African Scientist Vol. 20, No. 3 September 30, 2019 Printed in Nigeria 1595-6881/2019 \$10.00 + 0.00 © 2019 Nigerian Society for Experimental Biology http://www.niseb.org/afs

AFS 2019073/20304

GCMS Analysis of Leaves and Seeds of *Piper guineense* Shumach & Thoon

E. D. Kpomah^{*1}, D. A. Monday¹ and B. Kpomah²

¹Biochemistry Department, Federal University, Otuoke, Bayelsa State, Nigeria. ²Chemistry Department, Delta State of Education, Mosogar, Nigeria

*Corresponding author; Email:denniskpomah@yahoo.com, Tel: +234 (0) 803 417 3608

(Received August 16, 2019; Accepted in revised form September 24, 2019)

ABSTRACT: This study was undertaken to investigate the ethanolic extract of the leaves and seeds of *P. guineense* for its bioactive constituents using GCMS. The shade dried and pulverized leaves and seed were extracted with absolute ethanol by Soxhlet method to obtain a crude extract. The GCMS analysis was carried out on a GC; Clarus 500 Perkin Elmer System comprising of a AOC-20i Autosampler interfaced with a Mass Spectrometer. The result shows different peaks indicating the presence of thirty and thirty-eight phytochemical compounds in the leaves and seeds respectively. Some of the compounds found in samples include butyrolactone, organic acids like dodecanoic acid, tetradecanoic acid, n-hexadecanoic acid, oleic acid, and octadecanoic acid, alcohol, hydrocarbons (mainly terpenes) such as Beta-myrcene, elemene, caryophylene, dillapiol, linolool and copaene, other compounds include Cyclohexane, 1-ethenyl-1-methyl-2, 4-bis(1-methylethenyl), Pyridine, 3-(5-phenyl-4H-1,2,4-triazol-3-yl-,. These compounds are known for their, strong aroma, flavours, and spiciness. These phytochemicals have documented aphrodisiac, anti-fungal, anti-inflammatory, antibiotic, and skin conditioning properties. The present finding suggests that this plant have a promising potential phytopharmaceutical and nutritive value.

Keywords: Piper guineense, phytochemical compound, GCMS analysis

Introduction

All over the world herbal medicine has made an enormous impact on the overall health and wellbeing of man most especially in poorly developed economies where paltry sum of the annual financial budget is allocated to the health sector (Abada and Ugwunta, 2016) and which is further compounded by large scale misappropriate of the little allocated funds due to corruption (Kress *et al.*, 2016; Akokuwebe and Adekanbi, 2017).

Herbs are generally valued for their virtues as food as well as medicine (Balogun *et al.*, 2016), hence their overall importance cannot be overemphasized in that they are almost available year in and out, cheap, ease of accessibility and generally regarded as safe with little or no side effects when compared to synthetic drugs.

The leaves and seeds of *Piper guineense* Schumach & Thonn have long history of usage in ethnomedicine and as spices for enhancing the taste of food and beverage in many parts of Africa and Asia. It is commonly used as aphrodisiacs (Kpomah *et al.*, 2012a), the seeds are consumed by women after childbirth, (Mba, 1994) to enhance uterine contraction for the expulsion of placenta and other remains from the womb (Mbongue *et al.*, 2005) and also for the control of weight (Mensah *et al.*, 2008). They are also used for the treatment of cough, bronchitis, intestinal disease and rheumatism (Sumathykutty *et al.*, 1999; Okoye and Ebeledike, 2013). The seed and leaf extracts are capable of exhibiting a depolarizing neuromuscular activity (Ojinaka *et al.*, 2016). The antiparasitic, antimicrobial and antifungal activities of the leaf and seeds of *P. guineense* have also been well reported (Morebise *et al.*, 2002). *P. guineense* belong to the family *Piperaceae* and commonly called climbing pepper, it is a slender climber up to 12 m high with prominent nodes and clasping roots, the leaves are elliptic in shape about 15 cm long and 7 cm broad, the flowers are small, borne on common stalk as cluster opposite to the leaves, the fruits are red and turns black when dry (Kpomah *et al.*, 2012b)

