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Full Length Research Paper

Phytochemical screening, gas chromatography-mass spectrometry (GC-MS) analysis of phytochemical constituents and anti-bacterial activity of *Aerva lanata* (L.) leaves

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The present study is aimed to determine the phytochemical screening and anti-bacterial activity of the extracts (acetone, ethyl acetate and ethanol) of medicinal plant, *Aerva lanata* leaves against bacterial strains. Acetone, ethyl acetate and ethanol extracts of *A. lanata* leaves were prepared using Soxhlet apparatus. These extracts of *A. lanata* leaves were checked for their anti-bacterial activity by well diffusion, minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC) techniques against bacterial strains. Preliminary phytochemical screening and gas chromatography-mass spectrometry (GC-MS) analysis of phytochemical constituents of *A. lanata* leaves were performed. Preliminary phytochemical screening of different extracts of *A. lanata* revealed the presence of alkaloids, proteins, amino acids, flavonoids, tannins, phenolic compounds, saponins, quinone, terpenes and coumarins. Among the three different extracts tested, acetone extract of *A. lanata* leaves showed maximum anti-bacterial activity. The bioactive components of acetone fraction of *A. lanata* leaves were evaluated by GC-MS analysis which showed the presence of sixteen chemical compounds. The extracts of *A. lanata* leaves have a broad spectrum of anti-bacterial activity and support the traditional use of these plants as medicines.

Key words: *Aerva lanata,* phytochemical screening, anti-bacterial activity, gas chromatography- mass spectrometry, minimal inhibitory concentration index.

INTRODUCTION

Plants have been widely used to treat various ailments, since ancient times. World Health Organization (WHO) has estimated that nearly 80% of the total population in the developing countries relies on medicinal plants for health care (Muruganantham et al., 2009). Therefore, such plants should be examined to understand their

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medicinal properties, safety and efficiency (Ellof, 1998).

Antibiotics are one of our most important weapons in fighting microbial infections and have greatly benefited the health-related quality of human life (Fransworth, 1993). Although, antibiotics have been widely used in last decades, the development of microbial resistance to them is also increased (Cohen, 1992). In order to overcome this problem, scientists deviated their research towards anti-microbial compounds of medicinal plants as an alternative solution. The demand for herbal medicine is due to their wide biological activities, higher safety compared to synthetic drugs and low cost (Grabley and Thiericke, 1999).

Aerva lanata is a medicinal plant and belongs to Amaranthaceae family. The whole plant of *A. lanata* is used as diuretic, anti-helminthic, anti-diabetic and expectorant and also used in the treatment of lithiasis (Gupta and Neeraj, 2004.). Traditionally, leaves of *A. lanata* are used as sap for eye-complaints; an infusion is given to cure diarrhea and kidney stone and the root is used in snake bite treatment. A leaf decoction preparation is used as gargle for treating sore throat and is also used in various complex treatments against guinea-worm. They are also used as an antidote for scorpion sting (Vijaya Kumar and Pulliah, 1998), spermatorrhoea and urinary troubles and as an anti-rheumatic (Kakrani and Saluja, 1994).

In addition to the traditional uses, *A. lanata* has a number of pharmacological activities including demulcent (Pullaiah and Naidu, 2003), immunomodulatory and anti-tumor activity (Nevin and Vijayammal, 2003.), anti-inflammatory (Vetrichelvan et al., 2000), diuretic (Udupihille and Jiffry, 1986), expectorant, hepatoprotective (Manokaran et al., 2008), nephroprotective (Shirwaikar et al., 2004), anti-diabetic (Vetrichelvan and Jegadeesan, 2002), anti-hyperglycemic (Deshmukh et al., 2008) , anti-microbial, cytotoxic (Chowdary et al., 2002), urolithiatic (Rao, 1985), hypoglycemic, anti-hyperlipidemic (Appia Krishnan et al., 2009), anti-helmintic and anti-parasitic (Anantha et al., 2010) and anti-asthmatic activities (Deepak Kumar et al., 2009; Rajesh et al., 2011). Considering the medicinal importance of *A. lanata*, an attempt has been made to investigate the phytochemical and anti-microbial activities of acetone, ethanol and ethyl acetate extracts from leaves of *A. lanata*. Furthermore, the phytochemical constituents were identified from acetone extract of this plant by using gas chromatography-mass spectrometry (GC-MS) analysis.

