

GC-MS analysis, Antioxidant and Antimicrobial Properties of *Eclipta prostrata* leaves

Marili F. Zubair¹, Sunday O. Ajibade², Abubakar Z. Lawal³, Sodiq A. Yusuf⁴, Joseph B. Babalola⁴, Abdulrahman A. Mukadam⁴, Abdulmumeen A. Hamid^{4*}

¹Department of Industrial Chemistry, Faculty of Physical Sciences, University of Ilorin, Ilorin, Nigeria, ²Department of Chemistry, Faculty of Natural Science, Plateau State University, Boko, Plateau State, Nigeria, ³Department of Medical Biochemistry, Faculty of Basic Medical Sciences, University of Ilorin, Ilorin, Nigeria and ⁴Department of Chemistry, Faculty of Physical Sciences, University of Ilorin, Ilorin, Nigeria

Abstract

Subsequent to extraction and phytochemical screening, n-hexane, ethyl acetate and methanol extracts of the leaves of *Eclipta prostrata* were screened against certain strains of bacteria (*Escherichia coli*, *Klebsiella pneumoniae*, *Salmonella typhi*, *Pseudomonas aeruginosa*, *Bacillus subtilis*, and *Staphylococcus aureus*). The antioxidant properties of the three (3) extracts were investigated as well. Significant antibacterial and antioxidant activities were exhibited by methanol and ethyl acetate extracts, while hexane extract did not show either antibacterial or antioxidant activities. Gas Chromatography, coupled with Mass spectrometry (GC-MS) analysis- which was used to identify the main chief chemical components of the extracts was used to explain the plant extracts' activities as antibacterial and antioxidant due to its revelation of the bioactive compounds in the plant.

Key words: Antibacterial activity, antioxidant activity, *Eclipta prostrata*, GC-MS

Full length article *Corresponding Author, e-mail: hamidmemo@gmail.com, hamid.aa@unilorin.edu.ng, Tel: +2347035931646

1. Introduction

A steady increase in bacteria that possess strong resistance against antibiotics may thwart the development of antimicrobial agents to control some bacterial diseases [1]. Folk medicine has been used for the treatment of infectious diseases produced by common pathogens such as bacteria and fungi [2]. Non-severe cases of infectious diseases caused by these pathogens are now rampant and medicinal plants might represent an alternative treatment in these diseases [3]. They can also be a possible source for new potent antibiotics to which pathogen strains are not resistant. In due course, plant derived drugs have been concluded to be beneficial as good source of antibiotics, antioxidants and anti-inflammatory agents [4, 5]. Medicinal plants possessing natural antioxidant, polyphenolics such as anthraquinones, flavonoids, aromatic acids and tannins have been shown to have reactive oxygen species scavenging and lipid peroxidation preventing effects [6].

Eclipta prostrata belongs to Asteraceae family, commonly known as false daisy, yerba de tago and bhringraj is a species of plant in the sunflower family. It is wide spread across much of the African and Asian countries. This species grows commonly in moist places and as a weed in

warm temperate to tropical areas worldwide. It is widely distributed throughout India, China, Thailand, and Brazil.

Several studies have showed that this herb is effective against liver injury and inflammation. The herb has been attributed to promote good functioning of the liver, cure of jaundice, fatty liver, hemorrhoids and indigestion. The herb has been used in the treatment of infective hepatitis in India [7] and snake venom poisoning in Brazil [8]. In addition, the crude form of the herb is reported to have anti-inflammatory, anti-fungal and anti-hepatotoxic properties [9]. In spite of the various work done on the plant, there is no report on the antioxidant effect and antibacterial activity of this plant to the best of our knowledge. The present study is intended to determine the antibacterial and antioxidant activities of crude extracts of the *E. prostrata* in solvents with different polarities (n-hexane, ethyl acetate and methanol).

2. Materials and Methods

2.1 Sample preparation and extraction

Eclipta prostrata leaves were obtained from the University of Ilorin campus, Ilorin, Kwara state, Nigeria. The leaves were identified at the Department of Plant Biology, University of Ilorin with voucher number (UILH/002/1233). The leaf samples were air dried at room temperature for about 14 days and the dried leaves were pulverized into the powdered form, using a mortar and pestle. Exhaustive serial extraction of the powdered plant was then carried out in three different solvents with varying polarities (n-hexane, ethyl acetate and methanol). After the 7th day of extraction, the extracts obtained were concentrated using a rotary evaporator to obtain the crude extracts of the plant.

2.2 Phytochemical screening

Preliminary phytochemical screening of the crude extracts was carried out using methods described by Geetha and Geetha, 2014 [10].

2.3 Antimicrobial studies

Cultures of six human pathogenic bacteria made up of four gram negative and two gram positive bacteria were used for the antibacterial assay. These include: *Salmonella typhi*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiellae pneumoniae*, *Bacillus subtilis* and *Staphylococcus aureus*. Each of the microorganisms used was fresh clinical strains from the Medical Microbiology laboratory (University College Hospital, Ibadan) and screened in the Laboratory of Pharmaceutical Microbiology Department, University of Ibadan, Ibadan, Nigeria.

2.4 Antioxidant determination

The ability of the plant extracts to scavenge 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radicals was assessed by a standard method [11], adopted with suitable modifications [12]. Stock solutions of the extracts were prepared in methanol to achieve the concentration of 1 mg/mL. Dilutions were made to obtained concentrations of (1000, 500, 250, 125, 62.5, 31.25, 15.62, 7.81, 3.90 and 1.99) µg/mL. Diluted solutions 2.0 ml each was mixed with 2.0 mL of methanol solution of DPPH in concentration of 2.0 mg/10mL. Following 30 minutes of incubation in darkness and at room temperature, the absorbance- which is the absorbance of control- was recorded at 517 nm. Percentage inhibition was calculated using equation (i) below, whilst IC50 values were estimated from the % inhibition versus concentration plot, using a non-linear regression algorithm. Ascorbic acid was used as standard drug. The IC50 values (Inhibition Concentration at 50%) were estimated from the % inhibition versus concentration plot, using a non-linear regression algorithm.

$$\% \text{ inhibition} = \left(\frac{A \text{ of control} - A \text{ of sample}}{A \text{ of control}} \right) \times 100 \dots (i)$$

Zubair et al., 2017

2.5 Antimicrobial test

Cultures of six human pathogenic bacteria made up of four gram negative and two gram positive were used for the antibacterial assay. These are; *Salmonella typhi*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Klebsiellae pneumoniae* and they belong to the gram-negative type of bacteria, while *Bacillus subtilis* and *Staphylococcus aureus* are gram-positive bacteria. The media used for preparation of the cultures include: nutrient agar, nutrient broth and tryptone soya agar, while n-hexane, ethyl acetate and methanol were used as negative controls in the assays. Furthermore, Gentamicin (10 µg/mL) and Tioconazole (0.7 mg/mL) were employed as standard reference antibiotic drugs in this study.

3. GC-MS analysis of the extract

The analysis of the chemical constituents of the extracts was carried out at Shimadzu Training Centre for Analytical Instruments Lagos, Nigeria. The analysis was done using GC-MS equipment. Each extract's components were eluted at different retention time from the gas chromatograph and the mass spectrometer captured, ionized, accelerated, deflected and detected each constituent separately. The data obtained from GC-MS result revealed thirty-three compounds for the n-hexane extract, thirty compounds for the ethyl acetate and thirty three compounds for the methanol extract.

4. Results and Discussion

4.1 Phytochemical screening

The phytochemical composition of crude extracts of the aerial parts of *Eclipta prostrata* investigated revealed the presence of certain bioactive compounds (Table 1). It has been previously observed that the extract exhibited strong activity with the increase in polarity (with reference to organic solvent), indicating that polyphenols or flavanone or flavanoids may play important roles in the activities. The present findings are in agreement with report of Tepe [14].

