain [3]. In last few decades, *Z. Officinale* is extensively studied for its medicinal properties by advanced scientific techniques and a

variety of several compounds has been isolated from the different parts of the plants and analysed pharmacologically. The plant is reported for antimicrobial activity anticancerigenous, antioxidative, antidiabetic activity, hepatoprotective activity, and anti-inflammatory activity and immunomodulatory activities.

The purpose of this study is to evaluate the phytochemical characterization of *Zingiber officinale* hexane extracts with ethyl acetate, methanol, and ethanol. On the other hand, evaluate antimicrobial activities of its essential oil.

EXPERIMENTAL SECTION

Plant materials

The plant material (rhizomes of Zingiber officinale) was collected from a producer on July 22, 2009 in Meiganga in the Adamaoua Region.

Sample Preparation

The rhizomes were washed, cut with a knife and then dried in sunlight for 2 weeks. The dried rhizomes were powdered using an electric grinder.

Analysis by Gas Chromatography (GC)

Chromatograph type GC-14B (Shimadzu) was used. The compounds in the essential oil were identified by GC-FID. For this, a mixture was carried out in a bottle in a defined proportion bottle of oil and hexane (1/10). By using a micro syringe, 0.2 µl of the mixture is injected into the chromatograph. The analysis was performed on a non-polar capillary column HP-5MS SULPELCO mark (5% and 95% phenyl methyl siloxane) and whose dimensions were: length (30 m), diameter (0.32 mm), thickness of the wire (0.25 mm). The applied temperature program was 40 to 200 ° C at 5 ° C / min, in the succession of 200 to 230 ° C at 10 ° C / min and then maintained at 230 ° C for 10 min. The injection was performed according to the split mode with a ratio of 1/10. The carrier gas was nitrogen at a pressure of 50 kPa and a flow rate of 80 mL/mn. The temperatures of the detector and the injector were maintained respectively at 250 and 200 ° C.

Phytochemical analysis of the plant extracts

Phytochemical properties of the plant extract were tested as follows: Terpenes and steroids Reagent Lieberman-Buchard: 1ml concentrated H_2SO_4, 20 ml of acetic anhydride, 50 ml of $CHCl_3$.

In a test tube, a small amount of product is dissolved in a suitable solvent. To the resulting solution, was added a few drops of reagents Lieberman-Buchard, triterpenes give sorting with the reagent, a purplish color and the sterols a bluish-green color.

Phenolic compounds: 1g extract was dissolved in 5 ml of a solution of 1.5% DMSO and placed in a water bath (37-40°C) for 15 minutes. The solution was filtered and 3 drops of potassium ferrocyanide (K_3 Fe (CN) $_6$) 5% is introduced into the filtrate. Obtaining a violet color indicates the presence of phenolic compounds.

Alkaloids

0.5 g of extract was dissolved in 10 ml of 2% sulfuric acid for 2 minutes and the mixture is filtered. 5 drops of reagent Meyer were added to 1 ml of the filtrate. The formation of a white precipitate indicates a positive test.

Flavonoids

1 ml of extract was dissolved in 2 ml of 1N NaOH. The presence of a yellow color disappears immediately after the addition of hydrochloric confirms the presence of flavonoids acid.

Obtaining essential oil

Two thousand grams (2000 g) of powder material were introduced (weighed using an electronic scale type Mettler-Toledo.PB602-9) in the hydro distillation device (clavenger type) with 4 liters. The mixture was put under heating for 4 hours at 100 ° C using a hot plate-type IRS France. After a while, the water vapors along with them the most volatile compounds (components of the essential oil) which are fused in contact with a refrigerant which flows from the tap water continuously. Heating is stopped after 4 hours. The essential oil was collected, dried over anhydrous

sodium sulphate (Na_2SO_4) and then stored at 4 ° C in a dark bottle tightly closed to avoid organoleptic deterioration caused by sunlight.

Antimicrobial tests

The activities of the essential oil were tested on bacterial and fungal strains. The antimicrobial test was done according to the method of agar disk diffusion. Agar counts (PCA) to the count of bacteria in canned milk and other materials of sanitary magnitudes (American Public Health Association. Inc.).

