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Phytochemical Studies and GC-MS Analysis of *Gongronema Latifolium* and *Piper Guineense*

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Abstract:
Aims: In this study, the proximate, phytochemical composition and GC-MS analysis of the leave extracts of Gongronema latifolium, and Piper guineense were carried out.
Methodology: The leaves were collected, washed, shade dried and powdered. Methanol extract were prepared by simple maceration process and soxhalation method. All the extracts were concentrated and analyzed using Gas Chromatography Mass Spectroscopy for the identification of biochemical components present in the leaves of Gongronema latifolium, and Piper guineense, tests of statistical significance was carried out-using one-way ANOVA.
Results: The results showed that the plants are rich in alkaloids, flavonoids, cardiac glycosides, tannins,phenolic compound and saponins. The proximate analysis showed the following percentage values: moisture 80±0.35%,62±0.42% , Ash 14.96±0.5%,12.83±0.12% , Crude Fibre 7.68±0.1g,1.616±0.34g , Crude protein8.40±0.61 %,8.57±0.22 %, Crude lipid 60.23±0.44%, 4.13±0.33%, and Carbohydrate 36±0.26%, 51.02±0.67 % for G.latifolium and Piper guineense respectively. In the GC-MS results also revealed that thirty five bioactive compounds, were identified in both plants:18 in Gongronema latifolium, and 17 in Piper guineense comprising a range of fatty acids, and heterocyclic compounds. The six most abundant compounds in G.latifolium are Ooleic acid,3-beta acetate lup-20(29)-en-3-ol,14-methyl 8-hexadecenal,acetate-19-cyclolanost-24-en-3-ol,oleic acid chloride,and 3-alpha-12-oleanen-3-ylacetate with percentage values of 14.1,11,11.00,8.5 and 5.1 respectively while in Piper guineense they are 9-octahydro-2,5a-dimethyl-2H-benzo(F) oxireno (2,3-E) benzofuran 8 (H)-one,1,2-dipiperonyl-3-imino-1,2,4-triazine,Friedoolean 6-ene,1-piperonyl-3-amino-1,2,4-riazine and methyl ester 9-octadecenoic acid with percentage composition of 36.645,21.9,11.7,5.98 and 4.48 respectively. These compounds which are known for their anti- fungal, anti- inflammatory, antibiotic, and skin conditioning properties.
Conclusion: The present finding suggests that these plants have a promising potential phytopharmaceutical value.

Keywords*: GC-MS,Gongronema latifolium,Piper guineense, Phytochemical compounds, medicinal plants,n-hexadecanoic acid, oleic acid*

1. Introduction

Medicinal plants have had a crucial role in human culture and civilization. For millennia, people around the world have healed the sick with herbal derived remedies, and handed down through generations [1]. Traditional medicine is the sum total of knowledge, skills and practices based on the theories, beliefs and experiences indigenous to different cultures that are used to maintain health, as well as to prevent, diagnose, improve or treat physical and mental illness [2]. Various types of traditional medicine and other medical practices referred to as complementary or alternative medicine are increasingly used in both developing and developed countries. Potentially valuable treasures in medicinal plants remain unexplored. *G.latifolium* and *Piper guineense* are among such plants [3]. Gas Chromatography mass spectroscopy, a hyphenated system which is a very compatible technique and the most commonly used technique for the identification and quantification purpose. The unknown organic compounds in a complex mixture can be determined by interpretation and by matching the spectra with reference spectra [4].

Gongronema latifolium is an herbaceous nonwoody plant from the family of Asclepiadaceae. It has milky or clear latex, widespread in the tropical and subtropical regions especially in Africa and South America, with a moderate representation in Northern and South Eastern Asia [5]. In South Eastern and South Western Nigeria, *Gongronema latifolium* (whose leaves are bitter) is commonly called

"utazi" and "arokeke", respectively, and is primarily used as spice and vegetables [6]. Earlier reports on extract from this plant have focused mainly on their medicinal properties [7]. Reports have shown it use as a bittering agent in brewing to produce the characteristic flavour, foam stability, and preservative properties in beer [8].

