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Chemical Information from GCMS Analysis of Acetone-Ethanol Extract of *Piper guineense* Leaf. Part 2

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Abstract *The need to know the different constituents of Piper guineense leaf extract through the use of different solvent is remarkable because solvents play significant role in the constituent that can be extracted from planr materials. GCMS analysis of Acetone-ethanol extract of Piper guineense leaves indicated twenty one peaks which represented 2,3,6,7,7a-hexahydro-7a-methyl-5H-indene-5-one, alpha-cubebene, 1-ethenyl-1-methyl-2,4-bis(1-methylethenyl)-cyclohexane, caryophyllene, alpha caryophyllene, megastigma-7(E),9, 13-triene, eudesdma-4(14),11-diene, azulene, epsilon muurolene, di-t-butylacetylene, -cyclopentene -1-ethanol, 2,24-trimethyl, 2,6,6- trimethyl-3-(phenylthiol)cyclohep-4-enol, hexadecenoic acid, nonadecanoic acid, octadecanoic acid, cyclohexylidenecyclohexane,, pyrrolo[3,2-c]pyridine-4(5H)-one, 4,5-dihydrobenzo[1,2-c:3,4-c']bis[1,2,5]oxadiazole, piperidine, 3,7-diacetamidophenoxathin and 4-(6-methoxy-3-mythyl-2-benzofuran)-3-buten-2-one respectively. The most abundant components of the extract was di-t-butylacetylene (17.36%) and ,5-dihydrobenzo[1,2-c:3,4-c']bis[1,2,5]oxadiazole (12.95%). Most of the identified compounds were reported to possess strong biological activities. Compounds that has not been reported from GCMS analysis of the plant leaves using other solvent were also observed which confirmed that the missed solvent (acetone-ethanol) is more effective in extracting phytochemicals in Piper guineense leaves than either of the solvent.*

Key Words: *Piper guineense, leaves, acetone-ethanol extract, biological activity, effect of solvent.*

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1.0 Introduction

The leaves of *Piper guineense* are used as leafy vegetable and as flavor in most African soups. According to Usanga (2020), West African black pepper (*Piper guineense*) is an important plant used in traditional medicine and as spice. (Wasswa *et al*., 2017). The medicinal value of *Piper guineense* leaves have been attributed to the phytochemical constituents of the plant which include flavonoids, tannins, anthraquinones, steroids, phlobatannins, coumarins, and proteins while saponins, alkaloids, and volatile oils were not detected (Ebenso *et al*., 2008; Kabiru *et al*., 2016).

Imo *et al*. (2018) reported mean concentrations of proximate perimeters in ethanol extract of *Piper guineese t*o include moisture (6.11 %), crude protein (15.17 %), crude fibre (20.99), ash (11.98%), lipid (1.91%) and carbohydrate (43.86%). They also reported elemental composition including magnesium (13.311 ppm), calcium (47.127 ppm), manganese (0.284 ppm), chromium (0.109 ppm), copper (0.074 ppm), zinc (0.568 ppm), potassium (8.570 ppm), sodium (5.270 ppm) and phosphorus (1.290 ppm)

Ojinnaka *et al*. (2016) also stated that the concentrations of vitamins (mg/100g) in the leaves are vitamin A (9.31), vitamin B1 (0.028), vitamin B2 (0.028), vitamin B3 (0.0714), vitamin C (2.41) and vitamin E (0.06). Reported concentrations of antinutritional factors (mg/100g) in the leaves are tannins (0.34), phytate (0.29), saponin (3.26) and oxalate (0.55). Antinutritional factors are those constituents that can affect the availability of nutrients (Ekop and Eddy, 2006). Ojinnaka *et al*. (2016) also reported that the mean concentration of essential amino acids in the leaves is 240.14 µg/100g.

Although much has been documented on the chemical composition of *Piper guineense* leaves, there are still much that need to be investigated. In our previous study (Onyeije and Okop, 2020) we used GCMS to analysed acetone extract of the leaves of *Piper guineense* and found out that some new compounds that have not been reported by other works (using different solvents for the extraction) were detected indicating that solvent can play a significant role on the various fractions that can be obtained from GCMS analysis. Therefore, the present study is aimed at using equal mixture of acetone and ethanol to extract constituents of *Piper guineense* leaves for GCMS analysis.

2.0 Materials and Methods

Samples of *Piper guineense* leaves were purchase from Ikot Ekpene main market and transported to the Chemistry laboratory of the Michael Okpara University of Agriculture, Umudike. They were thoroughly washed with distilled water and allowed to dry. The leaves were sun dried for a week until the moisture content was reduced to minimum. The dried leaves were grounded to a powder form and soaked in a solution containing equal volume of ethanol and acetone solution. (Eddy *et al*., 2011a; Eddy and Odiongenyi, 2010). The mixed solvent was recovered using cold extractor, leaving behind, acetone/ethanol extract of *Piper guineense* leaves.

The produced extract was used for GCMS analysis using spectroscopically pure acetone solvent (Eddy *et al*., 2011b). The GCMS-QP2010 PLUS Schimadzu (made in Japan) instrument was used for the analysis. The analytical steps taken were plugger speed (high), syringe injection speed (high), viscosity/compression time (0.2 second), injection mode (normal), pumping time (5), injection port dwell time (0.3 second), terminated air cap (No), plugger washing speed (high), washing volume (8µl), syringe suction position (0), syringe injection position (0) and used three solvent vial (3). The operational setting of the GCMS instrument were column oven temperature (60°C), injection temperature (200°C), injection mode (split), flow control mode (linear velocity), pressure (100.2 kPa), total flow (6.2 ml/minute), linear velocity (46.3 cm/sec), purge flow (3.0ml/min) and split ratio (1.0). The high-pressure injection, carrier gas server and splitter hold functions were switch off. The initial rate of oven temperature program was 5 °C/min and was gradually increased to 140°C after which the temperature was increased to 280 °C at a rate of 10 °C/minute. Some heat unit and detector functions were checked in order to ensure consistency. These included column oven, SPL2, MS, SPL2 carrier, SPL2 purge and were ensured to be on. However, the APC setting was turned off.

Other setting functions of the machine were ion source temperature (200 °C), interface temperature (250 °C), solvent cut time ((2.50 minutes), detector gain mode (relative), detector gain (0.00kV), threshold (1000). The analytical start time was 3 minutes and the machine run for 45 minutes using ACQ scan mode at a scan speed of 769. However, mass/charge started at 50 and ended with 400 units.

