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GC–MS Analysis of Bioactive Compounds Present in Ethanol Extract of *Combretum Hispidum* (Laws) (Combretaceae) Leaves

O. V. Ikpeazu[1], I. E. Otuokere[2], K. K. Igwe[3]

[1]Department of Biochemistry, Abia State University, Uturu, Nigeria
[2]Department of Chemistry, [3]Department of Vet. Biochemistry and Animal Production,
[2,3]Michael Okpara University of Agriculture, Umudike, Nigeria

ABSTRACT

Phytoconstituents present in the ethanolic extract of Combretum hispidum leaves were explored by Gas Chromatography-Mass Spectrometry analysis. The compounds were identified by the gas chromatography coupled with the mass spectrometry. The molecular weight and structure of the compounds of Combretum hispidum leaves were ascertained by interpretation of the spectrum of GC-MS using the database of National Institute of Standard and Technology (NIST). GC-MS analysis of Combretum hispidum leaves revealed the presence of nineteen biological active compounds. The compounds are N-Tosyl-dl-3,4-dehydroprolylglycine, ethyl ester, 1-n-Butoxy-2,2,3,3-tetramethylaziridine, 2-Butenoic acid, 3-methyl-4-[tetrahydro-3,4-dihydroxy-5-[[3-(2-hydroxy-1-methylpropyl)oxiranyl] methyl]-2H-pyran-, Cobalt, octacarbonyl(zinc)di-, (2Co-Zn), 6''-Dehydroxy-2'',3',3'',4',4'',5,7-hepta-O-methylisoorientin, 2-naphthalenol, 3-[5-(3-nitrophenyl)-1,3,4-oxadiazol-2-yl]-, L-Proline, N-(1-naphthoyl)-, dodecyl ester, 3,6-Dispirocyclohexyl-1,2,3,4,5,6,7,8-octahydro-1,8-acridinedione, Sarcosine, N-(2-chloroethoxycarbonyl)-, heptadecyl ester, Cycloocta[1,2-b:4,3-b':5,6-b'':8,7-b''']tetrakis[1]benzothiophene, Butanoic acid, 2-chloro-3-methyl-, 4-(5-heptyl-2-pyrimidinyl)phenyl ester, Friedooleanan-1-one, 3,24-dihydroxy-, 9-O-Methyl-4,5-deoxymaytansino, Ditelluride, di-1-naphthalenyl, tert-Butylstibinous acid thioanhydride, l-Leucine, N-methyl-n-pentadecafluorocarbonyl-, octadecyl ester, 2,5-Dichloro-N-ethyl-N-phenyl-benzamide, 2-Thiophenylacetic acid, 2,2,2-trifluoroethyl ester, Methyl 8-[5-(methoxycarbonyl)methyl-2-furyl]octanoate. It was concluded that the bioactive compounds support the use of C. hispidum leaves in the treatment of diseases like cancer, anaphylactic shock, renal failure, diabetes and hypertension.

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1. INTRODUCTION

Plants play a vital role in the treatment and prevention of diseases. They help in the prevention and reduction of the adverse side effects of conventional drugs [1]. They are sources biological and pharmacological important chemicals. It has been reported that plants are sources of successful drugs, and will continuously be in the frontline for screening novel lead compounds [2]. An essential part of organic chemistry and biochemistry of plant is the identification of the novel bioactive compounds present in plant leading to further biological and pharmacological studies [3–5].

Combretum hispidum (Laws) (Combretaceae) is a common climbing weed of exist in the forest and savanna region. It regrows rapidly after forest and grass fires. It has trailing branches. It produces from seeds and vegetatively from basal stumps. The leaves are opposite, oblong, elliptic, 10 – 25 cm long and 5 – 11 cm wide. It has a cylindrical woody stem that is covered with short bristly hairs. The pharmacological use of plants of the family Combretaceae is widely reported in the scientific literature [6-8]. Combretaceae families exist predominately in tropical and subtropical areas, for example, in Africa and Brazil. Pictorial view of the leaves is shown in Figure 1.