Piper guineense, popularly known as African black pepper or hot leave is widely consumed in some part of West Africa especially Nigeria and Ghana on account of its nutritional and medicinal properties [9]. It belongs to the family Piperaceae or Sapotaceae [10]. In traditional herbal medicine, the seeds are put into a variety of uses, for instance, in some parts of Nigeria, the seeds are consumed by women after child birth,[11] to enhance uterine contraction for the expulsion of placenta and other remains from the womb [12], as an adjuvant in the treatment of rheumatic pains and as an antiasthmatics [13] and also for the control of weight [14]. The seed and leaf extracts are capable of exhibiting a depolarizing neuromuscular activity in a concentration related manners [15]. The antiparasitic, antimicrobial and antifungal activities of the leaf and seeds of *P. guineense* have also been reported [15].The leaf is also used by traditional medical practitioners for the treatment of respiratory diseases [16] and correction of female infertility problems, and the seeds as an aphrodisiac [17].Though these plants are widely used for various therapeutic purpose locally, however, not much is known about their chemical composition. For instance there is no published report on the GC-MS analysis of the plants.

Gas chromatography / mass spectrometry (GC-MS) has become firmly established as a key technological platform for secondary metabolite profiling in both plant and non-plant species [18]. So, the present study was aimed to investigate the possible chemical components of *G. latifolium* and *Piper guineense* using GC-MS technique.

2. Materials and Methods

2.1. Preparation of extracts

The leaves of *Gongronema latifolium* and *Piper guineense* plants were purchased from Abakpa Market in Abakaliki and brought to Department of Biotechnology Laboratory Ebonyi State University, Nigeria washed and dried under room temperature (25^oC to 27^oC) for two weeks. After which the leaves were pulverized into coarse form with a milling machine and passed through a 2mm – mesh sieve and the extract was weighed and stored at room temperature.

2.2. Extraction of plant material

Twenty grams (20g) of each of *G latifolium* and *Piper guineense* powder *w*as macerated in 1liter of absolute ethanol, and left to stand for 48 hours; after which the extractive was filtered out with the help of a cotton wool and a white filter cloth. The resulting ethanol extract was concentrated and evaporated to dryness using a rotary evaporator at an optimum temperature of between 40^oC and 45^oC to avoid denaturation of the active ingredients.

2.3. Qualitative Phytochemical Screening of extracts.

The freshly prepared crude extract was qualitatively tested for the presence of biochemical constituents were carried out using the method of [19]

2.4. Quantitative phytochemical analysis of extract

Quantitative determination of phytochemicals composition of *G latifolium* were carried out using the method of [19]

2.5. Quantitative Proximate analysis of P.guineense and G.latifolium

Quantitative Proximate composition of *P.*guineense and G.*latifolium* were carried out using the method of A.O.A.C. (2005).

3. Gas chromatographic and mass spectral analysis of *G. latifolium* and *P. guineense* extracts

GC –MS analysis 2µl of the *G. latifolium* and *P. guineense* extracts was employed for GC-MS for analysis of different compounds. Instruments and chromatographic conditions GC-MS analysis was carried out on a GC clarus 500 Perkin Elmer system comprising a AOC-20i auto sampler and gas chromatograph interfaced to a mass spectrometer (GC-MS) instrument employing the following conditions: columnElite-1 fused silica capillary column (30 ×0.25 mm × ID x 1 µm of capillary column, composed of 100% Dimethyl poly siloxane), operating in electron impact mode at 70 eV; helium (99.999%) was used as carrier gas at a constant flow of 1ml/min and an injection volume of 0.5 EI was employed (split ratio of 10:1) inject or temperature 250°C; ion-source temperature280°C. The oven temperature was programmed from 110°C (isothermal for 2min), with an increase of 10°C/min, to200°C/min, then 5°C/min to 280°C/min, ending with a 9 min isothermal at 280°C.Mass spectra were taken at 70 eV; a scan interval of 0.5 s and fragments from 45 to 450Da. Identification of phytocompounds and interpretation on mass spectrum GC-MS was conducted using the database of National Research Institute Technology (NARICT) Zaria Nigeria having more than 62,000 patterns. The spectrum of the unknown component was compared with the spectrum of the known components using computer searches on a NARICT Ver.2.1 MS data library. The name, molecular weight, retention time and structure of the components of the test materials were ascertained and results shown below.

