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(RESEARCH ARTICLE)

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Phytochemical characterization, minerals and antioxidant properties of leaf extract of *Piper guineenses* schum thonn (piperaceae) obtained from Owerri environs

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Abstract

Piper guineense Schum Thonn (Piperaceae) *is* a common medicinal plant employed for its numerous values which has drawn a lot of attention and a lot of research is still ongoing about the usefulness of the plant. The phytochemical screening of the leaf extract revealed the presence of tannins. saponins, alkaloids, flavonoids, cardiac glycosides, steroids and phenols, determination of some the phytochemicals indicated that the extract contains tannins 12.50 mg/100 g, saponins, 30.60 mg/100 g ,alkaloids, 0.30 mg/100 g and flavonoids, 36.90 mg/100 g. Mineral determination revealed the presence of important minerals such as Phosphorus 0. 35%, Calcium 1.07%, Magnesium 0. 46%, Potassium, 2.60%, Sodium 82.52 ppm, Manganese 300.74 ppm, Iron 315. 99 ppm , Zinc 25. 20 ppm, and Copper 7. 72 ppm. GC/MS analysis gave 34 compounds with their molecular formulas, weights and structures. The antioxidant activities were measured by the ferric reducing antioxidant power (FRAP) and 2, 2-diphenyl-1-picrylhydrazl hydrate (DPPH) assay methods. The extract had 84.303% scavenging activity at concentrations of 100 mg/ml as against that of butylated hydroxytoulene 84.303% at concentration of 80 mg/ml. Similarly, it showed a 91. 495% inhibition of free radical at concentrations of 80 mg/ml as against 99.640% by gallic acid at same concentration. Quantification of antioxidant activity of the extracts showed a dose dependent antioxidant activity comparing favorably with those of standard antioxidant. The extract is a strong antioxidant and free radical scavenger and could be used as a natural antioxidant in processes requiring natural antioxidant.

Keyword; Antioxidants; Phytochemicals; Minerals; Free radicals

1. Introduction

Piper guineense (black pepper) belongs to the Piperaceae family. The fruit (pepper) is used as spices having pungent aromatic smell which is responsible for its use as a flavoring and seasoning agent [1]. *Piper guineense* leaves has been reported to be useful in treatment of pseudofolliculitis Barbae (after shave rash). [2] The presence of flavonoids, alkaloids, tannins, and saponins, has also been indicated in the plant. It contains essential vitamins, phytochemicals, and other nutrients [3][4]). Earlier Studies have shown *Piper guineense* as a potential candidate for bio-insecticide and could serve as an alternative to synthetic insecticides. [5]. In another reports, the constituents of the seeds extracts of *Piper guineense* has been shown to contains secondary metabolites such as carotenoids anthraquinones, steroids, phenols, flavonoids and terpenes. [6]. The plant is a promising therapeutic agent that could have potential for the treatment of various pathogenic diseases. The use of *Piper guineense* as a possible agent in managing brain aging has been discussed because of its implications for practical brain function. [7] The plant contains an alkaloid (piperine) reported to be an antioxidant, anti-depressant, and central nervous system stimulant. This alkaloid and other related compounds are neuroprotective agents that reduce lipid oxidation and inhibit tangles in the brain tissues. Further studies has revealed

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that consumption of aqueous leaf extract of *Piper guineense* causes dyslipidemia and elevation of liver enzymes but no significant effect on hematological indices. [8] The leafy vegetable contains varying amount of the proximate compounds, vitamins and phytochemicals [9]. Reports has documented the pharmaceutical evidence of *Piper guineense* on reproduction; which suggested that it is an essential therapeutic input on reproductive health. *Piper guineense* is an important ingredient for pharmaceuticals and could be used to ameliorate reproductive dysfunction. [10]. Researchers have reported that *Piper guineense* leaves are aseptic in nature, with the ability to relieve flatulence. It is also useful for treating intestinal diseases, cough, bronchitis and rheumatism. [11]. Studies have shown that the plant possesses antioxidant, anticonvulsant, anti-inflammatory, and neuropharmacological activities ([12]. All these reports are fascinating and made the study of the plant interesting. It is a known fact that the full constituents of this plant have not been fully elucidated. Our work is focused on determining the mineral, phytochemicals, and antioxidant properties of the leaves of our locally grown *Piper guineense*

2. Material and methods

2.1. Plant materials

The sample, which is the Leaves of *Piper guineense* (uziza) was collected from the farm within Owerri environs in Imo State of Nigeria. They were identified by Prof. Mbagwu of Department of Plant Science and Biotech, School of Agriculture And Agricultural Technology, Federal University of Science and Technology, Owerri. The leaves were washed and air dried at room temperature and pulverized. The powdered sample was stored in air tight container

