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HPTLC and HR-LCMS Phytochemical Analysis of Methanolic Extract of *Aerva lanata*

Research Article

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Abstract

Medicinal plants are a gift to humankind as they play a significant role in preserving, maintaining, and improving our health. *Aerva lanata* has been known for decades for its various pharmacological activities. Despite extensive research on its medicinal properties, there have been limited studies focusing on its hepatoprotective activity. This study aims to explore the bioactive components of *Aerva lanata* and investigate its potential hepatoprotective properties. The review utilized various scientific databases, including PubMed and ScienceDirect, to gather information on the pharmacological activities and bioactive components of *Aerva lanata*. The whole plant methanolic extract of *Aerva lanata* was subjected to preliminary phytochemical analysis, revealing the presence of alkaloids, flavonoids, phenols, glycosides, and tannins. High-Performance Thin-Layer Chromatography (HPTLC) was used to identify prominent compounds, followed by High Resolution Liquid Chromatography-Mass Spectrometry Orbitrap (HR-LCMS, Orbitrap) for detailed component analysis. The HPTLC chromatogram indicated significant spots at Rf values of 0.026, 0.075, 0.096, 0.46, and 0.82. HR-LCMS analysis revealed several medicinally important compounds, including norharman, 10-hydroxycanthin-6-one, 9-methoxycanthin-6-one, trigonelline, methylquinoline, 8-hydroxyquinoline, quercetin, kaempferol, rutin, and artemisinin. These findings support the traditional uses of *Aerva lanata* and suggest the potential for new drug discoveries. The study concludes that *Aerva lanata* contains several bioactive compounds with medicinal properties, justifying its traditional applications. Further research is needed to fully understand its hepatoprotective mechanisms and explore new therapeutic possibilities.

Keywords: *Aerva lanata*; HR-LCMS; Hepatoprotective; Medicinal Plants; Phytochemicals

Introduction

Aerva lanata (*A. lanata*) is a well-known medicinal plant belonging to the family *Amaranthaceae* which is found all over India as a common weed [1]. It is native to Africa, tropical Asia, Madagascar and Saudi Arabia [2]. *A. lanata* is named differently in different regions of India like Chhaya in West Bengal, Gorakhganja in Gujarat,Pindidonda in Telugu, Buikallan in Punjab, Bhuyi in Rajasthan, Ulinai in Tamiland Paunsia in Odissa [3]. It is known to contain various alkaloids, flavonoids, proteins, amino acids, quinones and carbohydrates in different solvent extracts [4]. In recent years, it has gained prominent importance in the field of medicine because of its different pharmacological properties. Numerous studies on its extraction ingredients from different plant parts reported its antidiuretic [5], antimicrobial [6-7], hepatoprotective [8], anti-inflammatory, anti-helminthic [9], anti-HIV [10] anti-cancerous [11-12], anti-diarrheal [13], anti-urolithiatic [14], hypolipidemic [15], nephroprotective [16], analgesic [17], antidiabetic [18] and anti-asthmatic activity [19].

Herbal ingredients are promising choice as medicines in Ayurveda and Unani treatment since ancient times because of fewer side effects and low costs [20-21]. The potential bioactive compounds from plant were isolated and selected based on the phytochemical, taxonomic and ethnomedicinal approach [22]. *A. lanata* is a gift from nature because it possesses plentiful healing properties [2, 23]. It

