Chemical Information from GCMS Analysis of Acetone Extract of *Piper guineense* Leaves. Part 1

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Received 25 June 2020/Accepted 29 July 2020/Published online: 30 July 2020

Abstract *Applications of plant leaves for various purposes is based on its chemical constituents which may include proximate, elemental, phytochemical, toxicant, amino acid and other toxicants. Knowledge of phytochemical constituents is significant for their pharmaceutical/medicinal values. This study was carried out to investigate the chemical constituents of acetone extract of Piper guineense leaves through phytochemical screening and GCMS analysis. Results obtained from phytochemical screening indicated the major constituents (those whose concentrations were greater than 1%) to include ,6-dimethyloxazolo(5,4-c)pyridazin-4-amine (31.80 %), 3-(1-methylethyl)-cyclohexene (20.99 %), 4-methoxy-N-(4-nitrobenzyl)-benzamide (12.82 %), alpha bisabolene (7.33%), 1,2,3,4,4a,5,6,8a-octahydro-7-methyl-4-methylene-1-(1-methylethyl)-napthalene (4.42 %), 3,7-diacetamidophenoxathin (4.10 %), 1,3,3-trimethyl tricyclo{2,2,1]heptane (2.98%), 3H-indazol-3-one (2.11%), 1H-indene,octahydro-1,7a-dimethyl-4-(1-methylethenyl)-1,4-methano-1H-indene (1.98%), piperidine (1.97%),2,4-disopropenyl-1-methyl-1-vinyl (1.70%), n-hexadecanoic acid (1.68%), eudesdma-4[14],11-diene (1.27%). The pharmaceutical values of the identified constituents were also analsed. The study reveals that acetone extract of Piper guneense contains constituents that are not visible with some other solvents*

Key Words: *Piper guineense leaves, Chemical constituents, phytochemicals, GCMS, phytochemical screening*

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1.0 Introduction

Piper guineense is an erect herbaceous climbing liana native to tropical Africa, *P. guineense* fruits are widely applied externally as a counter-irritant or in a stimulating ointment, internally as a stomachic and carminative.The leaves are useful in the treatment of wounds while the stems and twigs are for the treatment of coughs and bronchitis (Owolabi *et al*. 2013). Food and food materials can be assessed based on its chemical composition (Ekop and Eddy, 2006). According to Imo *et al*. (2018), *Piper guineense* seeds have higher percentage of dry matter (94.03±0.21), crude lipid (4.06±0.12) and carbohydrates (65.46±0.85) than the leaves while the leaves have higher percentage moisture (6.11±0.01), protein (15.17±0.39), crude fibre (20.99±0.16) and ash (11.98±0.03) than the seeds. Several extract of *Piper guineense* have been found to exhibit pharmaceutical and medicinal values. For example, methanol extract of *Piper guineense* was found to offered protection against infection that is comparable to that of Livolin forte with better efficacy when pre-treated with 400 mg/kg for 14 days prior to CCl_4-exposure (Oyinloye *et al*., 2017). Ekudayo *et al*. (1988) identified elemicin as the major essential oil constituent of the plant and stated that the plant has significant medicinal applications. Olonisakin *et al*. (2006) identified (1s)-(-1)-ÃŽÂ²-pinene (43.9%), D-Limonene (7.7%), caryophyllene (6.9%), car-2-ene (5.4%) and 1,6,10-dodecetrien-z-ol, 3, 7, 11-trimetyl (2.9%) and found that they did not display any antimicrobial activity against *Escherichia coli , Serratia , Salmonella typhi , Klebsiella sp., Citrobacter and Pseudomonas aeruginosa* due to the solvent he used. Chinwendu *et al*. (2016) identified alkaloids (0.86%), HCN (8.87%), saponins

(1.87%) and phenols (0.66%) in ethanol extract of *Piper guineense* leaves while Ebenso *et al*. (2008) also identified the presence of alkaloids, tannins, saponins, flavonoids, hydrogen cyanides and phenols in ethanol extract of the leaves. They observed that, *Piper guineense* (Uziza leave) contains some considerable amount of anti-nutrients which have medicinal benefits.

GCMS analysis of plant extract has been found to be one of the most powerful tool that is useful for identifying chemical constituents of plants (Eddy *et al*., 2011a,b; Ikpeazu *et al*., 2020). Ojinnaka *et al*. (201,6) identified 22 peaks from the GCMS spectrum of ethanol extract of *Piper guineense* leaves and found that the spectrum was dominated by acids and hydrocarbon while alcohol and ester were the least constituents. Recent study conducted by Usaman *et al*. (2020) indicated that the component extracted from plant parts depends on the type of solvent. However, most studies on *Piper guineense* leaves are done with aqueous and ethanol extract. Therefore, the present study is aimed at identifying the chemical constituents of acetone extract of *Piper guneense* leaves using 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 acetone solution. The solvent was recovered using cold extractor, leaving behind, acetone extract of *Piper guineense* leaves.