2.2. Phytochemical screening

The phytochemical analyses of the sample extracts were carried out using A. O. A. C official methods of analysis [13] The presence of tannins, alkaloids, flavonoids, saponins (steroids and terpenoids), phenols, and cardiac glycoside were tested. The procedure were as follow, 5g of the plant sample was soaked in 100 cm^3 of water and another 5 g of the sample was soaked in 20% acetic acid in ethanol and a third percolated in 80% ethanol, they were kept for 4 hours and extracted

2.2.1. Test for tannins

5 cm^3 of the water extract was measured into a 250 cm^3 beaker and a 1% gelatin solution containing sodium chloride was added. Formation of white precipitate indicates the presence of tannins

2.2.2. Test for alkaloids

5 cm^3 of the acetic acid in ethanol extract was treated with Meyers' reagent. Formation of yellow precipitate indicated presence of alkaloids.

2.2.3. Test for flavonoids

5 cm^3 of the water extracts was treated with few drops of sodium hydroxide solution. Formation of intense yellow colour, which becomes colourless on addition of dilute acid, indicates the presence of flavonoids. [14]

2.2.4. Test for saponins

2 cm^3 of distilled water was added to 1ml of the extract and shaken vigorously (in the presence or absence of olive oil). Formation of persistent frothing foaming indicated presence of saponin.

2.2.5. Test for steroids

5 cm^3 of the water extract was treated with chloroform and filtered. The filtrates were treated with few drops of acetic anhydride, boiled and cooled. Conc. Sulphuric acid was added. Formation of brown ring at the junction indicates the presence of sterols [14]

2.2.6. Test for Glycosides

5 cm^3 of the water extract was treated with equal amount of Fehling solutions of A and B and boiled. The appearance of a reddish brown precipitate indicates the presence of cardiac glycoside.

2.2.7. Test for phenols - ferric chloride test

5 $cm^3$of water extract was treated with 3 drops of $FeCl_3$. Formation of dark-brown precipitate indicated the presence of phenols.

2.3. Alkaloid determination

20g of the plant sample was weighed into a beaker and soaked with 400 cm^3 of 20% acetic acid in ethanol and covered to stand for six hours. It was filtered using watman no1 filter paper. The filtrates were concentrated using a water bath to one quarter of the original volume. The alkaloid in the extract was precipitated out using concentrated ammonium hydroxide which was added drop by drop until precipitation was completed. The solution was allowed to settle and cool. The precipitate was filtered using whatman filter paper, dried and weighed. [15]

2.4. Saponin determination

20g of the plant sample was weighed into a beaker and socked with 400 cm^3 of 20% ethanol and stirred using a glass rod. The mixture was allowed to stand for four hours and filtered using watman no 1 filter paper. This procedure was repeated thrice and the combined filtrate was heated over water bath to concentrate it with continuous stirring while the temperature was maintained at 55 °C. The mixture was extracted with 200 cm^3 of 98% diethyl ether and separated with separating funnel. The aqueous layer was collected while the ether layer was discarded. The procedure was repeated thrice and the combine aqueous phase was concentrated. It was then treated with 60 cm^3 of n-butanol and washed with 10 cm^3 of 5% sodium chloride. The solution was heated to dryness and weighed. The saponin content was calculated in percentages. [15]

2.5. Flavonoid determination

20 g of plant sample was weighed into a beaker and soaked with 400 cm^3 of 80% methanol with continuous stirring. The mixture was allowed to stand for four hours and filtered with whatman filter paper. The filtrates were heated over water bath to dryness and weighed [16]

2.6. Tannin determination

20 g of plant sample was weighed into a beaker and soaked with 400 cm^3 of distilled water. The mixture was stirred vigorously with a glass rod and allowed to stand for four hour. The mixture was filtered into a volumetric flask and heated over a water bath and concentrated to one quarter the original volume. The concentrated solution was allowed to cool and acidified with aqueous drops of hydrochloric acid and treated with ethyl acetate. The mixture was separated in a separating funnel. The procedures was repeated thrice, the aqueous layer were collected and heated to dryness and weighed. [17]

2.7. Mineral determination by ashing method

. The macro elements such as calcium, sodium, potassium, phosphorus and magnesium were determined according to the method described by previous. Researchers. [18]. 5.0 g of dried plant sample was weighed into a petri-dish. The sample was ashed for six hours at a temperature of 500 ^oC in a muffle furnace. The ash was cooled and extracted with 2-3 cm^3 of hydrochloric acid (SG 1-4) and evaporated to dryness. The residue was re-extracted with 20 cm^3 of 25% V/V HCl and transferred into a volumetric flask and diluted to the mark with water. The elementary determinations were achieved by direct aspiration of the ash solution into the flame of the AAS (Atomic Absorption Spectrophotometer) by the spray chamber through the capillary tube and nebulizer. Each metal was determined by using the metal's hollow cathode lamp. The readings were taken in triplicate and the mean value was obtained.

