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# Gas Chromatography-Mass Spectrometry (GC-MS) Analysis of Ethanolic Extracts of Aerva lanata (L.)

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#### Authors' contributions

This research work is part of first author RV's Ph.D work under the guidance of second author RU and it was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

#### Article Information

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**Original Research Article** 

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# ABSTRACT

**Aim:** To determine the phytochemical constituents present in the different parts of *Aerva lanata* using Gas Chromatography – Mass Spectrometry (GC-MS).

Study Design: GC-MS analysis of bioactive compounds in different parts of A. lanata.

**Place and Duration of Study:** Post Graduate and Research Department of Biochemistry, Government Arts College (Autonomous), Kumbakonam and Department of Food Safety and Quality Testing, Indian Institute of Crop Processing Technology, Thanjavur, Tamilnadu, India, between May 2011 to June 2012.

**Methodology:** 15 g of powdered plant material of leaf, flower and root were soaked with 60 mL of 95% ethanol for 24 hrs. After 24 hrs, the extract was filtered and the filtrate was concentrated to 1 mL by bubbling nitrogen gas into the solution. 2  $\mu$ L of ethanolic extracts of leaf, flower and root of *A. lanata* was used for GC-MS analysis.

Results: The GC-MS analyses showed that the presence of four different phytocompounds in

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ethanolic extract of leaf of *A. lanata*. The highest peak area of 74.73% for isophytol was identified in leaf of *A. lanata*. The ethanolic flower extract of *A. lanata* showed that the presence of twelve different phytocompounds. Flower extract contains the highest amount of phytocompound was 6, 9,12 –octadecatrienoic acid, phenyl methyl ester (z,z,z)- with the peak area of 25%. The root extract of *A. lanata* showed that the presence of eight different bioactive compounds. The root of *A. lanata* showed more quantity of lanost-9 (11)-en-12-one with the highest peak area of 45.11%. **Conculsion:** The present study confirmed that the presence of active compounds in different parts of *A. lanata* would be useful to find out the novel drugs.

Keywords: Phytocompounds; ethanolic extract; Aerva lanata; leaf; flower; root.

# **1. INTRODUCTION**

In the recent past, there has been growing interest in exploiting the biological activities of different ayurvedic medicinal herbs, owing to their natural origin, cost effectiveness and lesser side effects [1]. Medicinal plants are expensive gift from nature to human. The approval of traditional medicine is an alternative form of health care and the development of microbial resistance to the existing antibiotics has induced the researchers to scrutinize the antimicrobial and other biological activities of compounds from plants [2]. Herbal medicines are safer than synthetic medicines because the phytocompounds of the plant extract has no side effects. Medicinal plants have been used all over the world for the treatment and prevention of various ailments, particularly in developing countries [3]. Plant-based natural constituents can be derived from any part of the plant like bark, leaves, flowers, roots, fruits, seeds, etc [4]. The medicinal properties of plants unique to particular plant species or groups are consistent with the concept that the combination of secondary products in a particular plant is taxonomically distinct [5]. There is a growing awareness in correlating the phytochemical constituents of a medicinal plant with its pharmacological activity [6]. Screening of active compounds from plants has led to the invention of new medicinal drugs which has efficient protection and used for the treatment of various diseases.

Today natural products derived from plants are being tested for presence of new drugs with new modes of pharmacological action. A special feature of higher plants is the capacity to produce a large number of secondary metabolites [7]. Recent studies are involved in the identification and isolation of new therapeutic compounds of medicinal importance from higher plants for specific diseases [8,9]. Knowledge of the chemical constituents of plant is helpful in the

discovery of therapeutic agent as well as new sources of economic materials like oil and gums. The most important bioactive constituents of the plants are alkaloids, tannins, flavonoids and phenolic compounds. In India large number of plant species had been screened for their pharmacological properties but still a vast wealth of endangered species are unexplored. Medicinal plants are interested in the field of biotechnology, because most of the drug industries are depending on the plants for the production of pharmaceutical compounds [10].

Plants are rich source of secondary metabolites with interesting biological activities. In general these secondary metabolites are an important source with a variety of structural arrangements and properties [11]. Natural products from microbial sources have been the primary source of antibiotics, but with the increasing recognition of herbal medicine is an alternative form of health care. The screening of medicinal plants for active compounds has become very significant because they may serve as talented source of bulk antibiotic prototypes [12,13].