African Scientist Volume 20, No. 3 (2019)

Investigations of the chemical and biological activities of plants have yielded components for the development of modern synthetic organic chemistry and the emergence of medicinal chemistry as a major route for the discovery of novel and more effective therapeutic agents (Kpomah, 2019) and due to the wide usage of *P. guineense* as spice and traditional medicine, this present study is therefore aimed at investigating the GC-MS analysis of the leaves and seeds of *P. guineense*

Materials and Methods

Collection of plant material and preparation of extract: The leaves and seeds of *P. guineense* were bought from Otuoke, Ogbia Local Government Area, Bayelsa State, Nigeria. They were identified and confirmed at the Herbarium, Department of Plant Science and Biotechnology, University of Portharcourt, Rivers State. A voucher specimen was prepared and deposited in the herbarium of the same department with voucher No: UPH/V/1277. The leaves were carefully plucked with the aid of a kitchen knife, after which both the leaves and the seeds of *P. guineense* were thoroughly washed separately with distilled water to remove debris and other contaminants, they were air-dried until a constant weight was reached and then pulverized to fine powder with the aid of an electric blender (Saisho, Model S-742). The extraction was done by Soxhlet method. 200 g of the fine powder each of leaves and seed was extracted separately by wrapping 50 g per batch in a thimble and inserted into the extractor connected to 1 L Pyrex round bottom flask containing 500 ml of absolute ethanol and extracted at 60 °C for 24 h, the extract was evaporated to dryness using a rotatory evaporator (Model RE52-1).

GC-MS Analysis of *P. guineese* **Leaves and Seeds Extract:** GC-MS analysis was carried out on a Gas Chromatography (Clarus 500 Perkin Elmer system) comprising of a AOC-20i auto- sampler interfaced to a mass spectrometer instrument employing the following conditions: column Elite-1 fused silica capillary column (30 x 0.25 mm ID x 1 μ M df, composed of 100 % dimethylpoly diloxane), operating in electron impact mode at 70 eV; helium (99.999 %) was used as carrier gas at a constant flow of 1 mL/min and an injection volume of 0.5 μ L was employed (split ratio of 10:1) injector temperature 250 °C; ion-source temperature of 280 °C. The oven temperature was programmed from 110 °C (isothermal for 2 min), with an increase of 10 °C/min, to 200 °C, then 5 C/min to 280 °C, 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 40 to 450 Da. Total GC running time was 28 min.

Identification of Components: 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.

Results

GCMS chromatogram of the ethanol extract of the leaves of *P. guineense* (Figure 1) clearly showed thirty peaks indicating the presence of thirty phytochemical compounds while GCMS chromatogram of the ethanol extract of the seeds of *P. guineense*.

On the other hand, the GCMS chromatogram of the ethanol extract of the seeds of *P. guineense* Figure 2 clearly showed thirty eight peaks indicating the presence of thirty eight different phytochemical compounds. The identification of the phytochemical compounds was based on the peak area, retention time and molecular formula.



Figure 1: Chromatogram of the bioactive compounds in the leaves of P. guineense



Figure 2: Chromatogram of the biochemical compounds in the seeds of P. guineense

Table 1 shows the compound name with its molecular formula, retention time, peak area and % peak area. The result of the ethanol extract of *P. guineense* indicated that the seeds contain more bioactive compounds (thirty eight) when compared to the leaves (thirty).

African Scientist Volume 20, No. 3 (2019)

 Table 1:
 GC-MS analysis of P. guineense leaves and seed showing phytochemical compound's molecular formula, structure, molecular weight (M.Wt), retention time (RT) and percentage content (% C).

