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PROFILING BIO-ACTIVE COMPOUNDS OF *AILANTHUS EXCELSA* LEAVES ROXB. (SIMAROUBACEAE) FOR LIVESTOCK HEALTH

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Abstract: Plants have an array of active ingredients, gained research attention on the animal diets suitability in terms of feed intake, milk production and growth as a way of getting improved livestock health. One such plant is *Ailanthus excelsa*. A.excelsa is a soft wood, fast growing tree species native to India, known for its fodder values besides having economic value in terms of non wood biomass for match stick industry. GC-MS/MS analysis of methanol and n-hexane extracts of *A.excelsa* leaves revealed the presence phytoconstituents of 13 compounds in methanol extract and 6 compounds in n-hexane extract. Of which the major compounds present in the methanol extract are Octadec-9-enoic acid (31.5%), Octadecanoic acid (16.6%), gamma-Sitosterol (9.346%), Eupomatilone-3 (8.78%), Iron, monocarbonyl-(1,3-butadiene-1,4-dicarbonic acid, diethyl ester)a,a'-dipyridyl (7.6%), Ethyl linoleate (35.27%) and Methyl palmitate (29.538%) followed by 1,1'-Biphenyl, 2,2',5,5'-tetrachloro-(7.95%), Octadecanoic acid, methyl ester (7.23%) and Stigmastan-3,5-diene (6.92%) in *n*-hexane crude extract. These compounds found to have anticancer and anti-tumour activity along with other properties like anti-diabetic, antimicrobial, anti-inflammatory, and antiviral activity which have added advantages of *A.excelsa* leaves for use in animal feed for maintaining the livestock wealth.
Keywords: Ailanthus, livestock, methanol, n-hexane, compounds.

1.0 Introduction

In today's perspective, green feed is highly nutritious, palatable, and wealthy in minerals and vitamins and an economic source of macro & micro nutrients. It shows that accessibility of nutrients from green fodder which is considerably cheaper than the concentrate feed. The animal feed which is very much necessary for farm animals' managing during wintry weather. Feed and fodder accessibility is not adequate to effectuate the break in continuity between the claim and make available of feed stuff to the increasing ruminants. Hence, there is a need to investigate an alternate suitable new feed resources which would fulfil the nutrient requirement and to replace pasture/hey and conventional feed resources. Use of fodder tree leaves as green fodder during lean period for grazing animals is significant for livestock production since they are important source of protein and other dietary nutrients (Shrestha, 2005). One such fodder tree is *Ailanthus excelsa* which ensures the availability of

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good quality cattle forage throughout the year. In addition to various medicinal properties, it has an absolute immunity to grazing and also leaves are excellent nutritious fodder material. In view of this, identification of the biologically active compounds present in the plant is very much essential in the exploration of species as nutrient rich fodder. Therefore, this study is aimed to investigate and characterize the bioactive compounds in crude extracts of *A.excelsa* leaves by Gas Chromatography-Mass Spectrum (GC-MS/MS) analysis, since in the last few years, gas chromatography mass spectrometry (GC-MS/MS) has become firmly established as a key technological platform for secondary metabolite profiling which helps to evaluate the fodder quality and the bioactive molecules for livestock feeding, better production and health.

2.0 Methodology

2.1 Collection and processing of *A. excelsa* leaf sample

The leaf samples were collected from *A.excelsa* plantation raised with 64 assemblage of the genetic resources collected from different agro climatic zones of Tamil Nadu, Andhra Pradesh, Rajasthan and Uttarakhand, at Field Research station, Institute of Forest Genetics and Tree Breeding, Kurumbapatti, Salem. The leaves were washed thoroughly with tap water and shade dried for a week at room temperature (24 ± 2^{o}C). The dried samples were powdered to get coarse granules using an electric blender and stored in an airtight container for further analysis.

2.2 Plant Extraction

The powdered leaf samples were then extracted in Soxhlet Apparatus using two solvents viz. Polar (methanol) and Non Polar (n-hexane) solvent. Both the extracts were concentrated under reduced pressure in a rotary evaporator below 50°C, oven dried and the extracts were stored in airtight containers at 4°C temperature for further studies.