4.2 Antioxidant activity

The antioxidant activity of the hexane, ethyl acetate and methanol extracts of *E. prostrata* were determined and the values are presented in Table 2.

4.3 Antibacterial activities

Table 3 shows the antibacterial activities of hexane, ethyl acetate and methanol extracts of *E. prostrata* against tested organisms. The extracts exhibited remarkable activity at higher concentration. In this investigation, the methanol extract of leaves of *E. prostrata* recorded significant antibacterial activity against all the tested bacterial strains, while ethyl acetate and hexane extracts of the plant exhibited low activities against the test bacteria. Methanol extract showed broad spectrum of activity against all tested organisms (*E. coli*, *K. pneumoniae*, *S. dysenteriae*, *S. typhi*, *P. aeruginosa*, *B. subtilis*, *S. aureus*). *S. typhi* is highly sensitive to methanol extract followed by ethyl acetate extract.

Phytochemicals act in numerous ways to assist the body in combating diseases and health problems. They combine with some biomolecules to neutralize activity and

scavenging free radicals before they can cause damage within the body [14].

Table 1. Phytochemical screening results of the crude extracts of *Eclipta prostrata* leaves

Phytochemicals	Tests	n-hexane	Ethyl acetate	Methanol
Alkaloids	Dragendroff Wagner test	-	-	+
Saponins	Frothy	-	+	+
Flavonoids	Shinoda	-	-	-
Steroids	Lieberman-burchard	+	+	+
Terpenoides	Noller's test	+	+	+
Glycosides	Keller killiani	+	+	-
Phenols	Ferric chloride	-	-	+
Carbohydrate	Benedict's test	-	+	+
Protein	Buired test	-	-	-
Anthraquinone	Borntrager's test	-	-	+
Tannin	Braemer's test	-	-	-
Fat and oil	Salkowski's	-	-	-

(+) = Present and (-) = Absent

Table 2. Absorbance of Standard Compound of ascorbic acid as antioxidant standard

Conc. (µg/mL)	Absorbance	Absorbance	Mean ± SD	% inhibition
1.95	0.991	0.991	0.991±0	21.66
3.9	0.782	0.781	0.781±0.001	38.26
7.81	0.453	0.452	0.453±0.001	64.18
15.62	0.311	0.311	0.311±0	75.44
31.25	0.245	0.245	0.245±0	80.67
62.5	0.193	0.195	0.194±0.001	84.26
125	0.180	0.180	0.180±0	85.79
250	0.161	0.162	0.161±0.001	87.26

Table 3. Percentage inhibition of n-hexane extract of *Eclipta prostrate* leaves

Conc (µg/mL)	Absorbance	Absorbance	Absorbance	Mean + SD	% Inhibition
1000	0.056	0.056	0.057	0.056±0.0005	85.06
500	0.063	0.063	0.061	0.061±0.0015	83.7
250	0.083	0.081	0.081	0.081±0.0011	78.4
125	0.105	0.113	0.115	0.111±0.0052	70.4
62.50	0.133	0.136	0.137	0.135±0.0020	64.0
31.25	0.147	0.145	0.145	0.145±0.0011	61.3
15.62	0.132	0.131	0.137	0.134±0.0032	64.2
7.81	0.156	0.154	0.155	0.155±0.001	58.6
3.90	0.168	0.168	0.163	0.166±0.0028	55.7
1.99	0.172	0.173	0.174	0.174±0.001	53.8

Table 4: Percentage inhibition of ethyl acetate extract of *Eclipta prostrate* leaves

Conc(µg/mL)	Absorbance	Absorbance	Absorbance	Mean + SD	% Inhibition
1000	0.087	0.086	0.084	0.085±0.0015	75.9
500	0.118	0.119	0.116	0.117±0.0015	66.9
250	0.124	0.129	0.126	0.126±0.0025	64.3
125	0.135	0.136	0.133	0.134±0.0015	61.9
62.5	0.139	0.133	0.136	0.136±0.003	61.5
31.25	0.146	0.144	0.145	0.145±0.001	59.0
15.62	0.158	0.158	0.155	0.156±0.0017	55.9
7.81	0.176	0.179	0.171	0.175±0.004	50.4
3.90	0.182	0.181	0.183	0.182±0.001	48.5

1.99	0.191	0.193	0.193	0.192±0.0011	45.6
------	-------	-------	-------	--------------	------

Table 5: Percentage inhibition of methanol extract of *Eclipta prostrata* leaves

Conc(µg/mL)	Absorbance	Absorbance	Absorbance	Mean + SD	% Inhibition
1000	0.155	0.156	0.154	0.155±0.001	42.80
500	0.084	0.086	0.087	0.085±0.0015	68.63
250	0.068	0.066	0.065	0.066±0.0015	75.6
125	0.045	0.049	0.051	0.048±0.0030	82.28
62.50	0.027	0.027	0.027	0.027± 0	90.03
31.25	0.052	0.053	0.052	0.052±0.0005	80.81
15.62	0.076	0.081	0.078	0.078±0.0025	71.21
7.81	0.101	0.1	0.1	0.1±0.000577	63.09
3.90	0.11	0.108	0.109	0.109±0.001	59.77
1.99	0.112	0.111	0.11	0.111±0.001	59.04

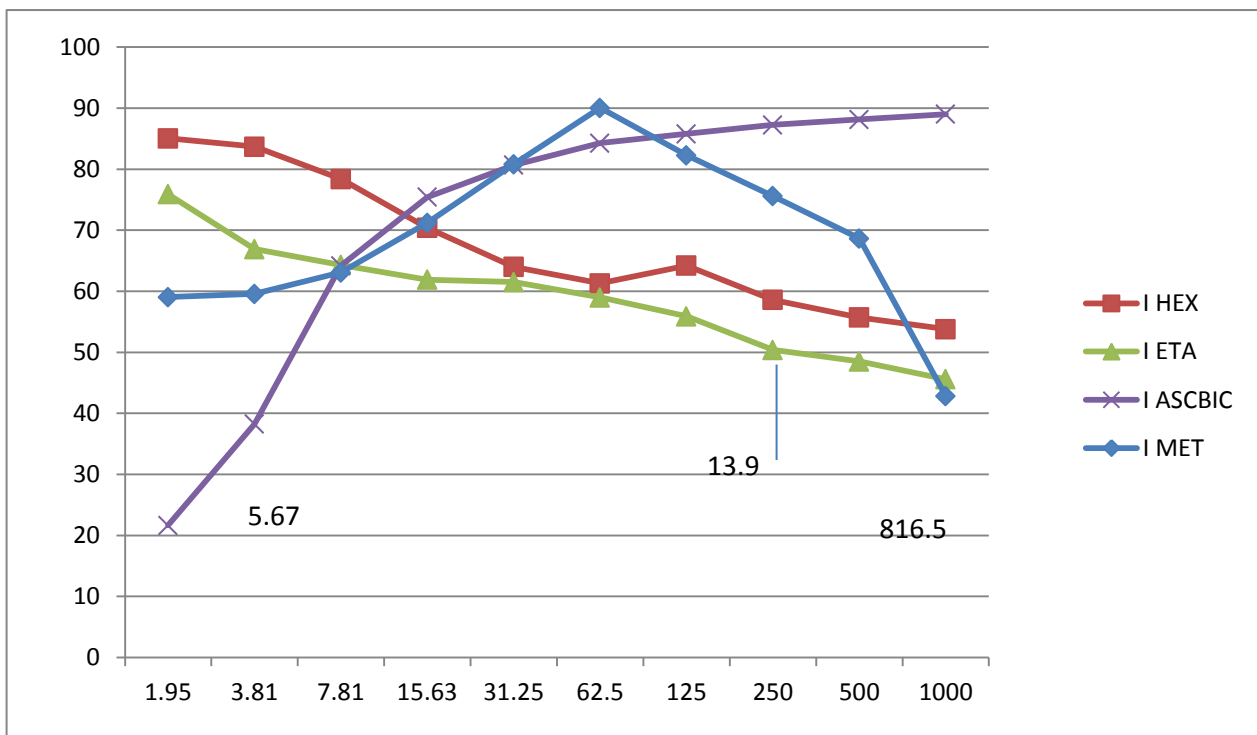


Fig. 1 IC₅₀ of the extracts of *Eclipta prostrata* with the IC₅₀ of the control

% I = Percentage Inhibition, IASCBIC = Ascorbic Acid Inhibition, IETA = Ethyl acetate Inhibition, IHEX = N – Hexane Inhibition and IMET = Methanol Inhibition