S/N		LEAVES	SEED							
	Nomenclature and Formula	Structure	M Wt	RT	% C	Nomenclature and Formula	Structure	M Wt	RT	% C
1	Butyrolactone C ₄ H ₆ O ₂		86	3.781	2.70	Butyrolactone $C_4H_6O_2$		86	3.69	5.20
2	Cyclopentanone, 2-methyl- C ₆ H ₁₀ O	O 98	98	3.699	4.75	2-Furanmethanol $C_5H_6O_2$	он	98	3.188	4.15
3	Propanoic acid, 2-hydroxy-, ethyl ester $C_5H_{10}O_3$	O OH OH	118	4.154	4.20	2-Propenoic acid, 2-methyl-, ethenyl ester $C_6H_8O_2$		112	3.514	0.91
4	Hydrazinecarboxylic acid, phenylmethyl ester C ₈ H ₁₀ N ₂ O ₂	NH2	166	4.766	2.68	Cyclohexanone C ₆ H ₁₀ O	°	98	3.812	6.68
5	4-Ethoxy-2-butanone C ₆ H ₁₂ O ₂		116	6.326	12.18	2-Pentanone, 4-methoxy-4- methyl- C ₇ H ₁₄ O ₂		130	4.130	1.44
6	Glycerin C ₃ H ₈ O ₃	ОН НООН	92	6.998	14.81	Glycerin C ₃ H ₈ O ₃	ОН НООН	92	7.393	6.66

Kpomah et al.

S/N		LEAVES	SEED							
	Nomenclature and Formula	Structure	M Wt	RT	% C	Nomenclature and Formula	Structure	M Wt	RT	% C
7	Phenyl venyl ether C ₈ H ₈ O		120	7.632	0.63	Oxalic acid, heptyl propyl ester C ₁₂ H ₂₂ O ₄	\sim	230	4.836	1.75
8	Benzoic acid, 2-methyl-3- nitro- C ₈ H ₇ NO ₄	O OH N OH	181	8.430	1.21	2,5-Dimethyl-4-hydroxy- 3(2H)-furanone C ₆ H ₈ O ₃	HO 0 128	128	5.383	3.47
9	Thyminose C ₅ H ₁₀ O ₄	OH OH OH	134	8.813	0.94	4H-Pyran-4-one, 2,3- dihydro-3,5-dihydroxy-6- methyl- C ₆ H ₈ O ₄	0 ОН	144	6.492	7.29
10	$\begin{array}{c} Cyclohexane, 1-ethenyl-1-\\methyl-2, 4-bis(1-\\methylethenyl)-\\C_{15}H_{24}\end{array}$		204	9.156	0.94	Cyclohexane, 1-ethenyl-1- methyl-2,4-bis(1- methylethenyl)- $C_{15}H_{24}$		204	9.155	1.67
11	1,3-Pentadiene,5(2,2- dimethylcyclopropyl)-2,4- dimethyl-,(Z or E)- C ₁₂ H ₂₀		164	9.674	3.09	4-Hydroxymethyl- benzaldehyde C ₈ H ₈ O ₂	H0 136	136	8.446	0.70
12	1,6,10-Dodecatriene, 7,11- dimethyl-3-methylene- (Z or E)- C ₁₅ H ₂₄		204	9.835	3.63	1,6,10-Dodecatriene, 7,11- dimethyl-3-methylene-, (Z)- C ₁₅ H ₂₄		204	10.602	10.13

African Scientist Volume 20, No. 3 (2019)

S/N	N LEAVES					SEED					
	Nomenclature and Formula	Structure	M Wt	RT	% C	Nomenclature and	Structure	M Wt	RT	% C	
						Formula					
13	1,2,3,4-Butanetetrol, [S- (R*,R*)]- C ₄ H ₁₀ O ₄	ОН НО ОН ОН	122	10.568	5.87	1-Hepten-6-one, 2-methyl- C ₈ H ₁₄ O	° –	126	8.599	1.09	
14	5-Allyl-4-[1-(p- aminophenyl)ethylidenehydra zono]-6-methyl-2- phenylpyrimidine C ₂₂ H ₂₃ N ₅		357	10.965	1.51	Gamma-Elemene C ₁₅ H ₂₄		204	9.678	1.72	
15	Asarone C ₁₂ H ₁₆ O ₃		208	11.259	6.93	Beta-Myrene $C_{10}H_{16}$		136	9.840	3.90	
16	2-Decanone, 5,9-dimethyl- C ₁₂ H ₂₄ O		184	11.51	1.55	2-Nitrostyrene C ₈ H ₇ O ₂		149	10.998	3.69	
17	Apiol (Dillapiol) C ₁₂ H ₂₄ O	207 0	222	12.19	7.65	1,3,6,10-Dodecatetraene, 3,7,11-trimethyl (Z,E)- (alpha-Farnesene) $C_{15}H_{24}$		204	11.263	2.22	
18	$\begin{array}{l} Spiro[2,4,5,6,7,7a-hexahydro-\\ 2-oxo-4,4,7a-trimethyl\\ benzofuran]-7,2-oxirane\\ (Elemicine) C_{12}H_{16}O_3 \end{array}$		208	13.02	1.60	Bicyclo(3.1.1)heptane-2,3- diol, 2,6,6-trimethyl- C ₁₀ H ₁₈ O ₂	он он	170	11.389	2.45	

