

PHYTOCHEMICAL AND GC/MS ANALYSIS OF THE RHIZOME OF ZINGIBER OFFICINALE PLANT GROWN IN EASTERN PART OF NIGERIA

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ABSTRACT: *Phytochemical and GC-MS analysis of zingiber offinale was carried out in the* laboratory and with the aid of SHIMAZU Japan Gas Chromatography 5890-11 with a fused GC column OV 101 coated with polymethyl silicon (0.25 mm x 50 m). The result obtained confirmed the presence of alkaloids, flavonoids, saponins, glycosides, tannins amd phenols in the plant. Twelve peaks were obtained from the he spectra of the GC-MS. peak 1 corresponds to Furan-3-carboxaldehyde with m/z 128 and molecular formulae $C_6H_8O_2$, peak 2 was identified as Benzene -1-(1,5-dimethyl-4-4hexenyl)-4-methyl m/z 202 with molecular formulae $C_{15}H_{22}$. Peak 3 as ,1-,3-cyclohexadiene-5- (,5 diethyl-4-hexenyl -2-methyl (zingiberene) m/z 204 with molecular formulae $C_{15}H_{24}$, peak 4 as Alpha farnesene m/z 204 with molecular formulae $C_{15}H_{24}$. Peaks 5,6,7,8,9,10,1,12 occurred at m/z; 220,204, 194,242,,256,296,282, 296 corresponding to butylated hydrotoulene, C₁₅H₂₄,,cyclohexene-3-(1,5-dimethyl-4-hexenyl-6- methelene $C_{15}H_{24}$, 2-butanone-4-(4-hydroxy-3-methoxyphenyl $C_{11}H_{14}O_3$, methyl tetra decanoate $C_{15}H_{32}O_2$, n-hexadecanoic acid $C_{16}H_{32}O_2$, 9-octadecenoic acid methyl ester $C_{09}H_{26}O_2$, Octadec-9-enoic acid, $C_{18}H_{34}O_2$, Gingerol $C_{17}H_{28}O_4$ and *Ricinoeic acid* $C_{18}H_{24}O_2$ *respectively*

KEYWORDS: Phytochemicals, Alkaloids, Tannins, Flavonoids, Zingiberene, Gingerol

INTRODUCTION

Zingiber officinale also known as ginger which is derived from the ancient Sanskrit Singabera, meaning 'shaped like a horn' is a common food spice grown around the world. its other names are Zingiber miaga, Alpinia galanga, Zingiber Zerumber, Asarum splendens and Alpinia caerules ^[1] It was introduced by the Spaniards to America and is now cultivated extensively in the West Indies and the Portuguese introduced it to West Africa. Zingiber officinale is used in China to reduce the toxicity of some herbs. The Chinese prescribe ginger tea for delayed menstruation. It is rich in vitamin C, and ward off scurvy. Ginger is a creeping perennial plant native to tropical south-east Asia and cultivated in the West Indies, Africa and China. The aromatic, knotty rootstock is thick and fibrous, and whitish or buff in color. It produces a simple, leafy stem covered with the leaf sheaths of the lanceolate-oblong to linear leaves, and reaches a height of 1.25 m. The leaves are up to 30 cm long and the sterile flowers are white with purple streaks and grow in small dense spikes. Ginger is a rain forest monocot about a meter high, with long, narrow leaves and spicate flowers. It has been grown in China since Antiquity. Seeds has never been found, ginger propagates through budding from its knotty rhizome. The fresh ginger rhizome is a versatile ingredient of the far eastern cuisine, and is now commonly used in most of the world. Its flavor is lemonybalsamic; the plant is used as carminative, expectorant and astringent. Ginger could be used



