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Antibacterial activity and phytochemical screening of ethanolic leaf, stem and flower extract of *Aerva lanata*

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Abstract
Screening of phytochemicals provides the potentiality for serving several illnesses. The current investigation was carried out the antibacterial activity and phytochemical screening of ethanolic extract of leaf, flower and stem of *Aerva lanata* commonly known as Sirupeelai through spectroscopic analysis. In this screening the major components are carbohyrates, aminoacids, alkaloids, flavonoids, phenols, terpenoids and cocumarins. The antibacterial activity showed maximum sensitivity in leaf extract of *Staphylococcus aureus* (16mm). The FTIR spectroscopic analysis revealed that the functional groups were alkene, alkyne, amines which showed major peaks. Based on antibacterial activity and functional group analysis, GC-MS was carried out in leaf extract of *A. lanata*. The current report indicated 27 bioactive compounds, in that Uridine is a major peak which showed antibacterial, anti-inflammatory, anti-diabetic, anti-cancerous activity etc.

Keywords: *Aerva lanata*, Antibacterial, FTIR, GC-MS, Phytochemical, *Staphylococcus aureus.*

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INTRODUCTION

The plant kingdom was treated as the benefit for diverse variety of potentialities. Earlier, many diseases were treated using plant derived secondary components (Gullo *et al.,* 2005). Abundantly 80% of drugs are derived from plant products. These medicinal plants contain convinced phytochemical compounds which serve as disorder for the infective diseases like bacterial, fungal, and viral and cancer disorders (Krishnamoorthi *et al.,* 2015).
The use of plant derived products can diminish negative impacts and the compounds present in plants have preventive consequence against many pathogenic microorganisms (Desai *et al*., 2008). Nearly all medicinal plants have immense potentiality for detection of new drugs of asset to humanity. Recently, a lot of approaches are reached for advanced active components in the medicinal herbs onto the production of secured medicines. The phytochemical components of the plants can cure human illness and these components are not toxic and consequently they are eco -friendly (Nostro *et al*., 2000). The chief metabolic compounds create phytochemicals. The main components are common sugars, protein and chlorophyll, and the minor components comprised of alkaloids, flavonoids, terpenoids, phenolic compounds, tannins, glycosides, gums and essential oils (Krishnaiah *et al*., 2007).
Aerva lanata is a medicinal herb reside the family Amaranthaceae. It is one of the plants counted in Dasapushpam, “the ten sacred flowers of Kerala”. The plant is used as food for people and animals. The whole plant, mainly the leaves are consumable as spinach. It has very rich pharmacological properties such as “anthelmintic, demulcent, anti-inflammatory, diuretic, expectorant, hepatoprotective, nephroprotective anti-diabetic activity, anti-hyperglycaemic activity in rats, antimicrobial, cytotoxic, urolithiatic, hypoglycemic, anti-hyperlipidaemic, anti-parasitic, anti-helmentic and antioxidant activities” (Muthukumaran *et al*.,2011). The plant is treated as a conventional medicine meant for snakebites. The crushed juice of *A. lanata* root is treated for jaundice therapy. It aids in controlling the blood glucose level. It is also a good herbal cure for stones in urinary bladder and kidney (Vijayakumar *et al*., 1998)
Hence the present research was initiated to study the antibacterial activity and phytochemical characterization of the ethanolic extracts of medicinal plant *A. lanata* for their therapeutic potential.

MATERIALS AND METHODS

Description of the study site: Karode, a village situated in Parassala Block within Thiruvananthapuram District of Kerala State, India. It resides to South Kerala Division. It is situated 30 km towards East from District headquarters Thiruvananthapuram and 3km from Parassala, 30km from State capital Thiruvananthapuram. It is close to the Tamil Nadu State border. It is nearby to Arabian sea. There is a chance of humidity in the weather. This place contains rich diversity of medicinal plants (Fig. 1). The plant *A. lanata* is commonly known as Sirupeelai in Tamil language, Cherula in Malayalam belongs to the family of *Amaranthaceae* (Fig.2). It is an erect herbaceous weed found all over in India.