Phytochemical analysis on the genus *Combretum* have revealed the presence of triterpenes, flavonoids and non-protein amino acids [9]. In the past few decades, numerous unusual phytocompounds have been isolated from *Combretum* species. It has been reported that 9,10-dihydrophenanthrenes and a substituted bibenzyl was isolated and characterized from *C. molle* [10]. Isolation of eleven triterpenes and their glycosides from *C. laxum* were reported by Bisoli and co-workers [11]. Cycloartane dienone lactone and alkaloids (combretine and betonicine) were isolated from *C. quadrangulare* [12], and *C. micranthum* [13]. Flavonoids such as rhamnoctrin, quercetin-5,3'-dimetylether, ramnazin and kaempferol were isolated from *C. erythrophyllum* [14].

Analysis of bioactive phytochemicals present in the leaves of *Combretum hispidum* is carried out for further reference of future studies on a common climbing weed in West Africa.

There are no published literatures that determine the bioactive compounds present in the ethanol extracts of *Combretum hispidum* leaves by gas chromatography–mass spectrometry (GC–MS) analysis. This study is aimed to investigate the compounds present in the leaves of *Combretum hispidum* by GC–MS analysis.

Figure 1: Leaves of *Combretum hispidum*

2. MATERIALS AND METHODS

2.1. Plant sample

Fresh leaves of *C. hispidum* were collected from Ngwa, Abia State Nigeria on 24th May, 2020. Sample of plant 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 leaves *C. hispidum* were air dried at room temperature for 3 days. The dried leaves were grounded using Wiley Mill Model No. 2 (Arthur H. Thomas Co., Philadelphia, USA). The powdered *C. hispidum* leaves were subjected to extraction using ethanol. The extract was then evaporated to dryness using Heidolph Rotavapor (Germany).

2.3. GC-MS Analysis

The GC–MS analysis of bioactive compounds of *C. hispidum* leaves extracts was done using agilent 6890N 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 30m x 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 °C for 0.4 min, increased to 200 °C at a rate of 25 °C/mins, then to 280 °C at a rate of 8 °C/mins and to a final temperature of 300 °C at a rate of 25 °C/mins, held for 2 mins . The identification time was based on retention time. Components with lower retention time eluted first before the ones of higher retention
time.

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.

