

Research Article

## Assessment of Bioactive Constituents by GC-MS of *Crotalaria longipes* Wight & Arn.: An Endemic Plant

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### ABSTRACT

*Crotalaria longipes* is one of the medicinally important plants belonging to the family Fabaceae, commonly known in English as "Rattlepod". The present investigation deals with the GC-MS determination of methanol extract of the above said plant. Twenty five phytochemical constituents have been identified by comparing the chromatogram, peak value of unknown compound with entries in NIST database. The presence of various bioactive compounds confirms the application of *C. longipes* for various ailments. However, isolation of individual phytochemical constituent may ensue to find a novel drug. This is the first report of identification of active constituents from aerial parts of *C. longipes*.

**Key words:** *Crotalaria longipes*, GC-MS, cis Z-11, 12-Epoxytetradecan-1-ol, Squalene

### INTRODUCTION

GC-MS is a technique used for screening/identification/quantification of many susceptible compounds in plant extracts. Gas chromatography (GC) is used to separate drugs that might be present in the sample. The retention time (RT) is an identifying characteristic of a drug. The detector for the GC is the mass spectrometry (MS). The fragmentation pattern for a drug is unique and therefore is an identifying characteristic of a drug. The identification of a drug by its retention time and fragmentation pattern, along with sample specific information afforded to make GC-MS the foremost confirmation method for analyzing herbal extract.

In recent years GC-MS studies have been increasingly applied for the analysis of medicinal plants. This technique has proved to be a valuable method for the analysis of non polar components and volatile essential oil, fatty acid, lipids and alkaloids<sup>1</sup>.

Presently, *Crotalaria* species are important because of their accumulation of pyrrolizidine alkaloid, a poisonous compound. Many species of the genus are reported to be toxic with epidemic outbreaks in some parts of the world<sup>2</sup>. Bras *et al*<sup>3</sup> stated that only few species of the genus *Crotalaria* have been studied and also that WHO have labelled the Pyrrolizidine alkaloids as a very important toxicant and have made a call that all species of plants that might contain the alkaloid should be studied ethnomedicinally, phytochemically and taxonomically, etc<sup>2</sup>. Despite the medicinal importance of this species and its likely danger because of the presence of the Pyrrolizidine alkaloids, information on the pharmacognostic parameters for identification of this species in whole and powdered form are unavailable. *C.*

*longipes* is a woody shrub growing upto 4m tall with bright yellow flowers endemic to Nilgiris and Kolli hills. However, perusal of literature survey reveals that GC-MS analysis of *Crotalaria longipes* is totally lacking and hence the present investigation was undertaken. The main objective of the present study is to analyze the various phytochemical constituents found in aerial parts of *Crotalaria longipes*.

### MATERIALS AND METHODS

**Collection of plant sample:** The aerial parts of *Crotalaria longipes* was collected from Kothagiri, Nilgiris Biosphere Reserve, Tamil Nadu. With the help of flora, voucher specimen were identified and preserved in the Ethnopharmacology Unit, Research Department of Botany, V. O. Chidambaram College, Tuticorin, Tamil Nadu for further references.

**Plant sample extraction:** The aerial parts of *Crotalaria longipes* were cleaned, shade dried and pulverized to powder in a mechanical grinder. Required quantity of powder was weighed and transferred to Stoppard flask and treated with ethanol until the powder is fully immersed. The flask was shaken every hour for the first 6 hours and then it was kept aside and again shaken after 24 hours. This process was repeated for 3 days and then the extract was filtered. The extract was collected and evaporated to dryness by using a vacuum distillation unit. The final residue obtained was then subjected to GC-MS analysis.