The produced extract was used for GCMS analysis using spectroscopically pure acetone solvent. 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), syring 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 presents the GCMS of acetone extract of *Piper guineene* while chemical names, retention time, molar mass and percentage concentrations of compounds deduced from each peak in the spectrum are recorded in Table 1. Fig. 2 shows the mass spectrum of the compounds.

The chemical constituents observed from the GCMS spectrum of the plant leaves were 3,6-dimethyloxazolo(5,4-c)pyridazin-4-amine (31.80 %), 3-(1-methylethyl)-cyclohexene (20.99 %), 4-methoxy-N-(4-nitrobenzyl)-benzamide (12.82 %), alpha bisabolene (7.33%), 1,2,3,4,4a,5,6,8a-octahydro-7-methyl-4-methylene-1-(1-methylethyl)-napthalene (4.42 %), 3,7-diacetamidophenoxathin (4.10 %), 1,3,3-trimethyl tricyclo{2,2,1]heptane (2.98%), 3H-indazol-3-one (2.11%), 1H-indene,octahydro-1,7a-dimethyl-4-(1-methylethenyl)-1,4-methano-1H-indene (1.98%), piperidine (1.97%),2,4-disopropenyl-1-methyl-1-vinyl (1.70%), n-hexadecanoic acid (1.68%), eudesma-4[14],11-diene (1.27%), tricosenoic acid (0.77%), alpha cubebene (H-ctclopenta[1,3]cyclopropal[1,2]benzene (0.71%), 2,6,6-trimethyl-3-(phenylthio)cyclohept-4-enol

(0.69%), bicyclo[7,2,0]undec-4-ene,4,11,11-trimethyl-8-methylene (caryophyllene (0.54%), 1H-cycloprop€azulen-4-ol,decahydro-1,1,4,7-tetramethyl-globulol (0.47%) and 2,6-dimethoxytoluene (0.30%).

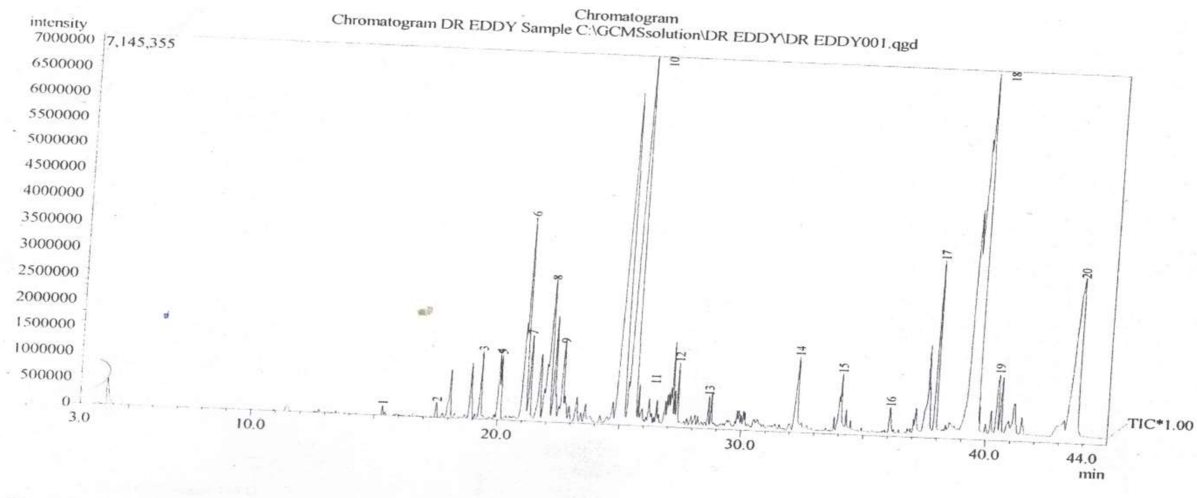
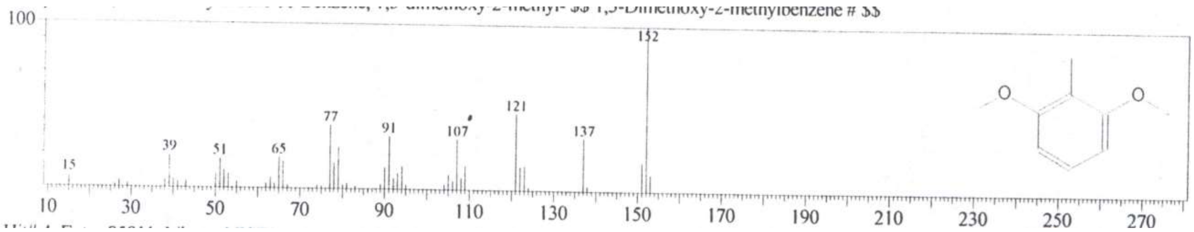


