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Gas Chromatography–Mass Spectrometric Analysis of Bioactive Compounds Present in Ethanol Extract of *Combretum hispidum* (Laws) (*Combretaceae*) Root

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Abstract *Knowledge of bioactive components of a given plant materia; is essential in furnishing information on possible applications. Therefore, this study was carried out to identify bioactive components in ethanol extract of Combretum hispidum roots using GCMS analysis. Results obtained from GCMS analysis of ethanol extract of Combretum hispidum roots indicated the presence of benzoic acid N'-(4,4,5,5,6,6,6-heptafluoro-3-oxo-1-phenyl-hex-1-enyl)-hydrazide, 1-(4-methyl-6-methoxy-2-quinolyl)-3,3'-dimethyl-(4,5'-bipyrazol)-5-ol, trans -1,2-Diphenyl-1-chloro-2-methylthio-ethene, 3-acetyl-3-demethylthiocolchicine, 1-anthracene-carboxaldehyde, 5,8,8a,9,10,10a-hexahydro-2,3-dihydroxy-10a-methyl-4-(1-methylethyl)-6-(4-methyl-, 1H-benzimidazole, 2-(2,2-dimethylpropyl)-, 1,3-benzoxathiol-2-one, 5-hydroxy-4,7-dimethyl-6-nitro-, bis(ditrifluoromethyldiphosphino)sulfide, benzeneethenylamine, 3,4-dihydroxy-N-isopropyl-, voachalotine oxindole, acetate (ester), ethanone, 1-(3-chloro-5,6-dihydro-1,4-oxathiin-2-yl)-, benzenetridecanoic acid, 3-chloro-4-methoxy-, 3-methoxy-2-(9-methyldecyl)-5-(4-methylpentyl)phenyl ester, ethyl 4,4-dimethyl-5-oxo-tetrahydrofuran-3-carboxylate, N-(4-bromophenylsulfonyl)aziridine-2,2-dicarboxylic acid diethyl ester, 1,3,5-triazin-2-amine, N,N-dihexyl-4,6-bis(2-naphthalenylthio)-, 1[5'-(hydroxymethyl)furfuryl] pyrrolidine, terephthalic acid, butyl cycloheptyl ester, 1H-pyrazole, 4,5-dihydro-1,3-diphenyl-, succinic acid, 2,2,3,3,4,4,5,5-octafluoropentyl 1-cyclopentylethyl ester. Each of the identified compounds in the extract have been documented to exhibit active pharmaceutical/medicinal activities. It was concluded that the bioactive compounds support the use of C. hispidum roots in the treatment of diseases like cancer, anaphylactic shock, renal failure, diabetes and hypertension.*

Keywords: *Bioactive compounds*, *Combretum hispidum, gas chromatography, mass spectrometry, roots*

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1.0 Introduction

Since creation, medicinal plants have been used as curative agents for diseases in individuals and communities (Hoareau & Da Silva,1999; Edeoga *et al*., 2005). Traditional medicine has been a source of economic growth in developing countries (Agra *et al*., 2007).Nigeria is blessed with many medicinal plants which are used traditionally to treat different diseases. Recently, Nigeria government is considering integrating herbal medicine as a degree program into the tertiary institution curriculum.

Combretun hispidum is a climbing weed of the forest with trailing branches. It reproduces from the seeds and vegetatively from basal stumps. It has a cylindrical woody stem that is covered with short bristly hairs. The leaves are arranged oppositely. The leaves are oblong, elliptic, 10 – 25 cm long and 5 – 11 cm wide. It is a common climbing weed of the forest and savanna regions.

The medicinal potentials of *combretaceae* is widely reported in several literatures and are attributed to some of its chemical constituents (Atindehou *et al*., 2004; Muthu *et al*., 2006; Gansané *et al*., 2010). For example, Aderogba *et al*. (2012) isolated cardamonin (chalcone) from *C. apiculatum*. Ellagic acid derivatives have been isolated from *C. kraussii* (Chaabi et al., 2008). Isolation of combretastatins, a group of stilbenes, from *Combretum* species have also been reported (Fyhrquist *et al.*, 2006).Phytochemical analysis of the genus *Combretum* revealed the presence of triterpenes, flavonoids, lignans and non-protein amino acids, among others (Pietrovski*et al.,* 2006).Antidiabetic effects of the aqueous leaf extract of *C. micranthum* have been reported by Chika and Bello (2010). They found that at the extract's concentration of 100 mg/kg significant hypoglycemic and antidiabetic activity were observed when compared to the standard drug (0.6 mg/kg glibenclamide). Consequently, they recommended the use of aqueous leaf extract of *C. micranthum* for both type 1 and type 2 diabetes remediation in Northwestern Nigeria.The antibacterial activity of *C. molle* against *Staphylococcus aureus* and *Helicobacter pylori* at different extract concentrations have been ascertained by Njume *et al*. (2011) reported (Njume *et al*., 2011). Lall and Meyer (1999) also deduced from their study that *C. molle*-exhibited significant activity against *Mycobacterium. Closely* similar activity against protozoal has also been documented by Asres *et al.* (2001). *In vitro* anthelmintic activity of *C. molle* (Combretaceae) against *Haemonchus contortus* ova and larvae have also have also recorded significant activity (Ademola and Eloff, 2010).