2.8. Preparation of samples for GC/MS analysis

Two hundred grams of sample was soaked in 400 cm^3 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.

2.8.1. System calibration OF AGILENT 7890A GC-MS

Prior to calibration, tables are selected from the sequence menu, sequence table is created by filling the sample name, sample type, the external standard mode for calibration is clicked for sequence parameters through the edit /sequence parameter. The sample is acquired by injecting 3 cm^3 of PAH standards of 0. 1, 0. 5. 1. 0, 5. 0, 10 mg/cm^3 in CH_2Cl_2 for a five level calibration. The GCMS automatically integrates the chromatograms of each component of the standards and label each peak with the corresponding name given by the manufacturer. Subsequently other levels of calibration were

added through the calibration /add level menu. The concentration of each calibration level is added in the calibration table or through the add level dialog box. The correlation factor is checked with the minimum set value at 0. 99. Finally the calibration is saved through the path file /save method

2.8.2. Analysis of sample extract

3 cm^3 dichloromethane is injected and used as a blank, the 3 cm^3 of extract after concentration into the injection port. If the peaks generated are above scale, the extract diluted with dichloromethane and re-analyzed. Surrogate quality control is run after every 20 samples of every batch , recovery should be between 80-120% ,if not, the system is checked

2.9. Data analysis

Integration is done automatically for all PAH components

2.10. Antioxidant evaluation of the plant

The antioxidant activity of the extract of the plant was carried out using the ferric reducing antioxidant power (FRAP) and 1, 1-Diphenyl-2-picrylhydrazl (DPPH) assay methods.

2.11. 2, 2-diphenyl-1-picrylhydrazl hydrate (DPPH)

2.11.1. DPPH spectrophotometric assay

The scavenging ability of the natural antioxidants of the leaves towards the stable free radicals was determined as follows; The leaf extract (40 cm^3) was added to 1 cm^3 of 0. 1 mM methanolic solution of DPPH and 0. 9 6cm^3 of methanol. The mixture was allowed to react at room temperature for 30 minutes. Methanol served as the blank and DPPH in methanol without the leaf sample served as the positive control, while butylated hydroxytoluene (BHT) served as reference. After 30 minutes of incubation, the discolouration of the purple colour was measured at 518nm in a spectrophotometer (Genesys 10-S, USA). The radical scavenging activity was calculated.

2.11.2. Ferric reducing antioxidant property

The principle focus of this assay was the quantification of ferric degradation product, by its condensation with the extract. The reducing property of the extracts was determined by standard method. 0. 50 cm^3 of the extract was mixed with 0. 50 cm^3 of 200 mM sodium phosphate buffer, pH 6. 6 and 0. 50 cm^3 of 1% potassium Ferro cyanide. The mixture was incubated at 50 ^oC for 20 minutes, thereafter 0. 50 cm^3 of 10% trichloroacetic acid was added and centrifuge at 2000 rpm for 10 minutes, 1 cm^3 of the supernatant was mixed with 1 cm^3 of distilled water and 0. 2 cm^3 of ferric chloride and absorbance was measured at 700nm.

3. Results and Discussion

Table 1 Result of phytochemical screening of the crude extracts

S/N	Phytochemicals	Inference
1	Saponin	+
2	Flavoniod	+
3	Alkaloid	+
4	Tannins	+
5	Cardiac Glycosides	+
6	Steroids	+
7	Phenols	+

Key; + = present - = negative

The phytochemical screening of the leaf extract revealed the presence of tannins. saponins, alkaloids, flavonoids, cardiac glycosides, steroids and phenols

Table 2 Result of phytochemical quantification

Phtochemicals	Quantity in Mg/100g
Tannins	12. 50
Flavonoid	36. 90
Saponins	30. 60
Alkaloid	0. 30