Table 6: Antibacterial activity of the n-hexane extracts of *Eclipta prostrata* leaves

Type	Microorganism			Mean zone of Inhibition (mm)				
	200	100	50	25	12.5	6.25	-ve	+ve
<i>S. aureus</i>	20	18	14	12	10	-	-	36
<i>E. coli</i>	18	14	12	10	-	-	-	38
<i>B. subtilis</i>	20	18	14	10	-	-	-	36
<i>P. aeruginosa</i>	16	14	10	-	-	-	-	36
<i>K. pneumoniae</i>	14	12	10	10	-	-	-	-
<i>S. typhi</i>	16	14	10	-	-	-	-	38
Extracts Conc. (mg/mL)	200	100	50	25	12.5	6.25	-ve	+ve

(+ve) = Gentamycin 10 µg/mL(bacteria), Tioconazole 70% (fungi) and (-ve) = Solvent of dilution

Table 7: Antibacterial activity of the ethyl acetate extract of *Eclipta prostrata* leaves

Microorganism	Mean zone of Inhibition (mm)							
	<i>S. aureus</i>	24	20	18	14	12	10	-
<i>E. coli</i>	20	18	16	14	14	-	-	28
<i>B. subtilis</i>	22	18	14	12	10	-	-	26
<i>P. aeruginosa</i>	20	18	14	10	-	-	-	24
<i>K. pneumoniae</i>	20	18	14	10	-	-	-	24
<i>S. typhi</i>	20	16	14	10	-	-	-	26
Extracts Conc. (mg/mL)	200	100	50	25	12.5	6.25	-ve	+ve

(+ve) = Gentamycin 10 µg/ml (bacteria), Tioconazole 70% (fungi) and (-ve) = Solvent of dilution

Table 8. Antibacterial activity of the methanol extract of *Eclipta prostrata* leaves

Microorganism	Mean zone of Inhibition (mm)							
	<i>S. aureus</i>	24	20	16	12	10	-	-
<i>E. coli</i>	24	20	16	12	10	-	-	38
<i>B. subtilis</i>	22	18	14	12	10	-	-	40
<i>P. aeruginosa</i>	20	18	14	12	10	-	-	38
<i>K. pneumoniae</i>	22	18	14	12	10	-	-	38
<i>S. typhi</i>	20	18	14	12	10	-	-	40
Extracts Conc. (mg/mL)	200	100	50	25	12.5	6.25	-ve	+ve

(+ve) = Gentamycin 10 µg/ml (bacteria), Tioconazole 70% (fungi) and (-ve) = Solvent of dilution

Table 9. Interpretation of the GC-MS analysis of the n-hexane extract of *Eclipta prostrata* leaves

S/N	Compound Name	Mol. Formula	Mol. Wt	Retention Time	% Abundance	Base peak
1	3,5-Dimethylcyclohexyl	C ₁₀ H ₁₇ ClO ₂	204	5.308	0.29	95.00
2	Pentyl 7-pentyl-7-azabicyclo[4.1.0]heptane-1-carboxylate	C ₁₇ H ₃₁ NO ₂	281	5.725	1.01	71.00
3	Analgit	C ₈ H ₈ O ₃	152	5.983	1.34	119.90
4	Neohexane	C ₆ H ₁₄	86	6.092	0.55	57.00
5	3,7-Dimethylundecane	C ₁₃ H ₂₈	184	8.758	1.88	57.05
6	1,2-Benzenediol, o-(4-butylbenzoyl)-o'-(2-methylbenzoyl)	C ₂₅ H ₂₄ O ₄	388	9.150	0.19	161.05
7	1-Phenyl-3-amino-4,5-dihydropyrazol-5-on	C ₉ H ₉ N ₃ O	175	9.517	0.31	175.00
8	3,7-Dimethylundecane	C ₁₃ H ₂₈	184	11.458	3.56	57.00
9	2,3,3-Trimethyloctane	C ₁₁ H ₂₄	156	14.700	2.09	57.00
10	1,1,2,2-Tetrafluoro-3-octanol	C ₈ H ₁₄ F ₄ O	202	14.800	0.47	82.95
11	Methyl tridecanoate	C ₁₄ H ₂₈ O ₂	228	16.258	0.75	73.95
12	Palmitic acid	C ₁₆ H ₃₂ O ₂	256	16.725	9.95	72.95
13	2,3,3-Trimethyloctane	C ₁₁ H ₂₄	156	17.133	1.45	57.00
14	Chloromethyl 6-chloroundecanoate	C ₈ H ₁₃ ClO ₂	176	18.075	0.05	67.00
15	Phytol	C ₂₀ H ₄₀ O	298	18.301	0.99	70.95
16	Linoleic acid	C ₁₈ H ₃₂ O ₂	280	18.567	10.82	55.00
17	Stearic acid	C ₁₈ H ₃₆ O ₂	284	18.808	3.19	57.95
18	Sulfurous acid, 2-ethylhexyl iso-hexyl-ester-iso-hexyl ester	C ₁₄ H ₃₀ O ₃	278	19.180	1.51	57.00
19	6-Methyl-1-octanol	C ₉ H ₂₀ O	144	19.308	1.84	82.00
20	9,10-Dibromopentacosane	C ₂₅ H ₅₀ Br ₂	508	20.000	1.31	73.00
21	5,9-Dimethyl-3-decanol	C ₁₂ H ₂₆ O	186	20.525	1.53	55.00
22	3,7-Dimethylundecane	C ₁₃ H ₂₈	184	20.908	0.99	57.00
23	1,3,3,3-Tetramethyldisiloxanyl tris(trimethylsilyl) orthosilicate	C ₁₃ H ₄₀ O ₅ Si ₆	444	21.233	0.81	73.00
24	Bisoflex 81	C ₂₄ H ₃₈ O ₄	390	22.058	5.13	148.95
25	Octadecamethyl-cyclononasiloxane	C ₁₈ H ₅₄ O ₉ Si ₉	666	22.375	1.42	73.00
26	Octadecamethyl-cyclononasiloxane	C ₁₈ H ₅₄ O ₈ Si ₉	666	23.433	2.05	73.00
27	(4Z,16Z)-4,16-Octadecadienyl acetate	C ₂₀ H ₃₆ O ₂	308	24.175	2.39	69.60
28	Octadecamethyl-cyclononasiloxane	C ₁₈ H ₅₄ O ₉ Si ₉	666	24.442	3.93	72.95
29	Sulfurous acid, octadecyl 2-propyl ester	C ₂₁ H ₄₄ O ₃ S	376	24.717	5.32	57.00

30	Ascorbyl 6-stearate	C ₂₄ H ₄₂ O ₇	442	25.492	5.14	57.00
31	9,10-Dibromopentacosane	C ₂₅ H ₅₀ Br ₂	508	25.642	4.37	72.95
32	Octadecanal	C ₁₈ H ₃₆ O	268	25.975	11.43	57.05
33	2-methyltetracosane	C ₂₈ H ₅₂	352	26.658	11.98	57.00