GC-MS analyses have been used by several researchers to identify compounds in plants (Otuokere *et al*., 2016; Igwe *et al*., 2016; Yan-qun *et al*., 2013). In our previous publication we reported on the application of GC-MS in analysis of ethanol extract of *Combretum hispidum leaves* (Ikpeazu *et al*., 2020). The results obtained were comparable to those reported from other analytical methods. Therefore, the present study seeks to analyses ethanol extract of *Combretum hispidum root using GCMS*. The expected results shall provide baseline information on bioactive components of the plants that can pass through GCMS column. To the knowledge of the authors, literature is scanty on GCMS analysis of the root of *Combretum hispidum,*

Fig. 1: *Combretum hispidum* root

2. 0Materials and Methods

2.1. Plant sample

Fresh roots of *C. hispidum* were collected from Obi-Ngwa, Abia State Nigeria on 24th May, 2020. Sample of plant roots was identified by a Botanist at the Department of Plant Science and Biotechnology, College of Natural Sciences, Michael Okpara University of Agriculture, Umudike, Nigeria.

2.2. Extraction of crude extracts

The roots of C. *hispidum* were air dried at room temperature for 3 days. The dried roots were grounded using Wiley Mill Model No. 2 (Arthur H. Thomas Co., Philadelphia, USA). The powdered sample, which was in powdered form, was soaked in ethanol for 48 hours after which the ethanol was removed from the solution using Heidolph Rotavapor (Germany).

2.3. GC-MS analysis

The GC–MS analysis of bioactive compounds of *C. hispidum* roots extracts was done using agilent6890N gas chromatography equipped with an

auto sampler connected to an Agilent Mass Spectrophotometric detector. A micro-litre of sample was injected in the pulsed spitless mode onto a 30 ×0.25 mm ID DB 5MS coated fused silica column with a film thickness of 0.15 micrometer. Helium gas was used as a carrier gas and the column head pressure was maintained at 20 psi to give a constant of 1ml/min. Other operating conditions were preset. The column temperature was initially held at 55 ºC for 0.4 min, increased to 200 ºC at a rate of 25 ºC/mins, then to 280 ºC at a rate of 8 ºC/mins and to a final temperature of 300 ºC at a rate of 25 ºC/mins, held for 2 mins

2.4. Identification of chemical constituents

The molecular weight and structure of the compounds of test materials were ascertained by the interpretation of mass spectrum of GC-MS using the database of National Institute of Standard and Technology (NIST). The mass spectra of the unknown compounds were compared with the spectra of the known compounds stored in the NIST library. Concentrations of the identified compounds were obtained as average of height and area normalizations.

3.0 Results and Discussion

The GCMS of ethanol extract of *Combretum hispidum* roots is presented in Fig. 2 while the mass spectra are presented in Fig. 3. Observed retention time, mass peak and estimated concentrations deduced from the several peaks are presented in Table 1.