4. Statistical Analysis

The results were expressed as mean ± SD and tests of statistical significance were carried out-using one-way ANOVA. The statistical package used was Statistical Package for Social Sciences (SPSS for windows, version 20).Data were expressed as mean± standard error of mean (SEM). Values of $p<0.05$ were considered significant.

5. Results

TEST	*G. latifolium* (utazi)	*P.guineense* (uziza)
Polyphenol	++	+++
Iodine	-	-
Biurate	++	++
Ninhydrin	-	-
Xanthoprotic	-	-
Alkaloid	++	+
Glycoside	++	+
Terpenoid	-	-
Steroid	-	-
Flavonoid	+	+++
Tanins	++	+
Saponins	++	+++
Oil	+	+

Table 1: Qualitative Phytochemical Screening of G.Latifolium and P.Guineense

- +++=Relative high Abundance of compound
- ++= Relative Abundance of compound
- += Relative low presence of compound
- = Not detected

Sample	Alkaloids (%)	Flavonoids (%)	Polyphenol (%)	Cardiac glycosides (%)	Tannins (%)	Saponins (%)
G.latifolium	3.79±0.05	1.01±0.02	37.03±0.00	39.56±0.04	5.04±0.5	2.14±0.03
P.guineense	1.67±0.35	2.93±0.05	39.10±0.06	35.33±1.5	0.64±0.	0.67±0.01

Table 2: Quantitative Phytochemical determination

Results are expressed as mean of three determinations ± SEM.

S/N	Parameters	*P.guineense*	*.G.latifolium*
1.	Moisture Content	80±0.35%	62±0.42%
2.	Ash content	14.96±0.5%,	12.83±0.12%
3.	Crude Fibre content	7.68±0.1%	1.616±0.34%
4.	Protein content	8.40±0.61 %,	8.57±0.22 %
5.	Lipid content Carbohydrate	60.23±0.44%, 36±0.26%,	41.3±0.33%, 51.02±0.67 %

Table 3: Quantitative Proximate analysis of P.guineense and G.latifolium

Data were expressed in mean and standard error (x±S.E) in triplical

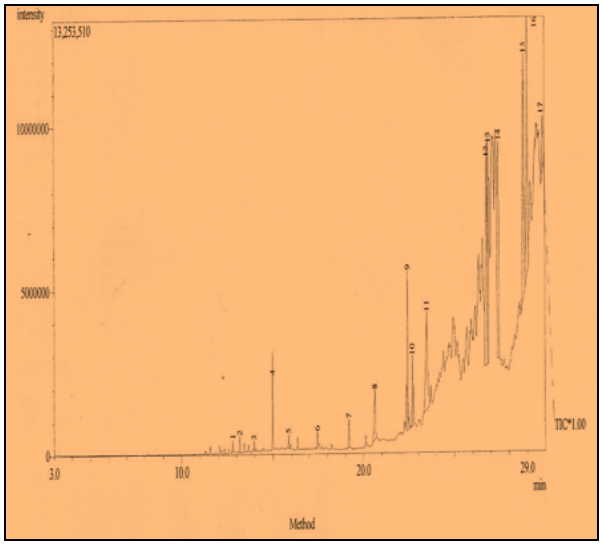
Peak	Compound	Molecular formular	Molecular weight	Retention	% Content
1	5-ethyl-4-methyl—3-heptanone	$C_{10}H_{20}O_2$	156	14.43	0.3
2	Methyl ester-tetradecanoic acid	$C_{15}H_{30}O_2$	242	15.85	0.1
3	Methyl ester hexadecanoic acid	$C_{17}H_{34}O_2$	256	19.183	0.7
4	Hexadecanoic acid	$C_{16}H_{32}O_2$	256	20.61	2,6
5	O,O-diethyl, O-(3,5,6-trichloro-2-pyridine ester-phosphorothioic acid	$C_{13}H_{11}Cl_{13}NO_3PS$	349	21.16	0.9
6	Ester-9-octadecanoic acid	$C_{19}H_{36}O_2$	296	22.41	13
7	3,7,11,15-tetramethyl-phytol-2-hexadecen-l-ol	$C_{20}H_{40}O$	296	22.66	1.8
8	Oleic acid	$C_{18}H_{34}O_2$	282	23.48	14.4
9	Octadecanoic acid	$C_{18}H_{36}O_2$	284	23.69	1.1
10	Chloromethyl 5-chlorodecanoate	$C_{11}H_{20}Cl_2O_2$	254	24.39	2.9
11	I-(hydroxymethyl)-1,2-ethanediylsss) ester hexadecanoic acid	$C_{35}H_{68}O_5$	568	25.00	3.0
12	3-Beta acetate lup-20 (29)-en-3-ol	$C_{32}H_{52}O_2$	468	26.15	14.1
13	14-methyl 8-hexadecenl	$C_{17}H_{32}O$	252	26.84	11
14	Acetate-19-cyclolanost-24-en-3-ol	$C_{32}H_{52}O_2$	468	27.58	11
15	4,4,6a,6b,8a,11,11,14b-octamethyl-1,4,4a,5,6,6a,6b,7,8,8a,9,10,11,12,12a,14,14a,14b-octadecahydro-2 H-picen-3-one	$C_{30}H_{48}O$	424	27.78	4.8
16	Friedoolan-6-ene	$C_{30}H_{50}O$	410	28.00	5.0
17	3-alpha-12-oleanen-3-ylacetate	$C_{32}H_{52}O_2$	468	28.80	5.1
18	Oleic acid chloride	$C_{18}H_{33}ClO$	300	29.16	8.5