A. lanata was collected from the natural habitats at karode, Thriuvanthapuram district, Kerala, India. The plants were identified and authenticated by NMC college herbarium, Marthandam, Tamil Nadu, India.

Preparation of leaf extracts: The freshly collected leaf, flower and stem samples were cleaned completely with distilled water and shade dried at room temperature for 30 – 45 days. Following drying, the samples were beached into fine debris using an electric blender then filtered using muslin cloth and were stored in air tight containers at ambient temperature until required. The finely powdered samples of 10 gm. were soaked in 100 ml of ethanol in 200ml sterile conical flask. The flask were covered with aluminium foil and kept for 48 hrs. at room temperature. Then it was filtered by using Whatman NO.1 filter paper. This extract was used for supplementary examination.

Determination of minimum inhibitory concentration (MIC): The antimicrobial activity of the extracts was evaluated by using well diffusion assay. 20 ml sterile Muller Hinton agar was flooded into petridishes and enabled for gelatination. The 24 hours pathogenic bacterial cultures were grown in nutrient broth such as

Escherichia coli and Staphylococcus aureus, Pseudomonas aeruginosa, Bacillus subtilis and Klebsiella pneumonia were seeded aseptically on the respective agar plates using cotton swab. Then 6mm diameter well was punched on the top of the agar plate using a sterile gel puncher. 50 µl of ethanolic leaf extract was filled into the wells and the plates were incubated at 37˚c for 24 hours. Following incubation the diameter of zone inhibition found throught every well measured and expressed in millimeters (mm).

The minimum inhibitory concentration (MIC) of ethanolic leaf, flower and stem extracts of *A. lanata* with respect to *S. aureus* was determined by broth dilution method.10 test tubes were taken and add nutrient broth in first and last tube contain 1.6ml and all other tubes were added with 0.9ml nutrient broth. In the first and last test tubes added 200µl extracts and 200 µl of DMSO solution and mix well. Then transfer 0.9 µl from the first tube to second and serially diluted till eight tubes and discard 0.9 ml from eighth tube. The ninth tube contain only nutrient broth (control). Then add 100 µl of 10 – 24 hours *S. aureus* in all tubes till eighth. The tubes were incubated at 37˚c for 18 – 24 hours. After incubation 100µl from first 3 to 4 tubes were sub cultured separately on Muller Hinton agar plates and incubated at 37˚c for 24 hours. The volume remained in the tubes after sub culturing were again added with 20µl of resazurin dye and incubated at 37˚c for 6-8 hours(Das *et al*.,2013).

Determination of minimal bactericidal concentration (MBC): The MBC of leaf, flower and stem extracts were conceived by counter culturing 10 µl of the test concentration from MIC tubes on to Muller Hinton agar plates. Cultured plates were then fostered at 37˚C for 24 hours. The highest intensity that prompted little or negative bacterial colony on the plates were documented as MBC (Pavithra *et al.,* 2010).

Phytochemical analysis: Phytochemical analysis of ethanolic extract of leaf, stem and flower for alkaloids (Wagner's test and Dragendorff's test), carbohydrates (Benedict's test, Fehling's test and Barfoed's test), aminoacids (Ninhydrin test), protein (Biuret test), flavonoids(Lead acetate test), phenols (Ferric chloride test and Lead acetate test), tannin, saponin, terpenoids and cocumarins was carried out to determine the presence of chemical compounds using the standard qualitative procedures (Sofowra, 1993; Harborne, 1998).

Fourier transform nfrared spectroscopic analysis (FTIR): FTIR analysis was carried out to reveal the functional group of the plant extract. Based on the sensitivity pattern of antibacterial studies FTIR was performed only in leaf extract. The dried 2mg leaf sample was mixed in the 100 mg KBR (FTIR grade) and then compressed to prepare salt disc the disk was medially kept in the sample holder and FTIR spectra were documented in the absorbance range between 1000 and 3500 cm^{-1}. All perusals were procured with a Shimdzu FTIR spectrometer.