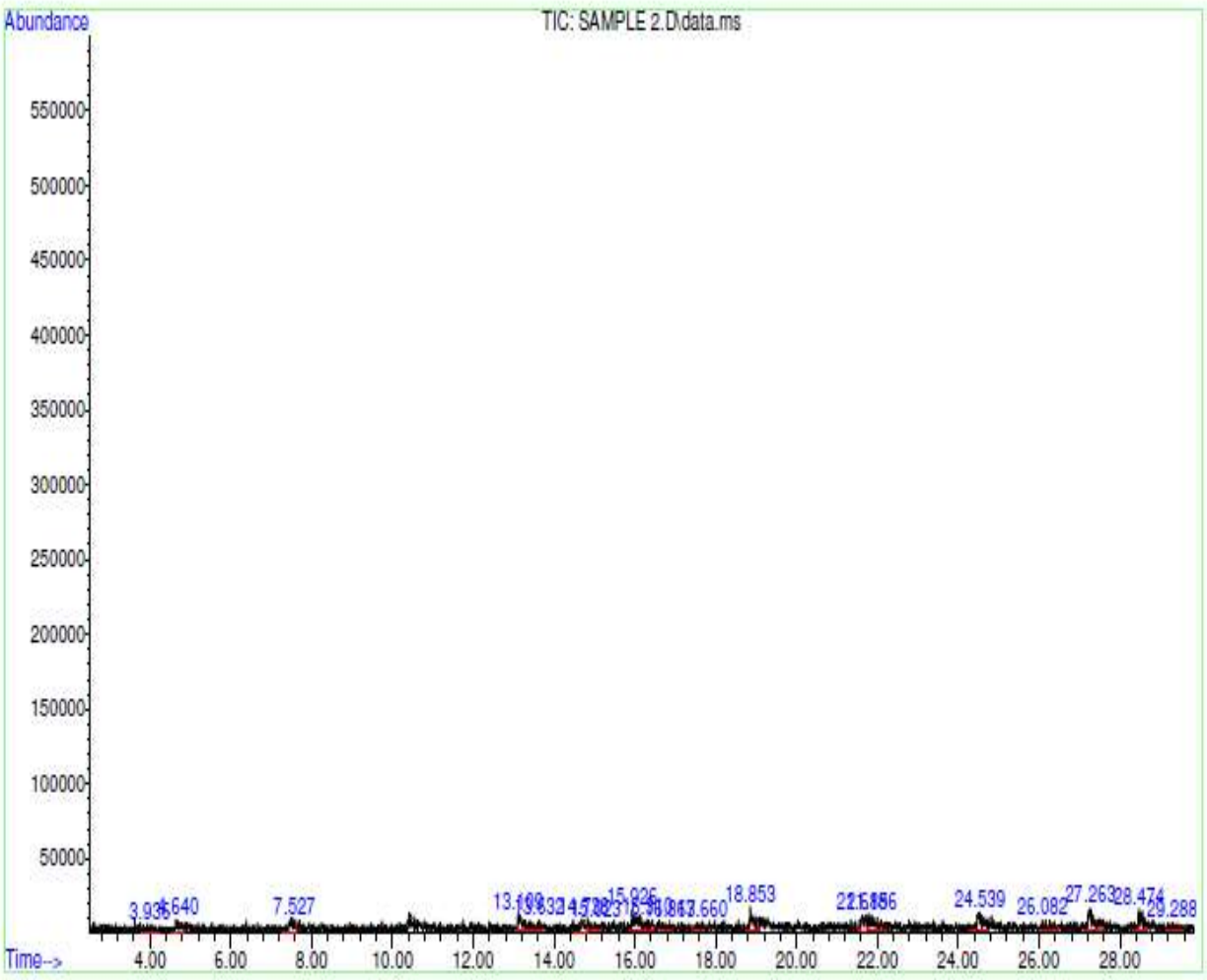
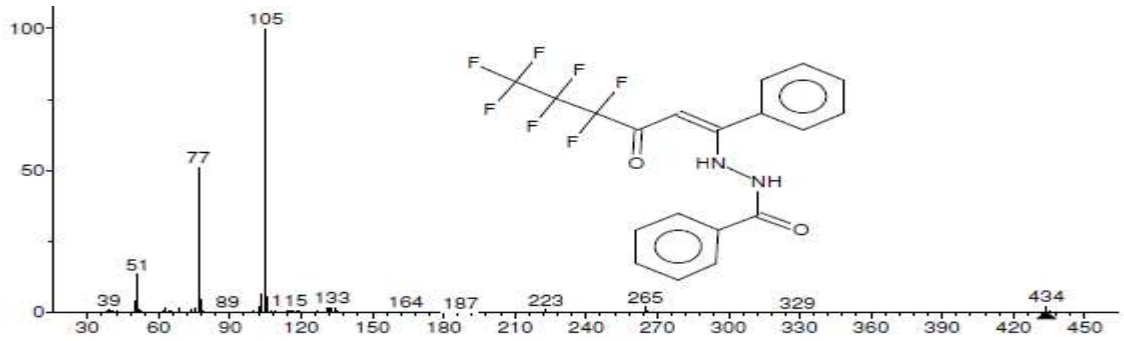


