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**Biochemical Analysis of *zingiber officinale rosc* (ginger rhizome) and
 Physicochemical Evaluation of its Oil**

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ABSTRACT

Biochemical and physicochemical analysis of ginger rhizome and its oil were respectively carried out to ascertain its health benefits. The mineral composition of the oil was determined using XRF. The active constituent in the oil was determined using GC-MS spectrophotometer, SHIMADZU GCMS-QP 2010 plus model. The metabolites present in the rhizome are alkaloids, tannins, saponins, flavonoids, glycosides, saponin, and steroids. Characterization of the oil showed 194.58mg/g, 123.35mg/g, 286.58mg/g, and 56.67meq/kg of acid, iodine, saponification and peroxide values respectively. The X-ray fluorescence (XRF) analysis showed the presence of Phosphorous, Silicon, Sulphur, Chloride, Calcium, Titanium, Manganese and Iron. GC-MS analysis showed the presence of gingerol and other fragmented compounds.

Keywords: Ginger rhizomes, XRF, GC-MS, Gingerol and Biochemical analysis

1.0 INTRODUCTION

Ginger is a perennial herb belonging to the family Zingiberaceae (Shaikh, *et al* 2013). It possesses pharmaceutical properties and originated from south-east Asia and exposed to other parts of the world (Burkill, 1996; Park and Pizutto, 2002). Ginger and its products can be traded globally for utilization in nutrients and pharmaceutical activities (Kanadea and Bhatkhandeb, 2016).

While many ginger-infused foods are trending, the use of ginger for all-round health benefits is nothing new (Kirkpatrick, 2020). Since primaeval times ginger rhizome has been utilized as anti-inflammatory, appetite stimulant, digestive and peripheral anti-inflammatory agent (Kizhakkayil and Sasikumar, 2012). *Zingiber officinale* can also be utilized for the treatment of illnesses such as high blood pressure, hypercholesterolemia and headaches in southeastern countries (Parakash *et al.*, 2013).

Ginger can be taken as fresh paste, dried powder and flavouring tea (Kizhakkayil and Sasikumar, 2012). Other applications can be found in cosmetics, perfumery and toiletries (Oluwatoyin, 2014).

1.1 taxonomy of *zingiber officinale*

Domain: Eukaryota

Kingdom: *Plantae*
 Phylum: *Spermatophyta*
 Subphylum: *Angiospermae*
 Class: *Monocotyledonae*
 Order: *Zingiberales*
 Family: *Zingiberaceae*
 Genus: *Zingiber*
 Species: *Zingiber officinale*



Fig 1: Ginger Rhizome

This work was aimed at identifying the elements present in ginger rhizomes, extract the essential oil from the ginger rhizome, characterize the oil and identify the active compounds present in ginger oil.

2.0 EXPERIMENTAL

2.1 Sample Collection and Preparation

Fresh Ginger rhizome was bought from Tudun Wada Market Gusau, authenticated by Malam Namadi Sanusi of the Department of Biological Science, Ahmadu Bello University Zaria, Nigeria. The Ginger rhizome was washed in running tap water to remove impurities and dirt that were attached to the rhizome after which it was weighed. The rhizome was then dried in an oven set at 105°C for two hours. The size of the ginger was then reduced using wooden mortar and pestle.

2.2 Phytochemical Screening

Phytochemical screening was carried out on the powdered rhizome using standard procedures as described by Harbone, (1984), Trease and Evans, (1978). Methanol and distilled water were used as solvents to obtain extracts for the screening.

2.3 Extraction of the Ginger Oil

194g of the powdered ginger rhizome was utilized in extracting the oil using soxhlet extraction with petroleum ether as the solvent for extraction. Extraction was carried out using AOAC (1984) method with little modification.

A 500mL round bottom flask with few anti-bumping chips inserted in it was weighed to give W_1 . 1L of petroleum ether was then poured into the flask and it was set with the solvent extraction unit. The solvent was allowed to reflux at steady state with the extraction lasting for six hours. The sample was removed from the soxhlet apparatus and the solvent was recovered. The round bottom flask was allowed to cool after which it was weighed to obtain W_2 . The percentage yield of the oil was calculated from using the expression;

$$\% \text{ crude lipid content} = \frac{W_2 - W_1}{\text{Weight of sample}} \times 100$$