Gas chromatogram and mass spectrum were automatically plotted and suggested chemical structures were obtained using the National Science Technology library installed in the machine. Percentage concentrations of each identified component was calculated using area normalization

3.0 Results and Discussion

Fig, 1 shows the GCMS spectrum of acetone/ethanol extract of *Piper guineense* leaves. The spectrum consists of twenty-one peaks whose respective mass spectrum is shown in Fig,.2. Identity of compounds deduced from the spectrum are shown in Table 1.

Table 1: Chemical identity of compounds deduced from GCMS spectrum of acetone-ethanol extract of *Piper Guineense* leaves.

Peak no	Name of compound	Retention time (minute)	Molecular weight	Mass peak	% Con.
1	1,2,3,6,7,7a-hexahydro-7a-methyl-5H-indene-5-one	15.325	150	33	0.52
2	Alpha cubebene	18.950	204	53	1.55
3	1-ethenyl-1-methyl-2,4-bis(1-methylethenyl)-cyclohexane	19.325	204	63	4.40

4	bicyclo[7,2,0]undec-4-ene,4,11,11-trime-thyl-8-methylene (Caryophyllene)	20.133	204	69	4.66
5	Alpha caryophyllene	21.150	204	62	4.66
6	Megastigma-7(E),9, 13-triene	22.175	204	75	7.51
7	Eudesdma-4(14),11-diene	22.390	204	78	5.70
8	Azulene	22.700	204	81	4.66
9	epsilon -muurolene (Naphthalene, deca-hydro-1,6-bis(methylene)-4-(1-meth-ylethyl)-(4.alpha.,4a.alpha., 8a.alpha.))	23.233	204	64	1.30
10	Di-t-butylacetylene	25.700	138	114	17.36
11	3-cyclopentene -1-ethanol, 2,24-trimethyl	27.383	154	75	2.85
12	2,6,6- trimethyl-3-(phenylthiol)cyclohep-4-enol	28.775	262	72	1.55
13	Hexadecanoic acid	31.725	270	61	2.07
14	Nonadecanoic acid	32.267	298	73	2.33
15	10, 13,Octadecanoic acid	33.550	294	136	8.29
16	Cyclohexylidenecyclohexane	37.575	164	107	2.07
17	Pyrrolo[3,2-c]pyridine-4(5H)-one	37.917	134	125	5.70
18	4,5-dihydrobenzo[1,2-c:3,4-c']bis[1,2,5]oxadiazole	39.558	164	159	12.95
19	Piperidine	40.329	285	98	2.07
20	3,7-diacetamidophenoxathin	40.525	314	114	1.55
21	4-(6-methoxy-3-mythyl-2-benzofuran)-3-buten-2-one	43.608	230	169	6.22

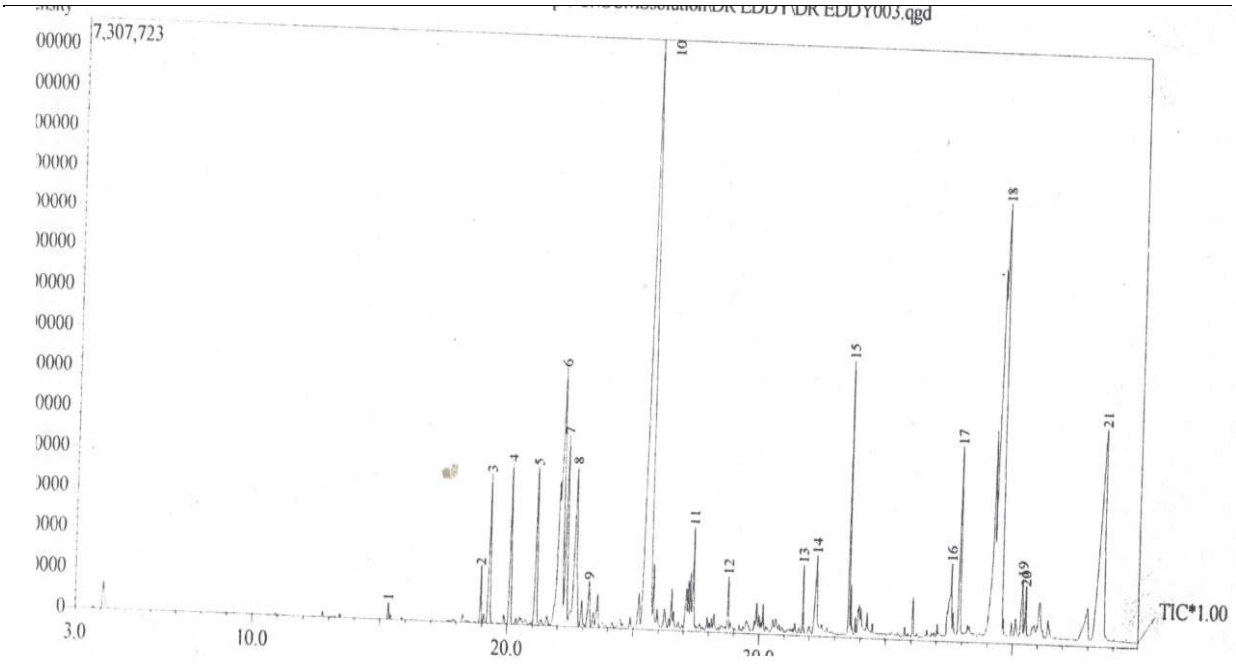
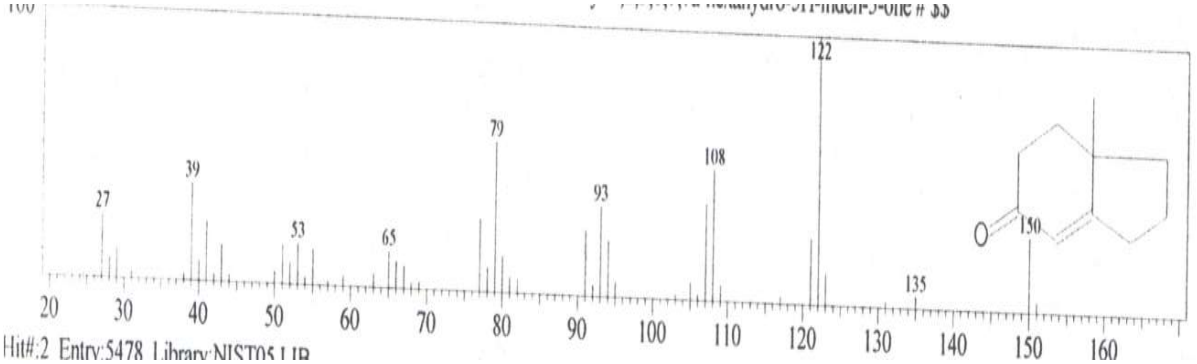
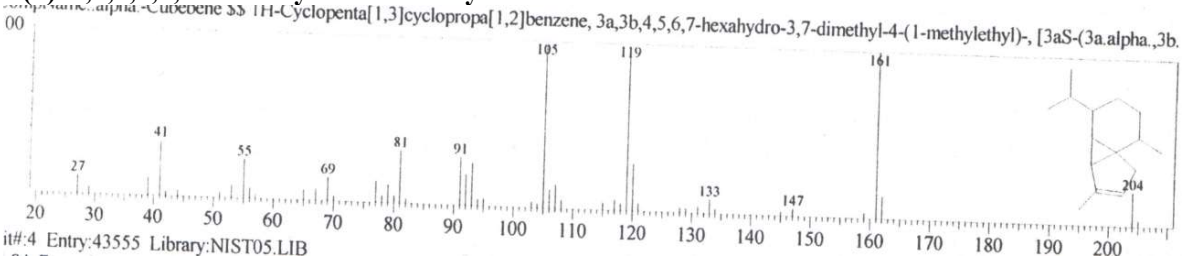


