

GC-MS Analysis of Bioactive Constituents of Ethanolic Extracts of *Aerva lanata* (Linn.)

ABSTRACT

Aim: To determine the phytochemical constituents present in the different parts like leaf, flower and root of *Aerva lanata* using Gas Chromatography – Mass Spectrum (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 600 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 µl of ethanolic extracts of leaf, flower and root of *A. lanata* was used for GC-MS analysis.

Result: The GC-MS analyses showed that the presence of four different phytochemicals in ethanolic extract of leaf of *A. lanata*, such as *γ*-linalool, 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%). The ethanolic flower extract of *A. lanata* showed that the presence of twelve different phytochemicals including Octanoic acid ethyl ester (1.19%), Phytol (4.76%), 6,9,12-Octadecatrienoic acid, phenyl methyl ester, (z,z,z)- (25.00%) and Octanal, 7-methoxy-3-7-dimethyl (14.29%). The root extract of *A. lanata* showed the presence of eight different bioactive compounds including Benzaldehyde, 4- (1-methylethyl)- (1.5%), Lanost-9 (11)-en-12-one (45.11%) and Cholest-22-ene-21-ol, 3,5-dehydro-6-methoxy-, pivalate (14.29%).

Conclusion: The present study confirmed that the presence of biological active compounds in different parts of *A. lanata*. In future, the isolation of individual phytoconstituents may proceed to find a novel drug.

Keywords: Phytochemicals; 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 compounds from plants [2]. Herbal medicines are safer than synthetic medicines because the phytochemicals 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 [6-12]. 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 [13]. Recent studies are involved in the identification and isolation of new therapeutic compounds of medicinal importance from higher plants for specific diseases [14, 15]. 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 [16].

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 [17]. 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 [18, 19].

Aerva lanata (Linn.) 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 eye 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 [20]. A variety of pharmacological functions of this plant like anti-inflammatory, diuretic, expectorant, hepatoprotective and nephroprotective activities were reported [21]. Alcoholic extract of shoots of *A. lanata* has shown significant antidiabetic and antihyperglycaemic activities [22]. Antimicrobial, cytotoxic, urolithiatic, antihyperlipidaemic, antiparasitic, antihelmentic activities of *A. lanata* were also reported [23, 24]. Although the preliminary phytochemical studies were conducted and revealed that the presence of various bioactive compounds, there is no detailed study on phytochemical profiling of different parts of *A. lanata*. So, the present study was aimed to analyze the phytochemicals of different parts like leaf, flower and root of *A. lanata* using Gas Chromatography-Mass Spectrum (GC-MS).

66 2. MATERIALS AND METHODS

67 2.1. Collection and Preparation of Plant Material

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

78 2.2 Plant Sample Extraction

79 15 g of the powdered plant material of leaf, flower and root were soaked in 600 ml of 95%
80 ethanol for 24 hrs. After 24 hrs, the extracts were filtered through Whatmann filter paper No.
81 1 along with 2 gm of sodium sulfate to remove the sediments and traces of water in the
82 filtrate. Before filtering, the filter paper along with sodium sulphate was wetted with 95%
83 ethanol. The filtrate was then concentrated to 1 ml by bubbling nitrogen gas into the solution.
84 From this, 2 ml of ethanolic extract of different parts of *A. lanata* was subjected to GC-MS
85 analysis [25].

87 2.3 GC-MS Analysis

88 GC-MS analysis of the ethanol extracts of different parts of *A. lanata* (leaf, flower and root)
89 were performed using a Perkin Elmer GC Clarus 500 system comprising AOC-20i auto-
90 sampler and a Gas Chromatograph interfaced to a Mass Spectrometer (GC-MS) equipped
91 with an Elite-5MS (5% Diphenyl / 95% Dimethyl poly siloxane) fused capillary column (30 x
92 250µm ID x 0.25µm df). For GC - MS detection, an electron ionization system was operated
93 in electron impact mode with ionization energy of 70eV. Helium gas (99.999%) was used as
94 carrier gas at a constant flow rate of 1 ml/min, and an injection volume of 2 µl was employed
95 (split ratio of 10: 1). The injector temperature was maintained at 250 °C, the ion-source
96 temperature was 200 °C, the oven temperature was programmed from 50 °C (isothermal
97 for 2 min), with an increase of 10 °C /min to 200 °C, then 5 °C /min to 280 °C, ending with a
98 9 min isothermal at 280 °C. Mass spectra were taken at 70eV; a scan interval of 0.5 seconds
99 and fragments from 45-450Da [26]. The solvent delay was 0 to 2 min and the total GC-MS
100 running time was 36 min. The relative percentage amount of each component was
101 calculated by comparing its average peak area to the total areas. The Mass detector used in
102 this analysis was Turbo-Mass Gold-Perkin Elmer and the software adopted to handle mass
103 spectra and chromatogram was a Turbo-Mass ver-5.2.