2.3 GC-MS/MS analysis

The GC-MS/MS analysis was performed using Varian 4000 Ion trap GC-MS/MS system equipped with a Fused silica capillary column of size 15m x 0.2 mm ID x 1µm linked to an EI detector. Helium gas (99.99% purity) was used as a carrier gas at a constant flow rate of 1ml/min and the sample injected was 1µl; the instrument was set to an initial temperature of 110 ºC, and maintained at this temperature for 2 min. At the end of this period the oven temperature was rose up to 280 ºC, at the rate of an increase of 5 ºC/min, and maintained for 9 min. Injection port temperature was ensured as 250 ºC. The ionization voltage was 70eV. The samples were injected in split mode as 10:1. Mass spectral scan range was set at 45-450

(m/z). Using computer searches on a NIST Ver.2.1 MS data library and comparing the spectrum obtained through GC-MS/MS compounds present in the plants sample were identified.

2.4 Identification of phyto-compounds

The active components in the extracts were identified by comparing their retention indices and mass spectra patterns with the spectrum of known components stored in the computer library and also with published literatures. Interpretation on mass-spectrum GC-MS/MS was conducted to match the identified components from the plant material using the database of National Institute of Standard and Technology (NIST), Wiley, Mainlib, Replib and Tutorial library sources having more than 62,000 patterns. The name, molecular weight and structure of the components of the test materials were ascertained.

3.0 Results

3.1 Bioactive compounds present in the extracts

A. excelsa leaf sample extracts were analyzed for the presence of different active compounds by Gas chromatography-Mass spectroscopy (GC-MS/MS) technique. The GC-MS/MS analysis of phytoconstituents in the leaves of *A.excelsa* revealed the presence of thirteen phytoconstituents in methanol extract and six phytoconstituents in n-hexane extract. The various components present in the entire *A.excelsa* methanol and n-hexane extracts were tabulated (Table 1 & 2). Their elution time, molecular weight, molecular formula and amount of the bioactive compounds were also presented. The chromatograms of the compounds present in polar and non polar solvent extracts of *A.excelsa* leaves presented in Figure 1 & 3 showed the retention time in the column and the detected peaks which correspond to the bioactive compounds present in the extract. The major compounds present in the methanol extract were Octadec-9-enoic acid (31.5%), Octadecanoic acid (16.6%) (Fig 2), gamma-Sitosterol (9.346%), Eupomatilone-3 (8.78%) and Iron, monocarbonyl-(1, 3-butadiene-1, 4-dicarbonic acid, diethyl ester) a, a'-dipyridyl (7.6%). The *n*-hexane crude extract contained Ethyl linoleate (35.27%) Methyl palmitate (29.538%) followed by 1,1'-Biphenyl, 2,2',5,5'-tetrachloro-(7.95%), Octadecanoic acid, methyl ester (7.23%) and Stigmastan-3,5-diene (6.92%) (Fig 4).

Table 1: Biologically active chemical compounds of methanol extract from *A.excelsa* leaves

S.No	Retention time (mins)	Name of the compound	Molecular formula	Molecular weight	Compound concentration (%)
1.	16.783	Hexadecanoic acid	$C_{16}H_{32}O_2$	256	5.6
2.	18.676	2,3-Dihydroxypropyl elaidate	$C_{21}H_{40}O_4$	356	2.6
3.	19.026	Octadecanoic acid, methyl ester	$C_{19}H_{38}O_2$	298	1.277
4.	19.282	Octadec-9-enoic acid	$C_{18}H_{34}O_2$	282	31.5
5.	19.59	Octadecanoic acid	$C_{18}H_{36}O_2$	284	16.6
6.	23.363	Oleoyl chloride	$C_{18}H_3ClO$	300	1.466
7.	26.345	Iron, monocarbonyl-(1,3-butadiene-1,4-dicarbonic acid, diethyl ester)a,a'-dipyridyl	$C_{21}H_{22}FeN_2O_5$	438	7.6
8.	28.966	Eupomatilone-3	$C_{23}H_{26}O_8$	430	7.395
9.	31.164	dimethyl 2-(1',4'-dimethoxy-9',10'-dioxo-5',6',7',8',9',10'-hexahydroanthracen-2'-yl)methylene]butane-1,4-dioate	$C_{23}H_{26}O_8$	430	6.273
10	31.488	Germacrane-a	$C_{15}H_{30}$	210	1.838
11	33.701	Morphine, bis(o-trimethylsilyl)	$C_{23}H_{35}NO_3Si_2$	429	5.394
12	36.060	gamma.-Sitosterol	$C_{29}H_{50}O$	414	9.346
13	36.956	Stigmast-5-en-3-ol, (3.beta.,24S)- (CAS)	$C_{29}H_{50}O$	414	3.183