W_1 = weight of the round bottom flask + anti-bumping chips

W_2 = weight of the round bottom flask containing the oil after extraction

2.4 Determination of acid value

The A.O.A.C. method of 1998 was used for the determination. 25 ml of diethyl ether was mixed with 25 ml of ethanol. 1 ml of 1% phenolphthalein solution was added to neutralize with 0.1 M sodium hydroxide solution. 10g of the oil was dissolved in the neutralized solvent mixture and titrated with 0.1M sodium hydroxide solution until pink colouration persists. The acid value was computed from the expression:

$$\text{Acid value (mg KOH/ml of oil)} = \frac{56.1 \times C \times V}{\text{weight of sample (ml)}}$$

Where C = concentration of KOH used

V = volume of KOH used

56.1 = molar mass of KOH

2.5 Determination of iodine value (A.O.A.C., 1980)

Fat (oil) dissolved in carbon tetrachloride (CCl_4), is shaken up with a known excess of iodine in the form of iodine chloride (Wijs Iodine). The remaining iodine which is not absorbed by the fat is then titrated against standard sodiumthiosulphate and, by difference; the amount of iodine taken up by the fat can be estimated.

0.2ml of oil sample was weighed into a 250ml conical flask and dissolved with 10ml carbon tetrachloride. 20ml of Wijs solution was added to the mixture. The flask was then stoppered (which has been moistened with potassium iodide solution) and gently shaken and placed in the dark for 30 minutes. The excess iodine was determined by adding 15 ml 10% (W/V) potassium iodide solution and 100 ml water. The mixture was well shaken and titrate with 0.1 M sodium thiosulphate using a starch indicator. The titration was continued until a blue colour just disappeared after vigorous shaking. A blank determination was carried out and the iodine value (IV) was determined.

$$\text{Iodine Value (gI/100mloil)} = \frac{1.269 \times C \times (V_1 - V_2)}{\text{weight of sample (ml)}}$$

C = Concentration of sodium thiosulphate used.

V_1 = Volume of sodium thiosulphate used for blank.

V_2 = Volume of sodium thiosulphate used for the sample.

2.6 Determination of saponification value

2g of the oil sample was weighed into a round bottom flask and 25ml of alcoholic potassium

hydroxide (0.5M KOH) was added in accordance to A.O.A.C, 1980 literature. To another flask, 25ml of the alcoholic potassium hydroxide only was added and used as blank. Both flasks were refluxed over boiling water bath for an hour with frequent shaking. 1ml of phenolphthalein indicator was added to each flask and titrated against 0.5M HCl with vigorous shaking, without delay to get the endpoint.

Saponification value is calculated from the equation: $SV = \frac{(S-B) \times C \times 56.1}{\text{sample weight (g)}}$.

S = sample titre value, B = blank titre value, C = concentration of HCl and 56.1 = molar mass of KOH.

2.7 Determination of peroxide value (A.O.A.C. 1980)

1g of oil sample was weighed into a clean dry boiling tube. 1g of powdered potassium iodide and 20 ml solvent mixture was added. The tube was placed in a boiling water bath for 60 seconds. The content was transferred into a titration flask containing 20 ml potassium iodide solution. The tube was washed twice with 25ml portions of water and the washings added to the titration flask. Titration was done with 0.002M thiosulphate using a starch indicator.

A blank determination was conducted. The peroxide value (PV) was computed from the relationship:

$$\text{Peroxide Value Meq/kg} = \frac{\text{titre value} \times N \times 100}{\text{weight of sample}}$$

2.8 GC-MS Analysis

The extracted oil was analyzed using gas chromatogram coupled to a mass spectrometer model GCMS-Qp 2010 Plus fitted with RTX5 column of dimension 30m X 0.5mm and film thickness of 0.25mm. The carrier gas used was helium at a flow rate of 4.67mL min⁻¹. The injection port and detector temperature are 25°C and 220°C respectively. The oven temperature was programmed from 60°C to 160°C at the rate of 10°C/min and ran for 3mins. The sample was injected into the spectrometer and mass spectra were acquired and processed with SHIMADZU MAT 300 data system.

2.9. Preparation of sample for XRF

Small amount of the oil was heated in an oven for about 5mins and spinned in a centrifuge at 200rpm. The portion to be measured was taken from 1/3 of the centrifuged sample and placed into a sample cup for analysis. The standard sample was measured and the relationship of x-ray intensity plotted as calibration curve and was used to quantify the oil sample. The oil sample was analyzed using XRF (EDX-7000/8000 SHIMADZU)

3.0 RESULTS AND DISCUSSION

Phytochemical screening was carried out on the ginger rhizome after which oil was extracted from the rhizome using AOAC method of 1980. The oil was then characterized using x-ray fluorescence spectrophotometric analysis (XRF) and Gas chromatography-mass spectrophotometric (GC-MS) analysis.