Table 10. Interpretation of the GC-MS analysis of the ethyl acetate extract of *Eclipta prostrata* leaves

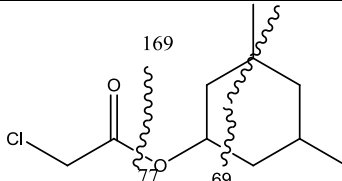
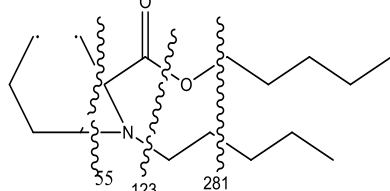
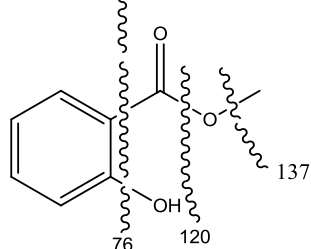
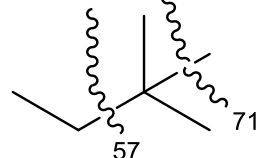
S/N	Compound name	Mol. Formula	Mol. Weight	Retention time	% Abundance	Base peak
1	Alcanfor	C ₁₀ H ₁₆ O	152	5.317	0.34	95.00
2	Menthol	C ₁₀ H ₂₀ O	156	5.725	0.16	70.95
3	1-(2-Methyl-1,3-oxathiolan-2-yl)ethanol	C ₆ H ₁₂ O ₂	148	6.575	0.35	102.90
4	Tripropylmethoxysilane	C ₁₀ H ₂₄ O ₅	188	7.975	0.24	57.00
5	Tetradecane	C ₁₄ H ₃₀	198	8.758	0.11	161.00
6	gamma.-Muurolene	C ₁₅ H ₂₄	204	9.150	0.04	57.00
7	Cetane	C ₁₈ H ₃₄	226	11.458	0.27	57.00
8	Myristic acid	C ₂₄ H ₂₈ O ₂	228	14.150	0.22	72.90
9	Tetradecane	C ₁₄ H ₃₀	198	14.700	0.20	57.00
10	9,10-Dibromopentacosane	C ₂₈ H ₅₀ Br ₂	508	15.253	0.28	57.00
11	Metholene 2216	C ₁₇ H ₃₄ O ₂	270	16.258	0.23	73.95
12	Palmitic acid	C ₁₆ H ₃₂ O ₂	256	16.858	10.42	72.95
13	Ethyl palmitate	C ₁₈ H ₃₆ O ₂	284	17.058	4.17	87.95
14	α-Bulnes	C ₁₅ H ₂₄	204	17.892	0.95	106.95
15	Phytol	C ₂₀ H ₄₀ O	296	18.308	1.61	70.95
16	Linolenic acid	C ₁₈ H ₃₀ O ₂	278	18.642	7.67	79.00
17	n-Propyl linoleate	C ₂₁ H ₃₈ O ₂	322	18.758	1.88	67.00
18	Ethyl linolenate	C ₂₀ H ₃₄ O ₂	306	18.825	5.45	78.95
19	Methyl 17-methyloctadecanoate	C ₂₀ H ₄₀ O ₂	312	19.075	1.65	87.95
20	Phytol, acetate	C ₂₂ H ₄₂ O ₂	338	19.325	8.18	68.00
21	alpha.-Selinene	C ₁₅ H ₂₄	204	20.908	5.28	189.00
22	2,4a,8,8-Tetramethyldecahydrocyclopropa[d]naphthalene	C ₁₅ H ₂₆	206	22.083	24.24	148.90
23	1,1,4,7-Tetramethyldecahydro-4a-cyclopropa[e]azulen-4a-ol	C ₁₅ H ₂₆ O	222	23.058	5.61	110.95
24	1.beta.,4.beta.H,10.beta.H-Guaia	C ₁₅ H ₂₄	204	23.217	7.65	161.00
25	2,4,16-Tribromopregn-16-ene-3,20-dione	C ₂₁ H ₂₇ Br ₃ O ₂	548	23.433	2.87	72.95
26	Octadecamethyl-cyclononasiloxane	C ₁₈ H ₅₄ O ₉ Si ₉	666	24.442	1.81	72.95
27	Heptadecafluorononanoic acid, nonyl ester	C ₁₈ H ₁₉ F ₁₇ O ₂	590	24.567	2.57	419.10
28	Z-7-Hexadecenal	C ₁₆ H ₃₀	238	24.725	2.91	57.00
29	Tetracosamethyl-cyclododecasiloxane	C ₂₄ H ₇₂ O ₁₂ Si ₁₂	888	25.625	1.81	72.95
30	Tetracontane	C ₄₀ H ₈₂	562	26.658	0.84	57.00

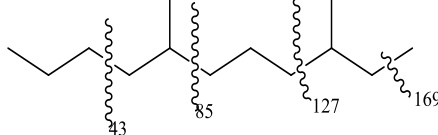
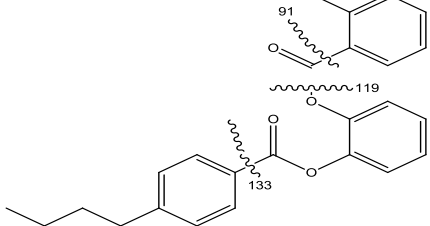
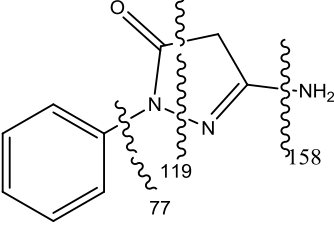
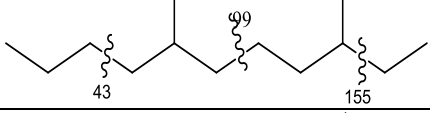
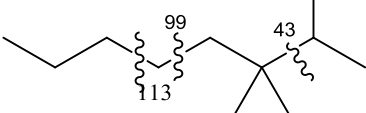
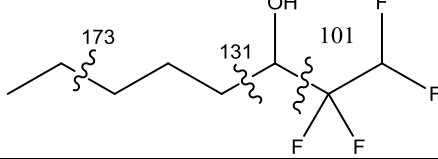
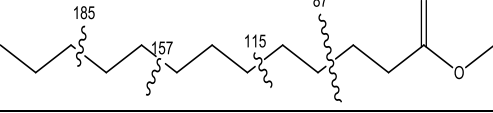
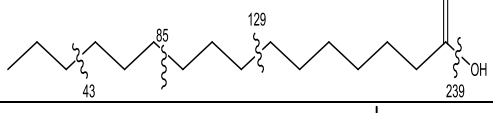
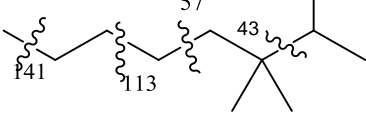
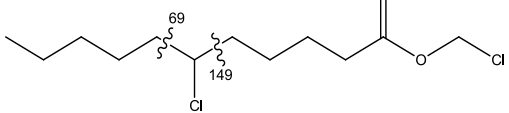
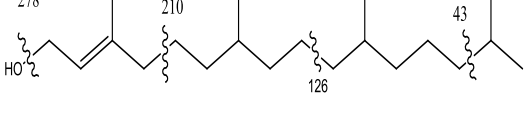
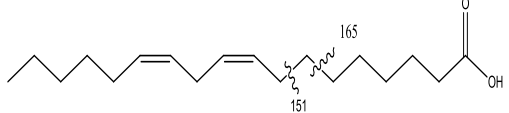
Table 11. Interpretation of the GC-MS analysis of the methanol extract of *Eclipta prostrata* leaves