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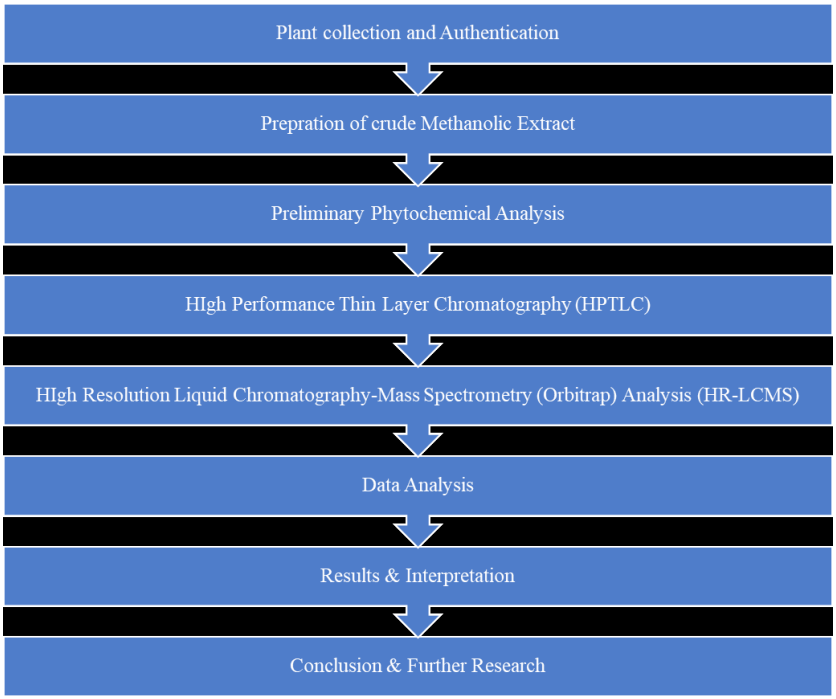
Execution of Study



has been observed that *A. lanata* has been used traditionally to treat common ailments like headache, burns, jaundice, cholera, measles, gall stones, diarrhea, cough and bronchitis, snake bites, arthritis [9]. Only few studies in literature evaluated significant hepatoprotective activity of *A. lanata* [8, 24-25]. In the present study, an attempt is made to explore the biological components of *A. lanata* and zooming on few components which can be the potential active components possessing hepatoprotective activity.

Materials and Methods

A. lanata was collected from natural habitats, Godhra, Gujarat, India. Plant authenticated by Dr. Bharat Maitreya (Professor, Department of Botany, Bioinformatics and Climate Change Impact Management, Gujarat University). The specimens collected was deposited for reference (No. GU/BOT/A/A21) at the herbarium, Gujarati University. The extraction of active ingredients from different parts of *A. lanata* was prepared using standard hot and dried methodology. Accurately Weighed 1gm of dried plant material was transferred to a 250 ml volumetric flask dissolving in 100 ml of solvent and kept on hot plate for 5 hours at 70 °C. The process was repeated until the solution become colorless. The content of the flask was filtered through Whatman No.1 paper (Merck, Mumbai, India). The residue was dried, concentrated and stored in desiccator for further analysis. The qualitative phytochemical analysis was carried out using standardized tests to identify polyphenols, tannins, flavonoids, steroids, alkaloids, saponins, terpenoids etc. [26].

HPTLC

An HPTLC system is equipped with a TLC scanner 3, Linomat V sample applicator and CATS V 4.06 software. All the solvents and chemicals procured were of HPTLC grade (Merck, Mumbai, India. The sample extract was prepared in 90% ethanol. The sample was applied as bands of 0.2 mm eachby CamagLinomat V sample applicator (Muttenz, Switzerland) equipped with 100 µl Hamilton syringe on 20 cm * 10 cm aluminum plates coated with silica gel $60F_{254}$ (Merck, Mumbai, India). Ascending development was performed at room temperature with Toluene: Ethyl acetate solution in ration of 9:1 (v/v) as a mobile phase in a Camag glass twin-trough chamber saturated with mobile phase vapor for 30 mins prior. The developed plates were air dried and scanned at 366 nm with a Camag TLC scanner with CATS V4.06 software, using deuterium lamp.

High Resolution Liquid chromatography-mass spectrometry (HR-LCMS) characterization

HR-LCMS was done to determine the bioactive compounds present in methanolic extract of *A. lanata*. The LC system consist of two pumps and an automated injector. Separation of components achieved in Syncronis C-18 column (Size: 5 µm, 100 mm x 4.6 mm) (Thermo Fisher Scientific, Massachusetts, USA). Before analysis, the sample was prepared by centrifuging at 12000 rpm for 10 mins. The separated components enter into QExactive Hybrid Quadrupole-Orbitrap mass spectrometer (Thermo Fisher Scientific, Massachusetts, USA) equipped with an electrospray ionization interface (ESI). This system has Xcalibur,Version 4.2.28.14 data acquisition software. Two mobile phases; A-0.1% formic acid in Milli-Q water and D-Acetonitrile in Milli-Q water were used as mobile phase at a flow rate of 500 µl min^{-1}.