Gas chromatography coupled with Mass spectroscopy (GC-MS): In order to determine the bioactive compound present in the leaf extract the experiment was performed in the Gas Chromatograph: A Shimdzu GC-2010 Plus gas chromatograph was assembled with a properly neutralised 2 mm direct injector liner and a 15m Alltech EC-5 column with a consistency of 250µ I.D., 0.25µ. A split injection was adopted for sample initiation and the split ratio was set to 10:1. The oven temperature was pre-set to start at 35°C, retain for 2minutes, at the rate of 20°C per minute to 450°C and retain for 5 minutes. Helium was used as a carrier gas was set to 2 ml/minute flow rate and

Figs. 1-3. *1. Map showing the study site Karode, 2. Plant A. lanata, 3 Leaf, stem and flower extracts of A. lanata.*

the findings of the components were corresponding their documented spectra with the data bank mass spectra of NIST library V 11 maintained by the instruments software. GC/MS metabolomics Database was used for the similarity search with retention index. The components were recognized by gas chromatography coupled with mass spectrometry.

RESULTS

Phytochemical screening: The qualitative screening of phytochemical constituents for the plant extracts of *A. lanata* indicated that the ethanolic extracts of leaf samples were positive for flavonoids and phenols. The ethanolic extract of stem sample showed positive for carbohydrates, alkaloids, flavonoids, phenol, terpenoids and cocumarins. And the ethanolic extract of flower was positive for aminoacids, alkaloids, flavonoids, phenol and terpenoids. The results of different extracts (Fig.3) and phytochemical analysis are shown Table 1 and Fig.4.

Antibacterial activity: In this study, the leaf sample of ethanol extract showed inhibitory activity against *S. aureus* (16 ± 0.2 mm) *E.coli* (14 ± 0.2 mm), *B. subtilis* (12 ± 0.5 mm), *P. auriginosa,* (11 ± 0.2 mm), *K. pneumonia* (10 ± 0.2 mm), and the stem sample showed inhibitory activity against *S. aureus* (11 ± 0.3 mm) *E.coli* (03 ± 0.1 mm), *B. subtilis* , *P. aeruginosa* and *K. pneumonia* also no zone of inhibition, and the flower sample showed inhibitory activity against *S. aureus* (11 ± 0.2 mm) *E.coli* (02 ± 0.2 mm), *B .cereus* (02 ± 0.2 mm), *P. auriginosa,* (09 ± 0.3 mm), *K. pneumonia* (08 ± 0.2 mm). Overall the leaf extract showed highly sensitivity against *S. aureus* as shown in Table 2 and Fig. 4.

Minimum inhibitory concentration (MIC)

Tube dilution assay: The tube dilution assay for MIC of the various extracts in terms of change in color of the tubes after addition of resazurin dye against the test strain *Staphylococcus aureus* are presented in table 3.

The growth of *S. aureus* was inhibited at 100 mg, 50 mg, 25mg and 12.5mg concentration of leaf and flower in the first to fourth tubes and showed no turbidity; and in stem concentration of 100 mg, 50 mg, 25mg showed no turbidity. Based on visual inspection it is claimed that 100 mg, 50 mg, 25mg and 12.5 mg would be the minimum inhibitory concentration (MIC) against *S. aureus*. For further confirmation of a bacterial growth inhibition resazurin dye (20 µl) was added as a growth indicator to tubes showing reduction of bacterial growth based

Table 1. Phytochemical constituents of *A. lanata.*

S.N.	Phytochemical constituents	Name of the test	Leaf	Stem	Flower
1	Carbohydrates	Benedict's test	-	-	-
2	Carbohydrates	Fehling's test	-	+	-
3	Carbohydrates	Barfoed's test	-	+	-
4	Aminoacids	Ninhydrin test	-	-	+
5	proteins	Biuret test	-	-	-
6	Alkaloids	Wagner's test	-	+	+
7	Alkaloids	Dragendorff's test	-	-	-
8	Flavonoids	Lead acetate test	+	+	+
9	Phenol	Ferric chloride test	+	-	-
10	phenol	Lead acetate test	+	+	+
11	Tannin	Tannin test	-	-	-
12	Saponin	Saponin test	-	-	-
13	Terpenoids	Terpenoids test	-	+	+
14	cocumarins	Cocumarins test	-	+	-