107 2.4 Identification of Components

108 The relative percentage amount of each component was calculated by comparing its
109 average peak area to the total areas. The detection employed by using the NIST (National
110 Institute of Standards and Technology) library ver. 2.0 (2005). The biological activity of
111 compound was predicted based on Dr. Duke's Phytochemical and Ethnobotanical
112 Databases created by Dr. Jim Duke of the Agricultural Research Service/USDA.
113 Interpretation of GC-MS was conducted using the database of NIST library having more than
114 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 molecular formula of the components of the test materials were ascertained.

3. RESULT

GC-MS is one of the best techniques to identify the constituents of volatile matter, long and branched cyclic hydrocarbons, alcohols, acids, esters etc. In the present study ethanol extract of leaf, flower and root of *A. lanata* revealed that the presence of twenty four phyto compounds that could be contribute to the medicinal quality of this plant. The identification of the phytocompounds based on the peak area, retention time and molecular formula was confirmed. The name of active principles are identified in the different parts of *A. lanata* with their retention time (RT), molecular formula (MF), molecular weight (MW) and peak area in percentage are presented in Tables 1, 2 and 3.

The leaf extract of *A. lanata* showed four phytocompounds 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%), 7-oxabicyclo(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%), 3,4-Hexanediol,2,5-dimethyl (2.38%), Octane,2,7-dimethyl (19.05%), Hexadecane (15.48%), 1,3-Bis-butyl peroxy-phthalan (5.95%), 6,9,12- Octadecatrienoic acid, phenyl methyl ester, (z,z,z)- (25.00%), Octanal,7-methoxy-3-7-dimethyl (14.29%) and Isopropyl,5,11-dihydroxy-3,7,11-trimethyl-2 dodecanoate (3.57%) (Table 2 and Fig. 2).

Eight phytocompounds were identified in root extract *A. lanata* by GC-MS analysis. The compounds Benzaldehyde,4-(1-methylethyl)- (1.5%), Cyclohexene,3-methyl-6-(1-methylethylidene)- (3.01%), 1,4-Dibromo-2-cyclohexylbutane (3.76%), 4-Vinylbenzoic acid (3.01%), Dihydrotachysterol (17.29%), Lanost-9(11)-en-12-one (45.11%), Cholest-22-ene-21-ol,3,5-dehydro-6-methoxy-,pivalate (14.29%), and Urs-12-en-24-oic acid,3-oxo-,methyl ester, (+)- (12.03%) were identified in root extract of *A. lanata* (Table 3 and Fig. 3).