Alkaloids are vast and vary a lot in their activity when ingested by man and livestock. Some alkaloids are useful and important in medicine and constitute most of the valuable drugs currently used by humans. Their amount in the sample is 0. 30 mg/100 g, . tables (1, 2). They are among the most efficient therapeutically significant plant substances. Pure isolated alkaloids and their synthetic derivatives are used by Etinomedicinal practitioners for their analgesic, antispasmodic and bactericidal effects [19]. They exhibit marked physiological activity when administered to animals; the high alkaloid content of these samples may be the reason for their use in the treatment of cough, wounds, and rheumatism and skin infections. Saponins was found to be available at 30.60 mg/100 g in the leaf of *the plant* tables (1 and 2). Some of the general characteristic of saponins includes; hemolytic activity and cholesterol binding properties. They boast several health benefits. They control immunological responses, protecting the body from bacteria, viruses, and fungi, having antibacterial, antiinflammatory, and immune-boosting properties. They are oxidative stress scavengers and antioxidants. Their hypoglycemic properties help to minimize insulin surges and maintain normal blood sugar levels. Saponins can reduce body fat and cholesterol, which helps to improve cardiovascular health, and are have molluscicidal, anthelmintic, insecticidal, analgesic, antiviral, anticancer, sedative, and anti-tumor activities. [20]

The flavonoid content of was found to be 30.90 mg/100 g as shown in Table (1, and 2). Flavonoids 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. . They possess free radical scavenging activities, having multiple biological activities including – vasodilatory, anti-carcinogenic, anti-allergic, antiviral, estrogenic effects as well as being inhibitors of phospholpase H-2, cycloxygenase, and glutathione reductase and xanthine oxidase. [21]. They possess anti-inflammatory and analgesic effect as well as anti-cancer properties. [22]

Tannins are a type of polyphenol that has several therapeutic and medical uses. 12. 5 mg/100 g was detected in the plant, tables 1 and 2. Their pharmacological qualities includes; the ability to heal wounds, prevent dysentery, fight bacteria, and reduce inflammation, been anti-toxic, anti-cancerous, antiallergic, and anti-inflammatory. [23]. Cardiac glycosides was detected in the extract, they are compounds that lower heart rate and increase heart muscle contraction force without increasing oxygen consumption. They are useful in the treatment of congestive heart failure and cardiac arrhythmia. . They do this by activating the heart muscle, which enhances cardiac output and efficiency. They also aid in the deceleration of irregular heartbeats. Apart from their beneficial effects on heart health, cardiac glycosides also exhibit anticancer capabilities. There is a growing interest in polyphenolic compounds as therapeutic agents against many diseases such as cardiac and cerebral ischemic, arteriosclerosis and rheumatic or pulmonary diseases. [24], [25] 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. Many polyphenolics are known to exhibit antioxidant properties; they are free radical's scavengers. Phenolic flavonoids are also excellent hydroxyl scavengers. These properties promote health, and prevent certain chronic disorders such as cancer, cardiovascular diseases, diabetics and arthritis. The presence of phenols means that the extract could act as antiinflammatory, anticlothing, antioxidants, immune enhancers and hormone modulators. Phenols have been the subject of extensive research as disease preventive agents. [24] [26]. The have the ability to block specific enzymes that causes inflammations. They modify the prostaglandin pathways and thereby protect platelets from clumping.

Table 3 Result of mineral element determination

Mineral elements	***Piper guineense*** **LAB ID: 202001529**
Nitrogen (N)	3. 88%
Phosphorous (P)	0. 35%
Calcium (Ca)	1. 07%

Magnesium (Mg)	0. 46%
Potassium (K)	2. 60%
Sodium (Na)	82. 52 ppm
Manganese (Mn)	300. 74 ppm
Iron (Fe)	315. 99 ppm
Zinc (Zn)	25. 20 ppm
Copper (Cu)	7. 73 ppm

Results obtained from the mineral determination revealed the presence of important minerals such as Nitrogen, Phosphorus, Calcium, Magnesium, Potassium, Sodium, Manganese, Iron, Zinc, and Copper, table(3). These minerals are vital to the body and are needed for its proper functions and development. Iron, magnesium and calcium contents of black pepper have been reported to be high *[3]*. Results also showed that the leaves were rich in calcium, magnesium and iron.