Fig. 1: GCMS of acetone extract of *Piper guineense*

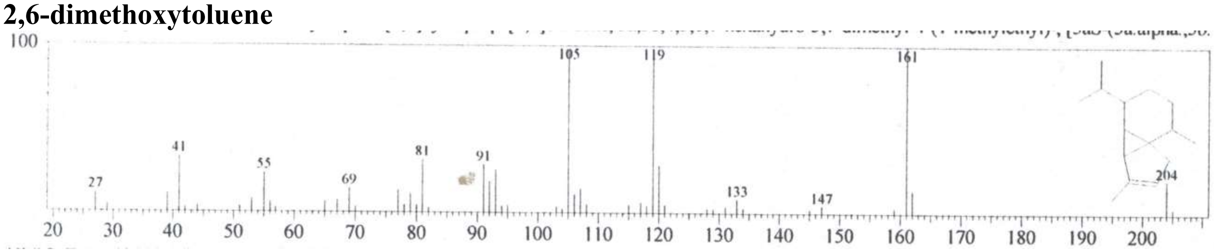
Table 1: Characteristics of compounds in acetone extract of *Piper guineense* leaves

Line No	Name	Mass peak	Retention time (minute)	Molar mass (g/mol)	Concentration (%)
1	2,6-dimethoxytoluene	32	15.333	152	0.30
2	alpha cubebene (H-ctclopenta[1,3]cyclopropal[1,2]benzene	40	17.517	204	0.71
3	2,4-disopropenyl-1-methyl-1-vinyl	62	19.308	204	1.70
4	1,3,3-trimethyl tricyclo{2,2,1]heptane (cyclofenchene)	45	20.033	136	2.98
5	bicyclo[7,2,0]undec-4-ene,4,11,11-trimethyl-8-methylene (caryophyllene)	65	20.108	204	0.54
6	Cis alpha bisabolene	82	21.133	204	7.33
7	eudesma-4[14],11-diene	69	21.308	204	1.27
8	1,2,3,4,4a,5,6,8a-octahydro-7-methyl-4-methylene-1-(1-methylethyl)-napthalene (gamma murolene)	73	22.125	204	4.42
9	1,2,3,5,6,7,8,8a-octahydro-1,4dimethyl-7-(1-methylethenyl)-azulene	74	22.65	204	1.37
10	3-(1-methylethyl)-cyclohexene	114	25.658	124	**20.99**
11	1H-cycloprop(e) azulen-4-ol,decahydro-1,1,4,7-tetramethyl-globulol	72	25.775	222	0.47
12	1H-indene,octahydro-1,7a-dimethyl-4-(1-methylethenyl)-1,4-methano-1H-indene	80	27.375	204	1.98
13	2,6,6-trimethyl-3-(phenylthio)cyclohept-4-enol	72	28.65	262	0.69
14	n-hexadecanoic acid	89	32.283	256	1.68
15	Tricosenoic acid	94	34.067	352	0.77

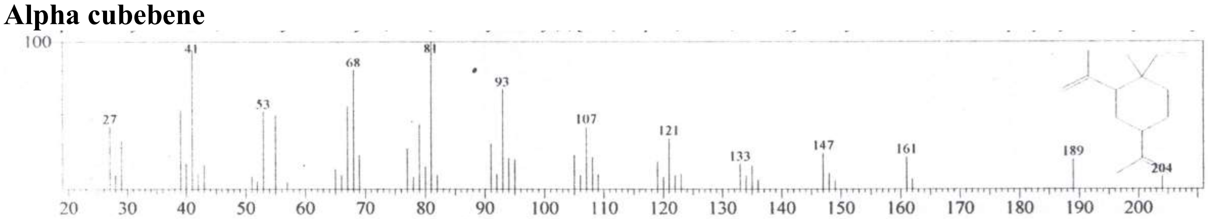
16	3H-indazol-3-one	61	36.075	134	2.11
17	4-methoxy-N-(4-nitrobenzyl)-benzamide	139	37.95	286	12.82
18	3,6-dimethyloxazolo(5,4-c)pyridazin-4-amine	153	39.667	164	**31.80**
19	piperidine	130	40.467	285	1.97
20	3,7-diacetamidophenoxathin	172	43.75	314	4.10