Table 4: GC-MS analysis and mass spectral data of G.latifolium fractions from the leaves of G.latifolium showing molecular formular, molecular weight, percentage content, retention time and base peak

Peak	Compound	Molecular formular	Molecular weight	Retention time	Percentage content	Base peak
1	1, 2, 3, 4, 5, 6, 7, 8- octahydro-1,4-dimethyl-7-azulene	$C_{15}H_{24}$	204	12.79	0,11	
2	1-methyl-4- (5-methyl-1-methylene-4-hexenyl-cyclohexene	$C_{15}H_{24}$	204	13.18	0.14	
3	3, 7, 11-trimethyl-1, 6, 10-do decatrien-3-0l	$C_{15}H_{26}O$	222	13.96	0.10	
4	1, 3-benzodioxole, 4, 7- dimethoxy-5-(2-propenyl)-apiol	$C_{12}H_{14}O_4$	222	14.99	1.41	
5	Octahydro-4-methyl-8-methylene-7- (1-mthylene) – 1, 4- methano- 1H-indene	$C_{15}H_{24}$	204	15.87	0.13	
6	Z Z -2, 15-octadecedien-1-ol acetate	$C_{20}H_{36}O_2$	308	17.46	0.26	
7	Ester hexadecanoil acid	$C_{17}H_{34}O_2$	270	19.20	0.53	
8	Hexadecanoic acid	$C_{16}H_{32}O_2$	256	20.63	1.45	
9	Methyl ester 9-octadecenoic acid	$C_{17}H_{36}O_2$	296	22.43	4.48	
10	2-hexadecen-1-ol3, 7, 11, 15-tetramethyl phytol	$C_{20}H_{40}O$	22.69	1.49	1.5	

11	Oleic acid	$C_{18}H_{34}O_2$	282	23.48	5.04	
12	9 (1,2-benzodioxol-5-ylmethyl) amino) methyl) octahydro-2,5a-dimethyl-2H-benzo(F) Oxireno (2, 3 –E) Benzofuran 8 (H)-one	$C_{23}H_{29}NO_5$	399	26.78	36.64.5	
13	13-octadecenal	$C_{18}H_{34}0$	266	26.86	4.49	
14	Lup-20(29)-en-3-ol	$C_{32}H_{52}O_2$	468	27.42	4.22	
15	1,2-dipiperonyl-3-imino-1,2, 4-triazine	$C_{18}H_{16}N_4O_4$	352	28.83	21.9	
16	1-piperonyl-3-amino-1,2, 4-riazine	$C_{10}H_{10}N4O_2$	218	29.04	5.98	
17	Friedoolean 6-ene	C_3OH_5O	410	29.89	11.7	

Table 5: GC-MS analysis and mass spectral data of P. guineense fractions from the leaves of P.guineense showing molecular formular, molecular weight, percentage content, retention time and base peak