Aerva lanata (L.) belongs to Amaranthaceae family, known as Polpula is a prostrate to decumbent, sometimes erect herb, found throughout tropical parts of India as a common weed in the fields and wasteland. Traditionally A. lanata leaves are used as sap for eve complaints, an infusion is given to cure diarrhea and kidney stone, and root is used in the treatment of snake bite. A leaf decoction is used as gargle for treating sore throat and also used in various complex treatments against guinea worm [14]. A variety of pharmacological functions of this plant like anti-inflammatory, diuretic, expectorant. hepatoprotective and nephroprotective activities were reported [15]. Alcoholic extract of shoots of A. lanata has significant antidiabetic shown and antihyperglycaemic activities [16]. Antimicrobial, urolithiatic, antihyperlipidaemic, cytotoxic,

antiparasitic, antihelmentic activities of *A. lanata* were also reported [17,18]. The preliminary phytochemical studies were conducted and revealed that the presence of various bioactive compounds. GC-MS analysis of acetone extract of leaves [19] and methanolic extracts of root, flower, stem and leaves of *A. lanata* [20] were reported. But, there is no phytochemical study on ethanol extract of different parts of *A. lanata*. So, the present study was aimed to analyze the phytocompounds of ethanol extract of different parts like leaf, flower and root of *A. lanata* using gas chromatography-mass spectrometry (GC-MS).

# 2. MATERIALS AND METHODS

#### 2.1 Collection and Preparation of Plant Material

The medicinal plant Aerva lanata was collected from in and around Mayiladuthurai at Nagapattinum District, Tamilnadu, India. The identified and authenticated plant was (RV/001/A.L Juss/2012) by Dr. S. John Britto, Director, Rapinat Herbarium and Centre for Molecular Systematics, Department of Botany, St. Joseph's College, Tiruchirappalli, Tamilnadu, India. The leaf, flower and root were separated and washed thoroughly in running tap water to remove soil particles and adhered debris and then finally washed with sterile distilled water. The parts leaf, flower and root of A. lanata were shade dried separately and ground well into powder. The powdered materials were stored in air tight containers at 4°C.

# 2.2 Plant Sample Extraction

15 g of the powdered plant material of leaf, flower and root were soaked in 60 mL of 95% ethanol for 24 hrs. After 24 hrs, the extracts were filtered through Whatmann filter paper No. 1 along with 2 gm of sodium sulfate to remove the sediments and traces of water in the filtrate. Before filtering, the filter paper along with sodium sulphate was wetted with 95% ethanol. The filtrate was then concentrated to 1 ml by bubbling nitrogen gas into the solution. From this, 2  $\mu$ L of ethanolic extract of different parts of *A. lanata* was subjected to GC-MS analysis [21].

# 2.3 GC-MS Analysis

GC-MS analysis of the ethanol extracts of different parts of *A. lanata* (leaf, flower and root) were performed using a Perkin Elmer GC Clarus 500 system comprising AOC-20i auto-sampler

and a Gas Chromatograph interfaced to a Mass Spectrometer (GC-MS) equipped with an Elite-1MS (100% Dimethyl poly siloxane) fused capillary column (30 m x 0.25 mm ID x 1EMdf). For GC-MS detection, an electron ionization system was operated in electron impact mode with ionization energy of 70eV. Helium gas (99.999%) was used as carrier gas at a constant flow rate of 1ml/min, and an injection volume of 0.5 EI was employed (split ratio of 10:1). The injector temperature was maintained at 250°C. the ion-source temperature was 280°C, the oven temperature was 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 9min isothermal at 280°C. Mass spectra were taken at 70eV; a scan interval of 0.5 seconds and fragments from 45-450Da [22]. The solvent delay was 0 to 2 min and the total GC-MS running time was 36 min. The relative percentage amount of each component was calculated by comparing its average peak area to the total areas. The Mass detector used in this analysis was Turbo-Mass Gold-Perkin Elmer and the software adopted to handle mass spectra and chromatogram was a Turbo-Mass ver-5.2.