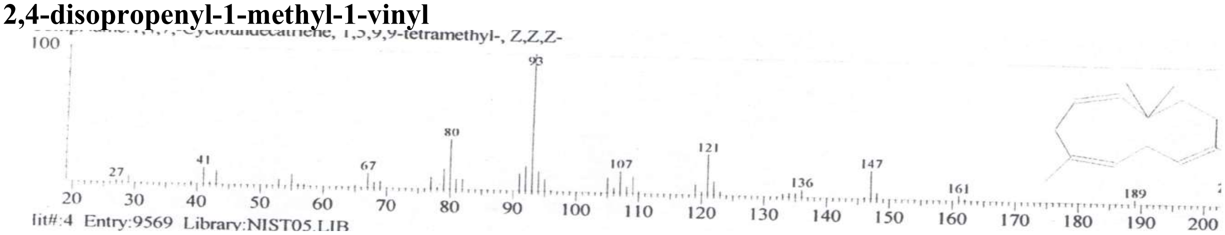
2,6-dimethoxytoluene



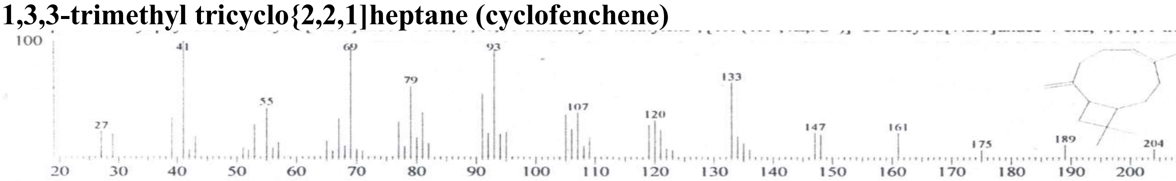
Alpha cubebene



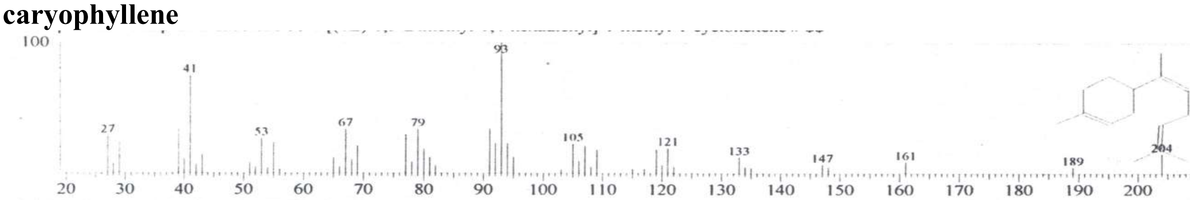
2,4-disopropenyl-1-methyl-1-vinyl



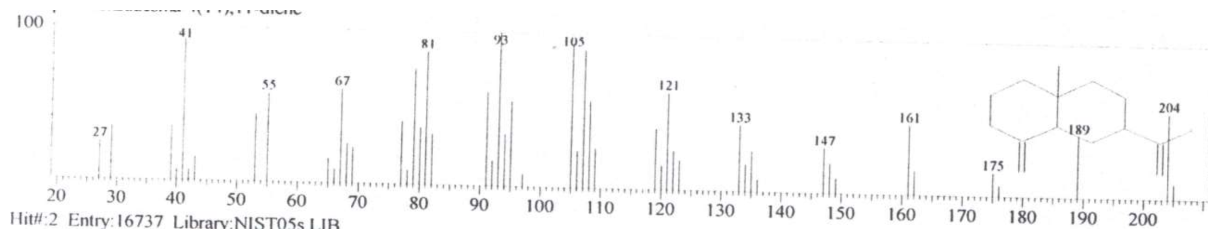
1,3,3-trimethyl tricyclo{2,2,1]heptane (cyclofenchene)