Fig. 4. *Antibacterial activity of extracts of different parts of A. lanata.*

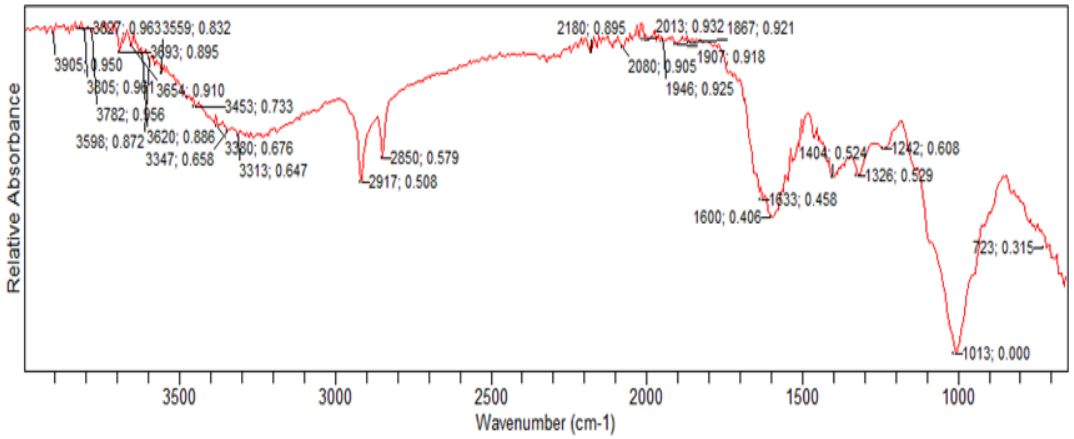

Fig. 5. *FTIR analysis of A. lanata –leaf.*

Table 2. Antimicrobial activity of *A. lanata*.

S.N.	Bacterial Strains	Zone of inhibition(mm)		
		Leaf	Stem	Flower
1.	*Staphylococcus aureus*	16±0.2	11±0.3	11±0.2
2.	*Escherichia coli*	14±0.2	3±0.1	2±0.2
3.	*Bacillus subtilis*	12±0.5	No zone	2±0.2
4.	*Pseudomonas aeruginosa*	11±0.2	No zone	9±0.3
5.	*Klebsiella pneumoniae*	8±0.2	No zone	8±0.2

Table 3. MIC of different extracts of *A. lanata.*

Bacterial strain	Plant extract	Plant extract concentration (mg/ml)									
		100	50	25	12.25	6.12	3.06	1.53	0.76	control	100
Staphylococcus aureus	Leaf	+	+	+	+	-	-	-	-	No turbidity	No turbidity
	Stem	+	+	+	-	-	-	-	-	No turbidity	No turbidity
	Flower	+	+	+	+	-	-	-	-	No turbidity	No turbidity

+ Absence of turbidity ; - Presence of turbidity

on turbidity. The pink color was indicative of presence of bacterial growth and blue color showed inhibition of bacterial growth. Here in leaf and stem extracts of first to fourth tubes showing blue color and rest of the concentration showed pink color indicating bacterial growth.