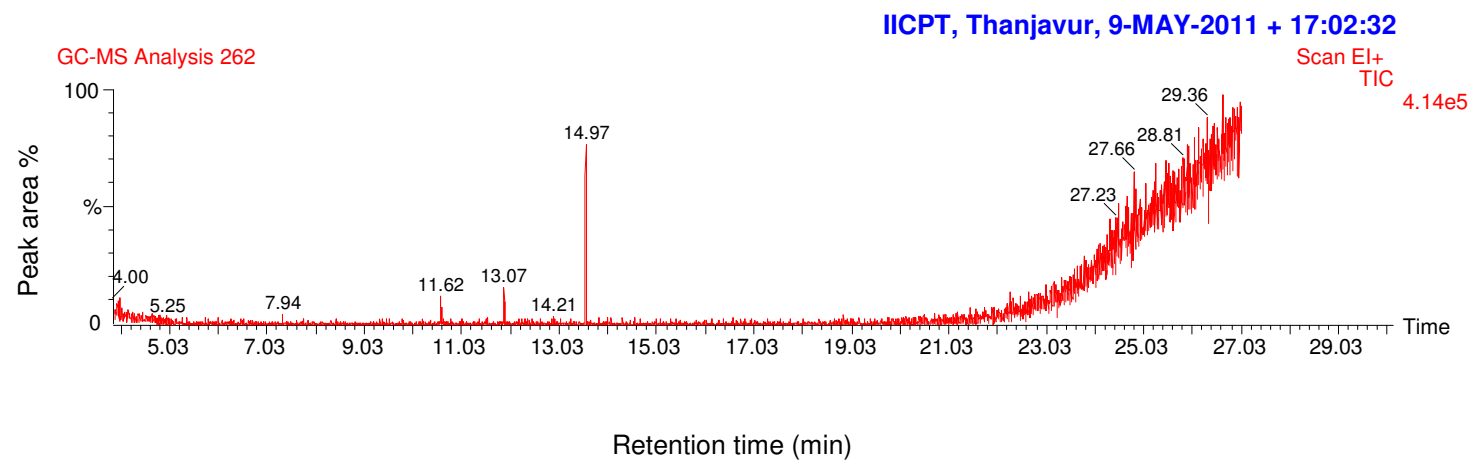
In this study, the phytocompounds were identified in the ethanol extract of *A. lanata* by GC-MS analysis possess many biological activities are predicted based on Dr. Duke's Phytochemical and Ethnobotanical Databases created by Dr. Jim Duke of the Agricultural Research Service/USDA.

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

Retention time	Name of the compound	Molecular formula	Molecular weight	Peak area %	Nature of compound	Activity*
7.94	Pyridine, 4-iodo-	C ₅ H ₄ IN	205	2.2	Iodo compound	Antimicrobial
11.62	1H-Pyrrole-2,5-dione, 1-ethenyl-	C ₆ H ₅ NO ₂	123	8.79	Alkaloid compound	Antimicrobial, Anti-inflammatory
13.07	Ethaneperoxoic acid, 1-cyano-1- [2-(2-phenyl-1,3-dioxolan-2-yl) ethyl] pentyl ester	C ₁₉ H ₂₅ NO ₅	347	14.29	Cyano compound	Antimicrobial, Anti-inflammatory
14.97	Isophytol	C ₂₀ H ₄₀ O	296	74.73	Diterpene	Antimicrobial, Anti-inflammatory, Diuretic, Anticancer

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

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

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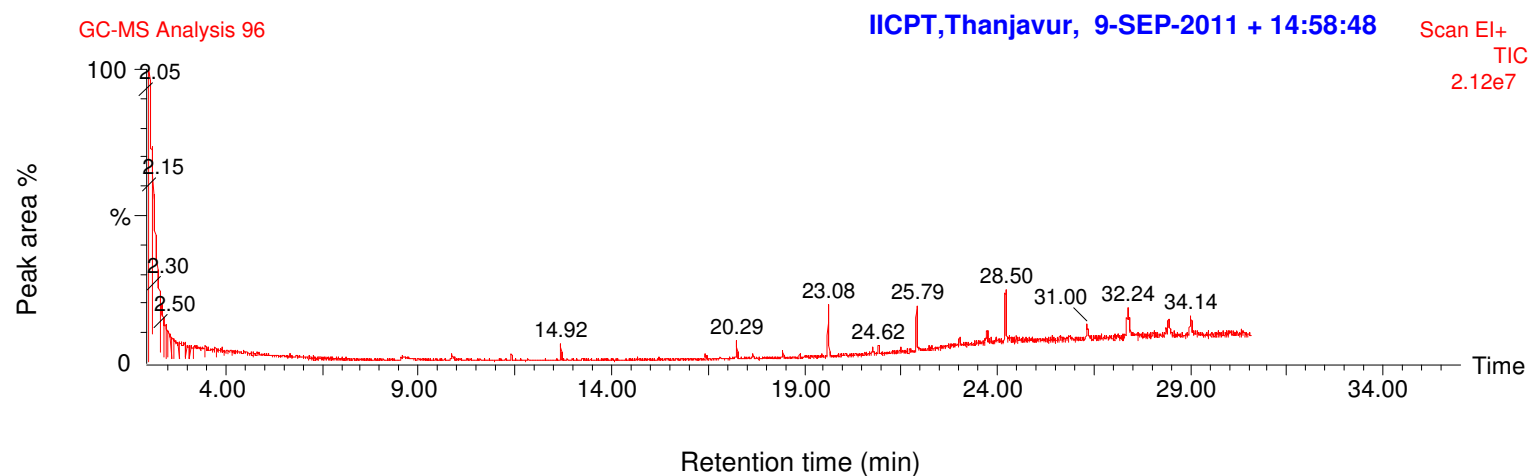
184 Table 2. List of identified phytocompounds in the extract of flower of *A. lanata* by GC-MS analysis