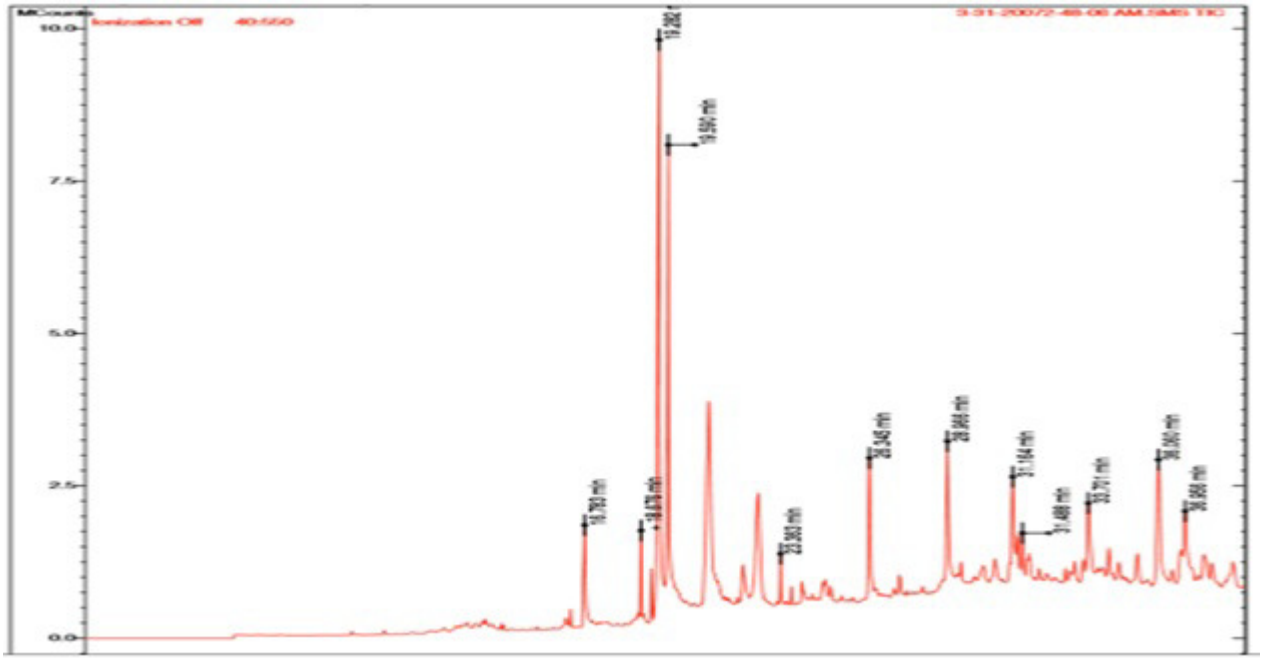


Fig 1. GC-MS/MS chromatogram of methanol extract of *A.excelsa* leaves

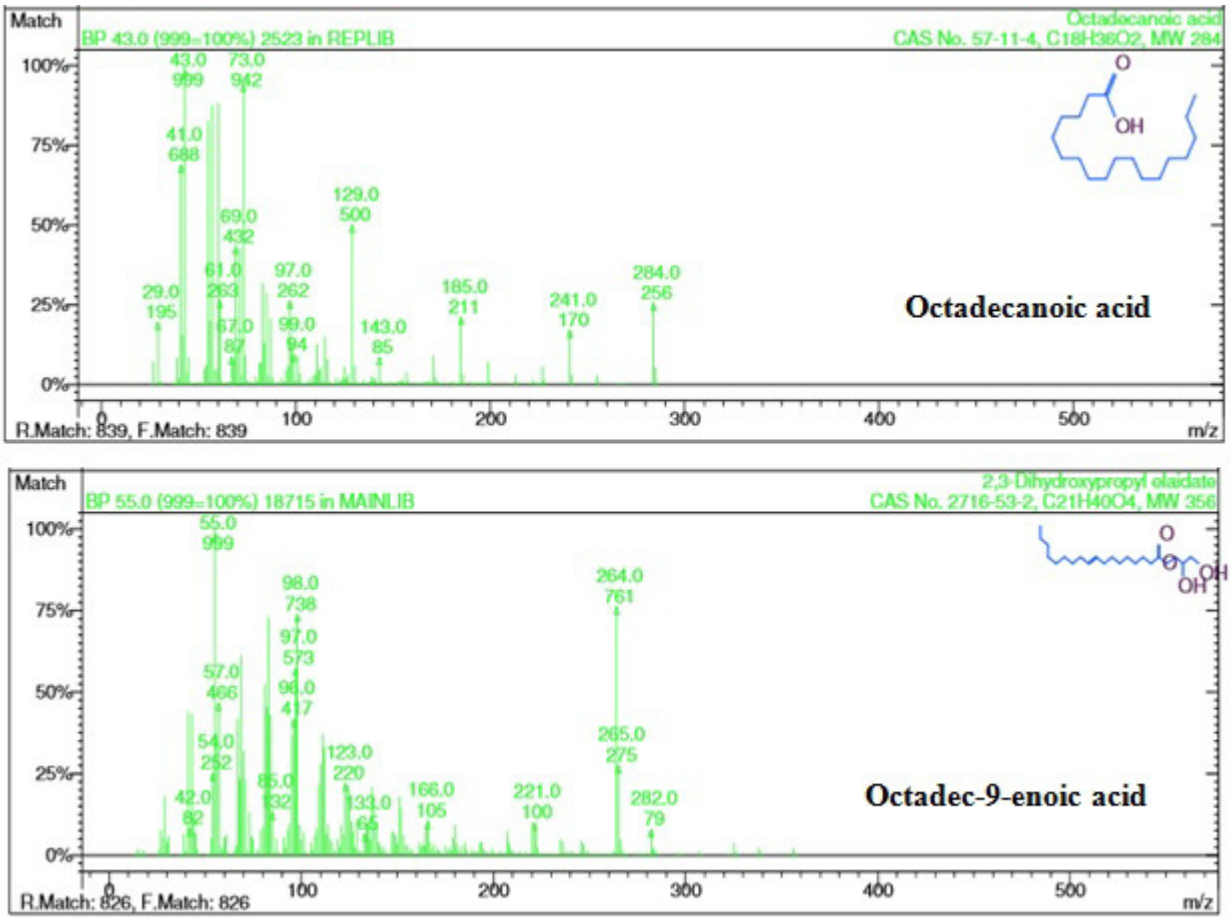


Fig 2. GC–MS/MS Spectra of the bioactive compounds reported in methanol extract of *A.excelsa* leaf

Table 2: Biologically active chemical compounds of n-hexane extract from *A.excelsa* leaves

S.No	Retention time (Mins)	Name of the compound	Molecular formula	Molecular weight	Compound concentration (%)
1	16.309	Methyl palmitate	C12H6Cl4	290	29.538
2	17.050	1,1'-Biphenyl, 2,2',5,5'-tetrachloro-	C12H6Cl4	290	7.945
3	18.588	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	C19H34O2	294	35.27
4	18.680	Ethyl lenoleate	C20H34O2	306	13.141
5	19.021	Octadecanoic acid, methyl ester	C19H38O2	298	7.232
6	19.903	Stigmastan-3,5-diene	C29H48	396	6.921