Fourier transform infrared spectroscopy (FTIR): In the leaf extract of *A. lanata*, 28 functional groups were observed. The peak values were 1013cm^{-1}, 723 cm^{-1}, 1242 cm^{-1}, 1326 cm^{-1}, 1404

Table 4. FTIR-functional group of leaf of *A. lanata.*

S.N.	Group	Stretching frequency
1	C – F	1013
2	C – Br	723
3	C – N stretching	1242
4	C – N stretching	1326
5	S = O stretching	1404
6	C = C alkene	1633
7	C = C aromatic	1600
8	Alkyne ≡ C - H	2180
9	Alkane (C – H)	2850
10	Alkane (C – H)	2917
11	Amine N – H	3313
12	Amine N – H	3380
13	Amine N – H	3347
14	O – H stretching	3782
15	O – H stretching	3620
16	O – H stretching	3589
17	O – H stretching	3654
18	O – H stretching	3693
19	O – H stretching	3559
20	Alcohol C – O	3453
21	C – H bending	1867
22	C – H bending	1907
23	C – H bending	1946
24	N = C = S stretching	2080
25	N = C = S stretching	2013
26	O – H alcohol	3805
27	NH amine	3905
28	NH amine	3827

cm^{-1}, 1633 cm^{-1}, 1600 cm^{-1}, 2180 cm^{-1}, 2850 cm^{-1}, 2917 cm^{-1}, 1867 cm^{-1}, 1907 cm^{-1}, 1946 cm^{-1}, 2080 cm^{-1}, and 2013 cm^{-1}, 3313 cm^{-1}, 3380 cm^{-1}, 3347 cm^{-1}, 3782 cm^{-1}, 3453 cm^{-1}, 3620 cm^{-1}, 3598 cm^{-1}, 3654 cm^{-1}, 3693 cm^{-1}, 3559 cm^{-1}, 3805 cm^{-1}, 3905 cm^{-1}, 3827 cm^{-1} (Fig. 5). The appropriate class of functional groups and its stretching frequency are given in Table 4.

Gas chromatography coupled with mass spectrometry: The phytochemical constituents present in plant *A. lanata* identified by gas chromatography coupled with mass spectrometry showed that the ethanol leaf extract of contained 27 phytochemical components (Fig. 6 and Table 5). The major components were Uridine (38.94%), Batilol (14.80%), Friedelan (10.91%), Adenosine (8.66%), 2-tetrahrdrofurylmethyl ester(6.55%), Ledene oxide(5.71%), Tetrahydro(3.05%), methyl (2.09%), propanetriol(1.56%), Iso-valeraldehyde (0.89%), Isopropyl (0.83%), Epoxy (0.74%), 9-Octadecenal(0.71%), Methyl octyl ether(0.70%), Isocyclocitral (0.54%),Alpha-methyl-alpha(0.48%), Batiloloctadecylether (0.44%),Cedrene(0.39%), Tetraphenylhydrazine(0.34%), Cysteine (0.31%), N-Acetyl(0.27%), 2-Aminomethyl(0.25%), Verrucarol (0.24%), Pyridol(0.22%), Picrotoxin (0.21%), D-Alanine(0.16%), 4-Methyleneproline(0.02%). This results showed that the major compounds possess the following biological activities viz**.,** antibacterial, antineoplastic, antiviral, anticarcinogenic, antiurolithic, anti-inflammatory, antiseptic, antitoxic, antihelmintic properties.

DISCUSSION

Antibacterial activity of *A.lanata* plant extract was observed against some pathogenic bacteria which possess increased activities. Earlier studies revealed *A. lanata* extracts of leaf, stem flower and root extracts possess different medicinal properties viz, antimicrobial activity, antidiabetic activity, antioxidant activity (Kumar *et al*., 2013). Recently many solvents such as ethanol, ethyl acetate, chloroform, acetone, water and methanol were administered for different phytochemical analysis and established the occurrence of flavonoids, glycosides, tannins, steroids, saponins, phenolics, terpenoids and alkaloid hold constituents were employed for the screening of phytochemical constituents (Ramana *et al*.,2015). In the present investigation we used the ethanol as solvent sources for the extraction of the metabolites. From table 1, it is clear that the chemical constituents of *A. lanata* are flavonoids, phenols, carbohydrates, aminoacids, terpenoids and cocu-