as an anti-thrombotic and anti-inflammatory, antioxidant and anticancer agent. ^[2,3] ginger exerts many direct and indirect effects on blood pressure and heart rate, it can be used to treat migraine, diabetes, retinopathy, ulcer and cancer, it can also treat elephantiasis, its tonic helps in memory improvement, preserves liver health. It can be used to treat paralysis and jaundice, dyspepsia, headache and arthritis. It is also useful in the treatment of filariasis, clear blood blockage and reduce high blood pressure ^[4]. It, reduces and relieves hemorrhoids and pains associated with it, and in treatment of asthma. The effectiveness of ginger in emesis due to motion sickness and cancer chemotherapy has also been reported. Ginger has been revealed as being useful in preventing post-operative nausea and vomiting in humans^[5]. Ginger has anti-oxidant properties, the anti-oxidant action of ginger has been proposed as one of the major possible mechanisms for the protective actions of the plant against toxicity and lethality of radiation and a number of toxic agents such as carbon tetrachloride, and as an anti-ulcer drug. Extracts and fractions of Z. officinale has been shown to protect against chemically-induced tissue damage, it has been shown that pretreatment of rats with an ethanol extract of the rhizome of Z. officinale and oil extracted from the plant were effective in ameliorating carbon tetrachloride and acetaminophen (paracetamol) -induced acute heptatotoxicity^[6]. It is also a known fact that ginger extracts mitigate the neurobehavioral effects of gamma radiation-induced conditioned taste aversion in rats ^[7]. Ginger contains gingerol, shogaol, zingiberene, paradol, zingerone 1,8- cineole, alpha linolenic acid, stach, protein, trace metals, wax or lipids, crude fibre and oleoresin. The root is rich in inulin, alantoluction, Ingenols and Singerones ^[8,1]. It gives special flavor to foods, Sonal ^[9]. The plant contains calcium, iron, magnesium, phosphorus, sodium, potassium, zinc, manganese. And copper It is rich in thiamine, riboflavin, niacin, panthenic acid, vitamin B6, follate vitamin C and vitamin E.^[10]. It has been shown to inhibit Heliobacter, Pylori, E. coli, Bascillus carellus, Clostridium, listeria, Entercoccus and Staphylococcus species and S. typhi, its extracts kills oncomelaris hupensis^[11,12]. It inhibits Aspergillus niger, S. cerevisiae, Mycoderma spp, L. acidphilus, streptococcoi and staphylococci and Coliform *bascillus* ^[13, 14].

MATERIALS AND METHOD

Sample Collection and Preparation

The rhizome of *Zingiber. officinale* was obtained from Ihiagwa market, Owerri North L.G.A. and was brought to the laboratory. The sample was sliced into bits and room dried, ground into powder and stored. It was kept in an air-tight container afterwards before analysis^[15]

Frothing test for Saponins

This test is based on the ability of the saponins to produce froth in aqueous solution. 5g of the plant extract was weighed into a test tube and 100cm^3 of water was added and extracted after 4 hours. The water extract was shaken vigorously in a conical flask. The production of a stable froth indicates the presence of saponins in the sample

Test for Flavonoids

5g of the sample was weighed into a 250cm³ beaker and 150cm³ of water was added and allowed to stand for 4 hours and then filtered. 10cm³ of the filtrate was measured into a



 50cm^3 and drops of ammonia and 3cm^3 of concentrated H₂SO₄ was added. A yellow precipitate which disappears on storage indicates the presence of flavonoids.

Test for Alkaloids

5g of the sample was extracted using 20% acetic acid in ethanol $.5cm^3$ of the extract was treated with Wagner's reagent (iodine crystals and KI). A yellowish-brown precipitate indicates the presence of alkaloids.

Test for Tannins

5g of the root sample was weighed into a beaker and 50cm³ of water was added and allowed to stand for 4 hours and extracted. The extract was treated with drops of ferric chloride. A blue-black precipitate indicates the presence of tannins.

Test for Steroids

5cm³ of the water extract was treated with concentrated H₂SO₄ in acetic anhydride. The formation of a blue-green color indicates the presence of steroids.

Test for Phenols

 20cm^3 of the water extract was treated with 5cm^3 of concentrated sulphuric acid and drops of sodium nitrate (NaNO₃). 2cm^3 of sodium hydroxide was added to the mixture. A blue precipitate indicated the presence of phenols.

Test for Glycosides

20cm³ of the water extract was treated with Fehling solutions of A and B in equal amount and boiled. A brownish red precipitate indicates the presence of glycoside.

Preparation of Samples for GC-MS Analysis

Two hundred grams of sample was soaked in ethanol for 48 hours and then extracted. The extract was re-extracted using chloroform to obtain chloroform soluble extract. This was centrifuged at 10,000 rpm for 20 minutes and the clear supernatant oil was subjected to GC-MS analysis.