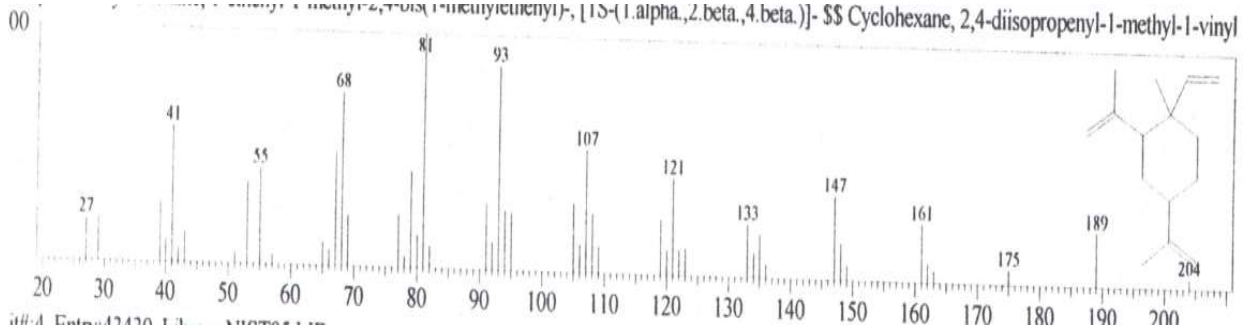
Fig. 1: GCMS of acetone-ethanol extract of *Piper guineense* leaf



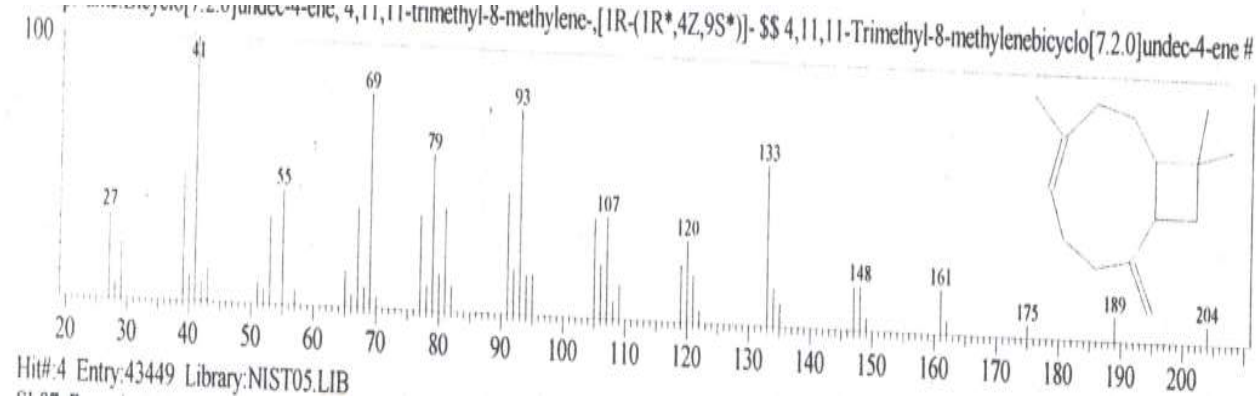
(1) 1,2,3,6,7,7a-hexahydro-7a-methyl-5H-indene-5-one