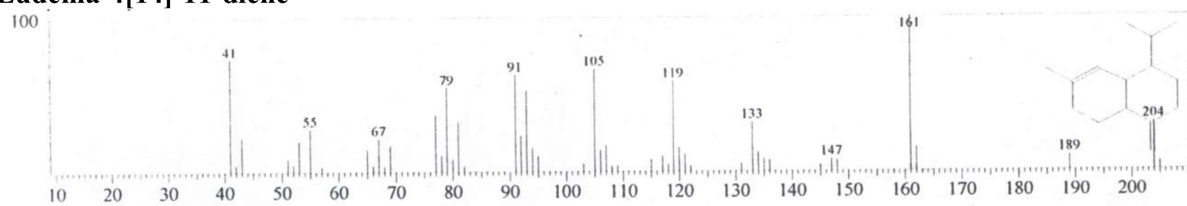
caryophyllene



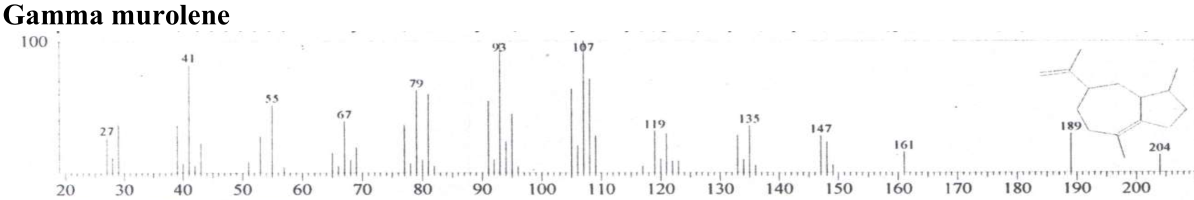
Cis-alpha bisabolene



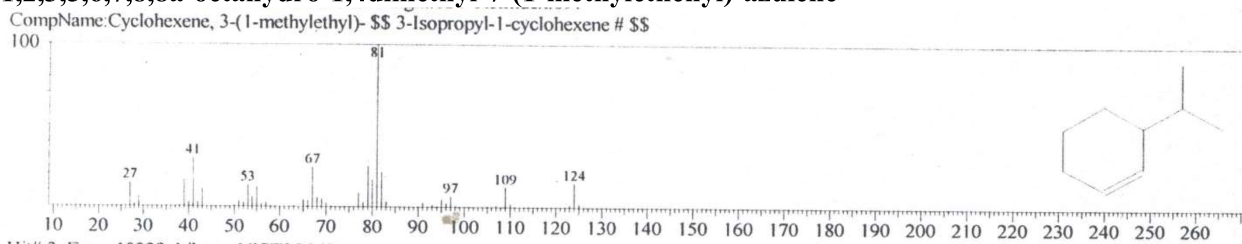
Eudema-4[14]-11-diene



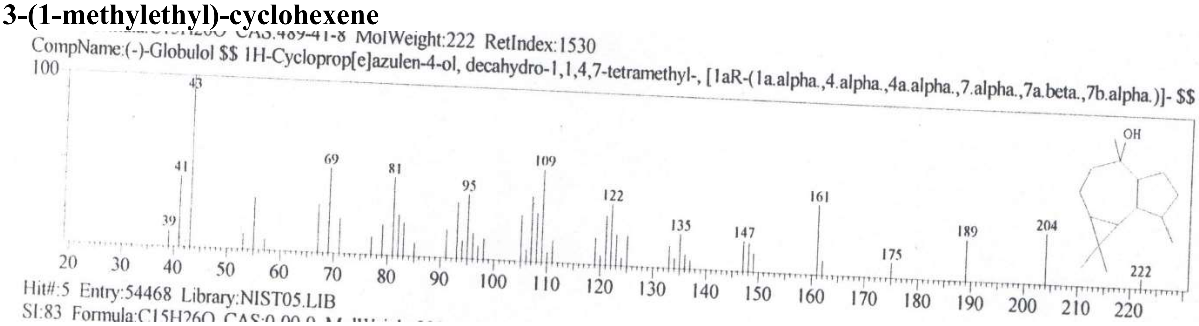
Gamma murolene



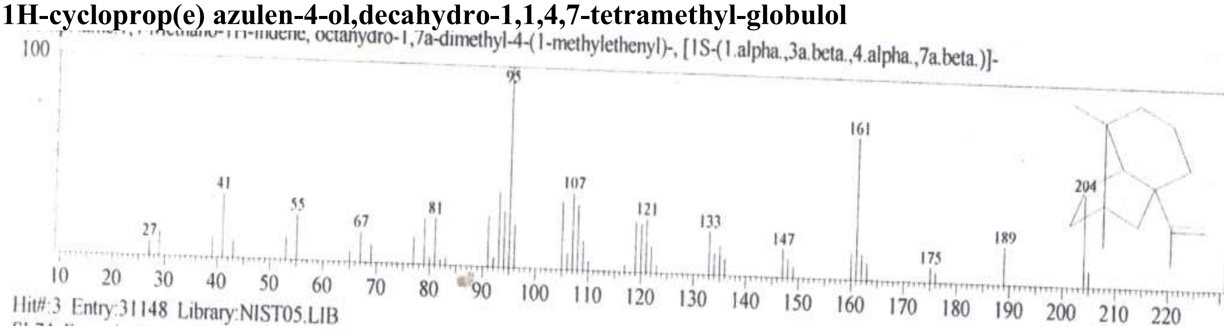
1,2,3,5,6,7,8,8a-octahydro-1,4dimethyl-7-(1-methylethenyl)-azulene