Fig. 2: Gas chromatogram of ethanol extract of *Combretum hispidum*roots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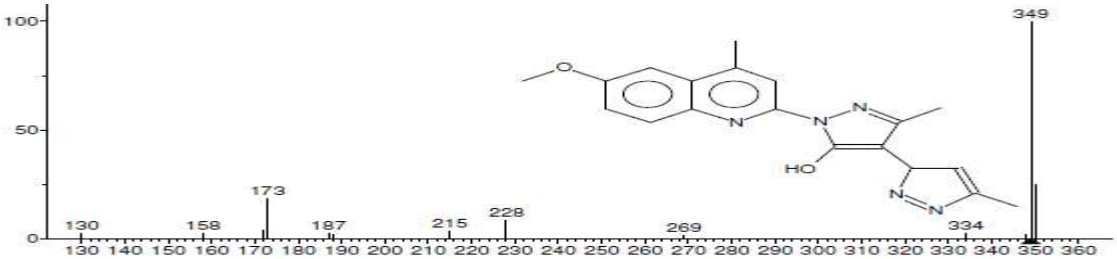
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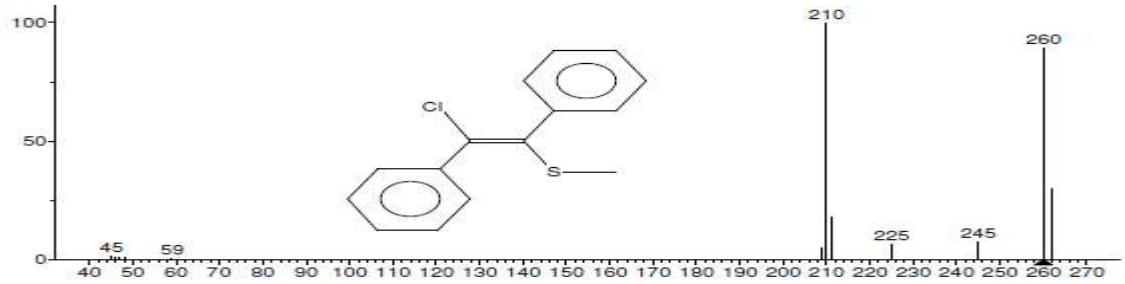
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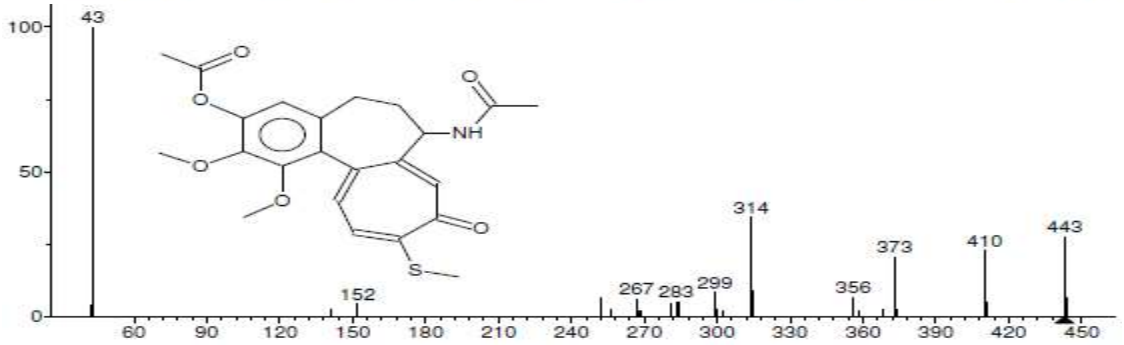