3.1. Result of GC/MC spectrum of n-haxane extract of *Piper guineense* leaf

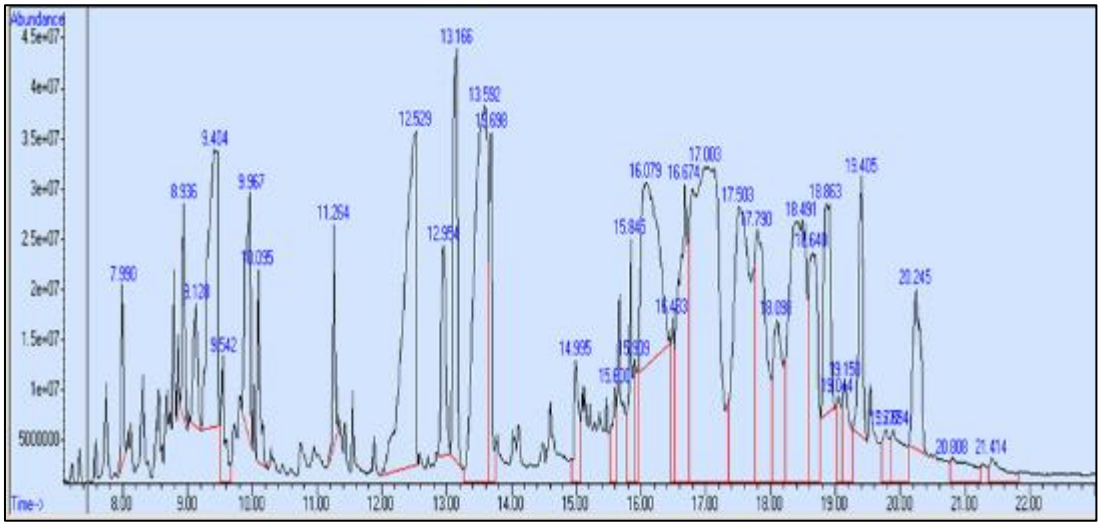


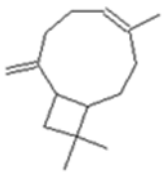
Figure 1 GCMS spectrum of sample

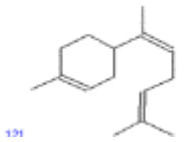
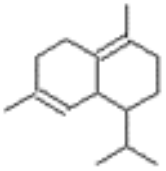
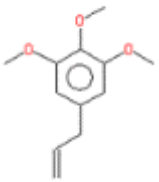
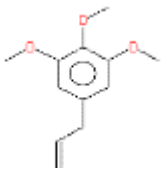
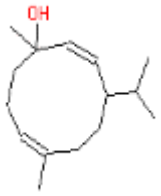
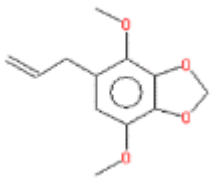
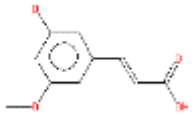
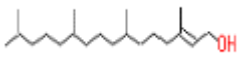
The GC/MS spectrum of the n-haxane extract of *Piper guineense* showed 34 major peaks, their intensity and elution time of bioactive compounds present in the extract. These compounds according to studies have medicinal values.

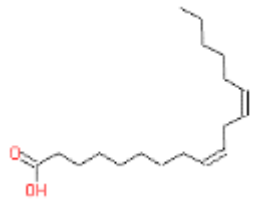
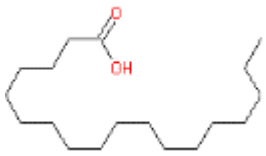
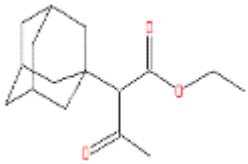
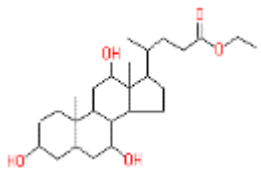
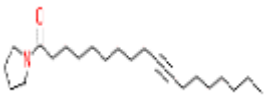
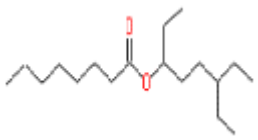
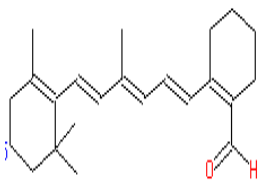
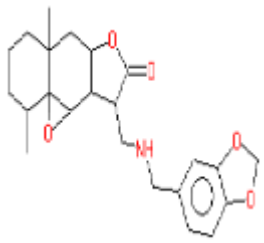
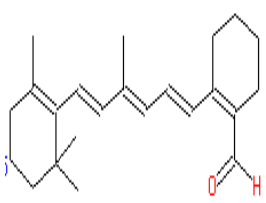
3.2. Compounds present in GC/MS analysis of n-haxane extracts of *Piper guineense*