# 2.4 Identification of Components

The relative percentage amount of each component was calculated by comparing its average peak area to the total areas. The detection employed by using the NIST (National Institute of Standards and Technology) library ver.2.0 (2005). The prediction of biological activity of compounds was based on Dr. Duke's Phytochemical and Ethnobotanical Databases created by Dr. Jim Duke of the Agricultural Research Service/USDA. Interpretation of GC-MS was conducted using the database of NIST library having more than 62,000 patterns. The spectrum of the unknown component was compared with the spectrum of the known the components stored in the NIST library ver. 2.0. The name, molecular weight and molecular formula of components of the test materials were ascertained.

# 3. RESULTS

The identification of the phytocompounds was carried out based on the retention time and molecular formula. The name of identified compounds in the different parts of *A. lanata* with their retention time (RT), molecular formula (MF), molecular weight (MW) and peak area percentage were represented in Tables 1, 2 and 3.

The leaf extract of *A. lanata* showed four phytocomponds such as pyridine,4-iodo-(2.20%), 1H-pyrrole-2,5-dione,1-ethenyl (8.79%), ethaneperoxoic acid,1-cyano-1-[2-(2-phenyl-1,3-dioxolan-2-yl) ethyl] pentyl ester (14.29%) and isophytol (74.73%) (Table 1 and Fig. 1).

The twelve different phytocomponents were identified in flower extract of A. lanata such as α-D-xylofuranoside, methyl-5-O-methyl (1.19%), 7oxabicyclo (4.1.0)heptan-2-one (1.19%), octanoic acid ethyl ester (1.19%), phytol (4.76%), dodecane,2,6,11-tri methyl (5.95%), 3.4hexanediol,2,5-dimethyl (2.38%), octane,2,7dimethyl (19.05%), hexadecane (15.48%), 1.3bis-butyl peroxy-phthalan (5.95%), 6,9,12octadecatrienoic acid, phenyl methyl ester, (z,z,z)-(25.00%), octanal,7-methoxy-3-7-dimethyl (14.29%) and isopropyl,5,11-dihydroxy-3,7,11trimethyl-2 dodecanoate (3.57%) (Table 2 and Fig. 2).

Eight phytocompounds such as benzaldehyde,4-(1-methylethyl)-(1.5%), cyclohexene,3-methyl-6-(1-methylethylidene)-(3.01%), 1,4-dibromo-2cyclohexylbutane (3.76%), 4-vinylbenzoic acid (3.01%), dihydrotachysterol (17.29%), lanost-9(11)-en-12-one (45.11%), cholest-22-ene-21ol,3,5-dehydro-6-methoxy-,pivalate (14.29%), and urs-12-en-24-oic acid,3-oxo-,methyl ester, (+)-(12.03%) were identified in the root extract of *A. lanata* (Table 3 and Fig. 3).

In this study, the phytocompounds were identified in the ethanol extract of *A. lanata* by GC-MS analysis and predicted their biological activities based on Dr. Duke's Phytochemical and Ethnobotanical Databases created by Dr. Jim Duke of the Agricultural Research Service/USDA [23].

# 4. DISCUSSION

The plant is endowed with various chemical components such as flavonoids, alkaloids, triterpenes, steroids, polysaccharides, tannins, saponins, proteins, amino acids, volatile oils, and free reducing sugars [24,25]. Six alkaloids were isolated and reported from A. lanata such as Canthin-6-one, 10- Methoxycanthin -6-one (methyl aervin), 10-Hydroxy canthin-6-one (aervin), 10-β –D- glucopyranosyl-oxy-canthin-6one (aervoside), β-Carboline-1-propionic acid 6-Methoxy-β-carboline-1-propionic and acid (aervolanin) [26]. The plant A. lanata contains αamyrin, campesterol, *β*-sitosterol, and its palmitate, chrysin and flavonoid glucosides were

reported [27]. The researchers reported that the GC-MS analysis of acetone extract of leaves [19] and the methanolic extracts of root, flower, stem and leaves [20] of *A. lanata* showed the presence of many phytocompounds.