The results of the phytochemical screening are shown in Table 1

Table 1: Phytochemical Screening of Ginger Rhizome

Tests	Solvents	
	Methanol	Distilled Water
Alkaloid	+	-
Tannins	+++	++
Saponins	+	-
Flavonoids	+++	+
Glycosides	+++	+
Saponin glyside	+++	+
Steroids	+++	+

The result of the phytochemical screening showed the presence of eight secondary metabolites.

Tannins, flavonoids, glycosides, saponin, glycosides, steroids and balsam are found to be

sufficiently present in methanol extract. Tannins, flavonoids, glycosides, saponin, and steroids were found to be moderately present in aqueous extract while alkaloids and saponins were absent from the aqueous extract (Table 1). The result of the phytochemical screening of the aqueous extracts in line with the works of Sarda *et al*; 2017 and Syed *et al* 2019.

The result of Physicochemical analysis showed Acid Value, Iodine Value, Saponification Value, and Peroxide Value as 194.48(mg/g),

123.35(mg/g), 286.58(mg/g), and 56.67(meq/kg) respectively (Table 2). Acid and peroxide values of ginger oil are high compared to codex standard; this makes it difficult for the oil to be stored over time as they become rancid with time. Also, the iodine value of 123.35mg/g is high indicating a high degree of unsaturation. However, the saponification value is sufficiently high thus it can be utilized in cosmetics industries for soap production.

Table 2: Result of Physicochemical Analysis of Ginger Oil

Acid Value (mg/g)	Iodine Value (mg/g)	Saponification Value (mg/g)	Peroxide Value (meq/kg)
194.48	123.35	286.58	56.67

The result of XRF Spectroscopy showed the presence of eight (8) elements in the oil (Table 3). They include Phosphorous (P), Silicon (Si), Sulphur (S), Chlorine (Cl), Calcium (Ca),

Titanium (Ti), Manganese (Mn), and Iron (Fe) with a concentrations of 621.879 mg/kg, 269.391 mg/kg, 172.574 mg/kg, 176.898 mg/kg, 337.365 mg/kg, 57.536 mg/kg, 7.616 mg/kg and 29.147 mg/kg respectively.

Table 3: Result of XRF Analysis Ginger Oil Extract

Elements	Concentration (Mg/Kg)
P	621.879
Si	269.391
S	172.574
Cl	176.898
Ca	337.365
Ti	57.536
Mn	7.616
Fe	29.147

The GC-MS analysis gave a total of sixty compounds which are mostly fragments. However, the compound gingerol present in ginger rhizome oil was identified based on GC-MS spectral library attached to the spectrometer (Fig 2). The mass spectra of the compound showed molecular ion peak $[M]^+$ at M/Z 294 which represent an intact

molecule and also give the molecular weight of the compound. Other fragment ion peaks occur at M/Z 276, 205, 194, 179, 161 and 137. The GC-MS analysis is in agreement with the works of Jaleel and Sasikumar, 2016 in identifying sixty compounds from the oil.

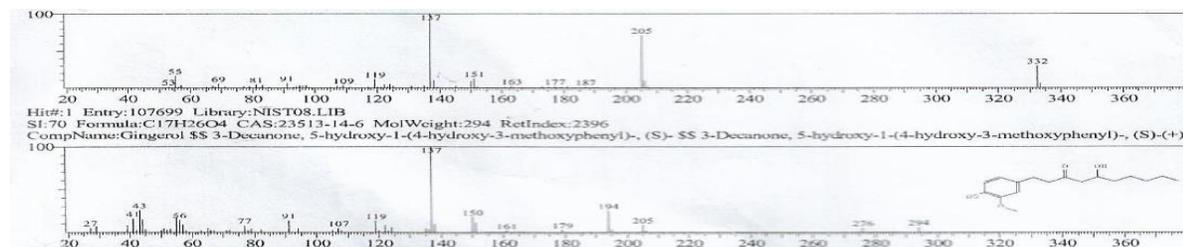


Fig 2: GC-MS Spectra of Gingerol

4.0 CONCLUSION

Phytochemical screening gave a result that justifies the ethnomedicinal use of ginger. Also present in ginger are several mineral elements which make them a good additive in food industries.

Gingerol was found to be the major compound in the ginger oil. Due to its high saponification value, ginger oil have found useful application in the production of soap and other cosmetics. Further research needs to be carried out on ginger oil to assess other physicochemical properties such as viscosity, fatty acids, volatiles, tocopherol and polyphenols.

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