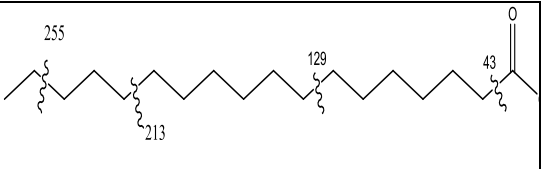
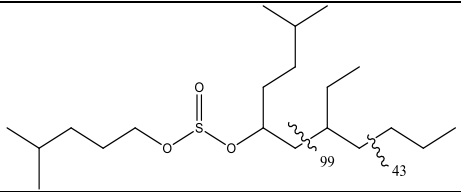
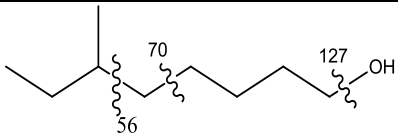
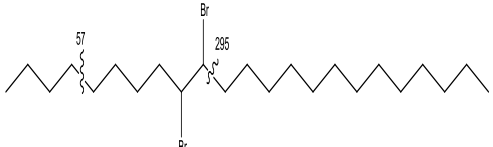
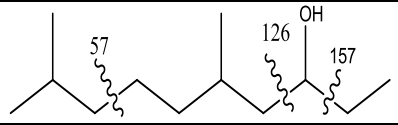
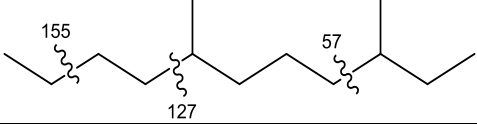
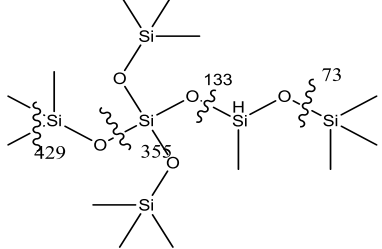
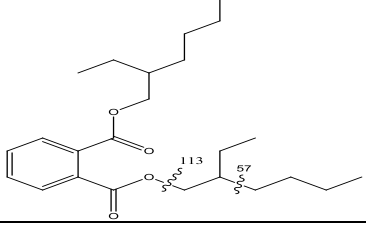
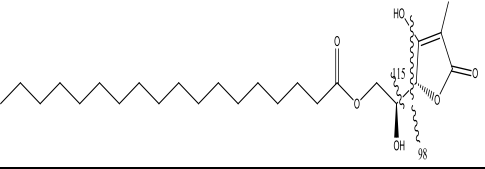
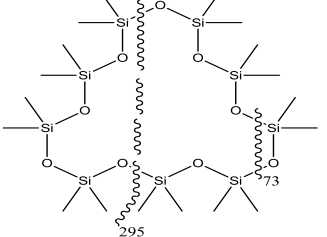
S/N	Compound name	Mol. Formula	Mol. Wt	Retention time	% Abundance	Base peak
1	Sorbitol	C ₆ H ₁₄ O ₆	182	5.175	0.52	85.95
2	Alcanfor	C ₁₀ H ₁₆ O	152	5.350	0.20	95.05
3	tert-Butyldimethylsilyl formate	C ₇ H ₁₆ O ₂ Si	160	6.608	4.88	102.95
4	Amyl 2-methylbutyrate	C ₁₀ H ₂₀ O ₂	172	7.967	0.01	102.95
5	3,7-Dimethylundecane	C ₁₃ H ₂₈	184	8.758	0.18	57.05
6	Phthalic acid, ethyl hexadecyl ester	C ₂₆ H ₄₂ O ₄	418	11.217	0.28	148.95
7	3,7-Dimethylnonane	C ₁₁ H ₂₄	156	11.450	0.20	57.00
8	2,5,6-Trimethyl-4-hepten-3-one	C ₁₀ H ₁₈ O	154	13.608	0.16	178.05

9	N-(4-Methylphenyl)hexopyranosylamine	C ₁₃ H ₁₉ NO ₅	269	14.117	0.15	72.95
10	3-Butyloctahydroindolizine	C ₁₂ H ₂₃ N	181	14.433	0.52	124.00
11	1-Chloro-3,3-dimethyl-2-butanone	C ₆ H ₁₁ ClO	134	14.692	0.14	57.00
12	Citronellyl isobutyrate	C ₁₄ H ₂₆ O ₂	226	15.217	0.67	95.00
13	Palmitic acid, methyl ester	C ₁₇ H ₃₄ O ₂	270	16.250	2.14	73.95
14	Palmitic acid	C ₁₆ H ₃₂ O ₂	256	16.733	14.18	72.95
15	Ethyl palmitate	C ₁₈ H ₃₆ O ₂	284	17.033	3.01	88.00
16	Ambrettolide	C ₁₆ H ₂₈ O ₂	252	18.067	1.05	67.00
17	Linolenic acid, methyl ester	C ₁₉ H ₃₂ O ₂	292	18.133	1.44	79.00
18	Phytol	C ₂₀ H ₄₀ O	296	18.292	3.14	71.00
19	Dichloroacetic acid, tridec-2-ynyl ester	C ₁₅ H ₂₄ Cl ₂ O ₂	306	18.558	7.39	79.00
20	Linolenic acid, ethyl ester	C ₂₀ H ₃₄ O ₂	306	18.800	1.58	79.00
21	Pentadecanal	C ₁₅ H ₃₀ O	226	19.300	2.58	68.00
22	Oleyl amide	C ₁₈ H ₃₅ NO	281	20.517	3.10	59.00
23	Palmitin, 2-mono-	C ₁₉ H ₃₈ O ₄	330	21.733	0.72	57.05
24	Diisooctyl phthalate	C ₂₄ H ₃₈ O ₄	390	22.042	4.04	148.9
25	2,2-Dimethylcholest-7-en-3-ol	C ₂₉ H ₅₀ O	414	22.608	10.16	414.3
26	Longifolene	C ₁₅ H ₂₄	204	23.158	1.38	61.05
27	beta.,4.beta.H,10.beta.H-Guaia	C ₁₅ H ₂₄	204	23.158	1.50	107.0
28	Urs-12-en-3-one	C ₃₀ H ₄₈ O	424	23.692	5.02	218.1
29	Cypermethrin	C ₂₂ H ₁₉ Cl ₂ NO ₃	415	24.108	8.41	162.9
30	Cypermethrin	C ₂₂ H ₁₉ Cl ₂ NO ₃	415	24.208	9.23	162.9
31	Aimcocyper	C ₂₂ H ₁₉ Cl ₂ NO ₃	415	24.325	7.42	162.9
32	3-Oxocholest-4-en-27-yl acetate	C ₂₉ H ₄₆ O ₃	442	25.475	3.89	441.2
33	17-Pentatriacontene	C ₃₅ H ₇₀	490	26.625	0.73	57.00

Table 12. Fragmentation of prominent peaks of n-hexane extract of *Eclipta prostrata* leaves

S/N	Compound name	Prominent peak	Structure
1	3,5-Dimethylcyclohexyl chloroacetate	169,95,77,69	
2	Pentyl 7-pentyl-7-azabicyclo[4.1.0]heptane-1-carboxylate	281,123,71,55	
3	Analgit	137,120,119,76	
4	Neohexane	71,57	

5	3,7-Dimethylundecane	169,127,85,57,43	
6	1,2-Benzenediol, o-(4-butylbenzoyl)-o'-(2-methylbenzoyl)	161,119,133,,91	
7	1-Phenyl-3-amino-4,5-dihydropyrazol-5-on	175,158 ,119,77	
8	3,7-Dimethylundecane	155,99,57,43	
9	2,3,3-Trimethyloctane	113,99,57,43	
10	1,1,2,2-Tetrafluoro-3-octanol	83,82,56,55	
11	Methyl tridecanoate	185,157,115,87,73	
12	Palmitic acid	239,129,85,72,43	
13	2,3,3-Trimethyloctane	141,113, 57,43	
14	Chloromethyl 6-chloroundecanoate	149,69,67	
15	Phytol	278,210,126,70,43	
16	Linoleic acid	165,151,55	