present study Similarly, in the many phytocompounds were identified in the ethanolic extract of root, flower and leaves of A. lanata. The identified compounds possess biological and pharmacological properties were predicted from Dr Duke's Phytochemical and Ethnobotanical Databases [21]. In this study, the identified phytocompounds from the ethanolic extract of leaf possess antimicrobial and anti-inflammatory 1H-Pyrrole-2,5-dione,1activities. ethenyl-(8.79%) is a alkaloid compound, which was observed in the leaf extract of A. lanata. Alkaloids are important defense system of the plant against pathogenic organisms and herbivores. It is a toxin for insects, which further modify that the alkaloids are incorporated them into their own defense secretion [28]. Similarly, the medicinally important phytocompound 1H-Pyrrole-2,5-dione,1- ethenyl- was observed in Acalypha indica [29]. The cyano compound Ethaneperoxoic acid, 1-cyano-1-[2-(2-phenyl-1,3dioxolan-2-yl) ethyl] pentyl ester (14.29%) was observed in the leaf of A. lanata, which possess antimicrobial, anti-inflammatory and insecticidal properties. Similarly, it was reported that the presence of this compound in the ethanolic extract of Peristrophe bicalyculata [30] and chloroform extract of Cocculus hirsutus [31].

Ethanolic extract of flower of A. lanata showed that the presence of twelve different phytocompounds. Among these, the four compounds such as octanoic acid ethyl ester (1.19%), phytol (4.76%), 6,9,12-octadecatrienoic acid. phenyl methyl ester, (z, z, z)- (25%) and octanal,7-methoy-3-7-dimethyl(14.29%) possess pharmacological activities. Octanoic acid ethyl ester possesses insecticidal, anticandidal and antifungal activities. Similarly, Prabhadevi et al. [32] reported that the presence of octanoic acid ethyl ester in the ethanolic extract of stem of Allamanda cathartica by GC-MS analysis. In this study, the compound 6,9,12-Octadecatrienoic acid phenyl methyl ester, (z,z,z)- (Linolenic acid ester) was identified in the flower extract of A. lanata and it possess anti-inflammatory, hypocholesterolemic, insectifuge, cancer preventive. nematicide. hepatoprotective, antihistaminic, antieczemic, antiacne, 5-alpha reductase inhibitor, antiandrogenic, antiarthritic and anticoronary properties.

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Fig. 1. GC-MS chromatogram of ethanolic extract of leaf of A. lanata (L.)

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Fig. 3. GC-MS chromatogram of ethanolic extract of root of A. lanata (L.)

Retention time	Retention index	Name of the compound	IUPAC name	Molecular Formula	Molecular weight	Peak Area %	Compound nature	Activity*
7.94	1078	Pyridine, 4-iodo-	4-iodopyridine	C₅H₄IN	205	2.2	lodo compound	Antimicrobial
11.62	1163	1H-Pyrrole-2,5- dione, 1-ethenyl-	1-ethenyl-2,5-dihydro- 1H-pyrrole-2,5-dione	$C_6H_5NO_2$	123	8.79	Alkaloid compound	Antimicrobial, Antiinflammatory
13.07	2584	Ethaneperoxoic acid, 1-cyano-1-[2- (2-phenyl-1,3- dioxolan-2- yl)ethyl]pentyl ester	3-cyano-1-[2-phenyl, 1, 3 dioxolan-2-yl] Ethaneperoxoic acid	C <sub>19</sub> H <sub>25</sub> NO <sub>5</sub>	347	14.29	Cyano compound	Antimicrobial, Antiinflammatory
14.97	1945	lsophytol	3,7,11,15- tetramethylhexadec-1- en-3-ol	$C_{20}H_{40}O$	296	74.73	Diterpene	Antimicrobial, Antiinflammatory, Diuretic, Anticancer

#### Table 1. List of identified phytocompounds in the extract of leaf of A. lanata by GC-MS analysis

Source: Dr. Duke's Phytochemical and Ethnobotanical Database'