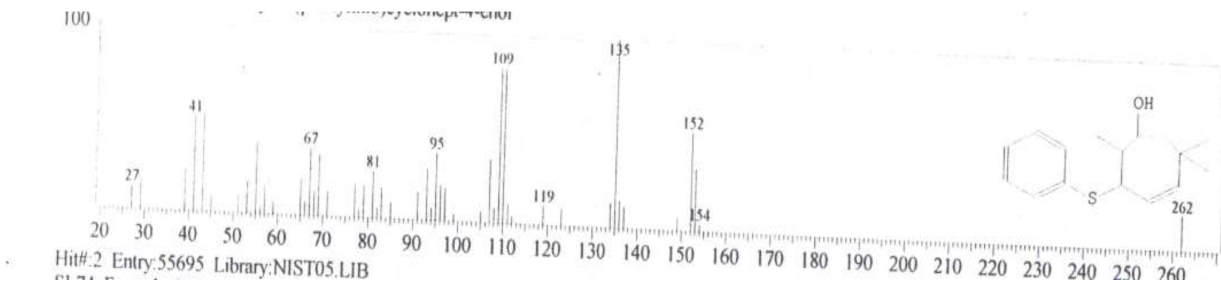
3-(1-methylethyl)-cyclohexene



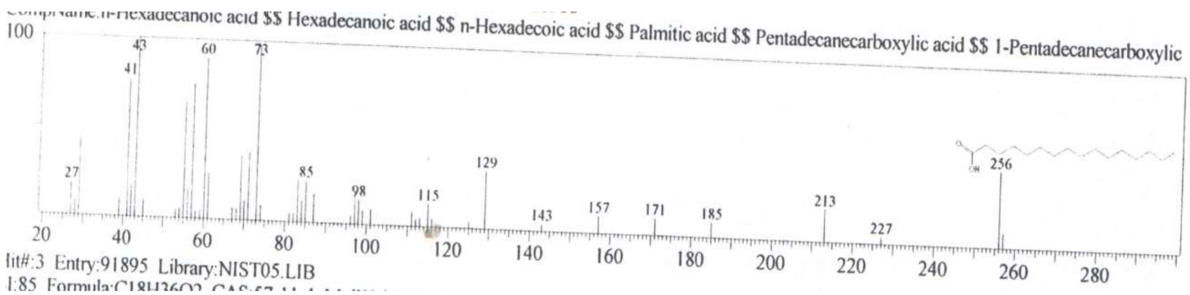
1H-cycloprop(e) azulen-4-ol,decahydro-1,1,4,7-tetramethyl-globulol



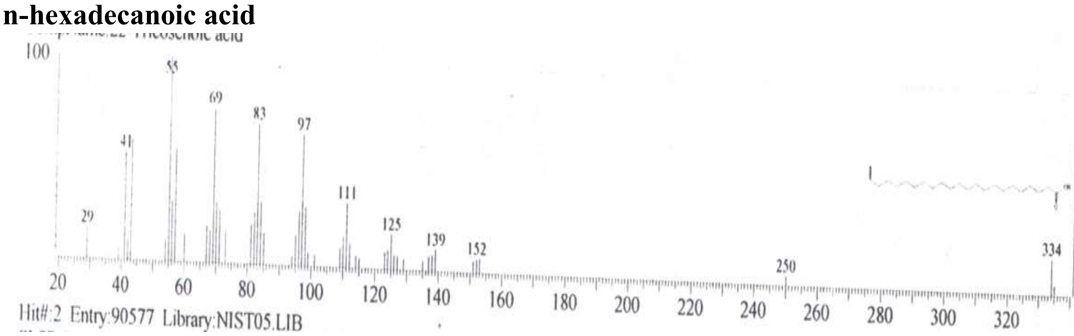
1H-indene,octahydro-1,7a-dimethyl-4-(1-methylethenyl)-1,4-methano-1H-indene