RESULTS AND DISCUSSION

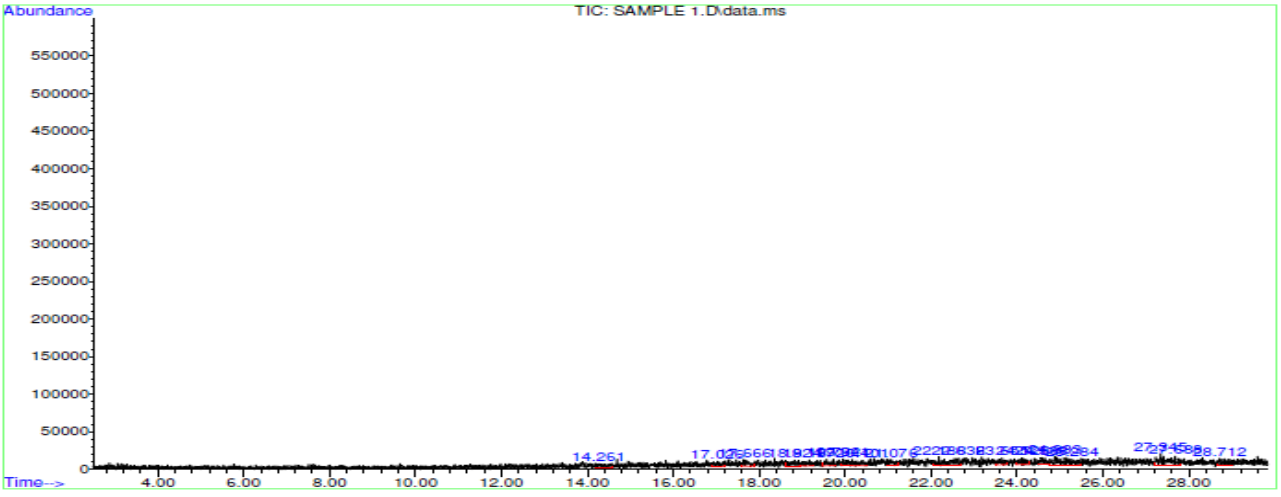