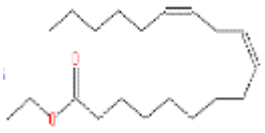
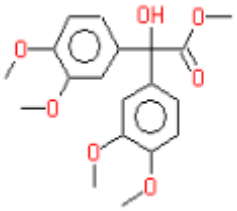
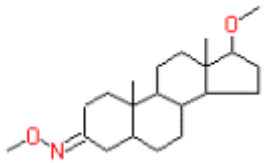
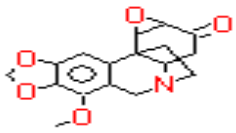
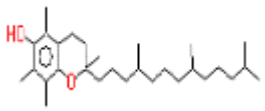
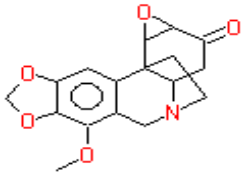
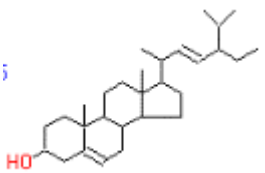
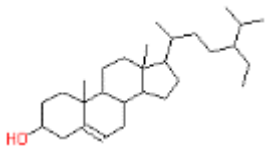
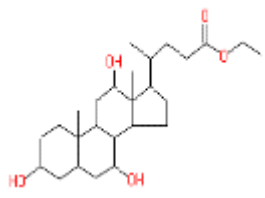
The major peaks were analyzed, the name of the compound, their molecular weight, molecular formula and retention time are structure

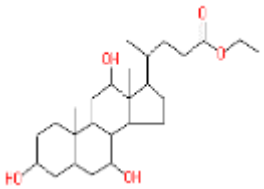
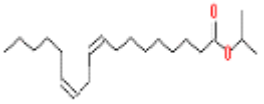
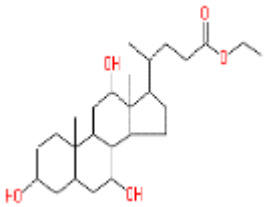
Table 4 Compounds obtained from GC-MS analysis of sample

S/N	Name of Compounds	Molecular weight (mw)	Molecular formula	Retention time RT(min)	Structure of the compounds
1	Cayophylene	204	$C_{15}H_{24}$	7. 990	

2	Cis-α-Bisebolene	204	$C_{15}H_{24}$	8. 309	
3	Naphthalene, 1, 2, 3, 5, 6, 8α-hexahydro-4, 7dimethyl-1-[1 methylethyl], (1Scis)	204	$C_{15}H_{24}$	8. 936	
4	Benzene, 1, 2, 3-trimethoxy-5-[2-propenyl]	208	$C_{12}H_{16}O_3$	9. 128	
5	Benzene, 1, 2, 3 trimethoxy-5- [2-propenyl]	208	$C_{12}H_{12}O_3$	9. 404	
6	1-Hydroxy-1, 7-dimethyl-4-isopropyl-2-7-dimethyl-4-isopropyl-2-7-cyclodecadiene	222	$C_{15}H_{26}O$	9. 542	
7	Apiol	222	$C_{12}H_{14}O_4$	9. 967	
8	3, 5-Dimethoxy cinnamic acid	208	$C_{11}H_{12}O_4$	10. 095	
9	3, 7, 11, 15-Tetramethyl-2-hexadecen-1-ol	296	$C_{20}H_{40}O$	11. 264	
10	n-Hexadecanoic acid	256	$C_{16}H_{32}O_2$	12. 529	
11	1-Eicosanol	298	$C_{20}H_{42}O$	12. 954	
12	Phytol	296	$C_{20}H_{40}O$	13. 166	

13	9, 12, Octadecadienoic acid	280	$C_{18}H_{32}O_2$	13. 592	
14	Octadecadienoic acid	284	$C_{18}H_{36}O_2$	13. 698	
15	Butanoic acid, 2, {1-adamantyl} -3-oxo, ethyl ester	264	$C_{16}H_{24}O_3$	14. 995	
16	Ethyl Iso-allocholate	436	$C_{26}H_{44}O_5$	15. 600	
17	Pyroidine-1-{1-oxo 10-octadecynyl}	333	$C_{22}H_{39}NO$	15. 664	
18	Octanoic acid, 6-ethyl-3-octyl ester	284	$C_{18}H_{36}O_2$	15. 845	
19	2-4-methy-6-(2, 6, 6-trimethylcyclohex-1-enylhexa-1, 3, 5) trienylcyclohex-1-en-1 carboxaldehyde	324	$C_{23}H_{32}O$	15. 909	
20	2H-Benzo[F]owireno[2, 3-E]benzofuran-B {9H}one, 9{1, 3benzodiowol-5-ylmethylamino}melhylocha	399	$C_{23}H_{29}NO_5$	16. 079	
21	2-4-methyl-6-{2, 6, 6-bimethylcyclohex-1-enyihewa-1, 3, 5-trienyl}cyclohex-1-en-1-carboxaldehyole	324	$C_{23}H_{32}O$	16. 484	