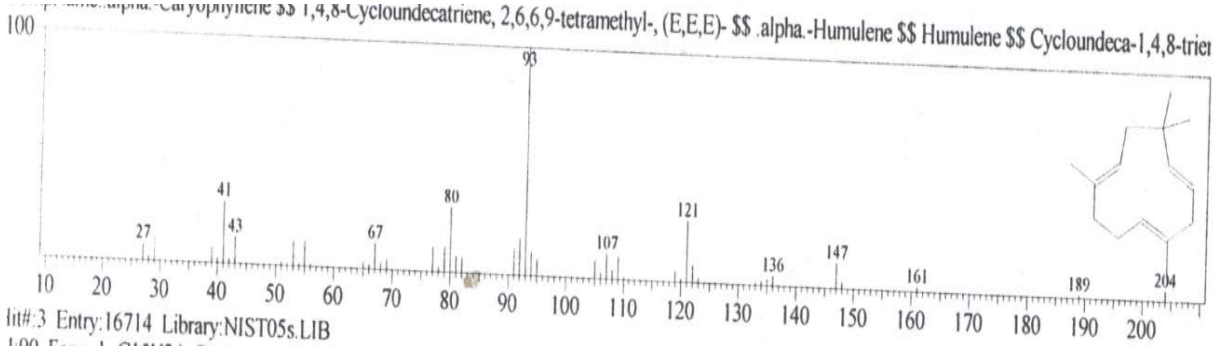
(2) Alpha cubebene



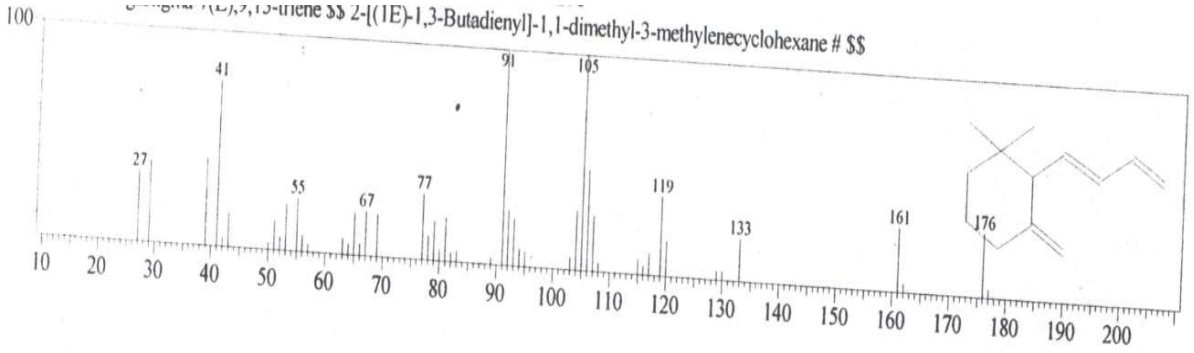
(3) 1-ethenyl-1-methyl-2,4-bis(1-methylethenyl)-cyclohexane



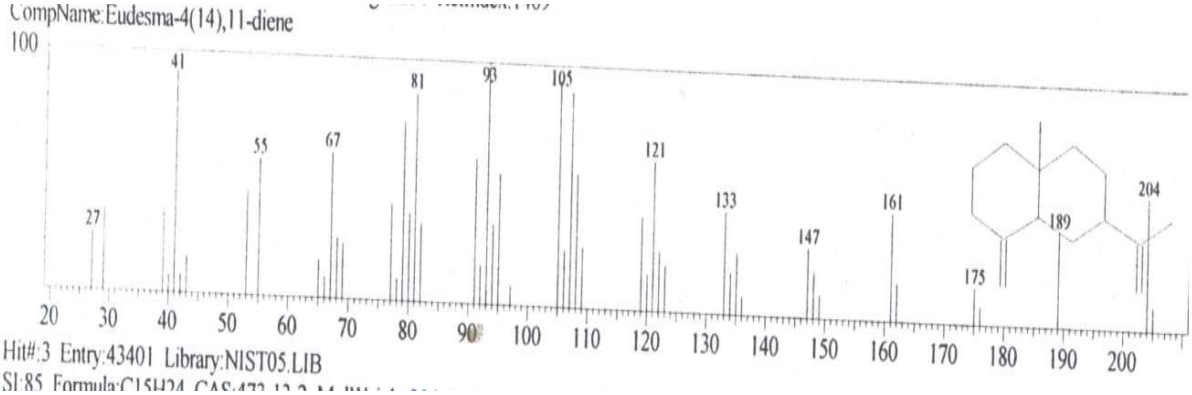
(4) bicyclo[7,2,0]undec-4-ene,4,11,11-trimethyl-8-methylene (Caryophyllene)