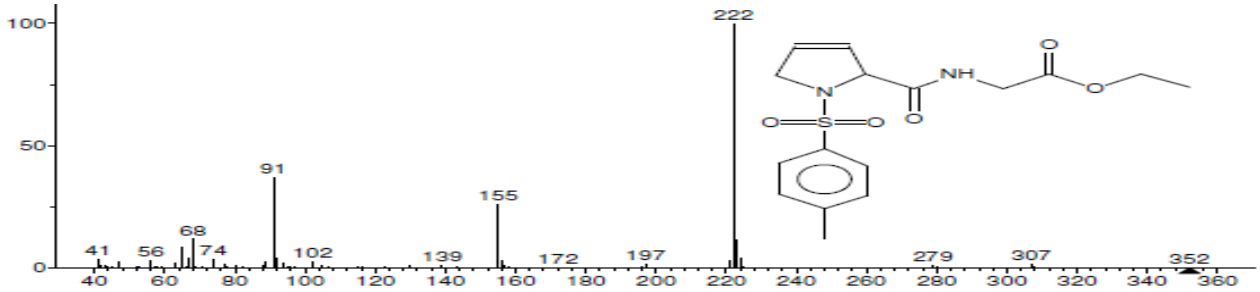
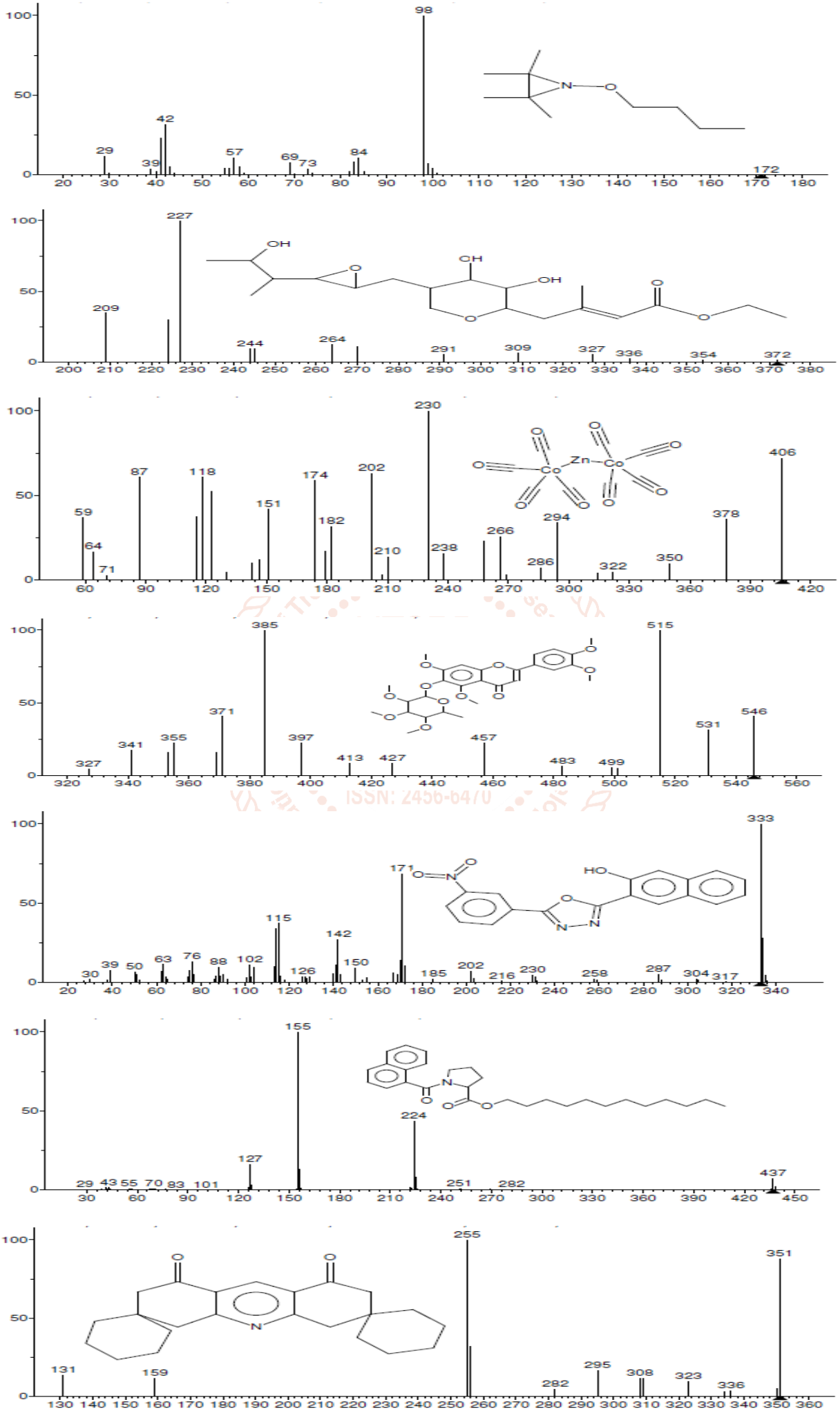