Kpomah et al.

S/N	/N LEAVES					SEED					
	Nomenclature and Formula	Structure	M Wt	RT	% C	Nomenclature and Formula	Structure	M Wt	RT	% C	
19	E-2-Tetradecen-1-ol C ₁₈ H ₂₄ O	CH CH	212	14.26	3.78	8-Hydroxy-2-octanone C ₈ H ₁₆ O ₂	HO	144	11.655	1.79	
20	5,8a-Trimethyl-3,5,6,7,8,8a- hexahydro-2H-chromene $C_{12}H_{20}O$		180	14.65	2.01	Decalin, anti-1-methyl-, cis- C ₁₁ H ₂₀ O	H H	152	16.580	2.86	
21	n-Hexadecanoic acid C ₁₆ H ₃₂ O ₂	0 0 0 0 0 0	256	16.89	0.66	n-Hexadecanoic acid C ₁₆ H ₃₂ O ₂	°	256	17.175	3.12	
22	Trifluoroacetic acid, n- heptadecyl ester C ₁₇ H ₃₁ F ₃ O ₂	~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~	324	18.72	1.78	Trifluoroacetic acid, n- heptadecyl ester C ₁₂ H ₁₄ O ₄		222	12.247	5.18	
23	Phytol C ₂₀ H ₄₀ O	***	296	19.19	4.37	9,12-Octadecadienoic acid, methyl ester, (E,E)- $C_{19}H_{34}O_2$		294	18.872	0.32	
24	Oleic acid C ₁₈ H ₃₄ O ₂	~	282	19.95	0.89	Oleic acid C ₁₈ H ₃₄ O ₂	چر	282	20.12	3.04	
25	3,4-(Methylenedioxy) toluene C ₈ H ₈ O ₂		138	23.36	0.62	Decane, 1-fluoro- $C_{10}H_{21}F$	F	160	21.646	1.40	

African Scientist Volume 20, No. 3 (2019)

S/N		LEAVES	SEED							
	Nomenclature and Formula	Structure	M Wt	RT	% C	Nomenclature and Formula	Structure	M Wt	RT	% C
26	Trans-Dodec-5-enal C ₁₂ H ₂₂ O	·////	182	23.50	0.71	9-Octadecenamide, (Z)- C ₁₈ H ₃₅ NO		281	22.638	0.51
27	$\begin{array}{l} 3-[2-(2-Methoxy-phenyl)-2-\\ oxo-ethyl]-5-phenyl-3H-\\ [1,3,4]oxadiazol-2-one\\ C_{17}H_{14}N_2O_4 \end{array}$		310	25.45	3.60	1-Piperonyl-3-amino-1,2,4- triazine C ₁₀ H ₁₀ N ₄ O ₂		218	23.441	1.48
28	Phenol, 2-(1,3-benzodioxol-5- ylmethyl)iminomethyl- C ₁₅ H ₁₃ NO ₃	C - OH N - C - O	225	25.63	1.35	9-Tetradecenal, (Z)- C ₁₄ H ₂₆ O	i de la constante de la consta	210	23.525	3.55
29	Pyridazo[4,5-b]pyrrole, 2,3- dihydro-1-methyl- C ₁₈ H ₃₄ O ₂		135	25.96	1.21	Octane, 4-ethyl- C ₁₀ H ₂₂		142	23.726	0.73
30	Z-6,17-Octadecadien-1-ol acetate $C_{20}H_{36}O_2$	~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~	308	26.94	1.83	Hexadecanoic acid, 2,3- dihydroxypropyl ester C ₁₉ H ₃₈ O ₄		330	24.043	0.63
31	Nil					1,3-Benzodioxole, 5-propyl- C ₁₀ H ₁₂ O ₂		164	25.382	1.74

Kpomah et al.