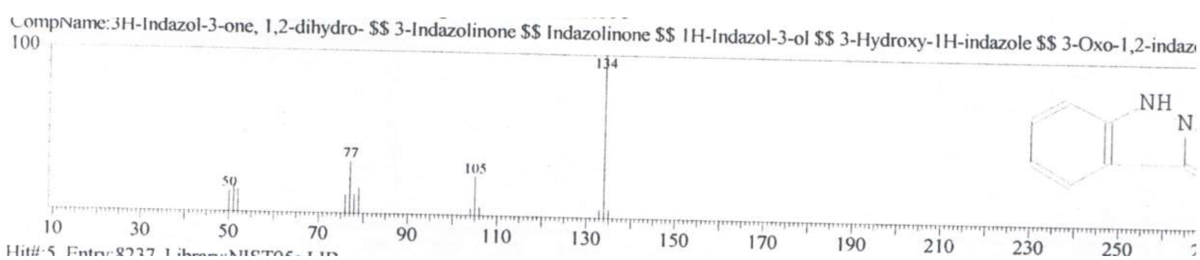
2,6,6-trimethyl-3-(phenylthio)cyclohept-4-enol



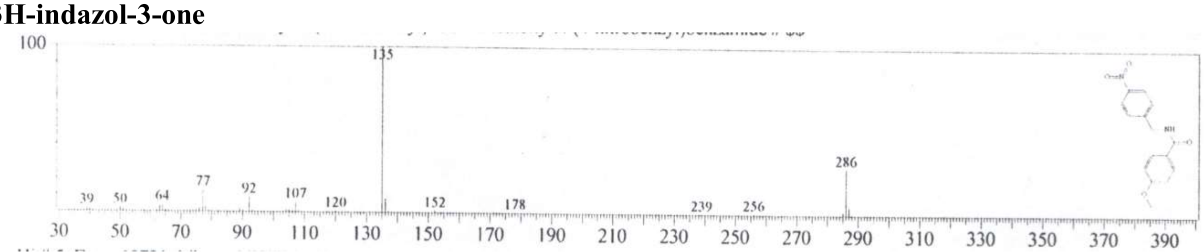
n-hexadecanoic acid



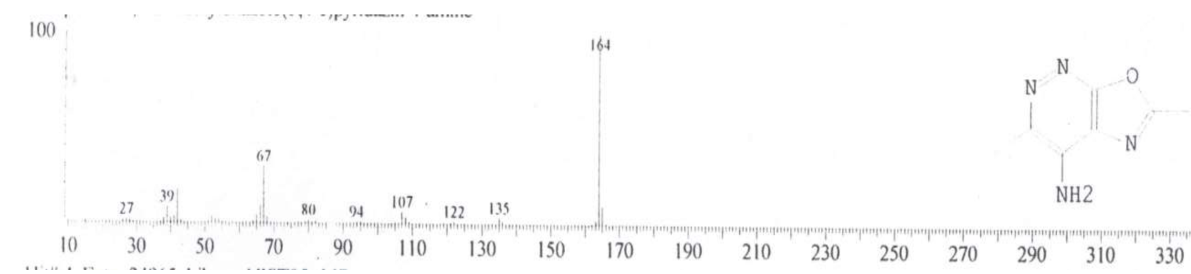
Tricosenoic acid



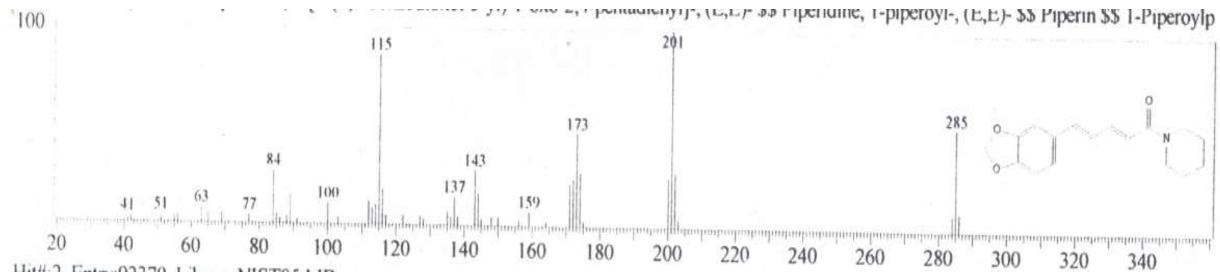
3H-indazol-3-one



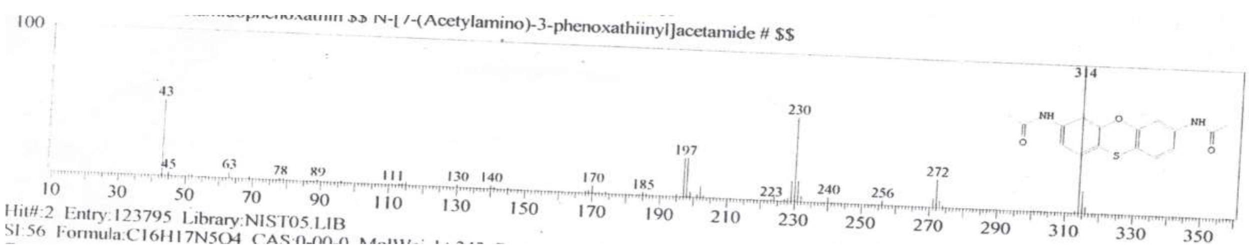
4-methoxy-N-(4-nitrobenzyl)-benzamide



3,6-dimethyloxazolo(5,4-c)pyridazin-4-amine



Piperidine



3,7-diacetamidophenoxathin

Fig. 2: Mass spectrum and chemical structures of compounds in acetone extract of *Piper guneense*

The mass spectrum of 3,6-dimethyloxazolo(5,4-c)pyridazin-4-amine was shown in Fig. 2. The compound is also call octocrinsghpfl-uhfffaoysa-N. Little is known of its bioactivity or other usefulness. However, the 5,4 derivatives of the compound has been implicated in the treatment of cancer tumor. Caryophyllene identified in line 5 (of the GCMS spectrum) contains several biological activities that are attributed to betacaryophyllene, such as anti-inflammatory, antibiotic, antioxidant, anticarcinogenicc and local anaesthetic. Piperidine (peak 19) has been confirmed to be active as antibacterial, analgesic and also exhibited anti-inflammatory activity (Mohammed *et al*., 2016). Alpha and beta cubebene were also reported in essential oils of *Annona salzmannii* and *A. pickelii* (*Annonaceae*) by Coataa *et al*. (2011) and were found to exhibit strong anti-bacterial activity. Boligon *et al*. (2012) also attributed antimicrobial activity of *Scutia buxifolia Reissek* leaves to the presence of cubebin. Haznedarogku *et al. (2001)* found that the essential oil of *Salvia tomentosa* contain 1,8-cineol (17%), β-caryophyllene (11%), cyclofenchene (10%) and δ-cadinene (6%). They attributed the antimicrobial activity of the essential oil to these constituents, which also inhibited the growth of tested Gram-positive and Gram-negative bacteria except for *Pseudomonas aeruginosa*. Alpha-cubebene, camphene, geraniol, limonene, myrcene, palmitic