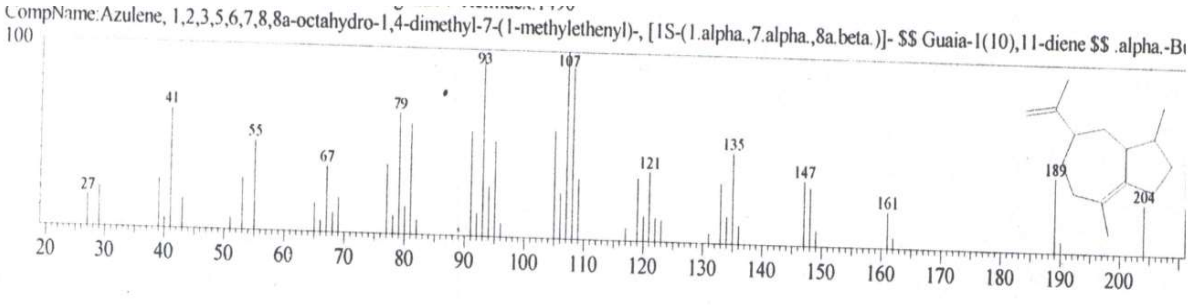
(5) Alpha caryophyllene



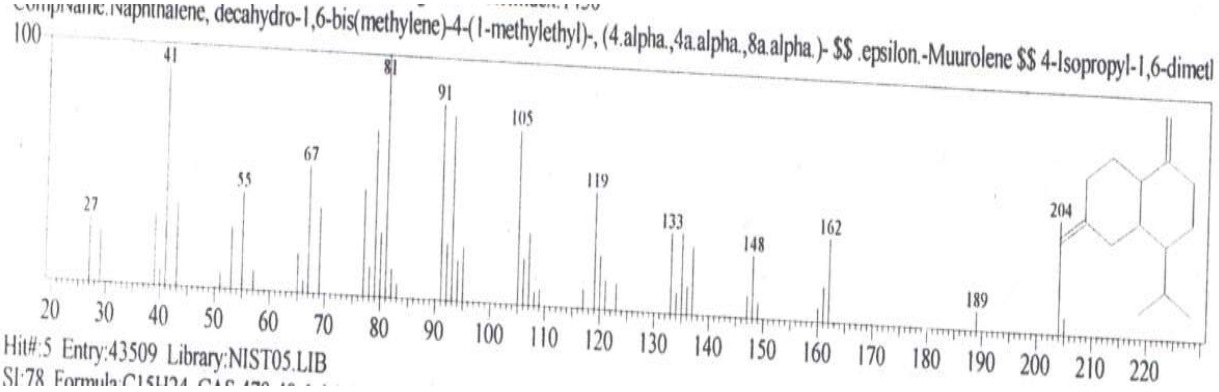
(6) Megastigma-7(E),9, 13-triene



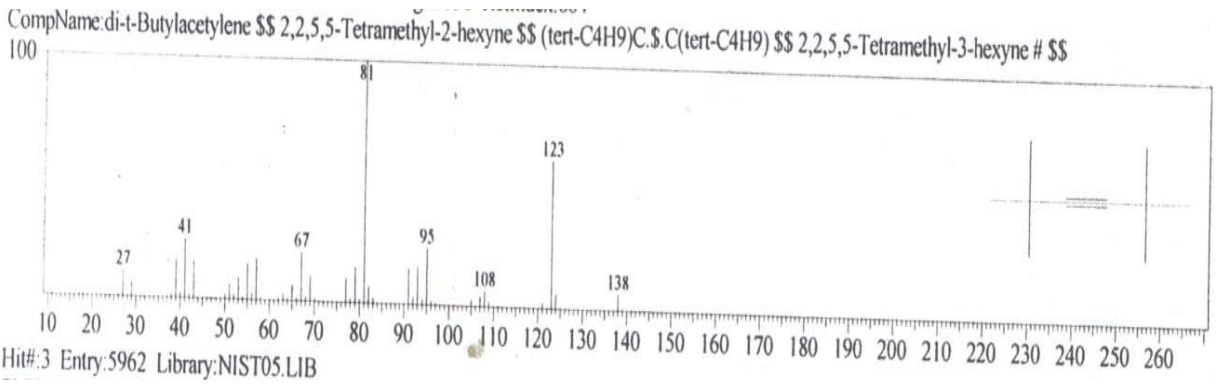
(7) Eudesdma-4(14),11-diene



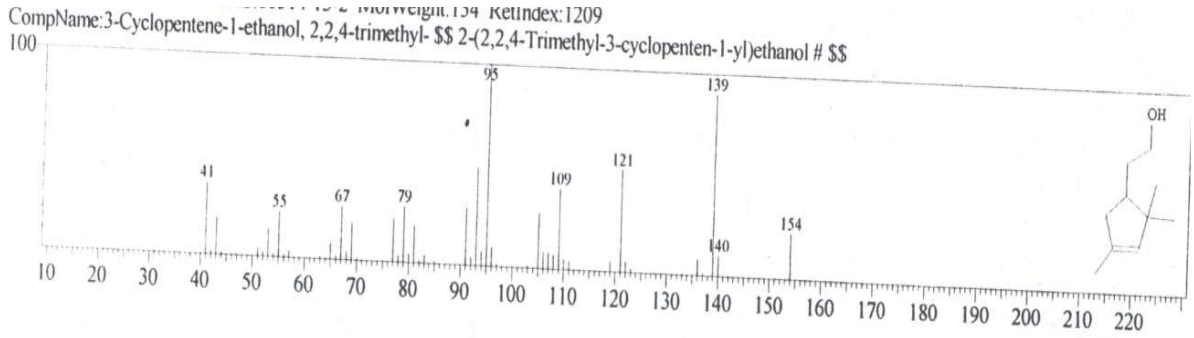
(8) Azulene



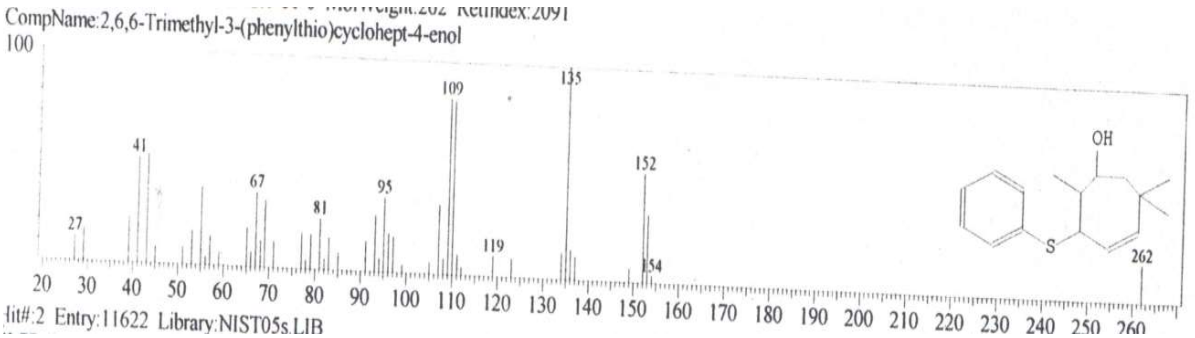
(9) Epsilon-muurolene



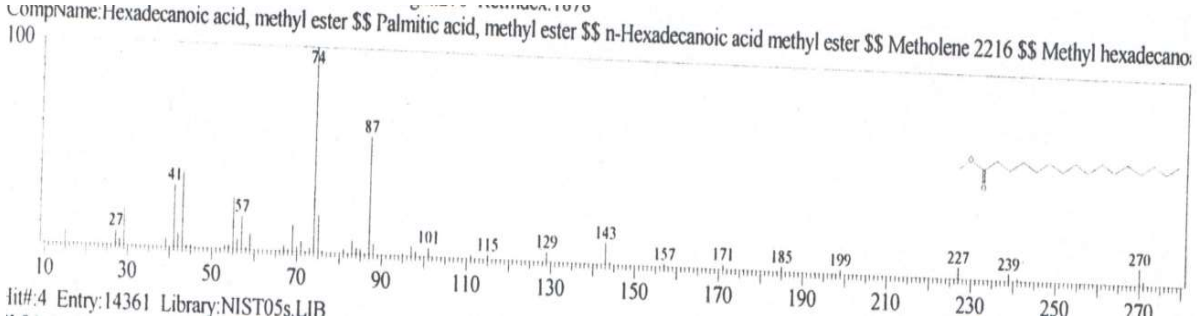
(10) di-t-butylacetylene



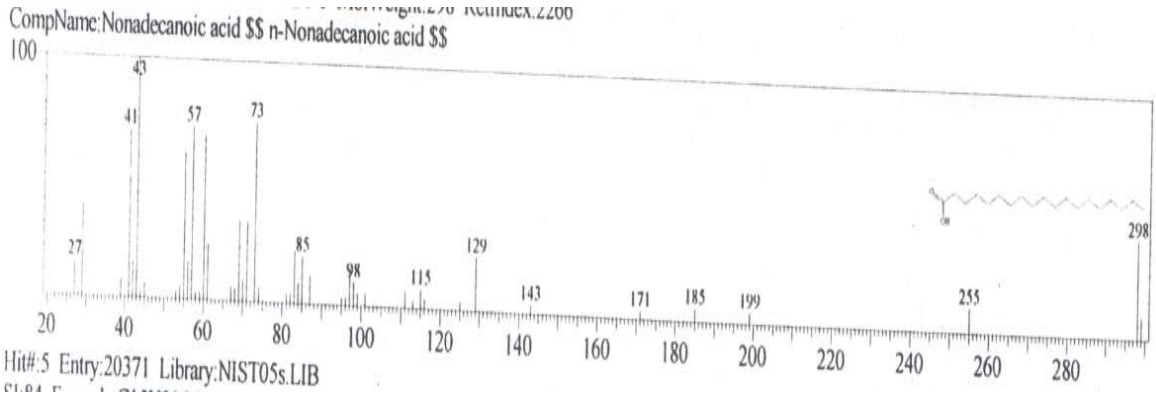
(11) 3-cyclopentene -1-ethanol, 2,24-trimethyl