Figure 3.1: Gas chromatogram of ethanol extracts obtained from *C. hispidum* leaves

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171
HO
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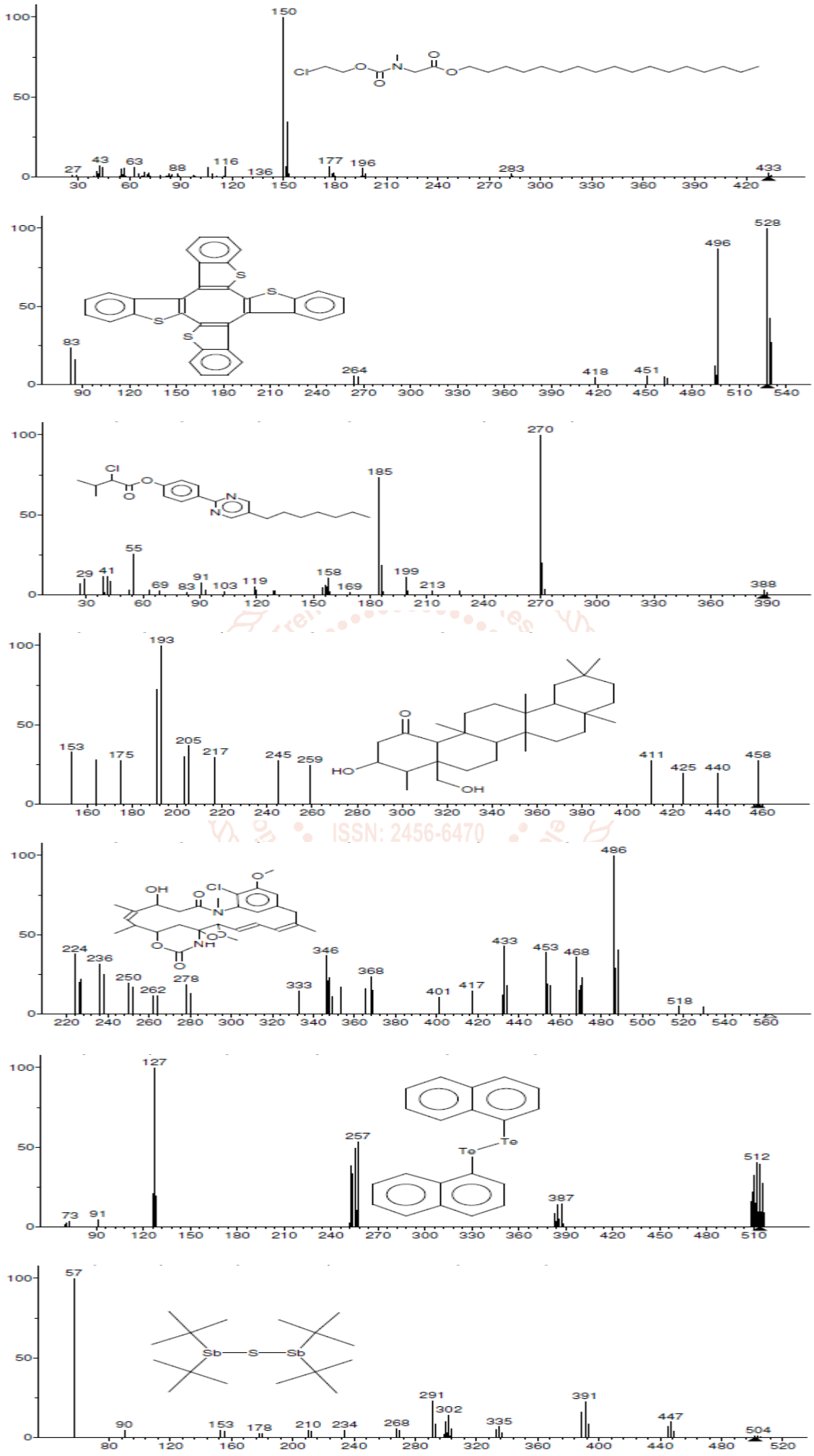