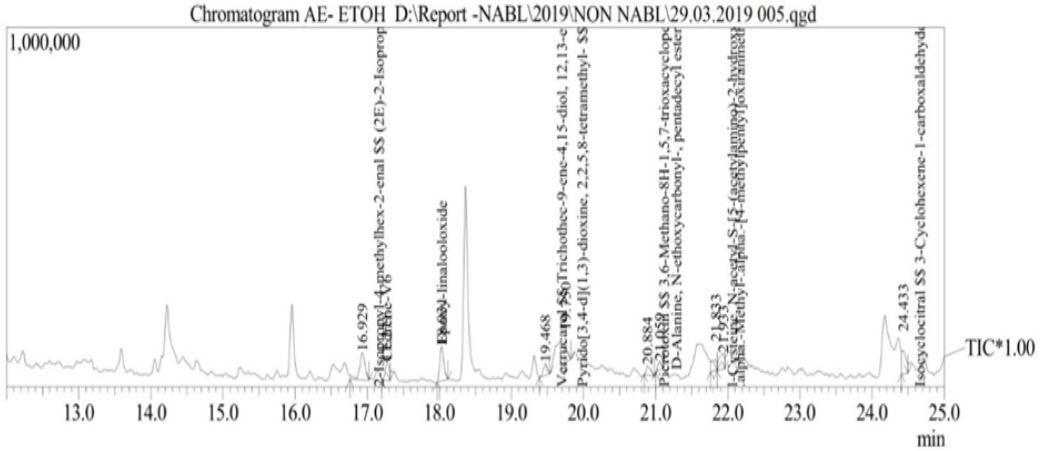

Fig. 6. *Gas chromatography coupled with mass spectrometry of leaf of A.lanata.*

Table 5. Gas chromatography coupled with mass spectrometry of leaf of *A. lanata*.

Peak Report TIC

Peak#	R.Time	Name	Area	Area%	Height	Height%
1	5.087	Adenosine	4257816	8.66	402705	7.70
2	5.453	1,2,3-PropanetriolMonoacetin $$ 2,3-D	766263	1.56	88364	1.69
3	5.730	Methyl octyl ether	342526	0.70	45697	0.87
4	6.746	N-Acetyl-dl-norleucine $$ N-Acetylnorleucine	131262	0.27	24853	0.48
5	6.975	2-aminomethyl-6-methyl-ester	121869	0.25	38055	0.73
6	7.300	2-tetrahydrofurylmethyl ester	3218396	6.55	439488	8.40
7	7.701	Uridine	19150188	38.94	1458902	27.90
8	8.094	Ledene oxide-(I)	2807453	5.71	358263	6.85
9	8.492	iso-Valeraldehyde propyleneglycol acetal	440028	0.89	102422	1.96
10	10.096	Tetrahydro[2,2']bifuranyl-5-one	1500536	3.05	299979	5.74
11	10.675	Tetraphenylhydrazine	164971	0.34	25189	0.48
12	11.763	Methyl 6-oxoheptanoate	1027678	2.09	159309	3.05
13	16.929	2-Isopropyl-4-methylhex-2-enal	406629	0.83	75850	1.45
14	17.271	Cedrene-V6	193425	0.39	50650	0.97
15	18.031	Epoxy-linalooloxide	365599	0.74	96810	1.85
16	19.468	Verrucarol	119838	0.24	32441	0.62
17	19.750	Pyrido[3,4-d](1,3)-dioxine	108463	0.22	38811	0.74
18	20.884	Picrotoxin	101429	0.21	25522	0.49
19	21.059	D-Alanine	78698	0.16	22263	0.43
20	21.833	l-Cysteine	153882	0.31	29666	0.57
21	21.933	.alpha.-Methyl-.alpha.-[4-methylpentyl]oxiran	234137	0.48	43103	0.82
22	24.433	Isocyclocitral $$ 3-Cyclohexene-1-carboxalde	264789	0.54	65778	1.26
23	26.892	4-Methyleneproline	7460	0.02	4913	0.09
24	32.565	9-Octadecenal	349819	0.71	71654	1.37
25	42.616	Batilol Octadecylether	214774	0.44	34424	0.66
26	42.829	Batilol $$ dl-Batyl alcohol $$ Bathyl alcohol $	7279345	14.80	703592	13.45
27	43.484	Friedelan-3-one	5365836	10.91	491233	9.39
			49173109	100.00	5229936	100.00

marins which serve as a main pharmaceutical product in curing ulcers, kidney stone, jaundice, head ache, stomatitis, fever, pain in liver, wounds in cattle, treatment of oedema and rheumatic joint pains etc.