#### Table 2. List of identified phytocompounds in the extract of flower of A. lanata by GC-MS analysis

Retention time	Retention index	Name of the compound	IUPAC name	Molecular Formula	Molecular weight	Peak Area %	Compound nature	Activity*
10.11	Nf	α-D- xylofuranoside,methyl-5- O-methyl,	methyl, 5-O-methyl, α- D-xylofuranoside	$C_7H_{14}O_5$	178	1.19	Sugar compound	No activity
11.62	902	7- oxabicyclo(4.1.0)heptan- 2-one	7- oxabicyclo[4.1.0]hepta n-2-one	C <sub>6</sub> H <sub>8</sub> O <sub>2</sub>	112	1.19	Ketone compound	No activity
13.42	1190	octanoic acid ethyl ester	ethyl octanoate	$C_{10}H_{20}O_2$	172	1.19	Fatty acid ester	Insecticide, Antifungal, Anticandidal
14.92	2122	Phytol	(2E,7R,11R)- 3,7,11,15- tetramethylhexadec-2- en-1-ol	C <sub>20</sub> H <sub>40</sub> O	296	4.76	Diterpene	Antimicrobial, Antiinflammatory, Diuretic, Anticancer
20.29	1275	Dodecane,2,6,11-tri methyl	2,6,11-tri methyl dodecane	$C_{15}H_{32}$	212	5.95	Alkane compound	No activity

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Retention time	Retention index	Name of the compound	IUPAC name	Molecular Formula	Molecular weight	Peak Area %	Compound nature	Activity*
21.7	1013	3,4-hexanediol, 2,5- dimethyl	2,5-dimethylhexane- 3,4-diol	$C_8H_{18}O_2$	146	2.38	Alcoholic compound	Antimicrobial
23.08	929	octane,2,7-dimethyl	2,7-dimethyl octane	$C_{10}H_{22}$	142	19.05	Alkane compound	No activity
25.79	1600	hexadecane	hexadecane	C <sub>16</sub> H <sub>34</sub>	226	15.48	Alkane compound	No activity
31	1889	1,3-bis-butyl peroxy- phthalan	1,3-Bis[(2-methyl-2- propanyl)peroxy]-1,3- dihydro-2-benzofuran	$C_{16}H_{24}O_5$	296	5.95	Oxy compound	No activity
32.24	2774	6,9,12-octadecatrienoic acid, phenyl methyl ester,(z,z,z)-	benzyl octadeca- 6,9,12-trienoate	C <sub>25</sub> H <sub>36</sub> O <sub>2</sub>	368	25	Linolenic acid ester	Anti-inflammatory, Hypocholesterolemic Cancer preventive, Hepatoprotective, Nematicide, Insectifuge, Antihistaminic Antieczemic, Antiacne, 5-Alpha reductase inhibitor Antiandrogenic, Antiarthritic, Anticoronary, Insectifuge
33.47	1230	Octanal,7-methoxy-3-7- dimethyl	7-methoxy-3,7- dimethyloctanal	$C_{11}H_{22}O_2$	186	14.29	Aldehyde compound	Antimicrobial, Anti- inflammatory
34.14	Nf	lsopropyl,5,11- dihydroxy-3,7,11- trimethyl-2 dodecanoate	Isopropyl,5,11- dihydroxy-3,7,11- trimethyl-2 dodecanoate	C <sub>18</sub> H <sub>34</sub> O <sub>4</sub>	314	3.57	Hydroxy compound	No activity