Results

The present study was designed to evaluate the phytochemical

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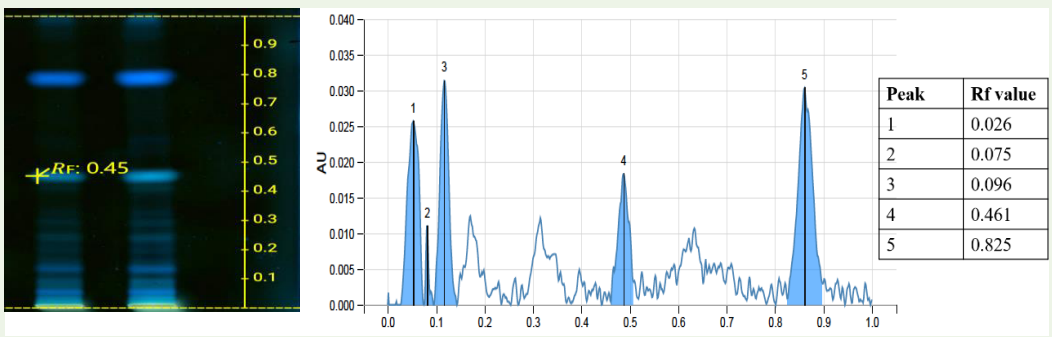
screening and HR-LCMS analysis of the crude methanolic extract fraction of *A. lanata*. In plant extraction process, the percentage yield of aqueous and methanolic extract of *A. lanata* whole plant was obtained 5.5% and 5.2%, for leaves 4.2% and 2.7%, for stem 2.5% and 3.7%, and for flower 3.4% and 2.9% w/w, respectively.(Table 2) showed the preliminary qualitative phytochemical analysis of methanolic extract of *A. lanata* and its plant parts, which confirm the presence of Alkaloids, carbohydrates, glycosides, phenols, protein, amino acids, and flavonoids in whole plant extract. Numerous solvent compositions were tested for HPTLC analysis, from which Toluene: Ethyl acetate (v/v) gave the desired aim results. HPTLC chromatogramof whole plant methanolic extract of *A. lanata* determined high resolution and reproducible peaks under 366 nm, which revealed the presence of five prominent bands with Rf value ranged from 0.026-0.82 (Figure 1).

HR-LCMS analysis of methanolic extract of *A. lanata* lead to the identification of 47 bioactive compounds at different retention time that could attribute towards the pharmacological properties (Table 3). The big molecules fragments into small compounds giving rise to appearance of peaks at different m/z ratio. We have identified these components by comparing with the authentic m/z spectra available in HR-LCMS data library. These identified components correspond to 80 % of the chromatogram (Figure 2). The first predominant peak observed with less retention time 0.97 was identified as Adenine whereas Hexadecanamide was the last compound identified which took longest retention time 26.37. HR-LCMS data of *A. lanata* confirms that it comprises of active alkaloids such as Norharman, aervine (10-hydroxycanthin-6-one), 9-methoxycanthin-6-one and Trigonelline. The heterocyclic compounds such as 6-Methylquinoline and 8-Hydroxyquinoline also been observed. *A. lanata* comprises of flavanols like Quercetin and Kaempferol (Table 3). This plant also has flavonoid glucoside named rutin and artemisinin which is a terpene. Additionally, we have identified carbohydrates, few phenolic compounds, and amino acids like phenyl alanine and tyrosine.

Discussion

Phytochemicals play vital role in treating various diseases and are still used in modern medicine. The identification of new constituents is not only important for therapeutic agents but also helps in finding new economically important components like oil, saponins, tannins, flavonoids, gums etc. [27-29]. Nowadays it becomes easier with different methods to isolate and identify the bioactive compounds present in plant. Hot and dried method of plant extraction is very old method. Among 28 species of *Aerva genus*, only three species *A. lanata*, *A. javanica*, *A. persica* are known to have the medicinal properties [30].