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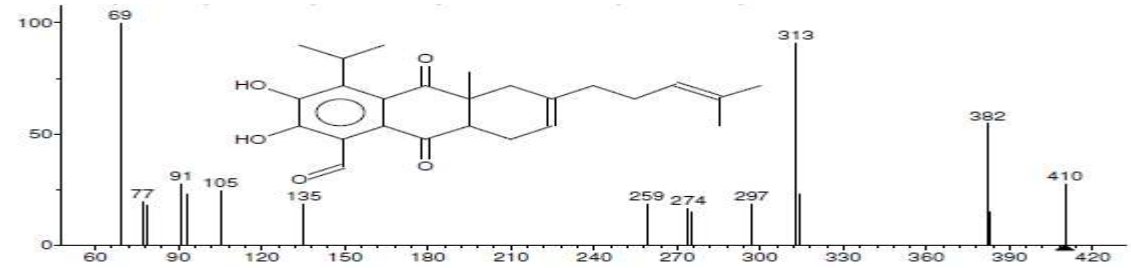
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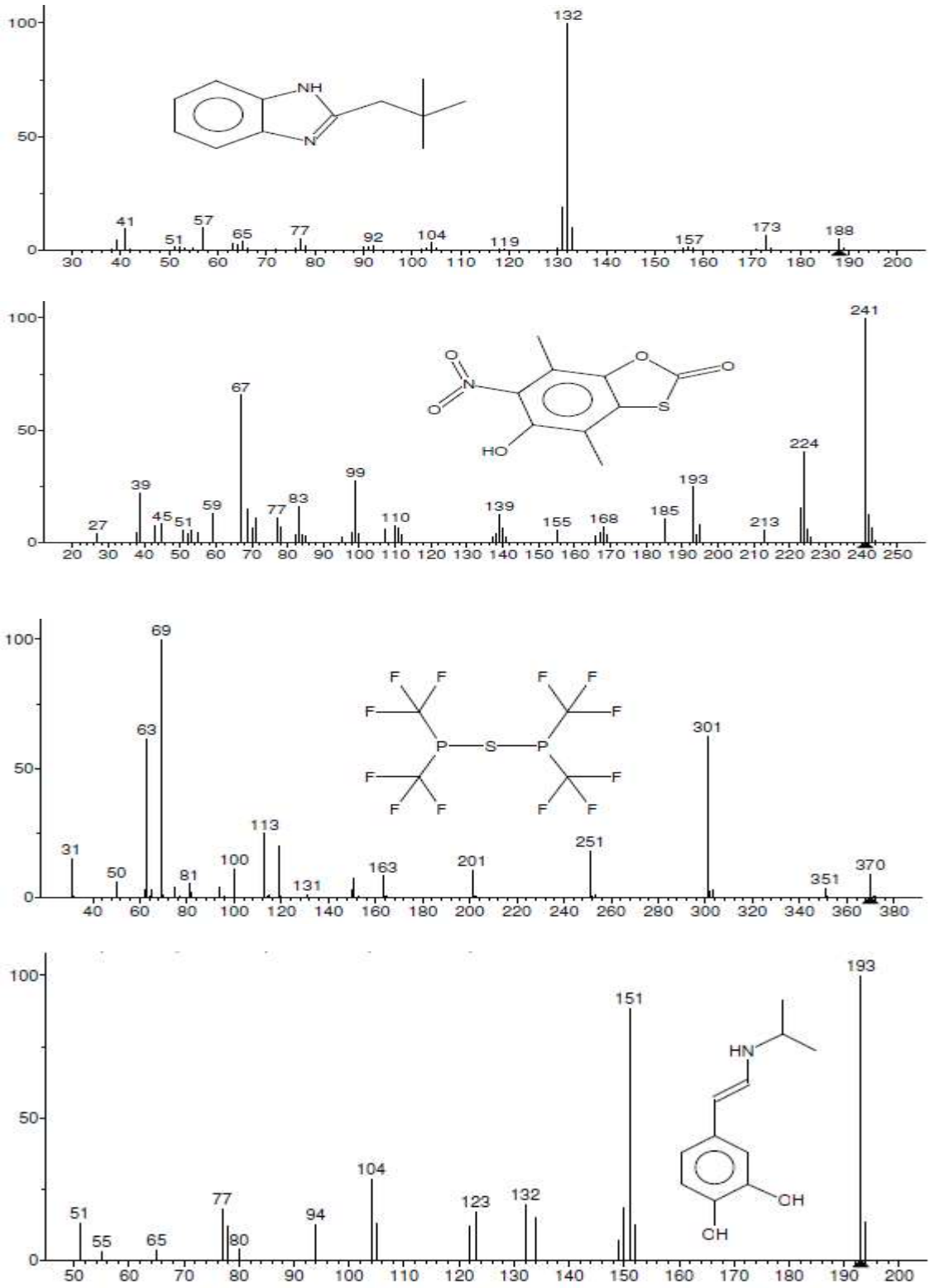
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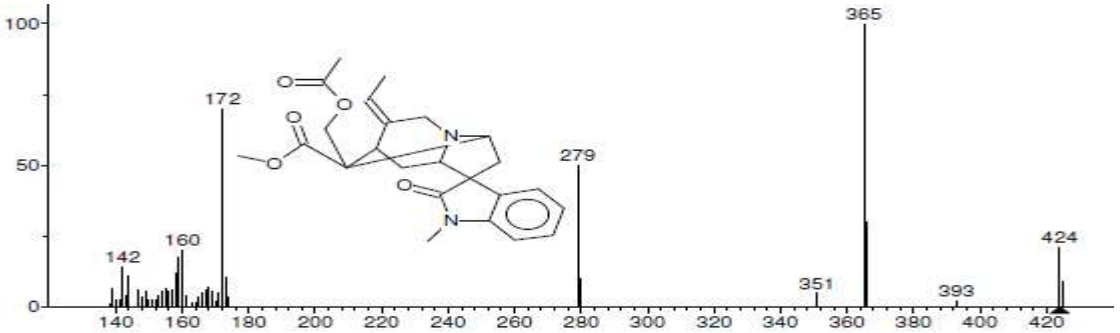
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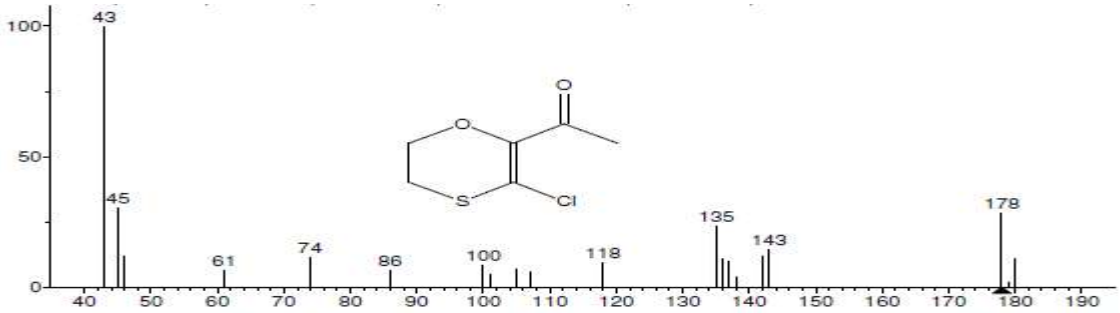