**GC-MS Analysis:** GC-MS analysis of these extracts were performed using a Perkin-Elmer GC Clarus 500 system and Gas Chromatograph interfaced to a Mass Spectrometer (GC-MS) equipped with a Elite-1, fused

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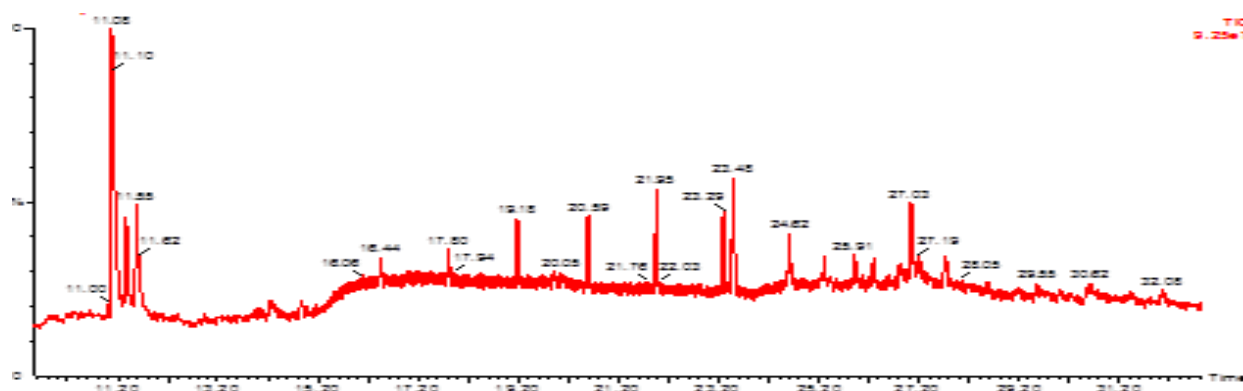


Fig. 1 GC-MS chromatogram of the ethanol extract of aerial parts of *Crotalaria longipes*

Table 1: Phytochemicals detected in aerial parts of *Crotalaria longipes*

No.	RT	Name of the compound	Molecular Formula	MW	Peak Area %
1.	11.00	cisZ-11,12-Epoxytetradecan-1-ol	C <sub>14</sub> H <sub>28</sub> O <sub>2</sub>	228	26.11
2.	11.24	2,3-Anhydro-d-galactosan	C <sub>6</sub> H <sub>8</sub> O <sub>4</sub>	144	3.84
3.	11.45	Propenal, 3-hydroxy-2-(4-pyridyl)-	C <sub>8</sub> H <sub>7</sub> NO <sub>2</sub>	149	13.22
4.	12.73	n-Decanoic acid	C <sub>10</sub> H <sub>20</sub> O <sub>2</sub>	172	4.91
5.	14.13	Tetradecanoic acid, ethyl ester	C <sub>16</sub> H <sub>32</sub> O <sub>2</sub>	256	1.93
6.	14.57	D-Glucose, 4-O-à-D-glucopyranosyl-	C <sub>12</sub> H <sub>22</sub> O <sub>11</sub>	342	0.56
7.	14.71	9,12-Octadecadienoic acid, methyl ester, (E,E)-	C <sub>19</sub> H <sub>34</sub> O <sub>2</sub>	294	5.93
8.	14.78	Z-10-Tetradecen-1-ol acetate	C <sub>16</sub> H <sub>30</sub> O <sub>2</sub>	254	3.00
9.	15.12	Pentadecanoic acid, 2,6,10,14-tetramethyl-, methyl ester	C <sub>20</sub> H <sub>40</sub> O <sub>2</sub>	312	1.36
10.	16.43	2-Cyclopentene-1-undecanoic acid, (+)-	C <sub>16</sub> H <sub>28</sub> O <sub>2</sub>	252	1.91