22	Linoleic acid methyl ester	308	$C_{20}H_{36}O_2$	16. 674	
23	Bis-(3, 4-dimethoxyphenyl)-hydroxyacetic acid methyl ester	362	$C_{19}H_{22}O_7$	17. 003	
24	Androstane-3-one, 17-melhoxy-3-methoxine, {5α, 17β}	333	$C_{21}H_{35}NO_2$	17. 503	
25	Cinamidine, 3-oxo	315	$C_{17}H_{17}NO_5$	17. 790	
26	Vitamin E	430	$C_{29}H_{50}O_2$	18. 098	
27	Cinamidine, 3-oxo	315	$C_{17}H_{17}NO_5$	18. 491	
28	Stigmasterol	412	$C_{29}H_{48}O$	18. 640	
29	β Sitosterol	414	$C_{29}H_{50}O$	18. 863	
30	EthylIso-allocholate	436	$C_{26}H_{44}O_5$	19. 044	

31	EthylIso-allocholate	436	C_{26} H_{44} O_5	19. 150	
32	1-Heptahiacotanol	536	C_{37} H_{76} O	19. 405	
33	1-Propyl9, 12, octadecanadienoate	322	C_{21} H_{38} O_2	20. 245	
34	Ethyl Iso-allocholate	436	C_{26} H_{44} O_5	21. 414	

3.3. Antioxidant evaluation

Antioxidant evaluation on piper guineense was analyzed using Ferric reducing antioxidant power (FRAP) and 1, 1-diphyl-2-picrylhydrazl (DPPH) assay methods in order to determine the scavenging ability of the natural antioxidants of the leaves towards the stable free radicals (DPPH) and quantify the ferric degradation product by its condensation with the extracts. Efficient concentration (EC_{50}) in mg/ml is the amount of antioxidant necessary to decrease the initial DPPH radical concentration by 50%.

Table 5 Ferric reducing antioxidant power (FRAP)

Concentration (mg/ml)	Absorbance
0	0
2	0. 114
4	0. 256
6	0. 322
8	0. 433
10	0. 522

The result of antioxidant activity showed that increase in concentration (mg/ml) resulted to increase in the absorbance.