17	Stearic acid	255,213,129,59,43	
18	Sulfurous acid, 2-ethylhexyl isoheptyl ester	99,57,43	
19	6-Methyl-1-octanol	127,82,70,56	
20	9,10-Dibromopentacosane	295,73,57	
21	5,9-Dimethyl-3-decanol	157,126,57,55	
22	3,7-Dimethylundecane	155,127,57	
23	1,3,3,3-Tetramethyldisiloxanyl tris(trimethylsilyl) orthosilicate	429,355,133,73	
24	Bisoflex 81	148,113,57	
25	Octadecamethyl-cyclononasiloxane	295,73	
26	Octadecamethyl-cyclononasiloxane	221,147,73	


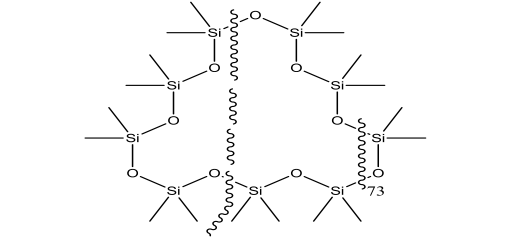
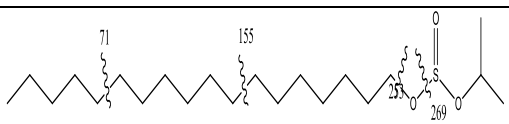
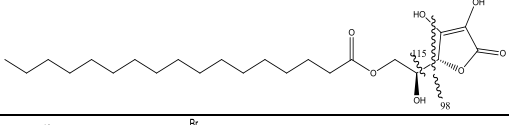
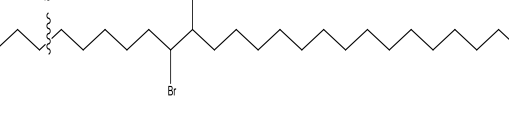
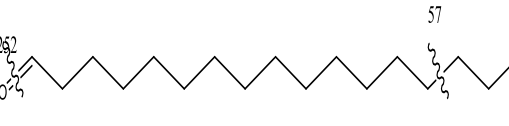
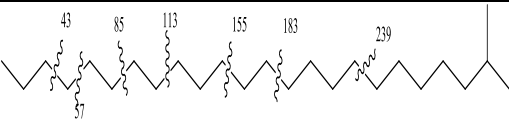
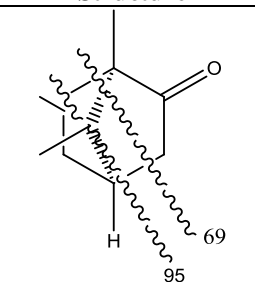
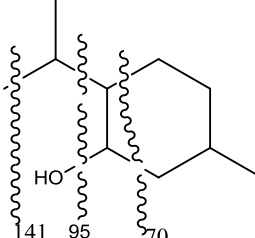
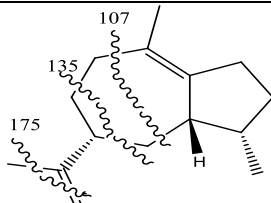
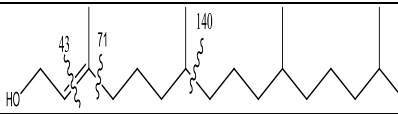
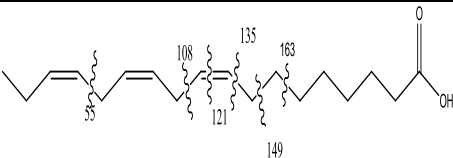
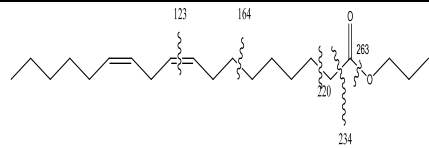
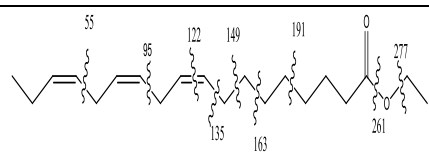
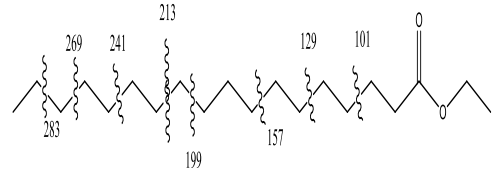
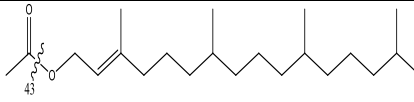
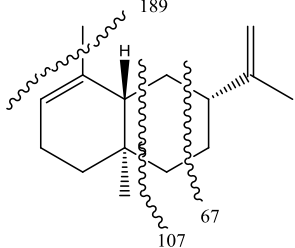
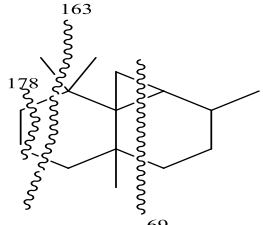
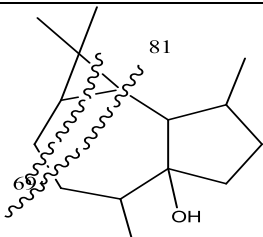
27	(4Z,16Z)-4,16-Octadecadienyl acetate	293,248,69,68,55	
28	Octadecamethyl-cyclononasiloxane	221,147,73,72	
29	Sulfurous acid, octadecyl 2-propyl ester	269,253,155,71,57	
30	Ascorbyl 6-stearate	115,98,57	
31	9,10-Dibromopentacosane	72,43	
32	Octadecanal	252,57	
33	2-methyltetracosane	239,183,155,113,85,57,43	

Table 13. Fragmentation of prominent peaks of ethyl acetate extract of *Eclipta prostrata* leaves

S/N	Compound name	Prominent peak	Structure
1	Alcanfor	95,69	
2	Methyl alcohol	141,95,70	

3	1-(2-Methyl-1,3-oxathiolan-2-yl)ethanol	103,102,88	
4	Tripropylmethoxysilane	102,88	
5	2,3,5-Trimethyldecane	169,141,113,99,71,57,43	
6	γ -Muurolene	161,119,93	
7	Cetane	183,155,127,99,71,57,43	
8	Myristic acid	211,183,85,72,43	
9	Tetradecane	169,155,141,127,113,99,85,71,57,43	
10	9,10-Dibromopentacosane	295,57	
11	Metholene 2216	239,73,57,43	
12	Palmitic acid	239,85,72,43	
13	Ethyl palmitate	269,255,239,87,57,43	

14	α -Bulne	175,135,107,106	
15	Phytol	140,71,70,43	
16	Linolenic acid	163,149,135,121, 108,79,55	
17	n-Propyl linoleate	263,234,220,164, 123,67	
18	Ethyl linolenate	277,261,191,163, 149,135,122,95,78, 55	
19	Ethyl stearate	283,269,241,213, 199,157,129, 101,87	
20	Phytol acetate	68,43	
21	α -Selinene	189,107,67	
22	2,4a,8,8-Tetramethyldecahydrocyclopropa[d]naphthalene	178,163,148,69	
23	1,1,4,7-Tetramethyldecahydro-4ah-cyclopropa[e]azulen-4a-ol	110,81,69	