The results of the present preliminary study on *A.lanata*'s whole plant methanolic extract evidenced the presence of alkaloids, flavonoids, phenols, terpenes and glycosides. Thus, this shows that whole plant extract prepared using methanol as a solvent had the maximum bioactive compounds. These phytoconstituents could be responsible for the medicinal properties of *A.lanata* [31-33]. Hence, for further confirmation of these different-phytoconstituents, we conducted HR-LCMS analysis for the methanolic whole plant extract. The obtained biological molecules in methanolic extract have therapeutic values. The hexadecanoic acid, which is methyl ester of palmitic acid, is already known for its anti-oxidant, hypo-cholesterolemic, antis-androgenic, hemolytic, and 5-Alpha-reductase inhibitor and pesticide activity [34]. Alkaloids are known for their role in plant defense system against pathogenic organisms. Similarly, *A. lanata* also contains many alkaloids like 10-hydroxycanthin-6-one (Aervine), 13-methoxy-1,6-diazatetracyclo hexadeca-heptaen-2-one, and Betaine. Presence of aervine, 9-methoxycanthin-6-one and β-carboline-like alkaloid has also been reported previously [35-36]. The compound 9-Oxo-10(E),12(E)-octadecadienoic acidfound in *A. lanata* as a significant role in prostaglandin biosynthesis and possesses other biological activities such as anti-inflammatory, anti-arthritic and hepatoprotective [37-38]. A previous study showed that *A. lanata* hydroalcoholic extract was effective in protecting liver damage in rats caused by paracetamol [8, 24]. Rutin, a flavanol, is known for its anti-inflammatory, anti-cancerous, neuro-protective, cardio protective and anti-oxidative activity [39-40]. One of the studies confirmed the hepatoprotective activity of alkaloid present in petroleum ether extract of *A. lanata* [12]. Considering the presence of different biologically active compounds in the methanolic extract of *A. lanata*, few compounds can be chosen for bioinformatic studies to select the best molecule which could be studied further in-vitro and in-vivo for hepatoprotective activity.



Peak	Rf value
1	0.026
2	0.075
3	0.096
4	0.461
5	0.825

Figure 1: HPTLC profile and Chromatogram of methanolic extract of A. lanata

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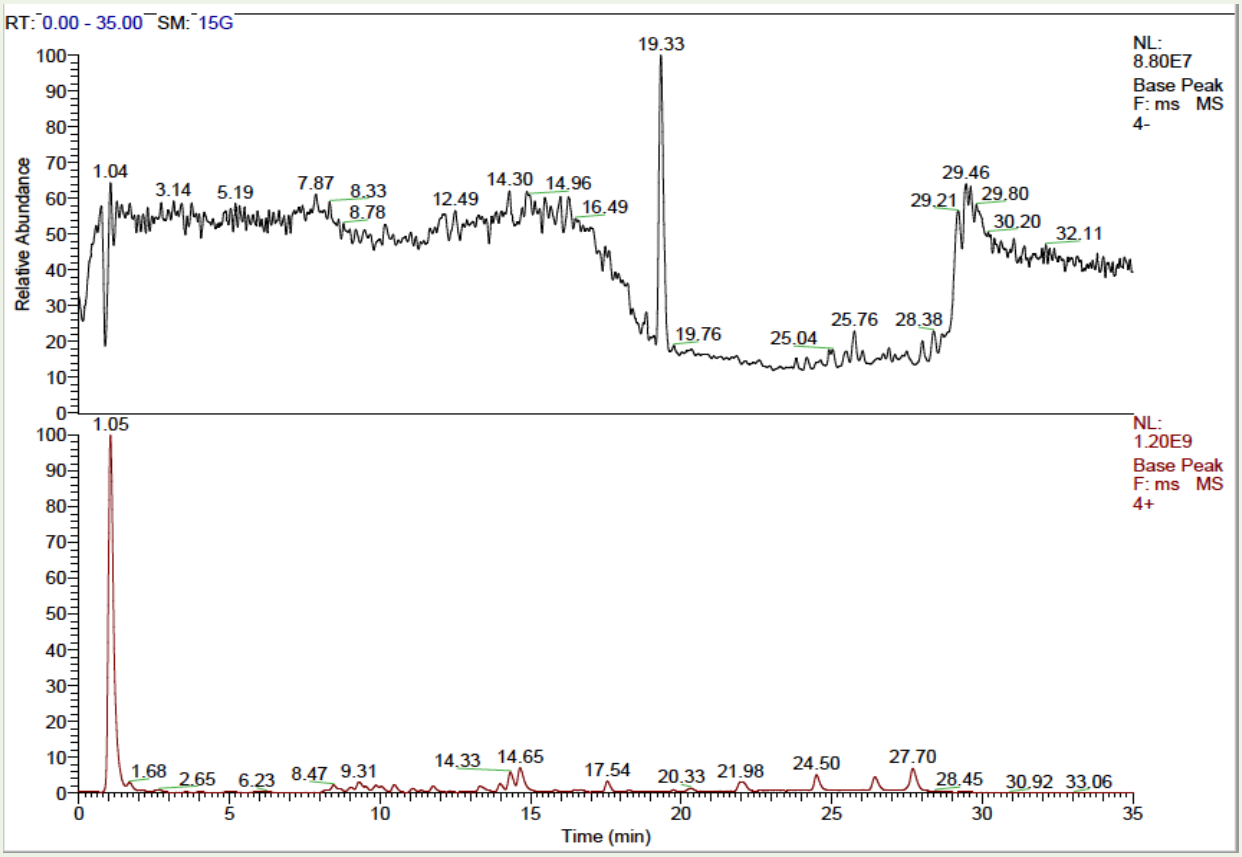