S/N		LEAVES	SEED							
	Nomenclature and Formula	Structure	M Wt	RT	% C	Nomenclature and	Structure	M Wt	RT	% C
32	Nil					Ethyl 2-piperonyl carbazate C ₁₁ H ₁₄ N ₂ O ₄		238	25.565	3.17
33	Nil					10-Undecanal C ₁₁ H ₂₀ O	~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~	168	26.673	1.89
34	Nil					1-Dycine C ₁₀ H ₁₈ O ₃	~~~~	138	26.300	0.36
35	Nil					S-Triazolo[4,3-a]pyridine-3- thiol, 8-methyl- C ₇ H ₇ N ₃ S		165	26.985	0.64
36	Nil					$1-(3,4-Dimethoxy-phenyl)-2-phenylethan-1,2-dione C_{16}H_{14}O_4$		270	27.217	0.95
37	Nil					Diethylene glycol C ₄ H ₁₀ O ₃	но	106	4.423	0.70
38	Nil					Isosorbide C ₆ H ₁₀ O ₄		146	9.303	1.17

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, flavonoids, saponins, and essential oils precursors for the synthesis of complex chemical substances (Gbadamosi et al., 2011). The results of GC-MS analysis of P. guineense leaves and seeds identified bioactive chemical constituents present in the plant (Table 1). The gas chromatogram showed the relative concentrations of various compounds getting eluted as a function of retention time. The heights of the peak indicate the relative concentrations of the compounds of the components present in the plant extract. The mass spectrometer analyzed the compounds eluted at different times to identify the natures and structure of the compounds. 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. A total of sixty-eight volatile constituents were identified in the leaves and seeds of P. guineense. (Thirty for the leaves and thirtyeight for the seeds) and these compounds comprises of acids, hydrocarbons esters and alcohols. Various acids were identified as a constituent group from the result shown and they constitute over 50% of total volatiles compounds in the leaves and seeds of P. guineense. Ali and Ibiam (2014) identified acids in their work on the phytochemical studies and GC-MS analysis of Gongronema latifolium and P. guineense, and Ojinnaka et al, (2016) also identified acids in their work on the volatile compounds in the leaves and seeds of P. guineense plant from South-East Nigeria, using GC-MS. Acids identified in the leaves and seeds of P. guineense include dodecanoic acid, tetradecanoic acid, n-hexadecanoic acid, oleic acid, and octadecanoic acid. Butyrolactone, glycerine, oleic acid and hexadecanoic acid were also identified in the leaves and seeds of P. guineense, this is in agreement with the work of Ojinnaka et al, (2016), that identified oleic acid and hexadecanoic acid only as against the work of Ali and Ibiam, (2014) that was not identified but rather was reported in the leaves of G. latifolium.

Owolabi *et al*, (2013) in their study of the aroma chemical composition of *P. guineense* from Lagos identified myrcene, alpha-copaene and beta-elemene in fruit (berries) of the plant. Hydrocarbons (mainly terpenes) were also identified in the leaves and seeds of *P. guineense* plant. The seeds of *P. guineense* had higher concentration of the hydrocarbons which were mainly terpenes. Dillapiol, beta-myrcene (monoterpene) was also identified only in the seeds of *P. guineense*. Gamma-elemene, and caryophylene (sesquiterpenes) was identified in both the leaves and the seeds of *P. guineense*. Other hydrocarbon sesquiterpenes identified only in the seeds of *P. guineense*. Other hydrocarbon sesquiterpene that was identified only in the leaves of *P. guineense*. Jirovetz *et al*, (2002) reported that monoterpenes, sesquiterpenes and benzenoids have been identified as the main compounds in *P. guineense* responsible for their characteristic flavour and spiciness. They also identified similar aroma compounds in their analysis of the seeds of black *P. nigrum*, white *P. guineense* and black *P. guineense*. Some of the similar terpenes identified include myrcene, gamma-elemene and copaene.