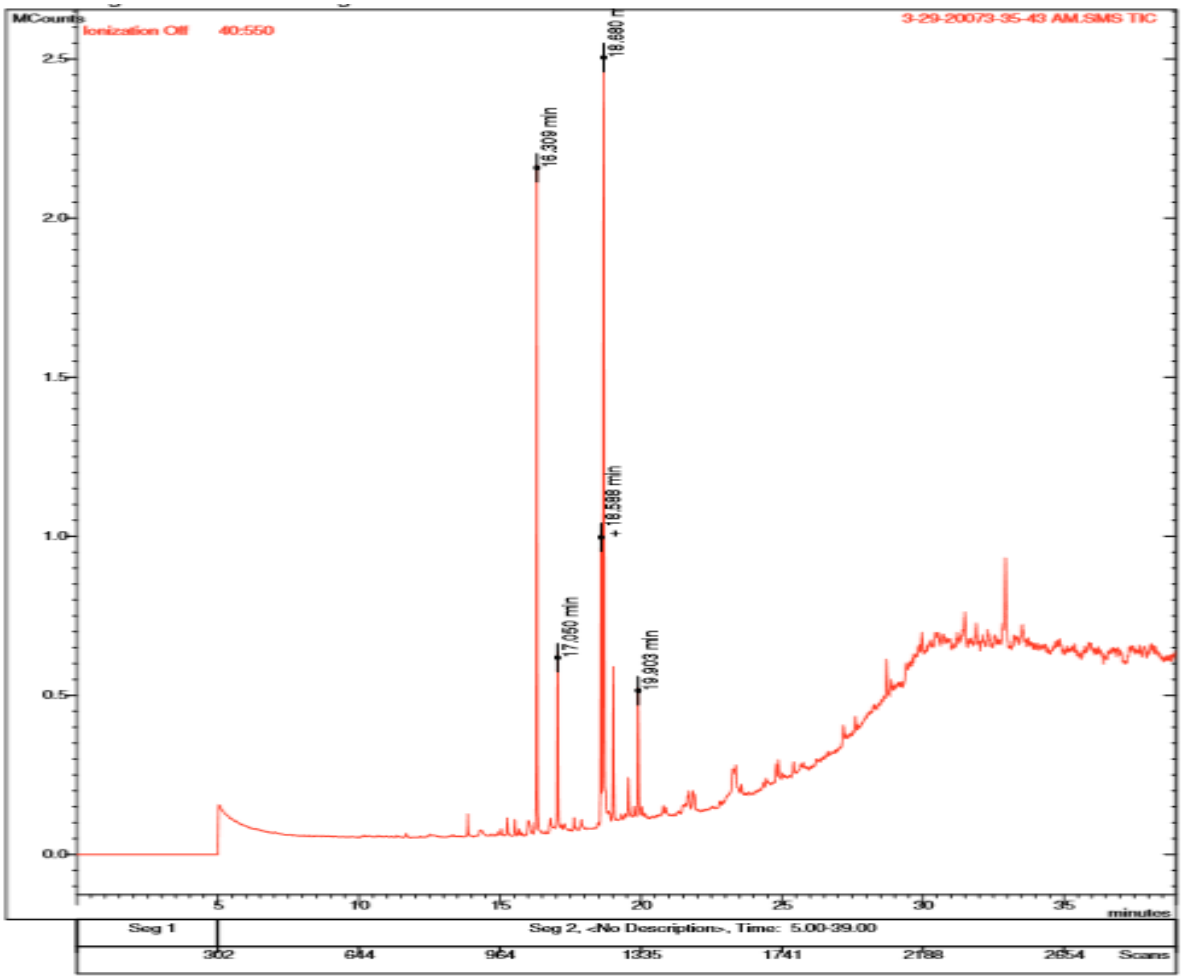
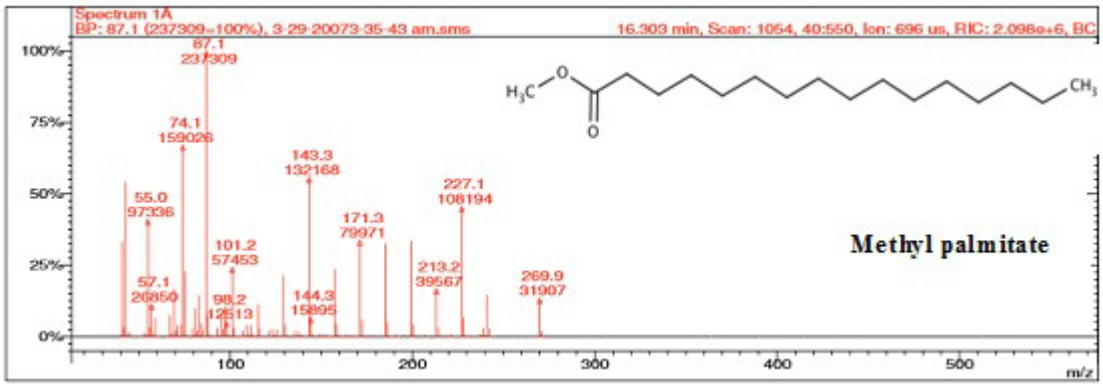