11.	16.93	9-Tetradecen-1-ol, acetate, (E)-	C <sub>16</sub> H <sub>30</sub> O <sub>2</sub>	254	4.02
12.	17.77	11-Tetradecen-1-ol, acetate, (Z)-	C <sub>16</sub> H <sub>30</sub> O <sub>2</sub>	254	3.53
13.	18.78	E-2-Tetradecen-1-ol	C <sub>14</sub> H <sub>28</sub> O	212	1.90
14.	19.15	3-Hexadecyloxycarbonyl-5-(2-hydroxyethyl)-4-methylimidazolium ion	C <sub>24</sub> H <sub>45</sub> N <sub>2</sub> O <sub>3</sub>	409	5.06
15.	19.82	7,8-Dioxabicyclo[4.2.2]decane	C <sub>8</sub> H <sub>14</sub> O <sub>2</sub>	142	3.01
16.	20.53	Valeric acid, 4-pentadecyl ester	C <sub>20</sub> H <sub>40</sub> O <sub>2</sub>	312	1.83
17.	21.88	Methoxyacetic acid, 3-tetradecyl ester	C <sub>17</sub> H <sub>34</sub> O <sub>3</sub>	286	1.97
18.	23.23	Heptadecane, 2,6,10,14-tetramethyl-	C <sub>21</sub> H <sub>44</sub>	296	0.85
19.	23.40	Squalene	C <sub>30</sub> H <sub>50</sub>	410	2.11
20.	23.83	13-Tetradecene-1-yn-1-ol	C <sub>14</sub> H <sub>24</sub> O	208	0.53
21.	26.74	1b,5,5,6a-Tetramethyl-octahydro-1-oxa-cyclopropa[a]inden-6-one	C <sub>13</sub> H <sub>20</sub> O <sub>2</sub>	208	2.07
22.	27.64	8,11,14-Eicosatrienoic acid, (Z,Z,Z)-	C <sub>20</sub> H <sub>34</sub> O <sub>2</sub>	306	0.88
23.	29.00	Z,Z,Z-1,4,6,9-Nonadecatetraene	C <sub>19</sub> H <sub>32</sub>	260	1.80
24.	29.44	Spiro[androst-5-ene-17,1'-cyclobutan]-2'-one, 3-hydroxy-, (3à,17à)-	C <sub>22</sub> H <sub>32</sub> O <sub>2</sub>	328	0.49
25.	30.44	5à-Androstan-16-one, cyclic ethylene mercaptole	C <sub>21</sub> H <sub>34</sub> S <sub>2</sub>	350	7.18