A review of the phytochemical compounds found in the ethanol extract of the leaves and seeds of *P. guineense* showed that they have potent biological and pharmacological activities and industrial applications. Gamma-butyrolactone (GBL) is a drug which acts primarily as central nervous system (CNS) depressants. (Schep et al., 2012). Documentary research showed that it is commonly used for improving athletic performance, sleep, sexual performance and pleasure. They also take it for relieving depression and stress, prolonging life, promoting clear thinking, causing relaxation, and releasing growth hormone. GBL is also used to trim fat and as a body- or muscle-builder. (Feigenbaum and Howard, 1996; LoVecchio et al., 1998; Sivilotti, 2001; Schneir et al., 2001; Mason and Kerns, 2002). Cyclopentanones and cyclopentenones are used in decorative cosmetics, fine fragrances, shampoos, toilet soaps and other toiletries as well as in non-cosmetic products such as household cleaners and detergents. (Belsito et al., 2012). Furanmethanol applications include use in flavours and fragrances. Moreover, furfuryl alcohol is also used as a laboratory reagent and as a chemical building block for drug synthesis (Sriram and Yogeeswari, 2010; European Commission, 2011; IHS Markit, 2016). The carbazates are peptide-based molecules, and are an important starting point for drug development, especially in the design of enzyme inhibitors, this is primarily due to their high affinity and specificity toward biological functions (Arun and Margherita, 2015). The carbamate's emerging role in medicinal chemistry is also due to its chemical stability and its capability to increase permeability across cellular membranes. These attributes of organic carbamates have been exploited in drug design. As a result, the carbamate motif is becoming the choice for peptide bond surrogates (Sureshbabu et al., 2007; 2008). 4-hydroxy-3-methoxyphenyl-2-butanone) is a non-toxic and inexpensive compound with varied pharmacological activities. It has potent anti-inflammatory, antidiabetic, antilipolytic, antidiarrhoeic, antispasmodic potentials. It is also known for enhancing growth and immune stimulation. It acts as an appetite stimulant, anxiolytic, antithrombotic, radiation protective, and antimicrobial. Also, it inhibits the reactive nitrogen species which are important in causing Alzheimer's disease and many other disorders (Ahmad et al., 2015). Alcohols such as 2-furanmethanol and glycerine are known to act as an antifungal and prevent food spoilage (Onyenekwe et al., 2012; Ojinnaka et al., 2016). Esters like propanoic acid, 2-hydroxy-, ethyl ester, trifluoroacetic acid, n-heptadecyl ester were also identified in the volatile compound analysis. The leaves of P. guineense had higher ester concentration than the seeds. P. guineense. Esters, mainly formed by esterification of carboxylic acids and alcohols were reported to determine the characteristic pleasant aromatic notes (Klesk and Qian, 2003). Hexadecanoic acid has anti-inflammatory properties, propanoic acid and butanoic acid has been used in various industries as a source of flavour and aroma, linolool and 3,4-dimethoxytoluene were shown to have sedative and anxiolytic effect on mice via inhalation (Joan and Michiho, 2013). Pentadecanoic acid is used as a flavouring agent while glycerine can serve as a skin conditioning agent. There is a growing awareness in correlating the phytochemical components and their biological activities (Ali and Ibiam, 2014).

Conclusion

In the present study, thirty and thirty-eight phytochemical constituents were identified from the ethanol extract of leaves and seeds of *P.guineense* by Gas Chromatogram - Mass Spectrometry (GC - MS) analysis. The presence of these phytochemical constituents justifies the use of this plant for various health challenges by traditional practitioners. The plant also contains phytochemical compounds which are of great pharmaceutical and nutritive value and should be exploited for health benefits and general socio-economic development of our nation.