MATERIALS AND METHODS

Plant material collection

The plant *A. lanata* was collected in and around Salem, Tamil Nadu. The collected plant species was identified and confirmed (LOT.NO-68; A/series/F/AMA) by Dr. R. Selvaraj, Professor, Department of Botany, Annamalai University, Annamalai Nagar-608 002, Tamil Nadu, India. The plant was washed with running tap water and finally washed with distilled water to remove the dirt and dried under shade for 7 days.

Preparation of plant extracts

Thoroughly washed and dried leaves of *A. lanata* were then crushed gently to make it into powder by using mixer grinder. About 25 g of crushed powder was filled into the thimble and was extracted separately with different solvents such as acetone, ethyl acetate and ethanol in Soxhlet apparatus for 24 h. The solvent present in the extracts was allowed to evaporate in open air and the extracts were stored in refrigerator until further use.

Phytochemical screening

The different extracts of plant *A. lanata* were subjected to preliminary phytochemical screening by using standard procedures (Harborne, 1993; Trease and Evans, 2002) for the detection of alkaloids, proteins, amino acids, anthraquinone glycosides, flavonoids, carbohydrates, saponins, terpenes, coumarin, quinone, tannin and phenolic compounds.

Gas chromatography-mass spectrometry (GC-MS) analysis

GC-MS analysis of acetone extract of *A. lanata* leaves was performed by using Thermo GC-Trace ultra version 5.0 gas chromatography interfaced to Thermo MS DSQ II mass spectrometer instrument employing the following conditions: DB5-MS capillary standard non polar column (30 × 0.25 mm × 0.25 µm) and helium gas was used as a carrier gas at a constant flow rate of 1 ml/min. The oven temperature was kept at 70°C and was programmed to reach 260°C at a rate of 6°C/min. Mass range was 50 to 650 (m/z). The total running time was completed within 43 min. The chromatogram obtained from gas chromatography was then analyzed in mass spectrometry to get the mass of all fractions. The identification of phytochemical components was achieved through retention time and mass spectrometry by comparing the mass spectra of unknown peaks with those stored in Wiley 9 GC-MS library.

Anti-bacterial activity of plant extracts

Anti-bacterial activity of plant extracts was determined by agar well diffusion method against microbial type culture collection (MTCC) bacterial strains such as *Staphylococcus aureus* (MTCC 796), *Escherichia coli* (MTCC 443), *Klebsiella pneumoniae* (MTCC 109), *Salmonella typhi* (MTCC 733), *Proteus mirabilis* (MTCC 422), *Pseudomonas aeruginosa* (MTCC 6750) and *Bacillus subtilis* (MTCC 441). All the bacterial strains used in the present study were obtained from MTCC, Chandigarh, India. Muller-Hinton agar (MHA) (HiMedia, India) plates were prepared and 16 h old bacterial culture was swabbed uniformly and was allowed to dry for 5 min. Four wells of 5 mm in diameter were made in each of these plates by using a sterile cork borer. The extracts were prepared at a final concentration of 30 mg/ml by dissolving them in their respective solvents. The different concentrations of the plant extract (300, 600, 900 and 1200 µg) were loaded onto the wells and were allowed to

diffuse for 10 min. Then, the plates were incubated at 37°C for 24 h. After the incubation, diameter of inhibition zone around the well was measured and recorded.

Determination of minimal inhibitory concentration (MIC)

Each plant extracts (100 mg) were suspended in 1 ml of their respective solvents and were kept as stock solutions. These were subjected to double fold serial dilution in peptone water to obtain ten different concentrations ranging from 100 to 0.19 mg/ml and added to the respective culture tubes containing 2 ml of peptone water. These tubes were then inoculated with 100 µl of 16 h old broth culture of bacterial strains followed by incubation at 37°C for 24 h. Three tubes containing peptone water, peptone water and extract and peptone water and inoculum were used as control (John et al., 2011).

Determination of minimal bactericidal concentration (MBC)

The MBC of each extract was determined by sub-culturing 10 µl of the test dilutions from MIC tubes on to Muller Hinton agar plates. Plates were incubated for 24 h. The highest dilution that yielded low or no bacterial colony on the plate was recorded as MBC (Pavithra et al., 2010).