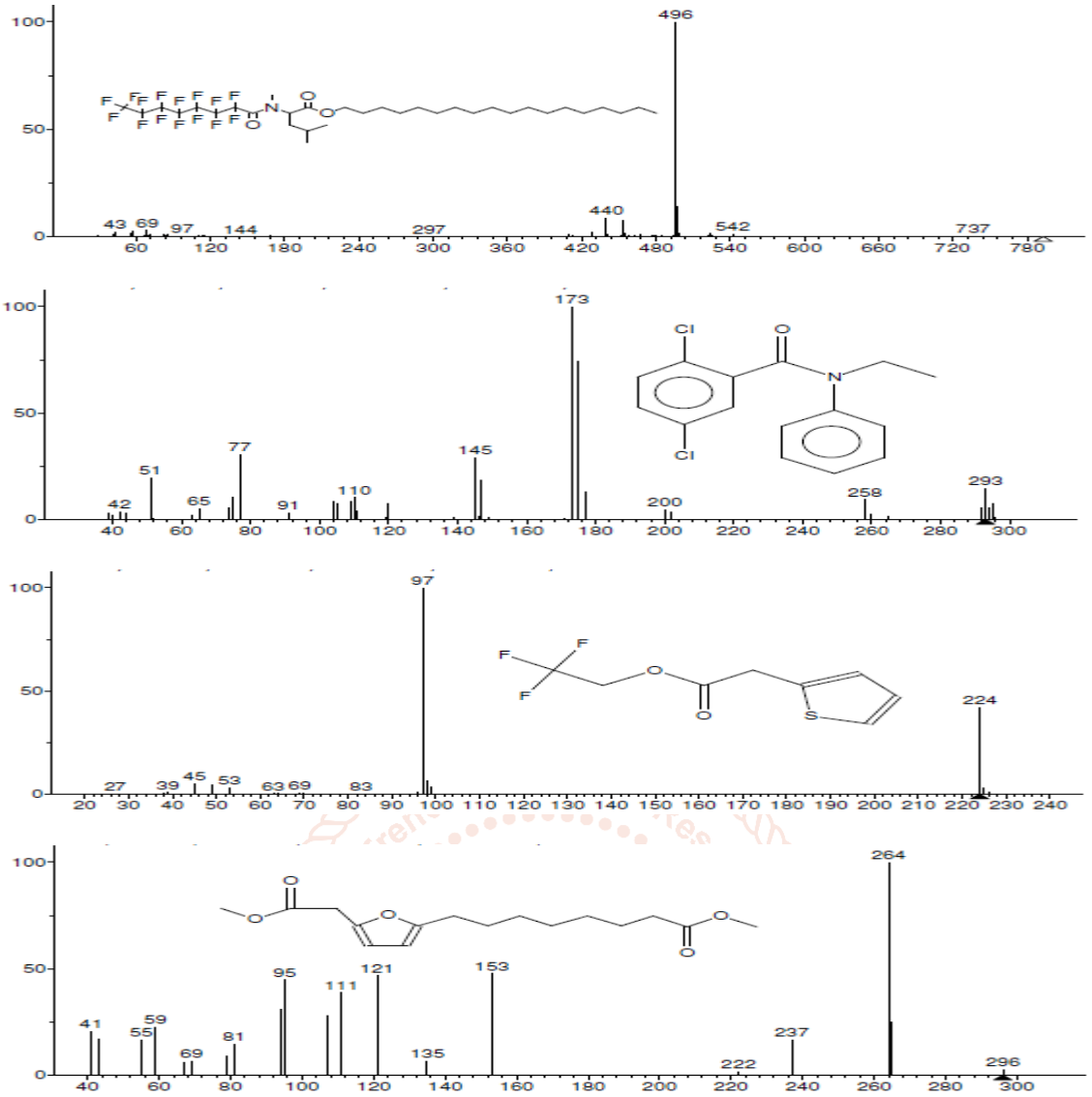

Figure 3.2: Mass spectra of ethanol extracts obtained from *C. hispidum* leaves

3.3. The bioactive compounds present in ethanol extracts obtained from *C. hispidum* leaves is shown in Table 1.

Table 1: Bioactive compounds present in ethanol extracts obtained from *C. hispidum* leaves

S/No	Compound	R.T (mins)	% of total	M.W (g/mol)	Biological activity
1	N-Tosyl-dl-3,4-dehydroprolylglycine, ethyl ester	14.261	4.872	352.10	Anaphylactic (antidote), antitumor (Nasopharynx),
2	1-n-Butoxy-2,2,3,3-tetramethylaziridine	17.026	4.635	171.16	Inhibit Production of Tumor-Necrosis-Factor, Increase natural killer cell activity,
3	2-Butenoic acid, 3-methyl-4-[tetrahydro-3,4-dihydroxy-5-[[3-(2-hydroxy-1-methylpropyl)oxiranyl]methyl]-2H-pyran-	17.666	5.073	372.21	Not found
4	Cobalt, octacarbonyl(zinc)di-, (2Co-Zn)	18.824	4.668	405.75	Increase Zinc Bioavailibility, Coronary-Dilator,
5	6''-Dehydroxy-2'',3',3'',4',4'',5,7-hepta-O-methylisoorientin	19.167	4.543	546.21	Down regulation of nuclear and cytosol androgen, Inhibit Production of Tumor Necrosis Factor, Inhibit Production of Tumor-Necrosis-Factor, Increase Osteocalcin, Inhibit Destruction of Glycosaminoglycans
6	2-naphthalenol, 3-[5-(3-nitrophenyl)-1,3,4-oxadiazol-2-yl]-	19.732	4.166	333.07	Not found
7	L-Proline, N-(1-naphthoyl)-, dodecyl ester	19.941	4.178	437.29	Anaphylactic (antidote=Neostigmine), Antitumor (Nasopharynx),