silica capillary column(30mm×0.25mm 10×1µMdf, composed of 100% Di methyl poly siloxene). For GC-MS detection an electron ionization system with ionizing energy of 70ev was used. Helium gas (99.999%) was used as the carrier gas at constant flow rate of 1ml/min and an injection volume of 2µ l was employed (split ratio of 10:1); injector temperature 250° C; ion-source temperature 280 ° C. The oven temperature programmed from 110 ° C (isothermal for 2 min) with an increase of

10 ° C /min to 200 ° C, then 5 ° C /min to 280 ° c, ending with a 9 min isothermal at 280 ° C, mass spectra were taken at 70ev; a scan interval of 0.5 seconds and fragments from 45 to 450Da, total GC running time was 36mlnutes. The relative % amount of each component was calculated by comparing its average peak area to the total areas. Software adopted to handle mass spectra and chromatograms was a Turbomass.

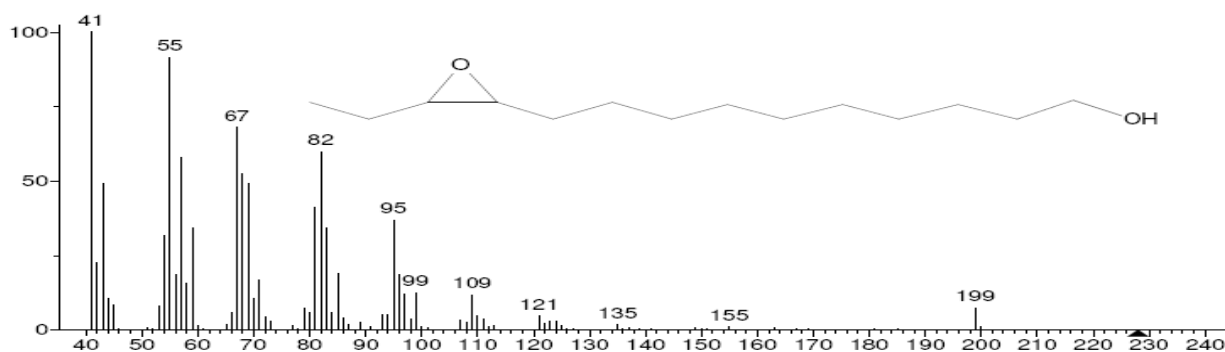


Fig. 2 Mass spectrum of cisZ-11,12-Epoxytetradecan-1-ol

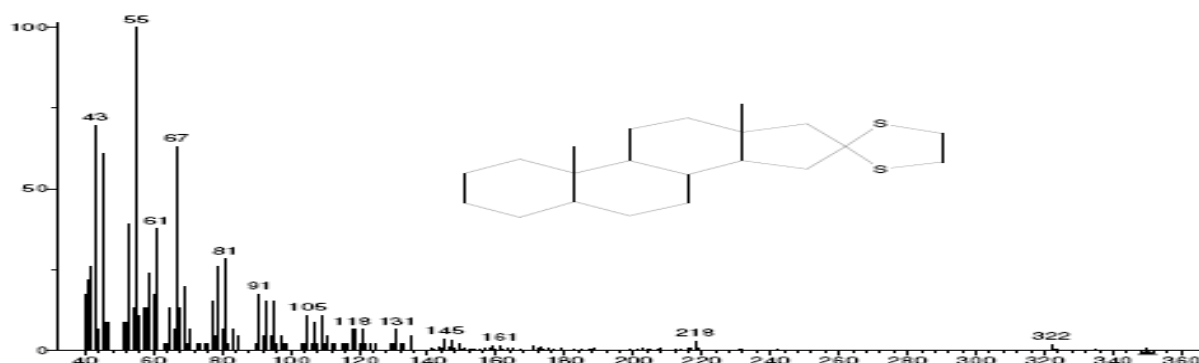


Fig. 3 Mass spectrum of 5α-Androstan-16-one, cyclic ethylene mercaptole

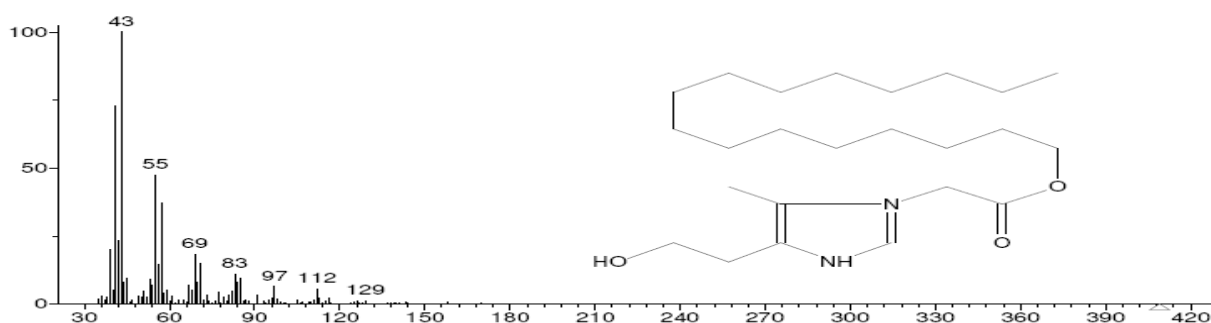


Fig. 4 Mass spectrum of 3-Hexadecyloxycarbonyl-5-(2-hydroxyethyl)-4-methylimidazolium ion