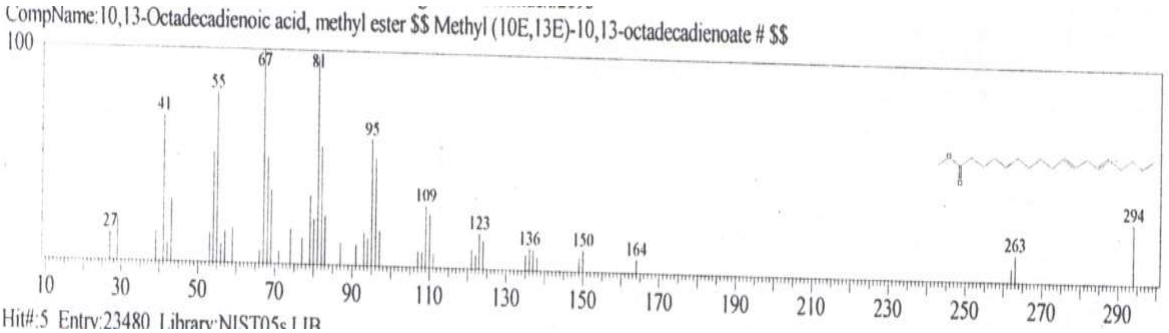
(12) 2,6,6- trimethyl-3-(phenylthiol)cyclohep-4-enol