Retention time	Name of the compound	Molecular formula	Molecular weight	Peak area %	Nature of compound	Activity*
10.11	α -D-xylofuranoside,methyl-5-O-methyl,	C ₇ H ₁₄ O ₅	178	1.19	Sugar compound	No activity
11.62	7-oxabicyclo (4.1.0) heptan-2-one	C ₆ H ₈ O ₂	112	1.19	Ketone compound	No activity
13.42	Octanoic acid ethyl ester	C ₁₀ H ₂₀ O ₂	172	1.19	Fatty acid ester	Insecticide, Antifungal, Anticandidal
14.92	Phytol	C ₂₀ H ₄₀ O	296	4.76	Diterpene	Antimicrobial, Anti-inflammatory, Diuretic, Anticancer
20.29	Dodecane,2,6,11-tri methyl	C ₁₅ H ₃₂	212	5.95	Alkane compound	No activity
21.7	3,4-Hexanediol, 2,5-dimethyl	C ₈ H ₁₈ O ₂	146	2.38	Alcoholic compound	Antimicrobial
23.08	Octane,2,7-dimethyl	C ₁₀ H ₂₂	142	19.05	Alkane compound	No activity
25.79	Hexadecane	C ₁₆ H ₃₄	226	15.48	Alkane compound	No activity
31	1,3-bis-butyl peroxy-phthalan	C ₁₆ H ₂₄ O ₅	296	5.95	Oxy compound	No activity
32.24	6,9,12-Octadecatrienoic acid,phenyl methyl ester,(z,z,z)-	C ₂₅ H ₃₆ O ₂	368	25	Linolenic acid ester	Anti-inflammatory, Hypocholesterolemic, Cancer preventive, Hepatoprotective, Nematicide, Insectifuge, Antihistaminic, Antieczemic, Antiacne, 5-Alpha reductase inhibitor, Antiandrogenic, Antiarthritic, Anticoronary, Antimicrobial, Anti-inflammatory
33.47	Octanal,7-methoxy-3-7-dimethyl	C ₁₁ H ₂₂ O ₂	186	14.29	Aldehyde compound	No activity
34.14	Isopropyl,5,11-dihydroxy-3,7,11-trimethyl-2 dodecanoate	C ₁₈ H ₃₄ O ₄	314	3.57	Hydroxy compound	No activity

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

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Fig. 2. GC MS chromatogram of ethanolic extract of flower of *A. lanata* (L.)