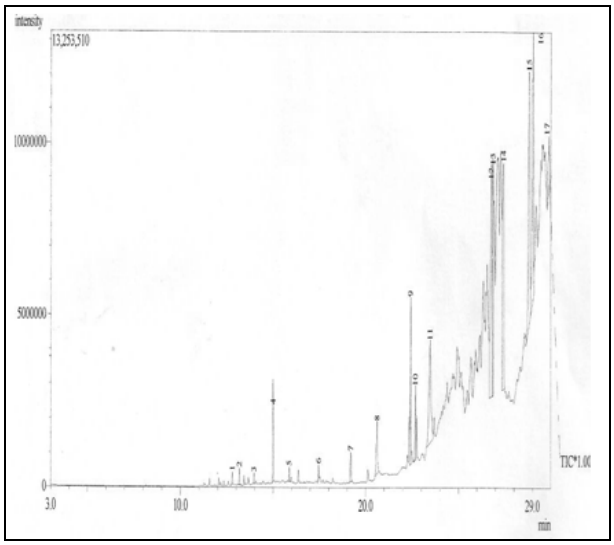


Figure 1: Chromatogram GC-MS analysis and mass spectral data of P.guineense
Figure 2: Chromatogram of GC-MS analysis and mass spectral data of G.latifolium

6. Discussion

Knowledge of the phytochemical constituents of plants is desirable, not only for the discovery of therapeutic agents, but also because such information may be of value in disclosing new sources of economic materials such as tannins, oils ,gums, flavonoids, saponins, and essential oils precursors for the synthesis of complex chemical substances[20].The result from the phytochemical studies and quantitative determination of the percentage yields of phytochemical constituents of *P. guineense* and *G. latifolium* indicated the presence of alkaloid, cardiac glycoside, flavonoids, phenols, saponins and tannins in both plants (Tables 1 and 2) . These phytochemicals exhibit a wide range of biological effects as consequence of their antioxidant properties. The presence of phenols, flavonoids, alkaloids, tannins etc suggest the dispersive nature of these phytochemicals present in the leaves of *P. guineense* and *G. latifolium.* These bioactive compounds have been reported to be free radical scavengers and inhibitors of lipid peroxidation[20] .The phenolic compounds are among the largest and most ubiquitous groups of plant metabolites [20]. They possess biological properties such as antiapoptosis, antiaging, anticarcinogen, antiinflammation, antiatherosclerosis, cardiovascular protection and improvement of endothelial function, as well as inhibition of angiogenesis and cell proliferation activities [21].The antioxidant properties of phenolic acids and flavonoids are due to their redox properties, ability to chelate metals and quenching of singlet oxygen[22].Several studies have described the antioxidant properties of medicinal plants which are rich in phenolic compounds [23]. Tannins bind to proline- rich protein and interfere with protein synthesis. Flavonoids are hydroxylated phenolic substances known to be synthesized by plants in response to microbial infection and they have been found to be antimicrobial substances against wide array of microorganisms in vitro [24]. Their activity is probably due to their ability to complex with extracellular and soluble proteins and to complex with bacterial cell wall [24]. They also are effective antioxidant and show strong anticancer activities [25]. The plant extracts were also revealed to

contain saponins which are known to produce inhibitory effect on inflammation [25]. Saponins have the property of precipitating and coagulating red blood cells. Some of the characteristics of saponins include formation of foams in aqueous solutions, hemolytic activity, cholesterol binding properties and bitterness [26]. Steroids have been reported to have antibacterial properties [27] and they are very important compounds especially due to their relationship with compounds such as sex hormones [28]. Alkaloids have been associated with medicinal uses for centuries and one of their common biological properties is their cytotoxicity [29].They are used as CNS stimulant, topical anaesthetic in ophthalmology, powerful pain relievers[29], and show antipuretic, antispasmodic and antibacterial properties[30]. Cardiac glycosides are known to lower the blood pressure according to many reports and functional for heart diseases [31]. The results obtained in this study thus suggest that *Glatifolium* and *Piper guineense* leaves are an increasingly valuable reservoir of bioactive compounds of potential substantial socioeconomic importance [32].