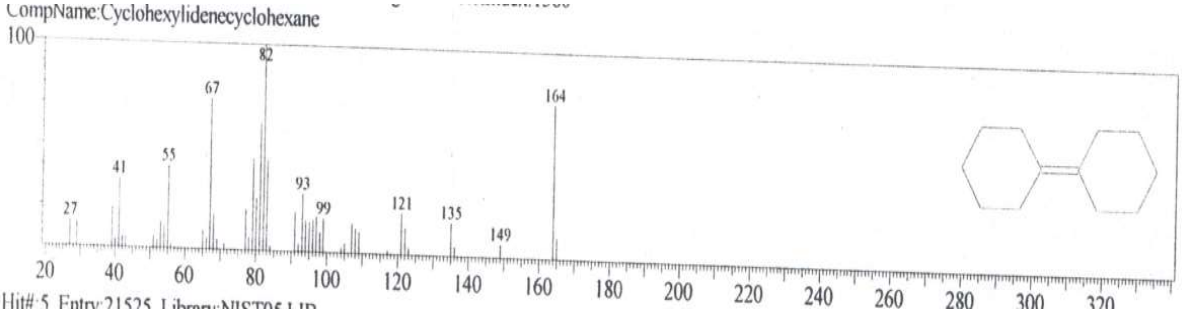
(13) Hexadecanoic acid



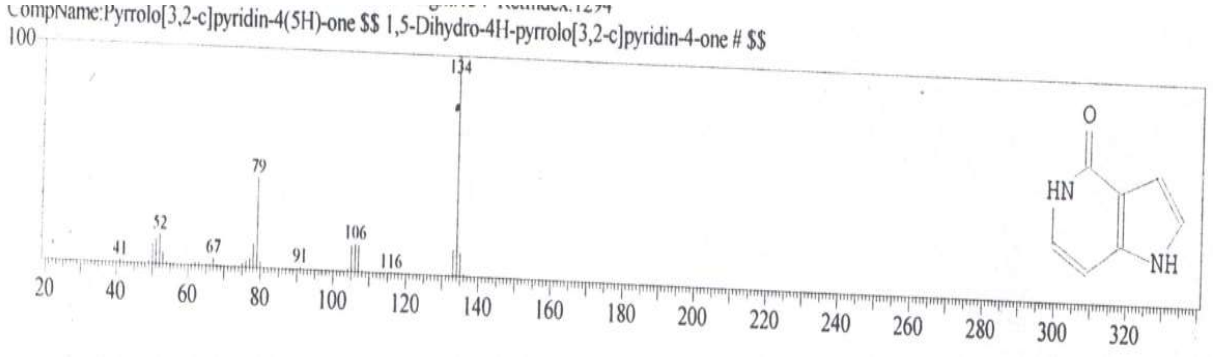
(14) Nonadecanoic acid



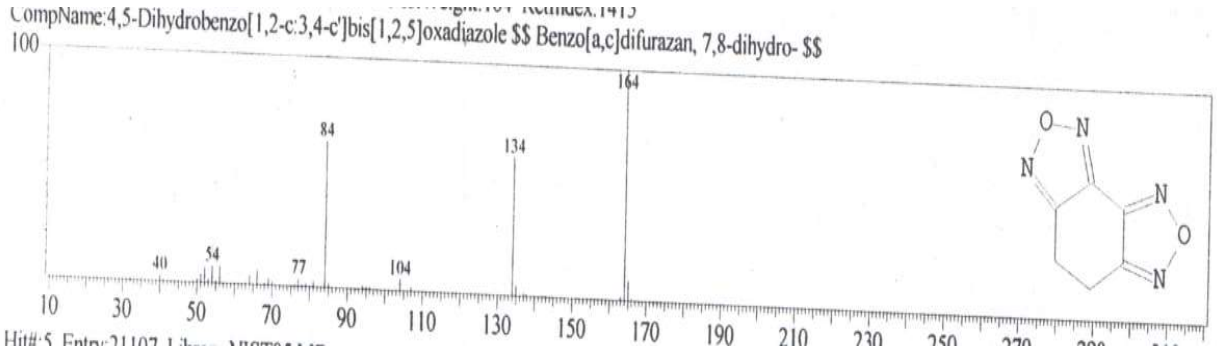
(15) 10, 13-octadecanoic acid



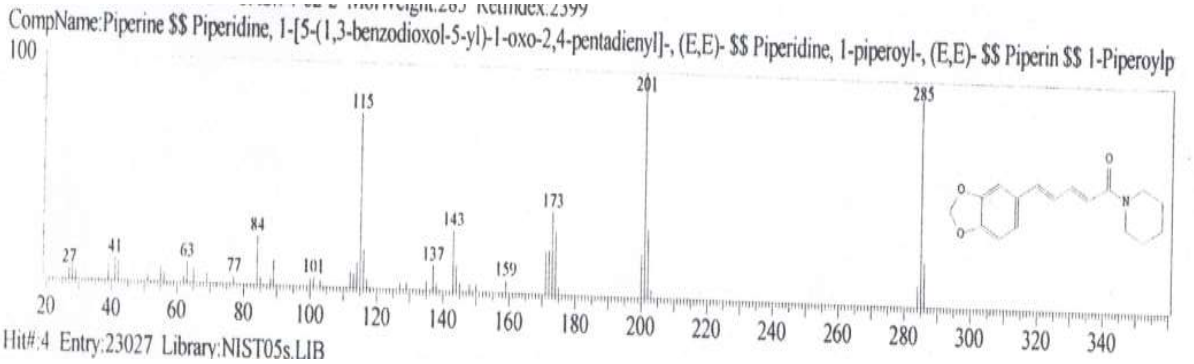
(16) Cyclohexylidenecyclohexane



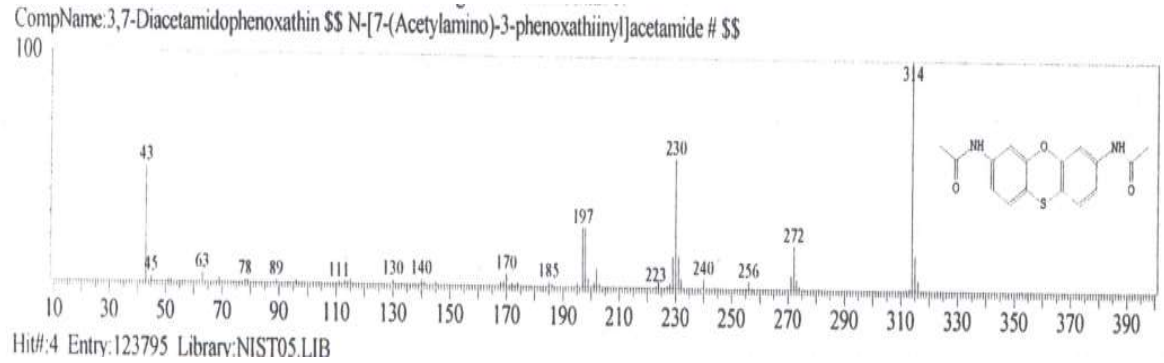
(17) Pyrrolo[3,2-c]pyridine-4(5H)-one



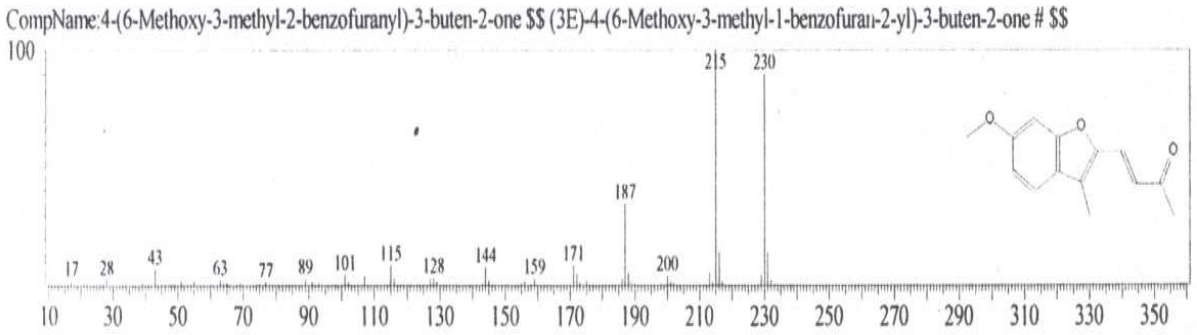
(18) 4,5-dihydrobenzo[1,2-c:3,4-c']bis[1,2,5]oxadiazole



(19) Piperidine



(20) 3,7-diacetamidophenoxathin



(21) 4-(6-methoxy-3-mythyl-2-benzofuran)-3-buten-2-one

1,2,3,6,7,7a-hexahydro-7a-methyl-5H-indene-5-one is an intermediate for the synthesis of isocomene, a sesquiterpene, which is a confirmed bioactive compound (Svante, 1982; Al-Hajj *et al*., 2014). Costa *et al.* (2011) identified alpha cubebene in *Annona salzmannii* and *A pickelli* and observed that it is a bioactive compound that exhibited antimicrobial properties. Martins *et al*. (2015) also attributed the antimicrobial and cutotoxic activities of *Kielmeyera coriacea Mart.* essential oil to the presence of alpha cubebene, alpha copene,(an isomer of cubebene) and other phytochemicals. Praskasia and Nair (2015) identified 1-ethenyl-1-methyl-2,4-bis(1-methylethenyl)-cyclohexane through hydrodistillation of *Glycosmis pentaphylla (Retz.) Correa* leaves. GCMS retention time of 12.42 minutes and concentration of 1.23 % were observed. The found that this compound, also called 2,4-disopropenyl-1-methyl-1-vinyl has strong antimicrobial activity. In our previous study in acetone extract of *Piper guinnesis*, we also identified this compound at a retention time of 19.308 minutes and concentration of 1.70 %. This gives some slight variation with what we have obtained in the present study, where the retention time for this compound is 19. 325 minutes and the concentration is 4.40% hence the choice of solvent is of essence in determining the chemical compound, retention and concentration that appears in GCMS spectrum.