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

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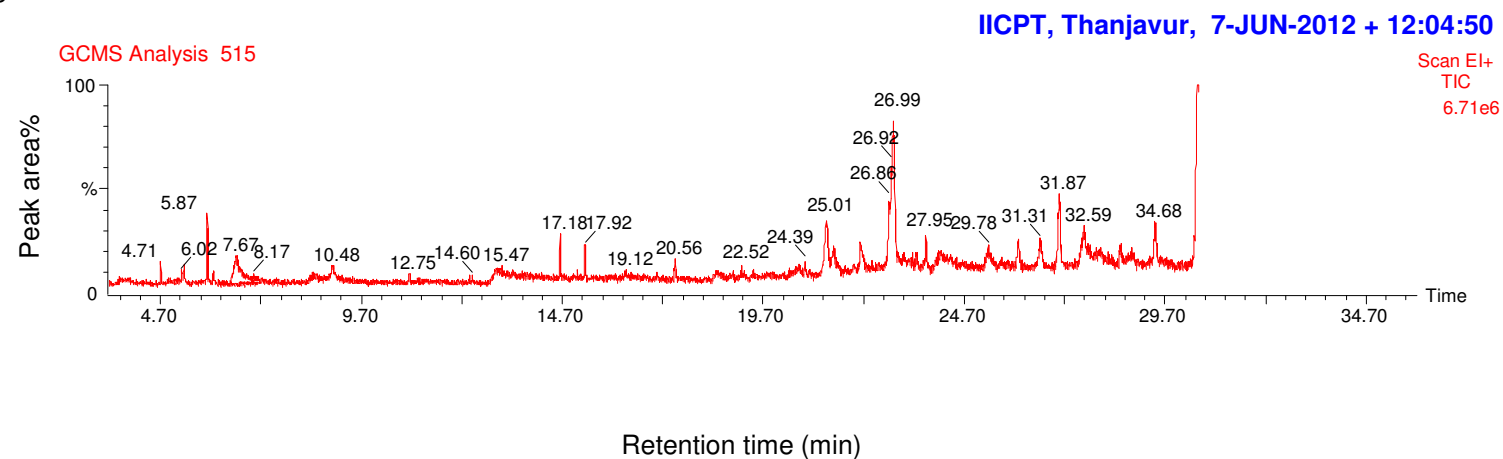
Retention time	Name of the compound	Molecular formula	Molecular weight	Peak area %	Nature of compound	Activity*
4.71	Benzaldehyde, 4- (1-methylethyl)-	C ₁₀ H ₁₂ O	148	1.5	Aldehyde compound	Antimicrobial, Anti-inflammatory

5.87	Cyclohexene, 3-methyl-6-methylethylidene)-	(1-	C ₁₀ H ₁₆	136	3.01	Aromatic compound	No activity
17.18	1,4-Dibromo-2-cyclohexylbutane		C ₁₀ H ₁₈ Br ₂	296	3.76	Bromo compound	Antimicrobial
17.92	4-Vinylbenzoic acid		C ₉ H ₈ O ₂	148	3.01	Aromatic Acid	Antimicrobial, Preservative
25.01	Dihydrotachysterol		C ₂₈ H ₄₆ O	398	17.29	Synthetic vitamin-D	Used in the mineralization of bone
26.99	Lanost-9 (11)-en-12-one		C ₃₀ H ₅₀ O	426	45.11	Steroid compound	Antimicrobial, Anti-inflammatory, Antiarthritic, Diuretic, Antiasthma
31.87	Cholest-22-ene-21-ol,3,5-dehydro-6-methoxy-, pivalate		C ₃₃ H ₅₄ O ₃	498	14.29	Steroid compound	Antimicrobial, Anti-inflammatory, Antiarthritic, Diuretic, Antiasthma
35.94	Urs-12-en-24-oic acid, 3-oxo-, methyl ester, (+)-		C ₃₁ H ₄₈ O ₃	468	12.03	Steroid compound	Antimicrobial, Anti-inflammatory, Antiarthritic, Diuretic, Antiasthma

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

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

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 [27, 28]. The plant has six alkaloids were reported earlier such as Canthin-6-one, 10-Methoxycanthin-6-one (methyl aervin), 10-Hydroxy canthin-6-one(aervin), 10-β-D-glucopyranosyl-oxy-canthin-6-one (aervoside), β-Carboline-1-propionic acid and 6-Methoxy-β-carboline-1-propionic acid (aervolanin) [29]. The plant contains α-amyrin, campesterol, β-sitosterol, and its palmitate, chrysin and flavonoid glucosides were reported [30]. ~~In the present study we characterized the phytochemical profile different parts of *A. lanata* using GC-MS. The gas chromatogram shows that the various compounds eluted at different retention time. The height of the peak indicates that the relative concentrations of the components present in the plant extract.~~