Preparation: we mixe 23.5 g of the medium in one liter of distilled water. Mix well. Heated with frequent agitation and boiled until complete dissolution. Disperse in appropriate containers and autoclave at 121 ° C for 15 minutes.

The essential oil was dissolved in DMSO (v / v: 1/9). 10 µL of the solution was deposited on each of three sterile 6 mm discs that were in each petri dish. The germ concentration pre culture was 10^5 UFC/mL. A negative control was performed with the dilution solvent, DMSO. The plates wer incubated at 37 ° C for 24 hours. The antifungal activity was tested in Sabouraud Dextrose Agar (Formulation: Balanced peptone No1, 10.0g / l, Dextrose, 40.0g / l Agar NO_2, 12.0g / l) as the culture medium. Mycelial fragments were transferred to Petri dishes containing SDA amended with essential oil and measure its effect on mycelial growth measured. The incubation lasted 48 hours at 27 ° C.

RESULTS AND DISCUSSION

Identification of the compounds

The essential oil was obtained by steam distillation with water and yielded 0.47%. This value is comparable to the results of Nguefack et al (2004) [7] who obtained a yield of 0.5% essential oil of ginger sample collected in another Region of Cameroon. The tiny difference could be explained either by geographic location or by harvest time. We tested the essential oil on bacterial and fungal strains provided by the Microbiology Laboratory of the ENSAI (Table 1).

Table 1: Diameter of bacterial growth inhibition after 24 h

Strain	Diameter of bacterial growth inhibition (mm)
E. coli	7.0±1.0
L.monocytogenes	5.0±0.81
P. fluorescens	4.0±0.81
S. aureus	4.0±0.80
B. cereus	23.0±082
E. fluorescens	5.0±0.81
B. subtilis	5.33±1.24
A. flavus	25.0±0.5
A. parasiticus	11.0±1.0
C. tropicalis	12.0±0.81
C. lusitaniae	7.5±0.5
C. neoformans	12.5±0.5
Controls	0.0±0.0

The values reported in the table are the average of three replicates followed by standard deviations

Analysis of the crude oil by GC-FID has led to the identification of twenty compounds (monoterpenes and sesquiterpenes). The identification was made by noting the retention times and by calculating the corresponding Kovats. The results were then compared with the data of the spectral library of essential oil of ginger.

Splitting on an opened silica column

Four fractions were obtained after fractionation on open silica column. 2 fractions, one of which exclusively containing hydrocarbon compounds and the other oxygenated compounds have been separated. The last two fractions were mixtures of hydrocarbon and oxygenated compounds. Fraction1 (1 mL) contains seven hydrocarbon products. Fraction2 (0.7 mL) contains twelve hydrocarbon and oxygenated compounds. Fraction3 (0.5 mL) contains six hydrocarbon and oxygenated compounds.Faction4 (0.6 ml) contains only six oxygenated compounds.

Table 2: Compounds identified in the crude essential oil

N°	RT	IK	Name	Molecular Weight	Molecular Formula
1	11.533	949	α-Pinene	136	$C_{10}H_{16}$
2	11.95	970	Camphene	136	$C_{10}H_{16}$
3	12.542	1000	ß-Myrcene	136	$C_{10}H_{16}$
4	13.117	1026	6-Methylhept-5-en-2-one	126	$C_8H_{14}O$
5	13.508	1044	Limonene	136	$C_{10}H_{16}$
6	13.667	1052	Sabinene	137	$C_{10}H_{17}$
7	13.817	1059	Cineole	154	$C_{10}H_{18}O$
8	15.642	1131	Nonan-2-one	142	$C_9H_{18}O$
9	15.817	1137	Linalol	154	$C_{10}H_{18}O$
10	17.625	1198	Citronellal	154	$C_{10}H_{18}O$
11	20.583	1277	α-Terpineol	154	$C_{10}H_{18}O$
12	21.85	1310	Neral	151	$C_{10}H_{15}O$
13	23.017	1338	Geranial	152	$C_{10}H_{16}O$
14	23.525	1350	Nerol	154	$C_{10}H_{18}O$
15	26.117	1413	Tridecan-2-one	162	$C_{15}H_{26}O$
16	30.233	1512	1-(1,5-dimethylhex-4-enyl)-4-methylbenzene	204	$C_{15}H_{24}$
17	30.475	1518	α-Zingiberene	204	$C_{15}H_{24}$
18	30.742	1524	ß-Bisabolene	204	$C_{15}H_{24}$
19	31.942	1553	E-ß-Farnesene	204	$C_{15}H_{24}$
20	34.242	1609	Nerolidol B	222	$C_{15}H_{26}O$