Figure 2: Chromatographic profile of (LC-ESI-MS/MS) of whole plant methanolic extract of A. lanata in both ESI (+) and ESI (-) ionization modes

Table 1: Materials Required and Procured with Authentic Sources

Material	Source	Authenticated by	Performed at
Aervalanta	Collected from natural habitat in Godhara, Gujarat, India.	Dr. Bharat Maitreya, Professor, Department of Botany, Bioinformatics and climate change Impact Management, Gujarat Univesity	-
Methanol	Merch, Mumbai, India.	-	-
Whatman No. 1 Filter paper	Merch, Mumbai, India.	-	-
HPTLC System	Camag, Muttenz, Switzerland	-	Gujarat Technical University, Gandhinagar, Gujarat.
HR-LCMS Orbitrap System	Q-Exactive Plus Biopharma, Thermo Scientific, Massachusetts, USA	-	IIT-Bombay, Mumbai, Maharashtra.

Table 2: Preliminary phytochemical screening of methanolic extract of *A. lanata* plant

	Phytochemicals	Whole Plant	Flower	Stem	Leave
1	Alkaloids	+	+	-	+
2	Flavonoids	+	+	+	+
3	Glycosides	+	-	+	+
4	Steroids	+	+	+	+
5	Phenolic	+	+	+	+
6	Protein	+	-	-	+
7	Carbohydrate	+	+	+	+
8	Tannin	+	_+	+	+

+ = Presence, - =absence

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Table 3: Phytochemical constituents of whole plant of A. lanata methanol fraction determined by LC-ESI-MS/MS