The identified compounds possess biological and pharmacological properties predicted from Dr. Datta's Phytochemical and Ethanobotanical Databases. For instance, Octanoic acid ethyl ester (R/T 13.42) was an ester compound possess insecticidal, anticandidal and antifungal activities. Similarly, Prabhadevi et al. [31] were identified octanoic acid ethyl ester compound from the ethanolic stem extract of *Allamanda cathartica* by GC-MS. The compound 9,12,15-Octadecatrienoic acid phenyl methyl ester,(z,z,z)- (Linolenic acid ester) (R/T 32.24) was identified in the flower extract of *A. lanata* and possess anti-inflammatory, insectifuge, hypocholesterolemic, cancer preventive, nematocidal, hepatoprotective, antihistaminic, antieczemic, antiacne, 5-α reductase inhibitor, antiandrogenic, antiarthritic and anticoronary properties. Arunkumar and Muthuselvam [32] were identified n-hexadecanoic acid, Phytol, 9, 12 - Octadecadienoic acid and 9, 12, 15-Octadecatrienoic acid in the ethanol leaf extract of *Aloe vera*. Similarly the compound 9, 12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)-, n-Hexadecanoic acid, were present in the ethanol extract of *Caesalpinia sappan* [33]. Phytol (R/T 14.92) is an antimicrobial, anticancer, anti-inflammatory and diuretic agent and also possess hepatoprotective and antiandrogenic properties [34].

Similarly, the presence of phytol in the leaves of *Lantana camara* [35, 36] and *Mimosa pudica* was reported [37]. Phytol reported to possess antibacterial activity against *Staphylococcus aureus* by causing damage to cell membranes [38]. In the present study, the compound Ethaneperoxoic acid, 1-cyano-1-[2-(2-phenyl-1,3-dioxolan-2-yl) ethyl] pentyl ester (R/T 13.90) was observed in the leaf of *A. lanata*, which act as antimicrobial and insecticidal agent. Similarly, it was present in the ethanolic extract of *Peristrophe bicalyculata* [39]. Most of the compounds were present in the root of *A. lanata* such as Lanost-9(11)-en-12-one, Cholest-22-ene-21-ol, 3, 5-dehydro-6-methoxyl- pivalate, urs-12-en-24oic acid, 3-oxo-methyl ester (+)- and possess antimicrobial, anti-inflammatory, antiarthritic, diuretic and antiasthma activities.

5. CONCLUSION

The results of the present investigation reveals that the presence of phytocompounds in the ethanolic extracts of different parts of *A. lanata* by GC-MS analysis. ~~In this study, the presence of phytoconstituents belongs to the different nature of compounds like acids, esters, alcohols, ethers, etc. The presence of such a variety of phytocompounds may be attributed to the medicinal characteristics of *A. lanata*. In future the isolation of individual phytocompounds of *A. lanata* may proceed to find a novel drug.~~

REFERENCES

1. Naik GH, Priyadarsini KI, Satav JG, Banavalikar MM, Sohani DP, Biyani MK, Mohan H. Comparative antioxidant activity of individual herbal components used in Ayurvedic medicine. *Phytochemistry*, 2003;63:97-104.