acid and sabinen were found to exhibit antioxidant (DPPH assay), anti-inflammatory (5-lipoxygenase assay), antimicrobial (disk diffusion) and anti-mosquito properties (insecticidal, larvicidal and repellency assays) by Naidoo *et al*. (2009). α-farnesene and bisabolene are known flavour ingredients and their catalytic hydrogenation gives the hydrocarbons, farnesane and bisabolane, respectively. These saturated derivatives are prospective industrial products as they have been singled out among the most promising biofuel candidates (Clarke, 2008). Sun *et al*. (2005) isolated four eudema and found that the compounds 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. Limberger *et al*. (2001) found gamma murolene (identified in ine 8) in *Blepharocalyx salicifolius* and linked it to some biological activities. Silva *et al.* (2009) also reported that gamma murolene is active against *Bacillus subtilis* and *Candida tropicalis*, including clinical strains. Tan *et al*. (2008) investigated. and obtained results which indicated that globulol (identified in line 11) is the main antimicrobial compound in the ethanol extract of *E. globulus* fruits. Hexadecanoic acid (palmitic acid), was identified in line 14 of the spectrum. It is a saturated long-chain fatty acid with a 16-carbon backbone which has been reported to have potential antioxidant, antitumor, anti-inflammatory, antibacterial and antifungal activities (Vasudevan *et al*., 2012).

4.0 Conclusion

GCMS of acetone extract of *Piper guineense* leaves reveals that the plant leaves contain organic acids, hydrocarbon, alcohol, ester, ketone and other aromatic compounds. Twenty compounds were identified and some of the compounds found in the spectrum differs from those reported by others. Most of the the identified compounds have industrial application and exhibit significant biological activities.

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