MIC index

The MIC Index (MBC/MIC) was calculated for each extract to determine whether an extract is bactericidal (MBC/MIC < 4) or bacteriostatic (MBC/MIC > 4) on growth of bacterial organisms (Chattopadhyay et al., 2007). The range of MIC Index value which is greater than 4 and less than 32 are considered as bacteriostatic (Cutler et al., 1994).

Statistical analysis

The experiments were repeated thrice and the average data were submitted to analysis of variance using ANOVA (Minitab version 15). A p value < 0.05 was considered statistically significant.

RESULTS

This study was designed to evaluate the phytochemical screening, GC-MS analysis of phytochemical constituents and anti-bacterial activity of *A. lanata*. Among the three extracts, acetone extract of leaves of *A. lanata* was found to have all the phytochemicals tested such as alkaloids, proteins and amino acids, anthraquinone glycosides, carbohydrates, flavonoids, saponins, coumarin, quinone, tannins and phenolic compounds. Ethyl acetate extract of leaf of plant showed the presence of flavonoids, terpenes, coumarin, tannins and phenolic compounds. Ethanol extract revealed the presence of alkaloids, proteins and amino acids, anthraquinone glycosides, flavonoids, terpenes, coumarin, quinone, tannins and phenolic compounds (Table 1).

GC-MS analysis of acetone extract of *A. lanata* leaves showed the presence of sixteen bioactive compounds that could contribute towards the medicinal properties to the plant (Figure 1 and Table 2). The first and predominant compound identified with less retention time (4.71) was (R)-(+)-ç-valerolactone (70.65%) whereas 5,14-di (*N*-butyl)-octadecane (1.47%) was the last compound identified which took longest retention time (44.83) for identification. The other prevalent compounds present in this extract were 9-octadecenoic acid (5.70%), 2-propynoic acid, methyl ester (5.46%) and 1-(2, 4, 6-trihydroxyphenyl)-3-(3-hydroxy-4-methoxy-6-ethylphenyl) propanone (4.24%). The remaining phytocompounds present in this extract were 2,3-trimethylsilyl-CC'-dimethyl-4,5-dicarbanido-hexaborane (1.67%), neophytadiene (1.62%), 5,14-di (N-butyl)-octadecane (1.47%), n-octacosane (1.31%), 2-hexadecen-1-ol, 3,7,11,15-tetramethyl-, [R-[R*,R*-(E)]]-phytol (1.28%), Trimethylamine borane (0.72%), 11-Hydroxy-8-oxo-13-tridecanolide (0.66%), n-heptacosane (0.58%), ethyl-2-bromo-3-hydroxy-3-methylpentanoate (0.43%), 1-hexadecene (0.43%), n-docosane (0.32%), and hexadecanoic acid, methyl ester (0.28%).

Acetone extract of *A. lanata* leaves exhibited the highest anti-bacterial activity against *P. mirabilis* followed by *S. aureus, S. typhi*, *B. subtilis, P. aeruginosa, K. pneumoniae* and *E. coli.* Ethyl acetate leaf extract of this plant showed anti-bacterial activity against *S. typhi, S. aureus* followed by *P. mirabilis, B. subtilis, E. coli, P.aeruginosa* and *K. pneumonia*e. Ethanol extract of leaf showed anti-bacterial activity against *E. coli* followed by *S. aureus*, *P. mirabilis, P. aeruginosa, S. typhi, K. pneumoniae* and *B. subtilis* (Table 3).