- 272 2. Sumathi P, Parvathi A. Antimicrobial activity of some traditional medicinal plants. J Med
273 Plant Res. 2010;4:316-321.
- 274 3. Zaidan MRS, Rain NA, Badrul AR. *In-vitro* screening of five local medicinal plants for
275 antibacterial activity using disc diffusion method. Trop Biomed. 2005;22:165-170.
- 276 4. Gordon DM. Geographical structure and host specificity in bacteria and the implications
277 for tracing the source of coliform contamination. Microbiology, 2001; 147:1079-1085.
- 278 5. Wink DA, Vodovotz Y, Grisham MB, DeGraff W, Cook JC, Pacelli R, Krishna M, Michell
279 JB. Antioxidant effects of nitric oxide. Methods Enzymol. 1999;301:413-424.
- 280 6. Prachayasittikul S, Buraparuangsang P, Worachartcheewan A, Isarankura-Na-Ayudhya
281 C, Ruchirawat S, Prachayasittikul V. Antimicrobial and antioxidant activity of bioreactive
282 constituents from *Hydnophytum formicarum* Jack. Molecules, 2008;13: 904-921.
- 283 7. Nogueira JC, Diniz Mde F, Lima EO. *In-vitro* antimicrobial activity of plants of acute *Otitis*
284 *externa*. Braz J Otorhinolaryngol. 2008;74:118-124.
- 285 8. Costa ES, Hiruma-Lima C A, Limo EO, Sucupira GC, Bertolin AO, Lolis SF. Antimicrobial
286 activity of some medicinal plants of Cerrado. Brazil Phytother Res. 2008;22:705-707.
- 287 9. Al-Bayati FA, Al-Mola HF. Antibacterial and antifungal activity of different parts of
288 *Trubulus terrestris* L. growing in Iraq. J Zhejiang Univ Sci. 2008;B9:154-159.
- 289 10. Chen IN, Chang CC, Wang CY, Shyu YT, Chang TL. Antioxidant and antimicrobial
290 activity of Zingiberaceae plants in Taiwan. Plant Foods Hum Nutr. 2008;63:15-20.
- 291 11. Pesewu GA, Cutler RR, Humber DP. Antibacterial activity of plants used in traditional
292 medicine of Ghana, with particular reference to MRSA. J Ethnopharmacol.
293 2008;116:102-111.
- 294 12. Turker AU, Usta C. Biological screening of some Turkish medicinal plants for
295 antimicrobial and toxicity studies. Nat Prod. 2008;22:136-146.
- 296 13. Castello MC, Phatak A, Chandra N, Sharon M. Antimicrobial activity of crude extracts
297 from plant parts and corresponding calli of *Bixa orellana* L. Ind J of Exp Biol
298 2002;40(12):1378-1381.
- 299 14. Erturk O, Kati H, Yayli N, Demirbag Z. Antimicrobial properties of *silene multifida*
300 (Adams) Rohrb. Plants extract. Turk J Biol 2006;30:17-21.
- 301 15. Kumar AR, Subburathinam KM, Prabaker G. Phytochemical screening of selected
302 medicinal plants of asclepiadaceae family. Asian J Microbial Biotechnol Environ Sci.
303 2007;9(1):177-180.
- 304 16. Velmurugan P, Kamaraj M, Prema D. Phytochemical constituents of *Cadaba Trifoliata*
305 Roxb. root extract. International Journal of Phytomedicine. 2010;2:379-384.
- 306 17. De-Fatima A, Modolo LV, Conejero LS, Pilli RA, Ferreira CV, Kohn LK, De-Carvalho JE.
307 Lactones and their derivatives, biological activities, mechanism of action and potential
308 leads for drug design. Curr Med Chem. 2006;13:3371-3384.
- 309 18. Koduru S, Grierson DS, Afolayan AJ. Antimicrobial activity of *Solanum aculeastrum*.
310 Pharm Biol. 2006;44:283-266.
- 311 19. Meurer-Grimes B, Mcbeth DL, Hallihan B, Delph S. Antimicrobial activity in medicinal
312 plants of the Scrophulariaceae and Acanthaceae. Int J Pharmacog. 1996; 34:243-248.
- 313 20. Krishnan Apai G, Rai VK, Nandy BC, Meena KC, Dey S, Tyagi PK, Tyagi LK.
314 Hypoglycemic and antihyperlipidaemic effect of ethanolic extract of aerial parts of *Aerva*
315 *lanata* Linn. in normal and alloxan induced diabetic rats. Int J Pharm Sci Drug Res.
316 2009;1(3):191-194.
- 317 21. Manokaran S, Jaswanth A, Sengottuvelu S, Nandhakumar J, Duraisamy R,
318 Karthikeyan D, Mallegaswari R. Hepatoprotective activity of *Aerva lanata* Linn. against
319 paracetamol induced hepatotoxicity in rats. Res J pharm Tech. 2008;1(4): 398-400.
- 320 22. Deshmukh T, Yadav BV, Badole SL, Bodhankar SL, Dhaneshwar SR.
321 Antihyperglycemic activity of alcoholic extract of *Aerva lanata*(L) leaves in alloxan
322 induced diabetic mice. J Appl Biomed. 2008;6:81-87.