GC-MS Experimental Procedures

GC-MS analysis was carried out with SHIMAZU Japan Gas Chromatography 5890-11 with a fused GC column OV 101 coated with polymethyl silicon (0.25 mm x 50 m) and the conditions are as follows: Temperature programming from 80 – 200°C held at 80°C for 1 minute, the rate is 5°C/min and at 200°C for 20 minutes. FID Temperature of 300°C, injection temperature of 250°C, carrier gas is Nitrogen at a flow rate of 1 cm³/min and split ratio of 1:75. GC-MS Gas chromatography, Mass spectrum analysis were conducted using GC-MS QP 2010 Plus Shimazu Japan with injector Temperature at 230°C and carrier gas pressure of 100kpa. The column length was 30 m with a diameter of 0.25 mm and the flow rate of 50m/min. The eluents were automatically passed into the Mass Spectrometer with a detector voltage set at 1.5kv and sampling rate of 0.2 seconds. The Mass Spectrometer was also equipped with a computer fed Mass Spectra data bank, HERMCE Z 233 M-Z centrifuge



Germany was used. Reagents and solvents such as Ethanol, Chloroform, Diethyl ether, hexane all of analytics grade was obtained from Merck Germany^[15,16]

RESULT AND DISCUSSION

The results obtained from the analysis of the rhizome of gingiber officinale are summarized the tables and figures below. Initial phytochemical analysis of the plant revealed the presence of alkaloids, glycosides, steroids, flavonoids, tannins and phenols and saponins table 1

Table 1: Phytochemical Constituents of the Plant Extract

Plant extract	Alkaloids	Glycosides	Steroids	Flavanoids	Tannins	Saponins	Phenols
Z. officinale	Present	Present	Present	Present	Present	present	Present

Alkaloids are regarded among the most efficient therapeutically significant plant substance ever known. Alkaloids and their synthetic derivatives are used by Etinomedicinal practitioners for their analgesic, antispasmodic and bactericidal effects ^[17]. They exhibit marked physiological activity when administered to animals. Most samples containing alkaloid are used in Nigeria for the treatment of malaria and fever ^[18],

Saponins are applied for their many antimicrobial properties. Some of the general characteristic of saponins includes formation of forms in aqueous solutions, hemolytic activity and cholesterol binding properties^[19, 20]. Saponin has the natural tendency to ward off microbes and this makes them good candidates for treating fungals and yeast infections. These compounds served as natural antibiotic, helping the body to fight infections and microbial invasion.

Flavonoid are distributed group of polycyclic compounds characterized by a common Benzo pyrone ring structure that has been reported to act as antioxidants in many biological systems. Their family encompasses flavonoids, flavones, chalcones, catchins, anthocyanidins and isoflavonoids^[21]. In addition to their free radical scavenging activities, Flavonoids have multiple biological activities including – vasodilatory, anti-carcinogenic, anti-allergic, antiviral, estrogenic effects as well as being inhibitors of phospholpase H₂, cycloxygenase, glutathione reductase and xanthine oxidase^[22,23,24], they support lactogenecity. These properties therefore support the use of *Pentaclethra Macrophylla* in cancer therapy ^[25]. Flavonoids in intestinal tracks lower the risk of heart diseases. As anti-oxidant, flavonoids provide anti-inflammatory actions.

There is a growing interest in poyphenolic compounds as therapeutic agents against many diseases such as cardic and cerebral ischemic, arteriosclerosis and rheumatic or pulmonary diseases ^[25,26]. The activated phagocytic cells are known to produce potentially destructive oxygen species like super oxide anion (O^{2-}), hydrogen peroxide (H_2O_2) and Hypochloric acid (HOCl) during chronic inflammatory disorder ^[21] Many polyphenolics are known to exhibit antioxidant properties, they are free radicals scavengers. Phenolic flavonoids are also



excellent hydroxyl scavengers. These properties promote health, and prevents certain chronic disorders such as cancer, cardiovascular diseases, diabetics and arthritis. The presence of phenols means that these extracts could act as anti-inflammatory, anti-clothing, anti-oxidants, immune enhancers and hormone modulators. Phenols have been the subject of extensive research as disease preventives ^[23,26]. They have the ability to block specific enzymes that causes inflammations. They modify the prostaglandin pathways and thereby protect platelets from clumping. Tannins have astringent properties, hastening the healing of wounds and inflamed mucors membrane ^[17]. The presence of Tannins in these samples supports their use in treating wounds, varicose ulcers, hemorrhoids, frost bites and burns in herbal medicine

The GC/MS result of the saple is shown in figure 1 below.the spectrum showed the presence of thirteen peaks