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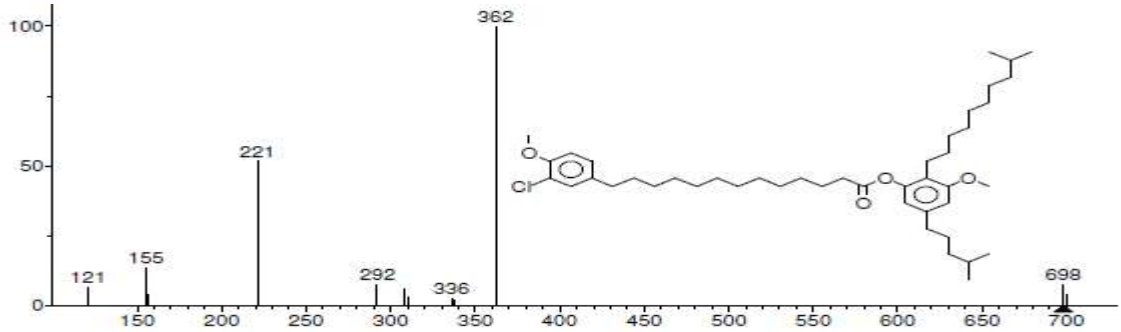
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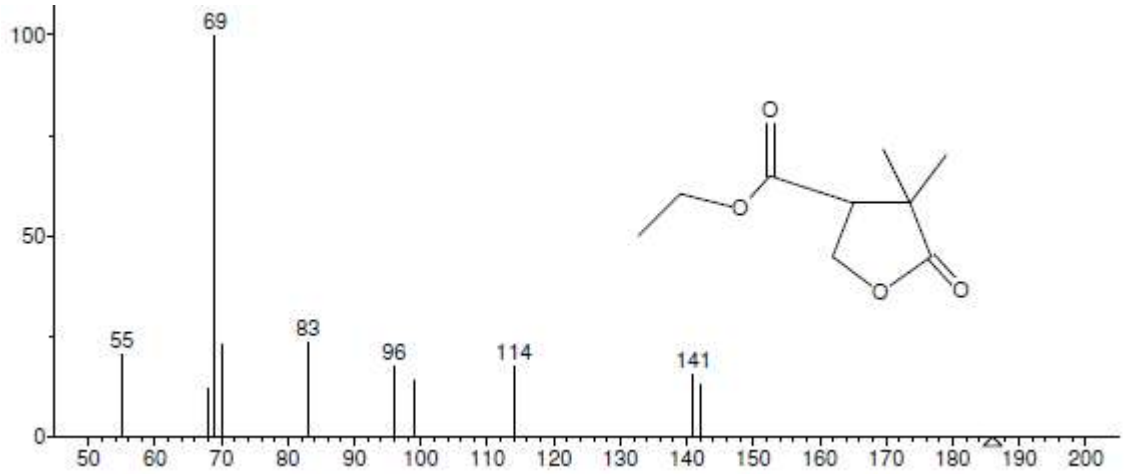
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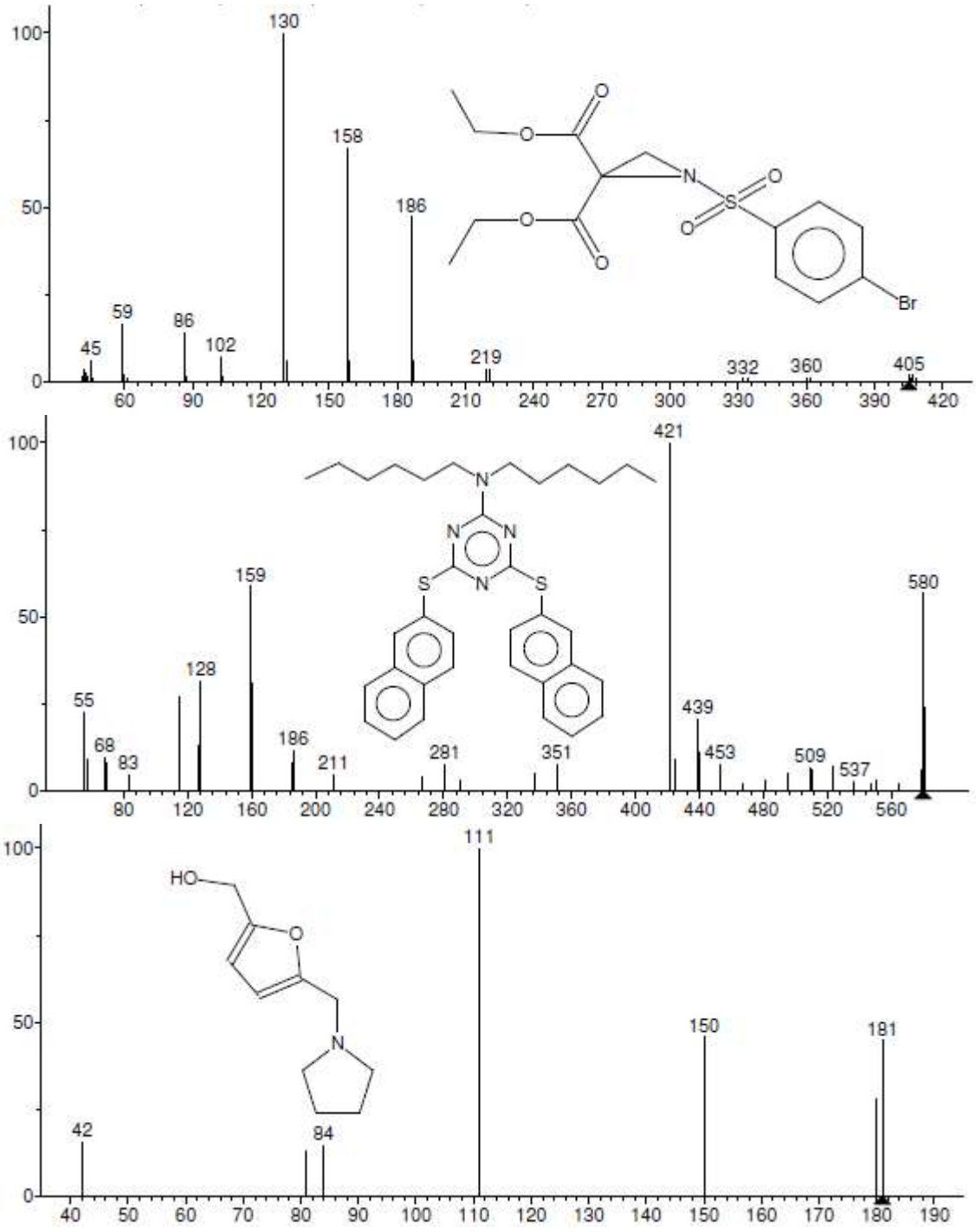
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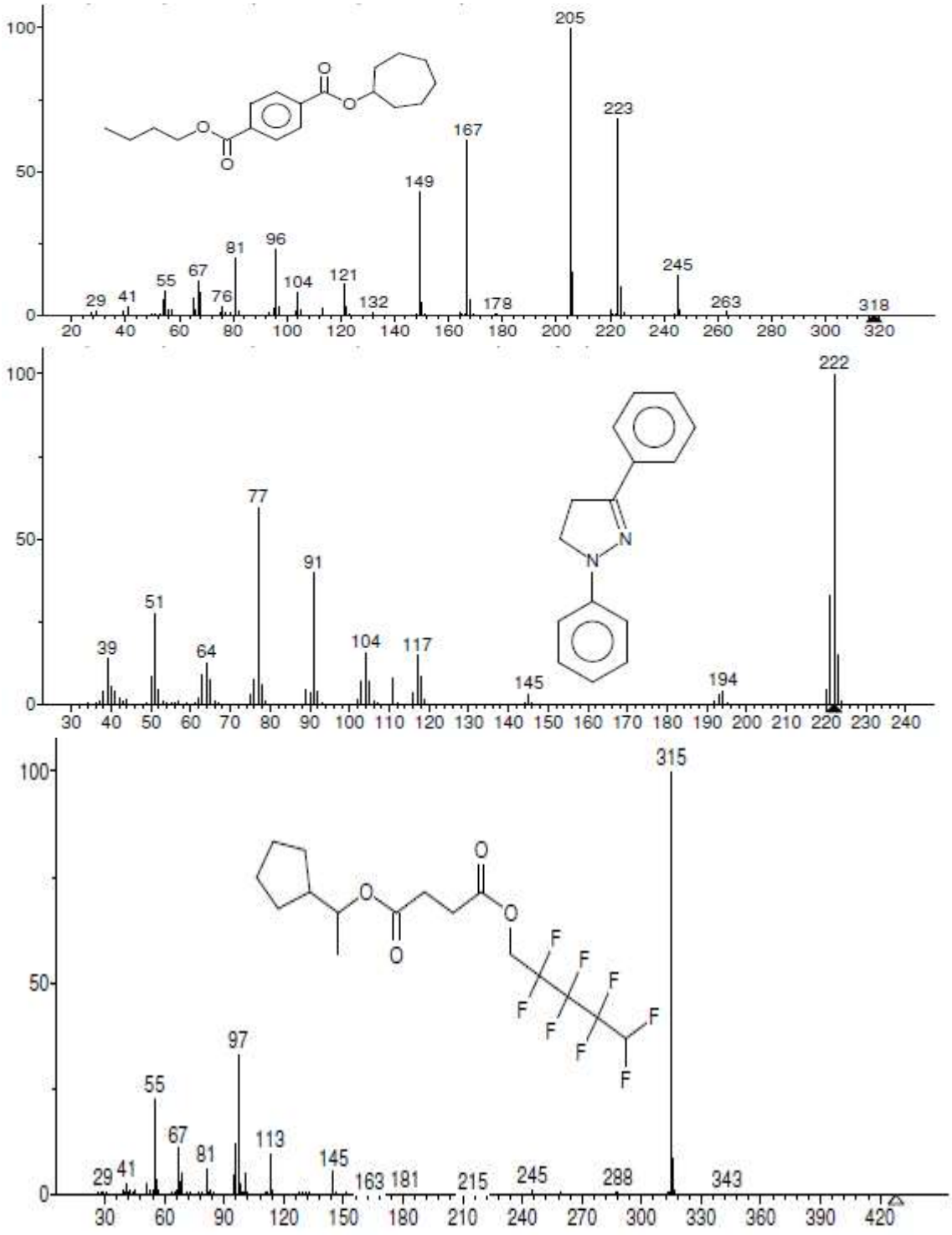