\*Source: Dr. Duke's Phytochemical and Ethnobotanical Databases; Nf-Not found

Retention time	Retention index	Name of the compound	IUPAC name	Molecular Formula	Molecular weight	Peak Area %	Compound nature	Activity*
4.71	1230	Benzaldehyde, 4- (1-methylethyl)-	4-(propan-2- yl)benzaldehyde	C <sub>10</sub> H <sub>12</sub> O	148	1.5	Aldehyde compound	Antimicrobial, Anti- inflammatory
5.87	1052	Cyclohexene,3- methyl-6-(1- methylethylidene)-	3-methyl-6-(propan-2- ylidene)cyclohex-1-ene	C <sub>10</sub> H <sub>16</sub>	136	3.01	Aromatic compound	No activity
17.18	1607	1,4-Dibromo-2- cyclohexylbutane	(1,4-dibromobutan-2- yl)cyclohexane	$C_{10}H_{18}Br_2$	296	3.76	Bromo compound	Antimicrobial
17.92	1353	4-Vinylbenzoic acid	4-ethenylbenzoic acid	$C_9H_8O_2$	148	3.01	Aromatic Acid	Antimicrobial preservevative
25.01	2843	Dihydrotachysterol	(5Z,7E,22E)-9,10- Sécoergosta-5,7,22-trién-3- ol	C <sub>28</sub> H <sub>46</sub> O	398	17.29	Synthetic vitamin- D	Used in the mineralization of bone
26.99	816	Lanost-9(11)-en- 12-one	4,4,10,13,14-pentamethyl- 17-(6-methylheptan-2-yl)- 1,2,3,5,6,7,8,15,16,17- decahydrocyclopenta[a]phe nanthren-12-one	C <sub>30</sub> H <sub>50</sub> O	426	45.11	Steroid compound	Antimicrobial, Anti- inflammatory, Antiarthritic, Diuretic, Antiasthma
31.87	2973	Cholest-22-ene-21- ol,3,5-dehydro-6- methoxy-, pivalate	(23E)-6-Methoxy-3,5- cyclocholest-23-en-22-yl pivalate	C <sub>33</sub> H <sub>54</sub> O <sub>3</sub>	498	14.29	Steroid compound	Antimicrobial, Anti- inflammatory, Antiarthritic, Diuretic, Antiasthma
35.94	2710	Urs-12-en-24-oic acid, 3-oxo-, methyl ester, (+)-	Methyl 3-oxours-12-en-24- oate	$C_{31}H_{48}O_3$	468	12.03	Steroid compound	Antimicrobial, Anti- inflammatory, Antiarthritic, Diuretic, Antiasthma

#### Table 3. List of identified phytocompounds in the extract of root of A. lanata by GC-MS analysis

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

Similarly, the researchers were reported that the presence of Phytol and 6.9.12-Octadecatrienoic acid in the ethanol extract of leaf of Aloe vera [33] and the compound 6,9,12-Octadecatrienoic acid, methyl ester, (Z,Z,Z)-, in the ethanol extract of Caesalpinia sappan [34]. Fatty acids in plants react with alcohols in an esterification reaction to form esters [35]. So, the compound 6,9,12 -Octadecadienoic acid phenyl ethyl ester (25%) is an important ester in the flower extract of A. lanata. Unsaturated fatty acids are important in the body for normal growth. These are vital in maintaining the integrity of cell structure as well as the unique ability to lower cholesterol levels of the blood [36]. Phytols possess antimicrobial, anti-inflammatory, anticancer. diuretic, hepatoprotective and antiandrogenic properties [22,37-40]. Similarly, the compound phytol was reported in Aristolochia krysagathra and the phytol constitute a promising novel class of pharmaceuticals for the treatment of rheumatoid arthritis and chronic inflammatory diseases [41].

Six phytocompounds has pharmacological activities out of eight compounds were observed in the root of A. lanata. Dihydrotachysterol (DHT) is an analog of Vitamin D and it was reported as systemic effectors of calcium metabolism and promotes calcification of bones. Vitamin D and DHT are administered in the case of hyperparathyroidism to activate calcification. However, high dose of DHT induces, the pathologic calcification leading to excessive accumulation of calcium [42]. The compounds such as lanost-9(11)-en-12-one, cholest-22-ene-21-ol, 3, 5-dehydro-6-methoxyl- pivalate, urs-12en-24oic acid, and 3-oxo-methyl ester (+)- were present in the root of A. lanata are steroid in nature and possess antimicrobial, antiinflammatory, antiarthritic. diuretic and antiasthma activities. Many steroids are used as medicine for the treatment of cancer, arthritis, allergy and in birth control [43,44]. Yamunadevi et al. [45] reported that the presence of different types of steroids in the methanolic extracts of root, stem, flower, leaves and seeds of A. lanata. Similarly, the steroidal compounds urs-12-en-24oic acid and 3- oxo-, methyl ester, (+)- were reported in the ethanolic extract of Canscora perfoliata [46] and also in ethanol extract of leaf of Barleria montana [47].

#### 5. CONCLUSION

The results of the present investigation revealed that the presence of phytocompounds in the ethanol extracts of different parts of *A. lanata* by

GC MS analysis. The phytoconstituents present in the different parts of *A. lanata* may be attributed to the medicinal characteristics. In future, the isolation and purification of above mentioned phytocompounds would be useful in the preparation of novel drugs for treating diseases.

### **COMPETING INTERESTS**

Authors have declared that no competing interests exist.

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