The results of proximate composition(Table 3) results show that *P.guineenese* and *G. latifolium* are nutritionally valuable .However, their high moisture contents (80%, 62%), respectively shows that they would be difficult to store. The high moisture content which would hinder the storage life which would be low. The moisture content of *P.guineense* is higher compared to that of *G.latifolium* indicating lower shelf life for the fresh *P.guineense,* hence long storage would lead to spoilage and high susceptibility to microbial attack. This supports the practice of storage in dry form by users. Moisture content is among the most vital and mostly used measurement in the processing, preservation and storage of food [33].

The ash content of both plants indicated that the leaves are rich in mineral elements, with *P.guieneense* having the highest ash content compared to *G.latifolium.* Ash in food constitutes the residue remaining after all the moisture and organic materials (fat, protein, carbohydrates, vitamins, organic acid etc) have been incinerated at a temperature of about 500^{o}C. Ash content is generally taken to be a measure of the mineral content of the original food [34].Crude fibre in food or plant is an indication of the level of non-digestible carbohydrate and lignin. The crude fibre obtained for *P. guineense* was 7.68±0.1% and that of *G.latifolium* was 1.616±0.34%. This low level in *G. latifolium* is considered appropriate, because it aids absorption of glucose and fat. Although crude fibre enhances digestibility, its presence in high level can cause intestinal irritation, lower digestibility and decreased nutrient usage [35]. Crude fibre is made up largely of cellulose together with a little lignin which is indigestible in human [36]. The crude lipid content obtained for *P. guineense* was 60.23±0.44% and *G. latifolium* 40.3±0.33%. Lipid provides very good sources of energy and aids in transport of fat soluble vitamins, insulates and protects internal tissues and contributes to important cell processes [37]. Moreso, it is good to add lipid (fat) to most of our diets, because many body functions depend on lipids [37].

The crude protein of *P.guineense* was 8.40% and that of *G. latifolium* was 8.58%. The recommended dietary allowance (RDA) for protein is 56g for individual weighing 70kg and 46g for adult weighing 50kg; children may consume 2kg/day [38]. The plant is a moderate source of protein [39]. *P. guineense* contained lower carbohydrate 36±0.26% than *G.latifolium* was 51.02±0.67% with respect to the Recommended Dietary Allowance (RDA) of 130g [40]. However, both plants can contribute to the caloric requirement of the body.

The results of GC-MS analysis of *P. guineense* and *G. latifolium* identified thirty five bioactive chemical constituents present in the leaf extracts of both plants .The gas chromatogram showed the relative concentrations of various compounds getting eluted as a function of retention time[Table 4 and 5;Fig 3 and 4 respectively]. The heights of the peak indicate the relative concentrations of the compounds of the components present in the plant extract [41]. The mass spectrometer analysed the compounds eluted different times to identify the natures – and structure of the compounds. The compounds which were identified by GC-MS analysis were 17 in *P. guineense* and 18 in *G. latifolium* respectively [42].

Identification of the bioactive compounds was carried out by comparison of their mass spectra and retention time with those of reference standard and published data [42]. Compounds identified in both *G.latifolium* and *Piper guineense* have many biological properties and have

reported to have an anti inflammatory,nematicide,insectifuge,hypocholesterolemic,anticancer,hepatoprotective[43].For instance ,Aceta te cyclolanost24en3ol,4,4,6a6b,8a,11,11,14b octamethy1,4,4a,5,6,6a,6b,7,8,8a,9,10,11,12,12a,14,14a,14b,octadecahydro-2 H-picen-3-one, has been used in various industries as source of flavor and aroma. There is growing awareness in correlating the phytochemical components and their biological activities [44].

Anti inflammatory compounds like hexadecanoic acid, fragrance and flavoring agents such as 2-octenoic acid, pentadecanoic acid etc are identified (Table 4 and 5). Flavouring agents like pentadecanoic acid, 2-chloroethyl linoleate, isoamyl laureate which is a skin conditioning agent. This study has highlighted further the phyto pharmaceutical importance *Gongronema latifolium* and *Piper guineense* leaves .The plants contain phytochemical which are of great pharmaceutical value and should be exploited for health benefits and general socioeconomic development of our nation.

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