- 323 23. Krishan GA, Rai VK, Nandy BC, Meena KC, Dey S, Tyagi PK, Tyagi LK. Hypoglycemic
324 and Antihyperlipidemic of ethanolic extract of aerial parts of *Aerva lanata* Linn. In normal
325 and alloxan induced Diabetic rats. Int J Pharm Sci Drug Res. 2009;1:557-563.
- 326 24. Anantha D, Israiel Kumar T, Santosh Kumar M, Manohar Reddy A, Mukharjee NS. *In-*
327 *vitro* Anthelmnetic activity of aqueous and alcoholic extracts of *Aerva lanata* seeds and
328 leaves. J Pharm Sci Res. 2010; 2(5):317-321.
- 329 25. Merlin NJ, Parthasarathy V, Manavalan R, Kumaravel S. Chemical Investigation of Aerial
330 Parts of *Gmelina asiatica* Linn. by GC-MS. Pharmacognosy Res. 2009;1:152-156.
- 331 26. Praveen Kumar P, Kumaravel S, Lalitha C. Screening of antioxidant activity, total
332 phenolics and GC-MS study of *Vitex negundo*. Afr J Biochem Res. 2010;4:191-195.
- 333 27. Khandelwal KR. Practical pharmacognosy technique and experiments. Nirali Prakashan,
334 Pune. 2000;2:149-156.
- 335 28. Kokate CK. Practical pharmacognosy, Vallabhprakashan, New Delhi. 1996;4:107-111
- 336 29. Zapesochneya G. Canthin-6-one and beta-carboline alkaloids from *Aerva lanata*. Planta
337 med. 1992;58(2):192-196.
- 338 30. Khare CP. Encyclopedia of India Medicinal Plants, Rational Western Therapy,
339 Ayurvedic and other traditional Usage. Botany, Springer-Verlag Berlin Heidelberg, New
340 York. 2007; 29-30.
- 341 31. Prabhadevi V, Sahaya Sathish S, Johnson M, Venkatramani B, Janakiraman N.
342 Phytochemical studies on *Allamanda cathartica* L. using GC-MS. Asian Pac J Trop
343 Med. 2012;S550-S554.
- 344 32. Arunkumar S, Muthuselvam M. Analysis of Phytochemical constituents and antimicrobial
345 activities of *Aloe vera* L. against clinical pathogens. World J Agricultural Sci. 2009;
346 5(5):572-576.
- 347 33. Sarumathy K, Vijayayakanthia T, Dhana Rajan MS. A Protective effect of *Caesalpinia*
348 *sappan* (CS) on acetaminophen induced Nephrotoxicity and oxidative stress in male
349 albino rats. J Pharmacology and Toxicology. 2011;1(2):11-21.
- 350 34. Praveen Kumar P, Kumaravel S, Lalitha C. Screening of antioxidant activity, total
351 phenolics and GC-MS study of *Vitex negundo*. Afr J Biochem Res. 2010;4(7):191-195.
- 352 35. Rani PMJ, Kannan PSM, Kumaravel S. GC-MS Analysis of *Lantana camara* L. Leaves.
353 JPRD. 2011;2(11):63-66.
- 354 36. Sridharan S, Meenaa V, Kavitha V, John Nayagam AA. GC -MS study and
355 phytochemical profiling of *Mimosa pudica* Linn. J Pharm Res. 2011;4(3):741-742.
- 356 37. Sathish Kumar M, Manimegalai S. Evaluation of larvicidal effect of *Lantana camara* Linn
357 against mosquito species *Aedes aegypti* and *Culex quinquefasciatus*. Advances in
358 Biology Res. 2008;2(3-4):39-43.
- 359 38. Inoue Y, Hada TA, Shiraishi K, Hamashima H, Kobayashi S. Biphasic effects of
360 Geranylgeraniol, Terpenone and Phytol on the growth of *Staphylococcus aureus*.
361 Antimicro Agen Chemother. 2005;49(5):1770-1774.
- 362 39. Janakiraman N, Johnson M, Sahaya Sathish S. GC-MS analysis of bioactive
363 constituents of *Peristrophe bicalyculata* (Retz.) Nees. (Acanthaceae), Asian Pac J Trop
364 Med. 2012;2(1):S46-S49.
- 365