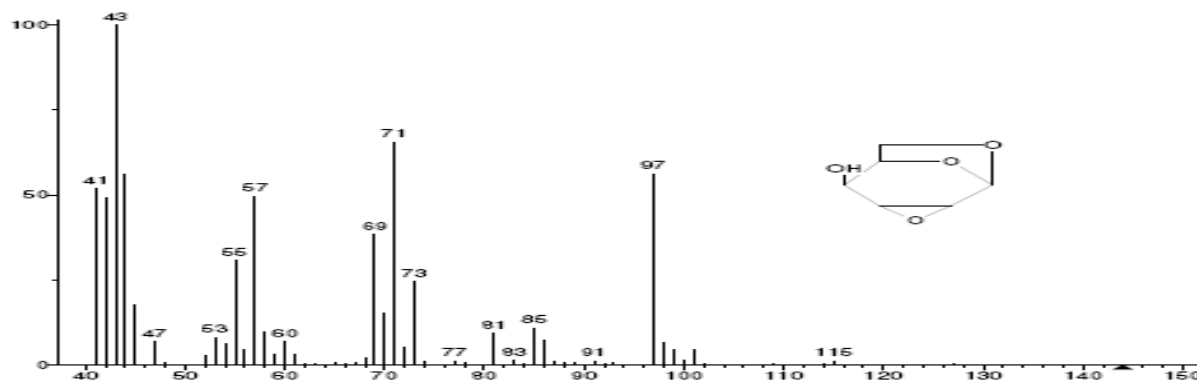


Fig. 5 Mass spectrum of 2,3-Anhydro-d-galactosan

Interpretation on mass spectrum GC-MS was conducted using the database of National Institute of Standard and Technology (NIST) having more than 62,000 patterns. The spectrum of the unknown component was compared with the spectrum of the known components stored in the NIST library. The name molecular weight and structure

of the components of the test materials were ascertained<sup>4,5</sup>.

## RESULTS

Table 2: Activity of phytocomponents identified in the ethanol extract of aerial parts of *Crotalaria longipes*

No.	RT	Name of the compound	Molecular Formula	MW	Peak Area %	Compound Nature	**Activity
1	11.00	cisZ-11,12-Epoxytetradecan-1-ol	C <sub>14</sub> H <sub>28</sub> O <sub>2</sub>	228	26.11	Epoxy compound	No activity reported
2.	11.24	2,3-Anhydro-d-galactosan	C <sub>6</sub> H <sub>8</sub> O <sub>4</sub>	144	3.84	Sugar moiety	Preservative
3.	11.45	Propenal, 3-hydroxy-2-(4-pyridyl)-	C <sub>8</sub> H <sub>7</sub> NO <sub>2</sub>	149	13.22	Nitrogen compound	Antimicrobial Anti-inflammatory
4.	12.73	n-Decanoic acid	C <sub>10</sub> H <sub>20</sub> O <sub>2</sub>	172	4.91	Fatty acid	Insecticide Fragrance compound
5.	14.13	Tetradecanoic acid, ethyl ester	C <sub>16</sub> H <sub>32</sub> O <sub>2</sub>	256	1.93	Myristic acid ester	Antioxidant Cancer preventive Cosmetic
6.	14.57	D-Glucose, 4-O-à-D-glucopyranosyl-	C <sub>12</sub> H <sub>22</sub> O <sub>11</sub>	342	0.56	Sugar moiety Linoleic acid ester	Nematicide Hypercholesterolemic Lubricant Preservative
7.	14.71	9,12-Octadecadienoic acid, methyl ester, (E,E)-	C <sub>19</sub> H <sub>34</sub> O <sub>2</sub>	294	5.93		Antiinflammatory, Hypocholesterolemic Cancer preventive, Hepatoprotective, Nematicide Insectifuge, Antihistaminic Antieczemic, Antiacne, 5-Alpha reductase inhibitor Antiandrogenic, Antiarthritic, Anticoronary, Insectifuge
8.	14.78	Z-10-Tetradecen-1-ol acetate	C <sub>16</sub> H <sub>30</sub> O <sub>2</sub>	254	3.00	Unsaturated alcoholic compound	No activity reported
9.	15.12	Pentadecanoic acid, 2,6,10,14-tetramethyl-, methyl ester	C <sub>20</sub> H <sub>40</sub> O <sub>2</sub>	312	1.36	Fatty acid ester compound	No activity reported
10.	16.43	2-Cyclopentene-1-undecanoic acid, (+)-	C <sub>16</sub> H <sub>28</sub> O <sub>2</sub>	252	1.91	Unsaturated cyclic fatty acid compound	No activity reported
11.	16.93	9-Tetradecen-1-ol, acetate, (E)-	C <sub>16</sub> H <sub>30</sub> O <sub>2</sub>	254	4.02	Unsaturated alcoholic compound	No activity reported
12.	17.77	11-Tetradecen-1-ol, acetate, (Z)-	C <sub>16</sub> H <sub>30</sub> O <sub>2</sub>	254	3.53	Unsaturated alcoholic compound	No activity reported
13.	18.78	E-2-Tetradecen-1-ol	C <sub>14</sub> H <sub>28</sub> O	212	1.90	Unsaturated alcoholic compound	No activity reported
14.	19.15	3-Hexadecyloxycarbonyl-5-(2-hydroxyethyl)-4-methylimidazolium ion	C <sub>24</sub> H <sub>45</sub> N <sub>2</sub> O <sub>3</sub>	409	5.06	Nitrogen compound	Antimicrobial
15.	19.82	7,8-Dioxabicyclo[4.2.2]decane	C <sub>8</sub> H <sub>14</sub> O <sub>2</sub>	142	3.01	Alkane compound	No activity reported