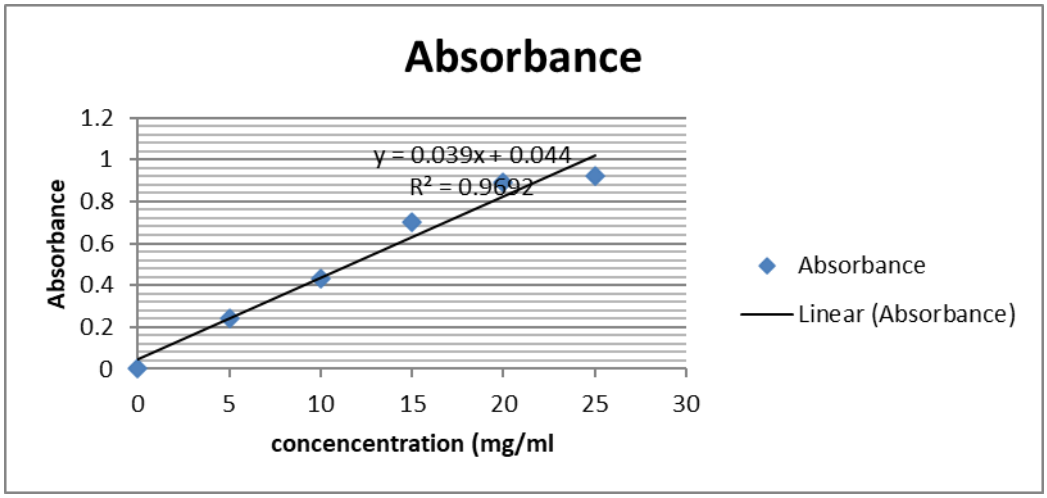


Figure 2 FRAP calibration curve

The curve showed strong antioxidant activities after 20 minutes of incubation at 50 °C and centrifuged at 200 rpm with 0. 25 ml of 10% trichloroacetic acid for 10 min, then the supernatant were mixed with water and ferric chloride, the absorbance were measured at 700 nm fig 2. The plant extract showed good antioxidant properties which increased as the concentration of the plant extract increased. An excellent antioxidant property is a measure of the scavenging power of the extract against free radicals. Correlating the increase in concentration of the extract with percentage inhibition of free radicles table(5), as the concentration doubles the inhibition of free radicles is double for the extract .The highest value been 79.436%, these values compared well with those of gallic acid, 99.611% being the highest inhibition for gallic acid with similar concentration, the higher the reducing power, the greater the antioxidant activity. Quantification of antioxidant activity of the extract showed a dose dependent antioxidant activity.

Table 6 FRAP analysis of the leaf extract of *Piper guineense*

Concentration of extract used	Absorbance	Concentration of samples mg/dL	% inhibition
PIPER-GUINEENSE			
0mg/ml	0. 326	7. 231	-0. 001
10mg/ ml	0. 128	2. 154	70. 212
20mg/ml	0. 090	1. 179	83. 695
40mg/ml	0. 071	0. 692	90. 430
80mg/ml	0. 068	0. 615	91. 495
GALLIC ACID			
10mg/ml	0. 109	1. 667	76. 946
20mg/ml	0. 062	0. 462	93. 611
40mg/ml	0. 049	0. 128	98. 230
80mg/ml	0. 045	0. 026	99. 640

Quantification of antioxidant activity of the extracts showed a dose dependent antioxidant activity. Table 7 revealed that increase in concentration result to increasee in percentage inhibition.

2, 2-diphenyl-1-picrylhydrazl hydrate **(DPPH)**

The scavenging activities of the sample were calculated As follows;

Scavenging Activity %

$$\frac{100 - \text{A518 (sample)} - \text{A518 (blank)}}{\text{A518 (blank positive control)}} \text{x } 100$$

Table 7 Free radical scavenging activities of the leaf extract of *Piper guineense*

Concentration of extract	Absorbance	% scavenging activity
PIPER-GUINEENSE (SAMPLE A)		
5mg/ml	0. 322	85. 730
10mg/ml	0. 298	87. 047
50mg/ml	0. 411	80. 845
100mg/ml	0. 348	84. 303
Blank (methanol)	0. 062	
Blank (positive control DPPH In methanol	1. 822	
REFERENCE BUTYLATED HYDROXYTOLUENE (BHT)		
10mg/ ml	0. 118	96. 926
20mg/ml	0. 103	97. 750
40mg/ml	0. 083	98. 847
80mg/ml	0. 092	98. 353
Blank (methanol)	0. 062	
Blank (positive control DPPH In methanol	1. 822	

The result of antioxidant activity obtained from the extracts of *Piper guineense* showed strong antioxidant activities after thirty (30) minutes of incubation with 50% efficient concentration values. The antioxidant activity was established on the free radical scavenging of DPPH. Also increase in concentration increases the percentage radical scavenging activity. Free radical scavengers (antioxidants) have potentials to prevent decay or ameliorate many of human chronic and ageing problems. ([27] Such diseases includes cancer, diabetes, heart disease, stroke, malaria and rheumatoid arthritis[28].

Similarly, the radical scavenging property of the extract compared favourably with that of DPPH, the highest value for the plant extract been 67.322% inhibtiion which is within the same range 93. 353% for DPPH. In most cases, The DPPH radical scavenging ability can be a measure of the antioxidant activities of most plants. The implication is that the plant extract is a good free radical scavenger. Therefore, the incorporation of *Piper guineense* in meals would enhance the nutritional value especially for the aged and people suffering from oxidative- stress related diseases including cardiovascular diseases. . The result showed that the leaves of this plant exhibited free radical scavenging effects. This could be attributed to the presence of phenolic compounds in the plant which is a major group of compounds that act as primary antioxidants or free radical scavengers [29].

4. Conclusion

The analysis of the sample has shown that the leaf of Piper guinenses is rich in phytochemical and minerals. The antioxidant activities also indicate that the leaf of the plant is readily natural antioxidant and a free radical scavenger. . The results justifies the inclusion of the Piper guinenses leaf in meals for aged persons who might be suffering from oxidative stress which leads to cancers and tumours, . It also justifies the consumption of the leaf of this plants by nursing mothers, as the array of minerals and phytochemicals could help prevent certain early growth sicknesses and act as a rich mineral supplement for the babies

Compliance with ethical standards

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Disclosure of conflict of interest

The authors declare that there is no conflict of interest

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