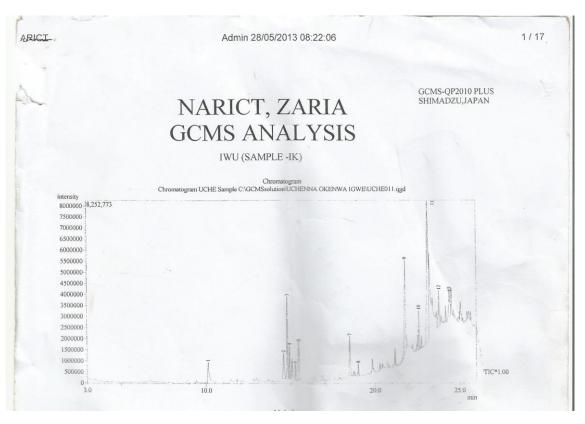


Figure 1: GC-MS Spectra of the Extract

These peaks are interpreted in (Table 3). Peak 1 occurred at m/z 128 with the molecular formula $C_6H_8O_3$ and is identified as Furan-3- Carboxaldehyde. Peak 2 appeared at m/z 202 with molecular formula $C_{15}H_{22}$ and identified as Benzene 1-(1, 5 dimethyl 4-hexenyl)-4-methyl. Peak 3 appeared at m/z 204, with molecular formula $C_{15}H_{24}$, and its name is 1,3-cyclohexadiene 5-(1,5-dimethyl-4-hexenyl)-2-methyl (Zingiberene). Peak 4 occurred at m/z 204 with the formula $C_{15}H_{24}$ and its name is Alpha Farnesene. Peak 5 occurred at m/z 220 with the formula $C_{15}H_{24}O$ and named as Butylated Hydroxytoluene. Peak 6 appeared at m/z 204 with formula $C_{15}H_{24}$ and named Cyclohexene 3-(1,5-dimethyl-4-hexenyl)-6-methylene.



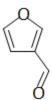
Peak 7 occurred at m/z 194 and its formula is $C_{11}H_{14}O_3$, and is named 2-Butanone 4-(4-Hydroxy-3-methoxyphenyl). Peak 8 occurred at m/z 242 with chemical formula $C_{15}H_{30}O_2$ and is identified as Methyl tetradecanoate. Peak 9 appeared at m/z 256; its formula is $C_{16}H_{32}O_2$ and is named n-Hexadecanoic acid. Peak 10 occurred at m/z 296; with the formula $C_{19}H_{36}O_2$ and its name is 9-Octadecanoicacid, methyl ester. Peak 11 occurred at m/z 282 with the chemical formula $C_{18}H_{34}O_2$ and is identified as Octadec-9-enoic acid. Peak 12 occurred at m/z 294 with chemical formula $C_{17}H_{26}O_4$ and is named Gingerol. And the last Peak 13 occurred at m/z 298; its formula $C_{18}H_{34}O_3$ and is identified as Ricinoleic acid.

Chromatographic			Molecular weight	
peak		formula		
1	Furan-3-carboxaldehyde	$C_6H_8O_3$	128	
2	Benzene 1-(1,5 dimethyl	$C_{15}H_{22}$	202	
	4-hexenyl)-4-methyl			
3	1,3 cyclohexadiene 5-	$C_{15}H_{24}$	204	
	(1,5-dimethyl-4-			
	hexenyl)-2-methyl			
	(Zingiberene)			
4	Alpha Farnesene	C15H24	204	
5	Butylatedhydroxytoluene	$C_{15}H_{24}O$	220	
6	Cyclohexene 3-(1,5-	$C_{15}H_{24}$	204	
	dimethyl-4-hexenyl)-6-			
	methylene			
7	2-Butanone-4-(4-	$C_{11}H_{14}O_3$	194	
	Hydroxy-3-methoxy			
	phenyl)			
8	Methyl tetradecanoate	$C_{15}H_{30}O2$	242	
9	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256	
10	9-Octadecanoicacid,	$C_{19}H_{36}O_2$	296	
	methyl ester			
11	Octadec-9-enoic acid	$C_{18}H_{34}O_2$	282	
12	Gingerol	$C_{17}H_{26}O_4$	294	
13	Ricinoleic acid	$C_{18}H_{34}O_3$	298	

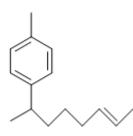
Table 2: GC-MS Analysis of Zingiber	Officinale Root
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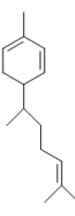
1. Furan-3-carboxaldehyde



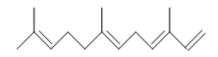
2. Benzene 1-(1,5 dimethyl 4-hexenyl)-4-methyl