Table 3: Compounds identified in fraction 1

N°	RT	IK	Name	Molecular Weight	Molecular Formula
1	11.533	949	α-Pinene	136	$C_{10}H_{16}$
2	11.95	970	Camphene	136	$C_{10}H_{16}$
3	12.542	1000	ß-Myrcene	136	$C_{10}H_{16}$
4	13.508	1044	Limonene	136	$C_{10}H_{16}$
5	13.667	1052	Sabinene	137	$C_{10}H_{17}$
6	30.475	1518	α-Zingiberene	204	$C_{15}H_{24}$
7	30.742	1524	ß-Bisabolene	204	$C_{15}H_{24}$

Table 4: Compounds identified in fraction 2

N°	RT	IK	Name	Molecular Weight	Molecular Formula
1	11.95	970	Camphene	136	$C_{10}H_{16}$
2	15.817	1137	Linalol	154	$C_{10}H_{18}O$
3	17.625	1198	Citronellal	154	$C_{10}H_{18}O$
4	20.583	1277	α-Terpineol	154	$C_{10}H_{18}O$
5	21.85	1310	Neral	151	$C_{10}H_{15}O$
6	23.017	1338	Geranial	152	$C_{10}H_{16}O$
7	23.525	1350	Nerol	154	$C_{10}H_{18}O$
8	26.117	1413	Tridecan-2-one	162	$C_{15}H_{26}O$
9	30.475	1518	α-Zingiberene	204	$C_{15}H_{24}$
10	30.742	1524	ß-Bisabolene	204	$C_{15}H_{24}$
11	31.942	1553	E-ß-Farnesene	204	$C_{15}H_{24}$
12	34.242	1609	Nerolidol B	222	$C_{15}H_{26}O$

Table 5: Compounds identified in fraction 3

N°	RT	IK	Name	Molecular Weight	Molecular Formula
1	23.017	1338	Geranial	152	$C_{10}H_{16}O$
2	26.117	1413	Tridecan-2-one	162	$C_{15}H_{26}O$
3	30.233	1512	1-(1,5-dimethylhex-4-enyl)-4-methylbenzene	204	$C_{15}H_{24}$
4	30.475	1518	α-Zingiberene	204	$C_{15}H_{24}$
5	30.742	1524	ß-Bisabolene	204	$C_{15}H_{24}$

Table 6: Compounds identified in fraction 4

N°	RT	IK	Name	Molecular Weight	Molecular Formula
1	15.817	1137	Linalol	154	$C_{10}H_{18}O$
2	17.625	1198	Citronellal	154	$C_{10}H_{18}O$
3	20.583	1277	α-Terpineol	154	$C_{10}H_{18}O$
4	21.85	1310	Neral	151	$C_{10}H_{15}O$
5	23.017	1338	Geranial	152	$C_{10}H_{16}O$
6	23.525	1350	Nerol	154	$C_{10}H_{18}O$

Biological activities of extracts

Table 8a: Diameter (mm) of inhibition obtained from extracts and essential oil

Extracts	*E. coli*	*L. monocytogenes*	*P. fluorescens*	*S. aureus*	*B. cereus*	*E. fluorescens*	*B. subitlis*
Essential oil	7.0±1.0	5.0±0.8	4.0±0.8	4.0±0.8	23.0±0.8	5.0±0.8	5.3±1.2
Aqueous extract	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
Ethanol extract	0.0±0.0	0.0±0.0	3.0±0.0	7.0±0.8	21.0±0.8	4.0±0.0	4.0±0.8
Hexane extract	3.5±0.5	2.0±0.8	3.3±0.5	5.0±0.8	4.3±1.2	7.3±0.5	2.0±0.0
Ethyl acetate extract	5.0±0.5	2.0±0.5	5.3±0.9	21.0±0.8	13.0±0.8	17.7±1.2	24.6±1.2
Methanol extract	0.0±0.0	3.7±0.5	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
Control	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0