\*\*Activity Source: Dr. Duke's Phytochemical and Ethnobotanical Databases

Table 2: Activity of phytocomponents identified in the ethanol extract of aerial parts of *Crotalaria longipes*

No.	RT	Name of the compound	Molecular Formula	MW	Peak Area %	Compound Nature	**Activity
16.	20.53	Valeric acid, 4-pentadecyl ester	C <sub>20</sub> H <sub>40</sub> O <sub>2</sub>	312	1.83	Ester compound	No activity reported
17.	21.88	Methoxyacetic acid, 3-tetradecyl ester	C <sub>17</sub> H <sub>34</sub> O <sub>3</sub>	286	1.97	Acetic compound	Antimicrobial
18.	23.23	Heptadecane, 2,6,10,14-tetramethyl-	C <sub>21</sub> H <sub>44</sub>	296	0.85	Alkane compound Triterpene	No activity reported
19.	23.40	Squalene	C <sub>30</sub> H <sub>50</sub>	410	2.11		Antibacterial, Antioxidant, Antitumor, Cancer preventive, Immunostimulant, Chemo preventive, Lipoxygenase-inhibitor, Pesticide
20.	23.83	13-Tetradecene-11-yn-1-ol	C <sub>14</sub> H <sub>24</sub> O	208	0.53	Unsaturated alcoholic compound	No activity reported
21.	26.74	1b,5,5,6a-Tetramethyl-octahydro-1-oxa-cyclopropa[a]inden-6-one	C <sub>13</sub> H <sub>20</sub> O <sub>2</sub>	208	2.07	Ketone compound	No activity reported
22.	27.64	8,11,14-Eicosatrienoic acid, (Z,Z,Z)-	C <sub>20</sub> H <sub>34</sub> O <sub>2</sub>	306	0.88	Unsaturated fatty acid compound	Cardio protective
23.	29.00	Z,Z,Z-1,4,6,9-Nonadecatetraene	C <sub>19</sub> H <sub>32</sub>	260	1.80	Unsaturated Alkene compound	No activity reported
24.	29.44	Spiro[androst-5-ene-17,1'-cyclobutan]-2'-one, 3-hydroxy-, (3á,17á)-	C <sub>22</sub> H <sub>32</sub> O <sub>2</sub>	328	0.49	Steroid compound	Antiarthritic Hepatoprotective Antiasthma Anti-inflammatory Diuretic Cancer preventive
25	30.44	5à-Androstan-16-one, cyclic ethylene mercaptole	C <sub>21</sub> H <sub>34</sub> S <sub>2</sub>	350	7.18	Steroid compound	Hepatoprotective Antiasthma Anti-inflammatory Diuretic Cancer preventive