8	3,6-Dispirocyclohexyl-1,2,3,4,5,6,7,8-octahydro-1,8-acridinedione	20.401	5.079	351.21	Not found
9	Sarcosine, N-(2-chloroethoxycarbonyl)-, heptadecyl ester	21.076	4.298	433.29	Increase Natural Killer (NK) Cell Activity
10	Cycloocta[1,2-b:4,3-b':5,6-b'':8,7-b''']tetrakis[1]benzothiophene	22.188	5.044	528.01	Not found
11	Butanoic acid, 2-chloro-3-methyl-, 4-(5-heptyl-2-pyrimidinyl)phenyl ester	22.636	5.592	388.19	Methyl-Guanidine-Inhibitor
12	d:a-Friedooleanan-1-one, 3,24-dihydroxy-	23.655	4.380	458.37	Not found
13	9-O-Methyl-4,5-deoxymaytansinol	24.126	4.807	562.24	Anticancer (Oral),
14	Ditelluride, di-1-naphthalenyl	24.568	4.755	513.92	Coronary-Dilator, Diaphoretic
15	tert-Butylstibinous acid thioanhydride	24.883	10.397	502.06	Arachidonic-Acid-Inhibitor, Increase Aromatic Amino Acid Decarboxylase Activity
16	l-Leucine, N-methyl-n-pentadecafluorocarbonyl-, octadecyl ester	25.284	4.207	793.35	Nauseant, NCS-Depressant
17	2,5-Dichloro-N-ethyl-N-phenyl-benzamide	27.345	5.810	293.03	Nephroprotective
18	2-Thiophenylacetic acid, 2,2,2-trifluoroethyl ester	27.688	8.208	224.01	Acidifier, Acidulant
19	Methyl 8-[5-(methoxycarbonyl)methyl-2-furyl]octanoate	28.712	5.292	296.16	Not found

The gas chromatogram showed the presence of 19 compounds (Figure 3.1). The mass spectra of the 19 compounds are shown in Figure 3.2. The bioactive compounds, retention time, percentage, molecular weight and suggested bioactivity are presented in Table 1.

N-Tosyl-dl-3,4-dehydroprolylglycine, ethyl ester has been reported as an anaphylactic (antidote) [15]. Anaphylactic shock is a rapid, potentially life-threatening condition that is caused by a severe allergic reaction to an allergen. Anaphylaxis is characterized by rapidly progressive cardiopulmonary compromise with acute respiratory failure and hypotension following exposure to a trigger [16]. Literature has also reported the anti-tumor activity of N-Tosyl-dl-3,4-dehydroprolylglycine, ethyl ester in human nasopharyngeal carcinoma cells [15]. Leucine, N-methyl-n-pentadecafluorocarbonyl-, octadecyl ester have been reported as central nervous system depressant agent [15]. These compounds can slow brain activity, making them useful for treating anxiety, panic, acute stress reactions, and sleep disorders Butanoic acid, 2-chloro-3-methyl-, 4-(5-heptyl-2-pyrimidinyl)phenyl ester function as methyl-guanidine-inhibitor [15]. 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 [17]. This means that methylguandine can attenuate the degree of inflammation and tissue damage associated with endotoxic shock. 2,5-Dichloro-N-ethyl-N-phenyl-benzamide acts as a nephroprotective agent . Nephroprotective agents are material that has potential to minimize the effects of nephrotoxic agents. Ditelluride, di-1-naphthalenyl are proven coronary dilators [15]. Coronary dilators reduces the myocardial need of oxygen by reducing contractibility of the myocardium and slowing the frequency of cardiac contactors. They cause the dilation of coronary arteries and increases coronary blood flow.

CONCLUSION

The results of the study clearly suggested the presence of bioactive compounds in the ethanol extracts of *C. hispidum* leaves. The bioactive compounds support the use of *C. hispidum* leaves in the treatment of diseases like cancer, anaphylactic shock, renal failure, diabetes and hypertension.

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