S. No	RT (min)	Measured m/z	Product ions (m/z)	Compounds	Type of Metabolites
1	0.974	135.0543	C5 H5 N5	Adenine	Nitrogen base
2	0.965	145.0849	C5 H11 N3 O2	4-Guanidinobutyric acid	Alkaloid
3	1.004	129.0789	C6 H11 N O2	Pipecolic acid	Polyphenol
4	1.167	129.07887	C6 H11 N O2	Vigabatrin	Amino acid
5	1.033	137.0475	C7 H7 N O2	Trigonelline	Alkaloid
6	1.045	117.0791	C5 H11 N O2	Betaine	Alkaloid
7	1.129	143.0945	C7 H13 N O2	DL-Stachydrine	Alkaloid
8	1.132	123.032	C6 H5 N O2	Nicotinic acid	Vitamin
9	1.141	161.1051	C7 H15 N O3	DL-Carnitine	Amino acid
10	1.201	129.0425	C5 H7 N O3	L-Pyroglutamic acid	Amino acid
11	1.201	136.0384	C5 H4 N4 O	Pyrazolo[1,5-a][1,3,5]triazin-4(1H)-one	Heterocyclic compound
12	1.273	159.1257	C8 H17 N O2	2-(Dimethylamino)ethyl butyrate	Ester
13	1.455	228.1472	C11 H20 N2 O3	Prolylleucine	Amino acid
14	1.572	194.0803	C8 H10 N4 O2	Caffeine	Alkaloid
15	1.69	167.0581	C8 H9 N O3	Pyridoxal	Vitamin
16	1.761	165.0789	C9 H11 N O2	L-Phenylalanine	Amino acid
17	1.947	223.0842	C11 H13 N O4	N-Acetyl-L-tyrosine	Amino acid and derivative
18	2.96	169.0737	C8 H11 N O3	Pyridoxine	Vitamin
19	3.571	113.08427	C6 H11 N O	Caprolactam	Amide
20	4.04	145.0527	C9 H7 N O	8-Hydroxyquinoline	Heterocyclic compound
21	4.95	143.0734	C10 H9 N	6-Methylquinoline	Heterocyclic compound
22	5.118	189.0424	C10 H7 N O3	Kynurenic acid	Quinoline carboxylic acid
23	8.226	168.0686	C11 H8 N2	Norharman	Alkaloid
24	8.404	226.1566	C13 H22 O3	5-(6-hydroxy-6-methyloctyl)-2,5-dihydrofuran-2-one	Butanolides
25	8.293	193.1102	C11 H15 N O2	NP-019401	Unknown
26	8.762	278.1151	C15 H18 O5	(3aR,4R,11aR)-4-hydroxy-10-(hydroxymethyl)-3-methylidene-2-oxo-2H,3H,3aH,4H,5H,8H,9H,11aH-cyclodeca[b]furan-6-carbaldehyde	Heterocyclic aromatic
27	9.368	210.1254	C12 H18 O3	Jasmonic acid	Hormone
28	9.393	302.04222	C15 H10 O7	Quercetin	Flavonols
29	9.942	286.0474	C15 H10 O6	Kaempferol	Flavonols
30	10.63	282.14635	C15 H22 O5	Artemisinin	Terpene
31	10.174	266.1516	C15 H22 O4	Verrucarol	Terpenoid
32	10.562	610.1532	C27 H30 O16	Rutin	Flavonol
33	11.911	262.1202	C15 H18 O4	(3aR,4aS,5R,8S,9aR)-5-hydroxy-4a,8-dimethyl-3-methylidene-2H,3H,3aH,4H,4aH,5H,6H,8H,9H,9aH-azuleno[6,5-b]furan-2,6-dione (Artemisin)	Sesquiterpene lactone
34	12.22	236.05831	C14 H8 N2 O2	10-hydroxycanthin-6-one (Aervine)	Alkaloid
35	13.392	335.1153	C20 H17 N O4	Berberine	Alkaloid
36	14.644	250.0738	C15 H10 N2 O2	13-methoxy-1,6-diazatetracyclo hexadeca--heptaen-2-one (9-methoxycanthin-6-one)	Alkaloid
37	14.762	292.2034	C18 H28 O3	12-oxo Phytodienoic Acid	Fatty acid
38	16.011	222.089	C12 H14 O4	Monobutyl phthalate	Ester
39	17.248	294.1827	C17 H26 O4	6-Gingerol	Phenolic
40	19.119	304.2036	C19 H28 O3	6ß-Hydroxytestosterone	Steroid
41	19.97	317.2925	C18 H39 N O3	2-Amino-1,3,4-octadecanetriol	Amino alcohol
42	20.874	272.23396	C16 H32 O3	16-Hydroxyhexadecanoic acid	Methyl ester
43	21.948	272.1772	C18 H24 O2	β-Estradiol	Fatty acid ester
44	21.958	294.219	C18 H30 O3	9-Oxo-10(E),12(E)-octadecadienoic acid	Methyl ester
45	22.037	278.1514	C16 H22 O4	Mono(2-ethylhexyl) phthalate (MEHP)	Ester
46	23.837	323.282	C20 H37 N O2	Linoleoyl Ethanolamide	Linoleic acid amide
47	26.379	255.2557	C16 H33 N O	Hexadecanamide	Fatty amide

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Conclusion

The developed HPTLC profile revealed fingerprint of phytochemicals present in *A. lanata* and confirming the purity and identity of the species. HR-LCMS revealed the presence of many diverse bioactive components which justifies the use of whole plant extract for treating different diseases in older times. However, a new area of research will open if isolation and identification of novel pharmacologically active compounds is conducted done. Further, Invitro and in-vivo study is needed to prove the hepatoprotective activity of those phytoconstituents present in *A. lanata*.

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