References

- Abada UD, Ugwunta OD: The effects of budgetary allocations on health sector reform agenda: evidence from Nigerian public sector. Online Journal of Arts, Management and Social Science 1(1):1-11. 2016
- Ahmad B, Rehman MU, Amin I, Arif A, Rasool S, Bhat AS, Afzal I, Hussain I, Bilal S, Manzoor ur RM: A Review on Pharmacological Properties of Zingerone (4-(4-Hydroxy-3-methoxyphenyl)-2-butanone). Sci. World J. 15:1-6. 2015
- Akokuwebe ME, Adekanbi DM: Corruption in the health sector and implications for service delivery in Oyo State public hospitals. Ilorin Journal of Sociology 9(1): 200-217. 2017
- Ali FU, Ibiam UA: Phytochemical Studies and GC-MS Analysis of *Gongronema Latifolium* and *Piper guineense*. Int. J. Innov. Res. Dev. 3:108-115. 2014
- Arun KG, Margherita B: Organic Carbamates in Drug Design and Medicinal Chemistry. J. Med. Chem. 58:2895-2940. 2015
- Balogun ME, Besong EE, Djobissie SF, Mbamalu OS, Obimma JN: A review of *Piper guineense* (African Black pepper). Int J Pharm Pharm Res 6: 369-384. 2016.
- Belsito D, Bickers D, Bruze M, Calow P, Dagli ML, Dekant W, Fryer AD, Greim H, Miyachi Y, Saurat JH, Sipes IG: A toxicologic and dermatologic assessment of cyclopentanones and cyclopentenones when used as fragrance ingredients. Food Chem Toxicol 50:S517-S556. 2012
- European Commission: Recommendation from the Scientific Committee on Occupational Exposure Limits for furfuryl alcohol. SCOEL/SUM/129, June 2011. Brussels, Belgium: European Commission. 2011
- Feigenbaum JJ, Howard SG: Gamma hydroxybutyrate is not a GABA agonist. Prog Neurobiol 50:1-7. 1995.
- Gbadamosi IT, Moody JO, Lawal AM: Phytochemical screening and proximate analysis of eight ethnobotanicals used as antimalarial remedies in Ibadan, Nigeria. J Appl Biosci 44:2967-2971. 2011.
- IHS Markit: Furfuryl alcohol and furan resins. Chemical economics handbook. IHS Markit. Available from: https://ihsmarkit.com/products/furfuryl-alcohol-and-furan-chemical-economics-handbook.html. 2016
- Jirovetz L, Buchbauer G, Ngassoum MB, Geissler M: Aroma compound analysis of *Piper nigrum* and *Piper guineense* essential oils from Cameroon using solid-phase microextraction–gas chromatography, solid-phase microextraction–gas chromatography–mass spectrometry and olfactometry. J. Chromatogr 97(6):265-275. 2002
- Joan MT, Michiho I: Inhalation of the essential oil of *P.guineense* from Cameroon shows sedative and anxiolytic-like effects in mice. Biol. Pharm. Bulletin 36(10):1608-1614. 2013
- Klesk K, Qian M (2003). Preliminary aroma comparison of Marion (*Rubus spp. Hyb*) and Evergreen (*R. laciniatus* L.) Blackberries by dynamic headspace/OSME Technique (RH Marion and Evergreen Volatiles. J. Food Sci. 68: 697-700.
- Kpomah ED, Arhoghro EM, Uwakwe AA: Biochemical and Histopathological changes in Wistar rats following chronic administration of diherbal mixture of *Zanthophylim leprieurii* and *Piper guineese*. Journal of Natural Science Research 2 (6):22-28. 2012
- Kpomah ED, Uwakwe AA, Abbey BW: Aphrodisiac studies of diherbal mixture of Zanthophylim leprieurii Guill. & *Perr.* and *Piper guineese* schumach. & thonn. On male wistar rats. Global J. Res. Med. Plants and indigenous Med 1(9): 381-390. 2012
- Kpomah ED: Biochemical Effects of Zanthoxylum leprieurii Guill. & Perr. on Reproductive Hormone, Liver Function and Plasma Enzymes Activity of Male Wistar Rats. International Journal of Basic Science and Technology 5(2):73-78
- Kress DH, Su Y, Wang H: Assessment of Primary Health Care System Performance in Nigeria: using the Primary Health Care Performance Indicator Conceptual Framework. Health Syst Reforms. 2(4):302-318. 2016.
- LoVecchio F, Curry SC, Bagnasco T: Butyrolactone-induced central nervous system depression after ingestion of RenewTrient, a dietary supplement. N Engl J Med 339:847-848. 1998
- Mason P, Kerns II W: Gamma Hydroxybutyric Acid (GHB) Intoxication. Acad Emerg Med 9:730-739. 2002
- Mba MA: Effect of dietary intake of *Piper guineense* on growth and indices of fitness in *Rattus rattus*. Int Seismological cent Inno 4:383-388. 1994
- Mbongue FG, Kamtchouing P, Essame OJ, Yewah PM, Dimo T, Lonts D: Effect of the aqueous extract of dry fruits of Piper guineense on the reproductive function of adult male rats. Indian J Pharmacol 7:30-32. 2005
- Mensah JK, Okoli RI, Ohaju-Obodo JO, Eifediyi K: Phytochemical, nutritional and medicinal properties of some leafy vegetables consumed by Edo people of Nigeria. Afr. J. Biotechnol. 7(14):2304-2309. 2008