\*\*Activity Source: Dr. Duke's Phytochemical and Ethnobotanical Databases

The components present in the ethanol extract of aerial parts of *Crotalaria longipes* was identified by GC-MS (Fig. 1). The active principles with their retention time (RT), molecular formula, molecular weight (MW) and concentration (%) in the ethanol extract of aerial part of *Crotalaria longipes* are presented in Table 1. Twenty five compounds were identified in the ethanol extract of aerial part of *Crotalaria longipes*.

The prevailing compounds were, cisZ-11,12-Epoxytetradecan-1-ol (26.11%), Propenal, 3-hydroxy-2-(4-pyridyl)- (13.22%), 5à-Androstan-16-one, cyclic ethylene mercaptole (7.18%), 9,12-Octadecadienoic acid, methyl ester, (E,E)- (5.93%), 3-Hexadecyloxycarbonyl-5-(2-hydroxyethyl)-4-methylimidazolium ion (5.06%), n-Decanoic acid (4.91%), n-Decanoic acid (4.02%), 2,3-

Anhydro-d-galactosan (3.84%), 11-Tetradecen-1-ol, acetate, (Z)- (3.53%), 7,8-Dioxabicyclo[4.2.2]decane (3.01%), Z-10-Tetradecen-1-ol acetate (3.00%), Squalene (2.11%), 1b,5,5,6a-Tetramethyl-octahydro-1-oxa-cyclopropa[a]inden-6-one (2.07%), Methoxyacetic acid, 3-tetradecyl ester (1.97%), Tetradecanoic acid, ethyl ester (1.93%), 2-Cyclopentene-1-undecanoic acid, (+)- (1.91%) and E-2-Tetradecen-1-ol (1.90%).

Figures (2-5) shows the mass spectrum of cisZ-11,12-Epoxytetradecan-1-ol, 5à-Androstan-16-one, cyclic ethylene mercaptole, 3-Hexadecyloxycarbonyl-5-(2-hydroxyethyl)-4-methylimidazolium ion and 2,3-Anhydro-d-galactosan.

Table 2 listed the major phytochemicals and its biological activities obtained through the GC-MS study of the aerial part of *Crotalaria longipes*.

#### DISCUSSION

The results pertaining to GC-MS analysis led to the identification of number of compounds from the GC fraction of the ethanol extract of *Crotalaria longipes*. These compounds were identified through mass spectrometry attached with GC. Twenty five compounds were identified in the ethanol extract of aerial part of *Crotalaria longipes*. Among the identified phytochemicals, Tetradecanoic acid and squalene have the property of antioxidant activity<sup>6,7</sup>. Recently it has been found that; squalene possesses chemopreventive activity against the colon carcinogenesis<sup>8</sup>. Many spiro compounds possess very promising biological activities as anticancer, anticonvulsant, antituberculosis, antialzheimer's, pain relief, antidermatitis and antimicrobial agents<sup>9</sup>. Recently spiro compounds have been also used as antioxidants<sup>10</sup>. Decanoate salt and esters of various drugs are available. Since decanoic acid is a fatty acid, forming a salt or ester with a drug will increase its lipophilicity and its affinity for fatty tissue<sup>11</sup>. The above said compounds found in the ethanol extract of *Crotalaria longipes* aerial parts are being used for the pharmacological work. Thus this type of GC-MS analysis is the first step towards understanding the nature of active principles in the medicinal plants and this type of study will be helpful for further detailed study. GC-MS method is a direct and fast analytical approach for identification of terpenoids and steroids and only few grams of plant material is required. The importance of the study is due to the biological activity of some of these compounds. The present study, which reveals the presence of components in *Crotalaria longipes* aerial parts suggest that the contribution of these compounds on the pharmacological activity should be evaluated.

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