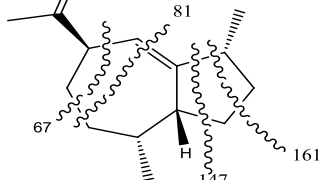
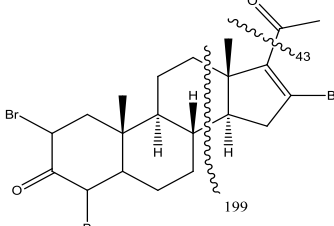
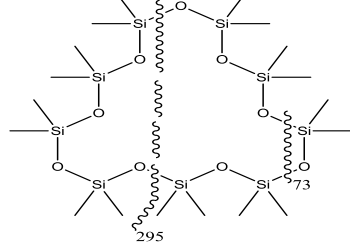
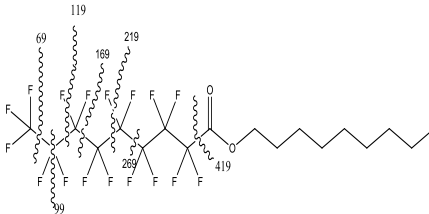
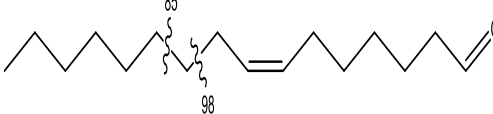
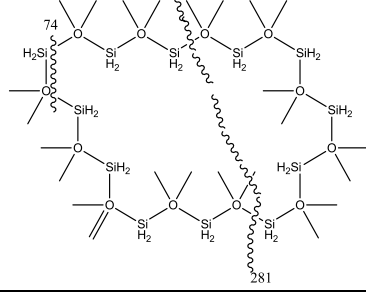
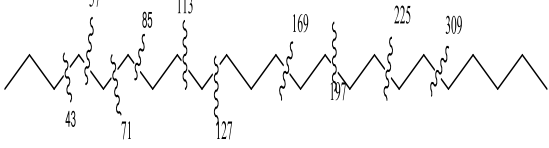
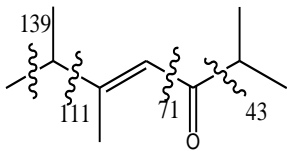
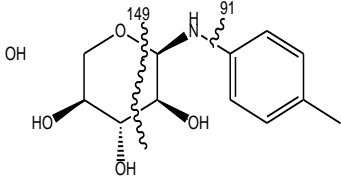
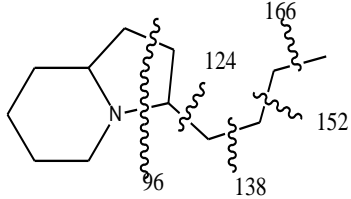
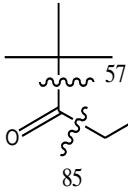
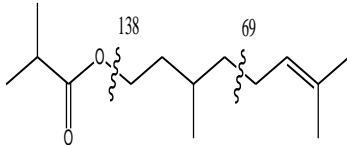
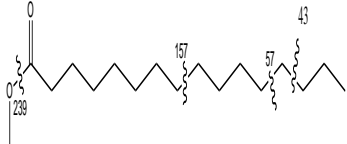
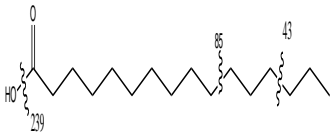
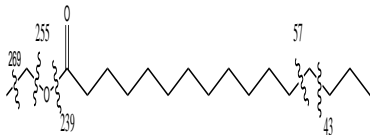

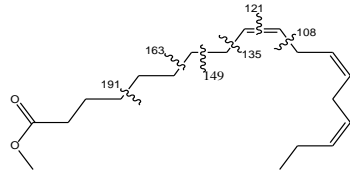
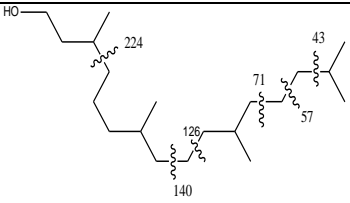
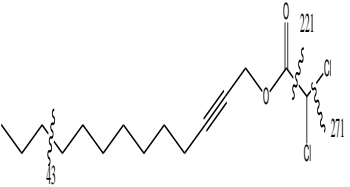
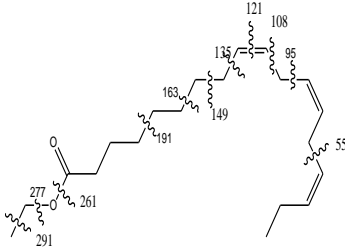
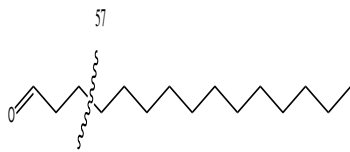
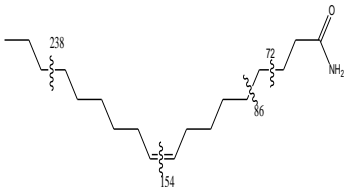
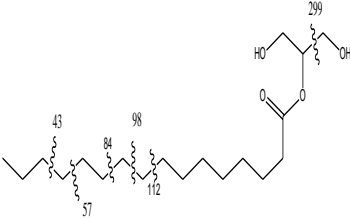
24	1.β.,4.β.H,10.β.H-Guaia	161,147,81,67	
25	2,4,16-Tribromopregn-16-ene-3,20-dione	199,72,43	
26	Octadecamethyl-cyclononasiloxane	295,7372	
27	Heptadecafluorononanoic acid, nonyl ester	419,268,219,169, 119,99,72,69	
28	Z-7-Hexadecenal	98,85,57	
29	Tetracosamethyl-cyclododecasiloxane	281,74,72	
30	Tetracontane	309,225,169,127, 113,85,71,57,43	

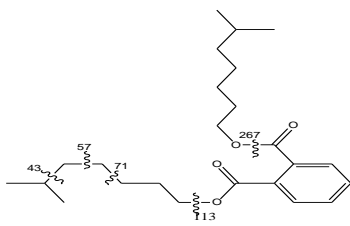
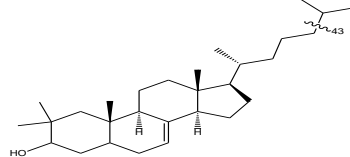
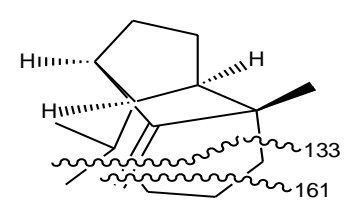
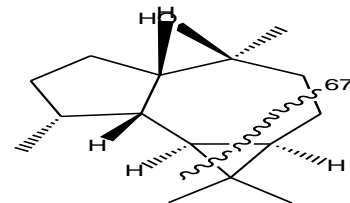
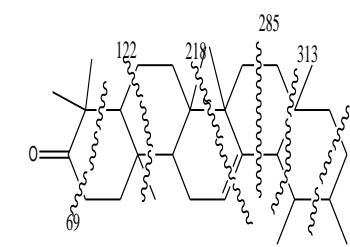
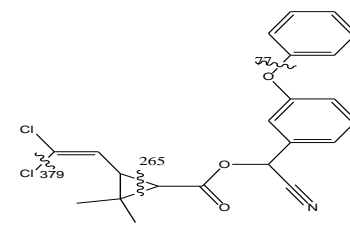
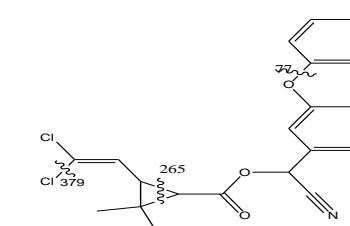
Table 14. Fragmentation of prominent peaks of methanol extract of *Eclipta prostrata* leaves