Fig 3. GC- MS/ MS Chromatogram of n-hexane extract of *A.excelsa* leaves



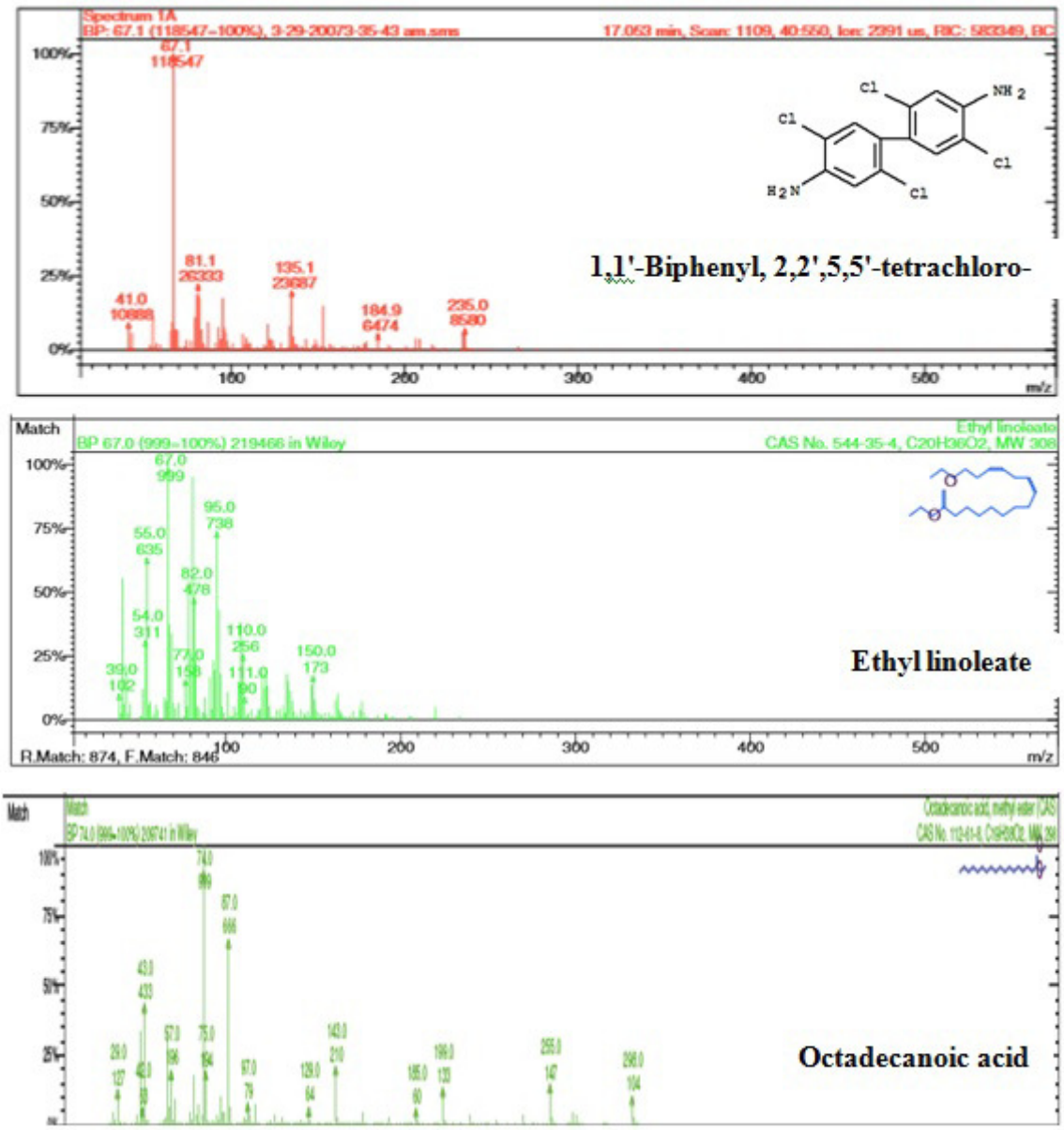


Fig 4. GC–MS/MS Spectra of the bioactive compounds reported in n-hexane extract of *A.excelsa* leaves