Table 8b: Diameter (mm) of inhibition obtained from extracts and essential oil

Extracts	*A. flavus*	*A. parasticus*	*C. tropicalis*	*C. lusitaniae*	*C. neoformans*
Essential oil	25.5±0.5	11.0±1.0	12.0±0.8	7.5±0.5	12.5±0.5
Aqueous Extract	-	-	-	-	-
Ethanol extract	-	-	-	-	-
Hexane extract	6.5±0.5	6.5±0.5	7.3±0.5	6.5±0.5	8.5±0.5
Ethyl acetate extract	-	-	-	-	-
Methanol extract	0.0±0.0	0.0±0.0	5.5±0.5	6.5±0.5	6.5±0.5
Control	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0

- : not tested. A. flavus : Aspergillus flavus, A. parasiticus : Aspergillus parasiticus, C. tropicalis : Candida tropicalis, C. lusitaniae : Candida lusitaniae, C. neoformans : C. neoformans : Cryptococcus neoformans.

Table 8c: Diameter (mm) of mycelial growth after 7 days

Extracts	Concentration (ppm)	*Aspergillus flavus*	*Aspergillus parasiticus*
Essential oil	500	10.5±0.5	16.5±0.5
Essential oil	750	0.0±0.0	7.5±0.5
Essential oil	1000	-	0.0±0.0
Control	0	84.5±0.5	87.5±0.5

- : minimum inhibitory concentration (MIC) reached before the value of the concentration

From the following results it can be noted that only essential oil and ethyl acetate extract are active against strains tested. We were expecting a good activity of ethanol extract (polar solvent) and hexane extract that showed the largest number of compounds in the TLC. This was not the case. The ethanol extract derived from hydro distillation residue. The heat treatment would have had an adverse effect on the activity of the compounds. The low activity of the hexane extract may result from interactions between the reactive sites of compounds that are not likely to inhibit bacterial growth or simply that these compounds do not have a particular biological activity. Activities exhibited by the essential oil and ethyl acetate extract have guided us in the choice of column chromatography to better search for valuable bioactive compounds in the development of an antimicrobial agent.

The minimum inhibitory concentration (MIC) was tested on two fungal strains: *Aspergillus flavus* and *Aspergillus parasiticus* with concentrations ranging from 500 to 1000 ppm. Mycelial growth of *Aspergillus flavus* was completely inhibited at 750 ppm after 7 days, while that of *Aspergillus parasiticus* is 1000 ppm.

Phytochemical screening

Table 8d: Quantitative phytochemical analysis of crude extract of *Zingiber officinale*

Chemical Tests	Hexane extract	AcOEt extract	Methanol extract	aqueous extract	Ethanol extract
Phenolic compounds	+++	++	-	+	+
Sterols et terpenoïds	-	++	++	+++	++
Alkaloids	-	-	-	-	-
Flavonoids	-	-	-	-	-

+: positive, ++: very positive, +++: abundant, - : absent

The phytochemical screening indicates that the extracts contain only phenolic, sterols and terpenoids compounds. This is consistent with data from the literature review. The alkaloids and flavonoids are absent in all the extracts. Phytochemical and pharmacological investigations done earlier on *Zingber officinale* focused only on the organic extracts and crude essential oil of the plant. This study takes into account not only the evaluation of antimicrobial properties of organic extracts, crude essential oil, but also the fraction of essential oil and pure strains isolated from products that have been the subject of any studies with extracts of *Zingiber officinale* in Cameroon.

Acknowledgements
The authors thank the Faculty of Science, University of Ngaoundere, Cameroon, for providing the necessary support for this study.

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