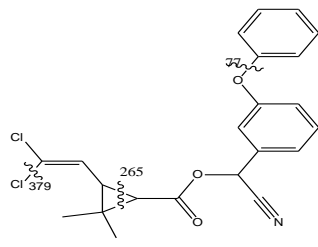
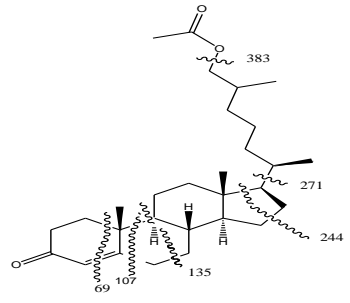
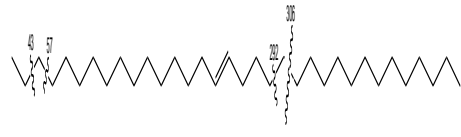
S/N	Compound name	Prominent peak	Structure
-----	---------------	----------------	-----------

1	Sorbitol	165,85,61	
2	Alcanfor	111,95,69	
3	tert-Butyldimethylsilyl formate	115,102,57	
4	Amyl 2-methylbutyrate	157,115,102,85,57, 43	
5	3,7-Dimethylundecane	169,155,127,99,85, 57,43	
6	Phthalic acid, ethyl hexadecyl ester	177,148,132, 104	
7	3,7-Dimethylnonane	141,127,99,85, 71,57	

8	2,5,6-Trimethyl-4-hepten-3-one	178,139,111,71,43	
9	N-(4-Methylphenyl)hexopyranosylamine	149,91,72	
10	3-Butyloctahydroindolizine	166,152,138, 124,96	
11	1-Chloro-3,3-dimethyl-2-butanone	85,57	
12	Citronellyl isobutyrate	138,95,69	
13	Palmitic acid, methyl ester	239,157,73,57, 43	
14	Palmitic acid	239,85,72,43	
15	Ethyl palmitate	269,255,239,88,57, 43	
16	Ambrettolide	210,67	

17	Linolenic acid, methyl ester	191,163,149, 135,121,108,79	
18	Phytol	224,140,126,71,57, 43	
19	Dichloroacetic acid, tridec-2-ynyl ester	271,221,79,43	
20	Linolenic acid, ethyl ester	291,261,191, 163,149,135, 121,108,95,79, 55	
21	Pentadecanal	68,57	
22	Oleyl amide	238,154,86,72, 59	
23	Palmitin, 2-mono-	299,112,98,84, 57,43	

24	Diisooctyl phthalate	267,148,113,71,57, 43	
25	2,2-Dimethylcholest-7-en-3-ol	414,43	
26	Longifolene	161,133	
27	β .4. β .H,10. β .H-Guaia	107,67	
28	Urs-12-en-3-one	381,313,285, 218,122,69	
29	Cypermethrin	379,265,162,77	
30	Cypermethrin	379,265,162,77	

31	Aimcocyper	379,265,162,77	
32	3-Oxocholest-4-en-27-yl acetate	441,383,271,244,135,107,69	
33	17-Pentatriacontene	306,292,57,43	

The GC-MS analysis of the n-hexane extract showed a total of thirty-three chemical compounds and the principal constituents are Linoleic acid, Octadecanal, 2-methyltetracosane with percentage abundances of 10.82, 11.43 and 11.98 percent respectively. The ethyl acetate extract mainly contains Palmitic acid (percent abundance 10.42) and 5-(7a-Isopropenyl-4,5-dimethyl-octahydroir (percent abundance = 24.24), while the methanol extract showed thirty-three compounds with Palmitic acid (percent abundance = 14.18), Cypermethrin (percent abundance = 9.23) and 2,2-Dimethylcholest-7-en-3-ol (percent abundance = 10.16) are the most abundant.

5. Conclusion

The grinded leaves of *Eclipta prostrata* has been investigated in this research and the preliminary phytochemical screening of the extracts indicate the presence of bioactive compounds of medicinal benefits. The leave extracts were subjected to antimicrobial activity. The n-hexane was found to have low activity against some strains of bacteria isolated at moderate to high concentration, while the ethyl acetate extract has a moderate activity against the tested bacteria at high concentration. GC-MS reveals various peaks of bioactive compounds of which the activity of the plant against the test bacteria can be attributed. The medicinal uses of the plant such as the treatment of liver, cure of jaundice, fatty liver and indigestion can be attributed to the bioactive compounds in

the extracts. It is mentioned to be a very good hair promoter as it prevents hair loss caused by bacteria.

References

- [1] J.C.A. Tanaka, C.C. da Silva, A.J.B. de Oliveira, C. Nakamura and B.P. Dias Filho. (2006). Antibacterial activity of iodole alkaloids from *Aspido spermaramifloram*. *Brazilian Journal of Medical and Biological Research*. 39(3): 387-391.
- [2] O. Wintola and A. Afolayan. (2015). The antibacterial, phytochemicals and antioxidants evaluation of the root extracts of *Hydnora Africana* Thunb used as antidysenteric in Eastern Cape Province, South Africa. *BMC Complementary and Alternative Medicine*. 15: 307.
- [3] J. Gonzalez. (1980). Antimicrobial activity of traditional medicinal plants. *Journal of Ethnopharmacology*. 2(43).
- [4] A. Mathur, G.B.K.S. Prasad and V.K. Dua. (2010). Screening of some Indian plants for their antibacterial and antifungal properties. *Flora and Fauna*. 16: 281-285.
- [5] A. Mathur, G.B.K.S. Prasad and V.K. Dua. (2011). Anti-inflammatory activity of leaves extracts of *Murraya koenigii*. *International Journal of Pharmacology and Biosciences*. 2(1): 541-544.
- [6] C.Y. Hong., C.P. Wang., Y.C. Lo and F.L. Hsu. (1994). Effect of flavan-3-ol tannins purified from *Camelliasinensis*

on lipid peroxidation of rat heart mitochondria. *American Journal of Chinese Medicines*. 22: 285-92.

[7] H. Wagner., B. Geyer., Y. Kiso., H. Hikino and G.S. Roa. (1986). Coumestans as the main active principles of the liver drugs *Eclipta alba* and *Wedeliaca lendulacea*. *Planta Medica*. 52: 370-374.

[8] P.A. Melo, M.C. Nascimento, W.B. Mors and G. Surez-Kurtz. (1994). Inhibition of the myotoxic and hemorrhagic activities of crotalid venoms by *Eclipta prostrata* (Astraceae) extracts and constituents. *Toxicon*. 32: 595-603.

[9] S.M. Wong, S. Antus, A. Gottsegen, B. Fessler, G.S. Rao, J. Sonnenbichler and H. Wagner. (1988). Wedelolactone and coumestan derivatives as new antihepatotoxic and antiphlogistic principles. *Arzneimittel-Forschung*. 38(5): 661-665.

[10] N. Geetha and C.S. Geetha. (2014). Phytochemical Screening, Quantitative Analysis of primary and secondary metabolites of *Cymbopogon citrates* (DC). *International Journal of PharmTech Research*. 6(2): 521-529.

[11] T. Tekao, N. Watanabe, I. Yagi and K. Sakata. (1994). A simple screening method for antioxidant and isolation of several antioxidants produced by marine bacteria from fish and shellfish. *Bioscience, Biotechnology and Biochemistry*. 58(45): 1780-1783.

[12] Y. Kumarasamy, M. Byres, P.J. Cox, M. Jasapars, L. Nahar and D. Sarker. 2007. Screening seeds of some Scottish plant for free radical scavenging activity. 21(1): 615-621.

[13] B. Tepe., D. Daferera., A. Sokmen., M. Sokmen and M. Polissiou. (2005). Antimicrobial and antioxidant activities of the essential oil and various extracts of *Salvia tomentosa* Miller (Lamiaceae). *Journal of Food Chemistry*. 90: 333-340.

[14] J.C. Okaka and A.N.C. Okaka. (2001). Food composition, spoilage and shelf life extension. *OCJANCO Acad. Publishers, Enugu, Nigeria*. 225-226.