Fig, 3.: Mass spectra of ethanol extracts obtained from *C. hispidum* roots

Table 1: Bioactive compounds identified by GCMS analysis of ethanol extract of *C. hispidum root*

S/N	Compound	M.W (g/mol)	R.T (mins)	%	Bioactivity
1	Benzoic acid N'-(4,4,5,5,6,6,6-heptafluoro-3-oxo-1-phenyl-hex-1-enyl)-hydrazide	434.08	3.936	3.238	Arachidonic acid inhibitor
2	1-(4-Methyl-6-methoxy-2-quinolyl)-3,3'-dimethyl-(4,5'-bipyrazol)-5-ol	349.15	4.640	4.567	Catechol-O-methyl-transferase inhibitor Methyl-guanidine-inhibitor
3	Trans-1,2-Diphenyl-1-chloro-2-methylthio-ethene	260.04	7.527	5.681	Increase glutathione-s-transferase activity
4	3-Acetyl-3-demethylthiocolchicine	443.14	13.109	5.739	Arylamine-N-Acetyltransferase-Inhibitor
5	1-Anthracenecarboxaldehyde, 5,8,8a,9,10,10a-hexahydro-2,3-dihydroxy-10a-methyl-4-(1-methylethyl)-6-(4-methyl-	410.20	13.632	4.287	Not found
6	1H-Benzimidazole, 2-(2,2-dimethylpropyl)-	188.13	14.738	5.293	Not found
7	1,3-benzoxathiol-2-one, 5-hydroxy-4,7-dimethyl-6-nitro-	241.00	15.023	3.551	17-beta-hydroxysteroid dehy-drogenase-Inhibitor
8	: Bis(ditrifluoromethyldiphosphino) sulfide	369.90	15.926	9.922	Not found
9	Benzeneethenylamine, 3,4-dihydroxy-N-isopropyl-	193.11	16.310	3.203	Neuromuscular-Blocker
10	Voachalotine oxindole, acetate (ester)	424.19	16.863	3.402	Not found
11	Ethanone, 1-(3-chloro-5,6-dihydro-1,4-oxathiin-2-yl)-	177.98	17.660	3.202	Not found
12	Benzenetridecanoic acid, 3-chloro-4-methoxy-, 3-methoxy-2-(9-methyldecyl)-5-(4-methylpentyl)phenyl ester	698.50	18.853	6.245	Urinary-Acidulant Urine-Acidifier
13	Ethyl 4,4-dimethyl-5-oxo-tetrahydrofuran-3-carboxylate	186.08	21.618	4.559	Not found
14	N-(4-Bromophenylsulfonyl)aziridine-2,2-dicarboxylic acid diethyl ester	404.98	21.856	5.850	Inhibit Production of Tumor Ne-crosis Factor
15	1,3,5-Triazin-2-amine, N,N-dihexyl-4,6-bis(2-naphthalenylthio)-	580.26	24.539	7.712	Not found
16	1[5'-(hydroxymethyl)furfuryl] pyrrolidine	181.11	26.082	3.879	Not found
17	Terephthalic acid, butyl cyclohep-tyl ester	318.18	27.263	9.426	Increase Aromatic Amino Acid Decarboxylase Activity
18	1H-Pyrazole, 4,5-dihydro-1,3-	222.11	28.474	6.199	5-HT-Inhibitor