Bicyclo[7,2,0]undec-4-ene,4,11,11-trimethyl-8-methylene (caryophyllene) was observed at retention time of 20.133 minutes and concentration of 4.66% but in acetone extract of *Piper guineense,* we observed this compound at 20. 108 minutes and its concentration was 0.54 % (Onyeije and Okop, 2020) Haznedarogku *et al. (2001)* identified cryophyllene as a major contributor to the antimicrobial activity of essential oil of *Salvia tomentosa* , which contain 1,8-cineol (17%), β-caryophyllene (11%), cyclofenchene (10%) and δ-cadinene (6%). Alpha caryophyllene was also observed at peak 5 at a retention time of 21.150 minutes but the presence of this isomer was not observed in our previous work (Onyeije and Okop, 2020). Alpha cryophyllene, a characteristics terpene is also called humulene, which is a ring open isomer of beta caryophyllene. Humulene possesses strong anti-inflammatory activity that is equal to dexamethasone. Humulene is a known and effective analgesic when consumed tropically, orally or by aerosol (Gupta, 2016). At a retention time of 23.694 minutes, humulene was also found at concentration of 1.79 % in *Artemisia absinthium* leaf extract by Mohiuddin *et al*. (2015), who also stated that it is an insect repellant.

In the sixth peak, megastigma-7(E),9, 13-triene was found at a retention time of 22.175 minutes. We did not identify this compound when we used acetone as a solvent (Onyeije and Okop, 2020). megastigma-7(E),9, 13-triene. Mohiuddin *et al*. (2015) identified, megastigma-7(E),9, 13-triene in *Artemisia absinthium* and stated that the compound is an alkene that has anticancer, antitumor and is a memory enhancer and expectorant.

In peak 7, eudesdma-4(14),11-diene was found at retention time of 22.390 minutes at a concentration of 5.70%. Similar compound was identified by us when we used acetone as a solvent. However, the retention time was 21.303 minutes and the mass peak was 69 and not 78 recorded in the present work. Eudesdma-4(14),11-diene extracted from plant extract by Sun *et al*. (2005) reportedly showed glucose consumption activity with an IC value of 10.7 microg/mL in a C2C12 muscle cell assay. The MIC value of this compound (100 mg/kg) in a db/db mice model was found to be equivalent to that of metformin in vivo. Eudesma-4(14),11-diene is a selinene

having a decahydronapthalene skeleton substituted by a isopropenyl group at number 7 position, a methyl group at position 4a and a methylidene group at position 1. It functions as a plant metabolite. Eudesma-4(14),11-diene has been documented to has antioxidant, antimicrobial and antiradical activities (Sacchetti *et al.* (2005). It is also a constituent of alcoholic beverage. Ye *et al*. (2012) also stated that eudesma-4(14),11-diene is used as a flavouring agent in yoghourt.

Azulene was observed in peak 8 with a characteristic's retention time of 22.700 minute and mass peak of 64. This compound has been found to exhibit strong anti-inflammatory activity by Guarrera *et al*. (2001). Its antifungal activity has also been confirmed (Rahman *et al*., 2014). Epsilon-murolene, a sesquiterpene, was observed in peak 9 at a retention time of 23.233 minutes and characteristics mass peak of 64. Although literature is scanty on biological activity of epsilon murolene, Abass (2018) has reported epsilon-muurolene as a potent antibacterial active compound. According to Sasikala *et al*. (2019), 3-Cyclopentene-1-ethanol, 2,2,4-trimethyl- which was resolved at retention time of 27.383 minutes exhibited varying levels of antimicrobial activity against 26 investigated bacteria. In peak 10 and at a retention time of 25.700 minute, di-t-butylacetylene was observed in the chromatogram. Antifungal and antibacterial activities of this compound has been reported by Rizwana (2018). 2,6,6-Trimethyl-3-(phenylthiol) cyclohep-4-enol was found in acetone/ethanol extract of *Piper guineense* leaves at a retention time of 25.700 minutes. The mass peak was 144 and its molar mass is 138 g/mol. This compound was not found in acetone extract of the same plant leaves. Literature is scanty on the biological activity of this compound indicating that there is need to carry out further study.

Hexadecanoic acid (palmitic acid), identified in line 13 of the spectrum (at a retention time of 31.725 minutes) is a long-chain fatty acid with a 16-carbon backbone and has been confirmed to exhibit remarkable antioxidant, antitumor, anti-inflammatory, antibacterial and anti- fungal activities (Vasudevan *et al*., 2012). Nanodecanoic acod and octadecanoic acid were detected in peaks 13 and 14 at retention time of 32.267 and 33.550 minutes respectively. Octadecanoic acid has been identified to have a strong binding affinity to MMP-2, hence enhancing its role as a proapoptotic factor in emancipation of inflammation apart from inducing apoptosis (Manivannan *et al*., 2017). Similarly, octadecanoic acid (stearic acid) has been reported to exhibit antibacterial and antifungal activities (Agoramoorthy *et al*., 2007). In our previous study, we did not detect octadecanoic and nonadecanoic acid in GCMS of acetone extract of *Piper guineense* leaves (Onyeije and Okop, 2020) indicating that the role of solvent in extraction of phytochemical is significant.

Piperidine (identified in peak 19 at retention time of 40.329) is known to be active as antibacterial, analgesic and anti-inflammatory agent (Mohammed *et al*., 2016). It is a heterocyclic amine with six-membered ring containing five methylene bridges (–CH_2–) and one amine bridge (–NH–). It is a colourless liquid with objectionable odour. Piperidine is also a good solvent for acid and base. Piperidine and its derivatives are ubiquitous building blocks in pharmaceuticals and fine chemicals. In acetone extract of *Piper guineense* leaves, piperidine was also detected at a retention time of 40.467 minutes and at a concentration, approximately the same. Piperidine is also known for its strong insecticidal properties (Bakhite *et al*., 2017)

Some compounds whose activities and detail chemical properties have not been fully established were also detected. These included **,**5-dihydrobenzo[1,2-c:3,4-c']bis[1,2,5]oxadiazole, (retention time, 39.558 minutes) 3,7-diacetamidophenoxathin (retention time, 40.4245 minutes) and 4-(6-methoxy-3-mythyl-2-benzofuran)-3-buten-2-one (retention time, 43.603 minutes).

4.0 Conclusion

Equal mixture of ethanol and acetone was used to extract phytochemicals from *Piper guneense* leaves. GCMS analysis of the extract indicated the present of different classes of compounds. Some of them were similar in identity (but different in concentration and retention time) to those obtained from acetone extract. New compounds that were not identify in acetone extract were also found. Therefore, the GCMS analysis of ethanol/acetone extract of *Piper guineense* leaves indicated the presence of compounds that were not identified through other solvents.

5.0 References

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