The antibacterial property of the leaf extract revealed extreme sensitivity on *S. aureus*(16mm), an prime cause of human infections and several illness by means of upper respiratory tract infection and skin infection etc. and it is also a chief cause of food poisoning. The minimum inhibitory concentration of leaf, flower showed inhibition on has first four tubes and in stem extract first three tubes showed inhibition and this findings revealed that this particular plant has high medicinal properties.

Based on the property of antibacterial activity, spectroscopic analysis carried out only in leaf extract of *A. lanata* showed more peaks of O-H stretching. Each peak indicated the phytochemical compound in the leaf extract. The FT-IR spectrum analysis of leaf powder of *A. lanata* showed that the existence of divergent functional groups vary from O – H stretching (3782 cm^{-1} , 3693 cm^{-1} , 3654 cm^{-1}, 3620 cm^{-1}, 3598 cm^{-1}, 3559cm^{-1}), amine N – H (3380 cm^{-1}, 3347 cm^{-1}, 3313 cm^{-1}) alkane (C – H) (2917 cm^{-1}, 2850cm^{-1}), N = C = S stretching (2080 cm^{-1}, 2013 cm^{-1}), C– H bending (1946 cm^{-1}, 1907 cm^{-1}, 1867 cm^{-1}), C = C alkene (1404 cm^{-1}), C – N stretching (1326 cm^{-1}, 1242 cm^{-1}), C – F (1013 cm^{-1}), C – Br (723 cm^{-1}).

Yamunadevi Mariswamy *et al*.,(2013) reported that the GC-MS analysis of methanol extract of whole plant of *A.lanata* has 23 different phytochemical compounds. The acetone extract of *A.lanata* leaves showed 16 bioactive compounds (Arun Thangavel *et al*., 2014). Venaktesh Prasad yadav *et al*.,(2019) reported the elevated parts of ethanolic extract of *A.lanata* revealed total 76 bioactive compounds, in that 40 known compounds which shows medicinal properties and 36 unknown compounds. In present study, total 27 bioactive compounds were isolated from the leaf extract of *A. lanata* in that, Uridine (38.94%) is a major compound, followed by other compounds (Table 5) which shows antiallergic, anti-

inflammatory, antimicrobial and anticancer activity as reported by Yamunadevi *et al*.,(2013), Arun Thangavel *et al*., (2014), Venaktesh Prasad yadav *et al*. (2019)) for alzheimer's disease, gaucher disease treatment, cystic fibrosis treatment, sickle-cell anemia treatment, cardiac treatment, rheumatoid arthritis etc.

This study suggests boundless activity for the major constituents of the ethanolic extracts of *A. lanata* leaves, flower and stem. The current research indicated that the ethanolic extract of leaf is a powerful remedial agent for many diseases. Because it is comparatively secure and can be used to dissolve many organic compounds and it contains polar O-H bond hence it is soluble in water. Additional requirement is involved to separate and recognize these bioactive compounds. For recognizing these bioactive compounds novel medicines can be developed to manage different ailments. The results of the current findings also supplemented with previous researchers.

Conclusion

To conclude from this investigation the ethanolic extracts of leaf, stem and flower can act as a good antibacterial activity and also good nutritive importance. The Spectroscopic analysis of *A. lanata* leaves showed 27 bioactive compounds with known medicinal properties. Identification of these bioactive compounds may serve as the possible health benefits to formulate new drugs with fewer side effects.

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