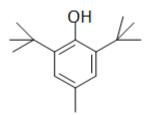
3. 1,3 cyclohexadiene 5-(1,5-dimethyl-4-hexenyl)-2-methyl (Zingiberene)



4. Alpha Farnesene

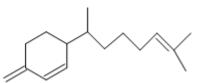


5. Butylatedhydroxytoluene

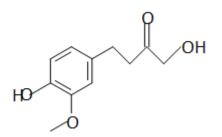




6. Cyclohexene 3-(1,5-dimethyl-4-hexenyl)-6-methylene



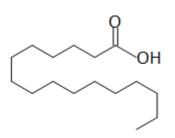
7. 2-Butanone4-(4-Hydroxy-3-methoxyphenyl)



8. Methyl tetradecanoate



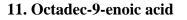
9. n-Hexadecanoic acid



10. a-Octadecanoicacid, methyl ester







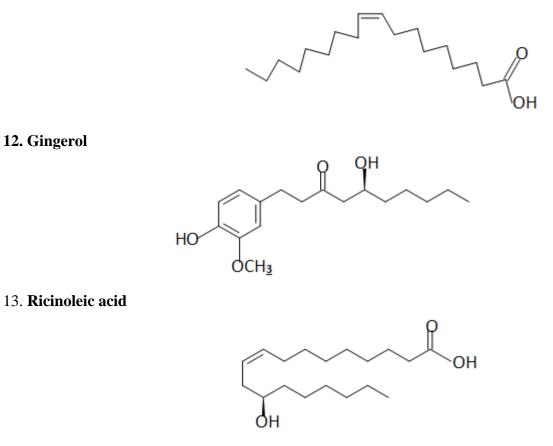


Fig 2. Structures of Compounds found in the Plant Extract

Most of these compounds has several medicinal applications, the pungency of fresh ginger results from a group of phenols, the gingerols, of which [6]-gingerol is most abundant. Fresh ginger also may contain a 5-deoxy derivative of ginger called paradol, there are also bioactive diarylheptanoids and zingerone that are believed to contribute to its purported health benefits. The major pharmacological activity of ginger appears to be due to gingerol and shogaol. Phenylalkylketones of ginger include 6-gingerol and 8- gingerol. Ginger has many active components. The [6]-gingerol, a major pungent ingredient of ginger has a potent antiangiogenic activity and [6]-gingerol may inhibit tumor growth and metastasis via its antiangiogenic activity^[27]. Topical application of [6]-gingerol inhibited COX-2 (cyclooxygenase-2) expression along with suppressed NF- kB DNA binding activity in mouse skin ^[28]. The proposed mechanisms of action of gingerol involved in anticancer and chemopreventive properties via multiple pathways that includes the inhibition of cyclooxygenase -2 (COX-2) expression by inhibiting p38 MAPK–NF-KB (mitogen activated protein kinase – necrosis factor kappa B) signaling pathway^[3] The [6]- gingerol is effective in suppressing growth of colon tumor. [6]- gingerol acts against skin cancer, breast cancer and ovarian cancer^[29]. The ginger constituents including [6] - shogaol, [6] - gingerol, [8] – gingerol and [10]-gingerol have shown certain pharmacokinetic properties of anticancer agents.^[30]. Another ginger compound [6]- paradol displays anticancer activity against skin cancer [31]. It reduces the elevated expression of tumor necrosis factor - alfa (TNF- α) and NF-Kb^[32]. Growth of colon



and lung cancer in mouse was suppressed and activates apoptosis by Zerumbone , a component of ginger ^[33]; Zerumbone inhibits NF-kB activation in o (6)-gingerol appears to be the antioxidant constituent present in ginger, as it was shown to protect HL-60 cells from oxidative stress . Ginger oil has dominative protective effects on DNA damage induced by H_2O_2 . Ginger oil might act as a scavenger of oxygen radical and might be used as an antioxidant ^[34]. Both (6)-shogaol and (6)- gingerol, and the gingerdiones, are reportedly potent enzymatic inhibitors of prostaglandin, thromboxane, and leukotriene biosynthesis, preventing, both joint inflammation and destruction. Non-gingerol components enhance the antiarthritic effects of the more widely studied [6]-gingerol^[35]

CONCLUSION

The rhizome of *Zingiber officinale* contains vital chemical compounds that have useful pharmacological properties which could be extracted and used as alternatives to synthetic drugs for the treatment of certain diseases, including arthritis, rheumatism etc.

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