4.0 Discussion

In the livestock industry, plants have been considered as a source of essential phytochemical which represent a variety of natural products which are the diets constituents of animals (Harborne, 2001; Wink, 2004). Of which some are nutritionally valuable but many have no nutritional value or antinutritional properties and are potential source of toxicity. Also there is also an escalating realization that plants may offer non-nutrient performance enhancing factors that benefit animal production (Greathead, 2003). Plants and their bioactive compounds can be delivered in numerous ways including, *in situ* grazing and supplemental

feeding. Awareness of health risks associated with synthetic chemicalsleads to find alternatives and balanced nutrient diet is very much essential for animal wealth and production. The present investigation leads to the chromatographic identification and quantification of active ingredients present in the leaves of *A.excelsa* could be suitable as an animal feed. Plant phyto compounds have an effect on feed intake, milk production and growth and there are numerous compounds that have physiological effects in animals and hence the phytochemical investigation of leaves of *A.excelsa* is very vital (Karimi and Jaafar, 2011) or use in animal feed. The bioactive compound hexadecanoic acid eluted at 16.783 min from methanol extract of the leaf extract *of A.excelsa* leaf found to have antioxidant activity. Palmitic acid (hexaecanoic acid) also intimately involved in the synthesis of milk and milkfat. Antioxidants has a great pancreatic role against the oxidative stress in the animal body due to which the defense mechanism gets weakened and causes oxidative damage to lipids, proteins and DNA (Dosek *et al*., 2007) and inhibits the initiation and propagation of ROS (Velioglu *et al*., 1998). Plants provide us with rich sources of natural antioxidants (Biswas *et al.,* 2005) and their present in the feed can protect the biologically important cellular components from oxidative processes. Other than antioxidant activity, n-hexadecanoic acid also reported to have antifibrinolytic, hemolytic and antimicrobial activities (Abirami and Rajendran 2011; Kala *et al.*, 2011). Octadecanoic acid eluted at 19.59 min found to have antitumor activity in addition to antimicrobial activity and the compound Eupomatilone-3 eluted at 29.969 also have antitumor activity. Ponnamma and Manjunath (2012) reported that octadecadienoic acid (Z, Z) found to have anti-inflammatory, hypocholesterolemic and antiarthritic activity. In addition to the compounds Octadecanoic acid and Eupomatilone-3, the bioactive compound squalene was also reported to have anticancer and anti-tumor along with other properties like anti-diabetic, anti-angeogenic, anticancer, antimicrobial, anti-inflammatory, antidiarrhoeal and antiviral activity (Katerere, *et al.,* 2003). *Evolvulusalsinoides* reported to have chemopreventive, anticancer, anti-microbial activity, antioxidant and antidiabetic activity due to the presence of secondary metabolites like piperine, octodeconoic acids, hexadecanoic acid and squalene reported in the ethanolic extract through GC-MS analysis (Gomathi, *et al.,* 2015). Germacrane is a sesquiterpenes, reported in low quantity in the leaves of *A.excelsa* found of have antimicrobial and insecticidal properties and typically produced in a number of plant species for such properties. The phytosterols such as α-Sitosterol and β-Sitosterol were reported to prevent cancer through targeting the mechanism which leads to cancer (Satyal, *et al*., 2012) and gamma-Sitosterol was reported in leaves of *A.excelsa*. γ –Sitosterol is a

phytosterol present in many plants possesses very strong antifungal, antibacterial and anti-angiogenic activity (Zhang Zhong-feng and Zhou Xia-yan, 2011). The bioactive compound ethyl linoleolate eluted at 18.680 min have antibacterial activity. Other than this compound squalene was also reported to have antimicrobial activity (Scortichini and Pia Rossi, 1991). The phytoconstituents compounds like caryophyllene, caryophyllene oxide, neophytadiene, beta.-Eudesmol, phytol and Beta-Tocopherol identified in the methanol extracts of *Eupatorium odoratum* by GC-MS (Venkata Raman, *et al*., 2012) also reported to have antimicrobial activity. The valuable beneficial compounds reported in the foliage provide better feed value than poor or dried-up pasture and thereby helping to improve animal welfare and boost their production. The study revealed that major bioactive compounds present in the extracts of *A.excelsa* leaves serves as the basis in determining the possible health benefits and leading to have physiological effects and diet constituents of animals.

Conclusion

The major bioactive compounds present in the extracts of *A.excelsa* containing antioxidant properties should be helpful for the sustaining of livestock animals. Several bioactive molecules were found in *A.excelsa* are consumed by livestock as a primary consumer and the biological activities of compounds support the medicinal application of the plant. Identification of these compounds in *A.excelsa* serves as the basis in determining the possible health benefits in livestock. Therefore, the leaves of *A.excelsa* with different bioactive compounds of antioxidant, anti-inflammatory, wound healing and other properties proving the assured use of this plant as suitable/palatable tree fodder.

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