- Morebise O, Fafunso MA, Makinde JM, Olajide OA, Awe EO: Antiinflammatory Property of the Leaves of *Gongronema latifolium*. Phytother Res 16(1):75–77. 2002
- Ojinnaka M C, Ubbor SC, Okudu HO, Uga U: Volatile compound analysis of the leaves and seeds of *Piper guineense* using gas chromatography-mass spectrometry (GC-MS). Afr. J. Food Sci. 10(11): 327-332. 2016
- Okoye EI, Ebeledike AO: Phytochemical constituents of *Piper guineense* (uziza) and their health implications on some microorganisms. Glob. Res. J. Sci 2:42-46. 2013
- Onyenekwe PC, Odeh C, Nweze CC: Volatile constituents of *Ogiri*, Soybean Daddawa and locust bean daddawa, three fermented Nigerian food flavour enhancers. Electronic J. Environ. Agric. Food Chem. 11(1): 15-22. 2012
- Owolabi MS, Lawal AO, Ogunwande IA, Hauser RM, Setzer WN: Aroma chemical composition of *Piper guineense* Schumach. &Thonn. From Lagos, Nigeria: a new chemotype. Am. J. Essent. Oils Nat. Prod 1:37-40. 2013
- Schep LJ, Knudsen K, Slaughter RJ, Vale JA, Megarbane B: The clinical toxicology of γ-hydroxybutyrate, γbutyrolactone and 1,4-butanediol. Clin Toxicol (Phila) 50(6):458-70. 2012.
- Schneir AB, Ly BT, Clark RF: A case of withdrawal from the GHB precursors gamma-butyrolactone and 1,4butanediol. J Emerg Med 21:31-33. 2001
- Sivilotti ML, Burns MJ, Aaron CK, Greenberg MJ: Pentobarbital for severe gamma-butyrolactone withdrawal. Ann Emerg Med 38:660-665. 2001
- Sriram D, Yogeeswari P: Medicinal chemistry. Second edition. Hyderabad, India: BITS Pilani. 2010
- Sumathykutty MA, Rao JM, Padmakumari KP, Narayanan CS: Essential oil constituents of some piper species. Flavours Fragr. J 14:279-282. 1999
- Sureshbabu VV, Hemantha HP, Naik SA: Synthesis of 1,2,4-oxadiazole-linked orthogonally urethane-protected dipeptide mimetics. Tetrahedron Lett. 49:5133–5136. 2008.
- Sureshbabu VV, Venkataramanarao R, Naik SA, Chennakrishnareddy G: Synthesis of tetrazole analogues of amino acids using Fmoc chemistry: isolation of amino free tetrazoles and their incorporation into peptides. Tetrahedron Lett. 48:7038–7041. 2007