	diphenyl-				
19	Succinic acid, 2,2,3,3,4,4,5,5-octafluoropentyl 1-cyclopentylethyl ester	428.12	29.288	4.046	Succinic-Dehydrogenase-Inhibitor

Benzoic acid N'-(4,4,5,5,6,6,6-heptafluoro-3-oxo-1-phenyl-hex-1-enyl)-hydrazide has been reported as arachidonic acid-Inhibitor (Duke, 2019). Arachidonic acid and its metabolites have recently generated a heightened interest due to growing evidence of their significant role in cancer biology. Consequently, inhibitors of arachidonic acid which have originally been implicated in the treatment of inflammatory conditions and certain types of cardiovascular disease, have now significant documentations attracting attention as an arsenal against cancer ((Hyde, & Missailidis, 2009). The compound, 1-(4-Methyl-6-methoxy-2-quinolyl)-3,3'-dimethyl-(4,5'-bipyrazol)-5-ol is a known catechol-O-methyl-transferase inhibitor (Duke, 2019). Catechol-O-methyltransferase inhibitors are a class of medications that are used along with carbidopa-levodopa therapy in the treatment of symptoms of Parkinson's disease. They can extend the effectiveness of carbidopa-levodopa therapy, and allow for lower doses of carbidopa-levodopa (Connolly & Lang, 2014). The compound, 1-(4-Methyl-6-methoxy-2-quinolyl)-3,3'-dimethyl-(4,5'-bipyrazol)-5-ol has been identified as methylguanidine inhibitors (Duke, 2019). Methylguanidine is a suspected uraemic toxin that accumulates in renal failure. However, it also exhibits anti-inflammatory effects. Recent evidence suggests that methylguanidine significantly inhibits nitric oxide synthase activity and tumor-necrosis factor (TNF) release (NLM, 2020). This means that methylguandine can attenuate the degree of inflammation and tissue damage associated with endotoxic shock. The compound, trans-1,2-diphenyl-1-chloro-2-methylthio-etheneis known for increasing glutathione-s-transferase activity (Duke, 2019). Glutathione transferase activity plays a critical role in cellular detoxification against xenobiotics and noxious compounds as well as against oxidative stress. The compound, 3-acetyl-3-demethylthiocolchicine has been reported as arylamine-N-acetyltransferase-inhibitor (Duke, 2019). Deletion of arylamine N-acetyltransferase (NAT) decreases mycobacterial cell wall lipids, particularly the distinctive mycolates, and also increases antibiotic susceptibility and killing within macrophage of *Mycobacterium bovis* (Westwood, 2010).The compound, 1,3-benzoxathiol-2-one, 5-hydroxy-4,7-dimethyl-6-nitro- is an inhibitor of 17-beta-hydroxysteroid dehydrogenase-inhibitor (Duke , 2019). Among all endocrine therapies for the treatment of breast cancer, inhibition of estrogen biosynthesis is becoming an interesting complementary approach to the use of antiestrogens. The enzyme 17-beta-hydroxysteroid dehydrogenase-Inhibitor (17beta-HSD) plays a critical role in the biosynthesis of estradiol catalyzing preferentially the reduction of estrone into estradiol, the most active estrogen. Consequently, this enzyme is an interesting biological target for designing drugs for the treatment of estrogen-sensitive diseases such as breast cancer (Martin, *et al*., 2005). 1H-Pyrazole, 4,5-dihydro-1,3-diphenyl- has been reported as 5-hydroxytryptamine receptors (5-HT-Inhibitor) (Duke , 2019). 5-HT_3 antagonists are most effective in the prevention and treatment of chemotherapy-induced nausea and vomiting

4.0 Conclusion

The research paper has justified the traditional use of *Combretum hispidum* roots for the treatment of various health problems. The pharmacological potential of the extracts, fractions and compounds isolated from *Combretum hispidum* roots is a promising new scientific research topic for investigation. With respect to biochemical studies, there is an increasing need for further *in vivo* investigations of toxicity as well as for insights into the possible mechanisms involved. Therefore, new research findings could lead to greater safety and benefits to people who use these species to treat diseases, contributing to a better access to health care and thereby a better quality of life.

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