MIC value of acetone extract of *A. lanata* leaves was found to be 1.53 mg/ml against *S. aureus, P. mirabilis*, *S. typhi* and 3.06 mg/ml against *E. coli, K. pneumoniae, P. aeruginosa* and *B. subtilis.* MBC value of this extract was 6.12 mg/ml against *S. aureus, S. typhi, P. mirabilis* and 12.25 mg/ml against *B. subtilis* (Table 4). MIC index value of acetone extract of plant leaves showed that bactericidal activity against *S. aureus, S. typhi, P. mirabilis* and *B. subtilis* and bacteriostatic activity against *E. coli, K. pneumoniae* and *P. aeruginosa* (Table 7). MIC value of ethyl acetate extract of *A. lanata* leaves was found to be 1.53 mg/ml against *S. typhi* and 3.06 mg/ml against *S. aureus, P. mirabilis*, *B. subtilis* and 6.12 mg/ml against *E. coli, K. pneumoniae* and 12.25 mg/ml against *P. aeruginosa.* MBC value of this extract was 12.25 mg/ml against *S. aureus, S. typhi*, *B. subtilis* and 25 mg/ml against *K. pneumoniae, P. mirabilis* and 50 mg/ml against *E. coli* and *P. aeruginosa* (Table 5). MIC index value of this extract proved that they are found to be bactericidal against *S. aureus, K. pneumoniae, P. aeruginosa, B. subtilis* and *P. mirabilis* and bacteriostatic against *E. coli* and *S. typhi* (Table 7).

MIC value of ethanol extract of *A. lanata* leaves was

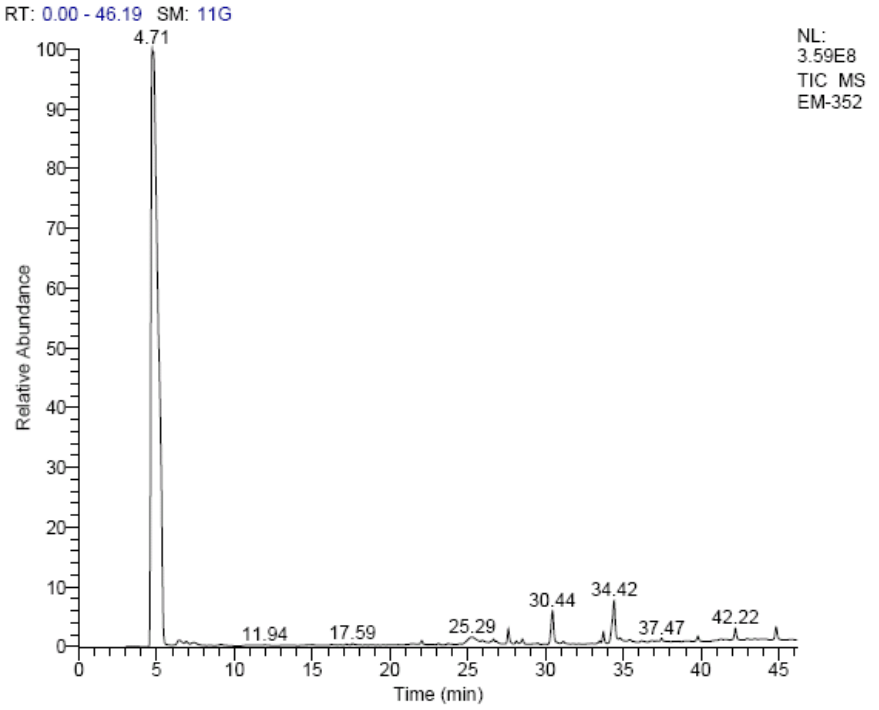


Figure 1. GC-MS chromatogram of acetone extract of *A. lanata* (L.) leaves.

found to be 1.53 mg/ml against *S. aureus, E. coli* and 3.06 mg/ml against *S. typhi, P. mirabilis* and 6.12 mg/ml against *K. pneumoniae* and *P. aeruginosa.* MBC value of this extract was 6.12 mg/ml against *S. aureus* and 12.25 mg/ml against *E. coli, S. typhi, P. mirabilis*, *B. subtilis* and 25 mg/ml against *K. pneumoniae* and *P. aeruginosa* (Table 6). MIC index value of this extract was also found to be bactericidal against *S. aureus, E. coli, P. aeruginosa, B. subtilis, S. typhi* and *P. mirabilis* and bacteriostatic against *K. pneumoniae* (Table 7). All these results proved that the acetone, ethyl acetate and ethanol extract of *A. lanata* leaves have bactericidal and also bacteriostatic effect on pathogenic microorganisms like against *S. aureus, E. coli, P. aeruginosa, B. subtilis, S. typhi* and *P. mirabilis* and *K. pneumoniae* and these effects were found to be varied from each solvent.

DISCUSSION

The antimicrobial activity of plants have been screened because of their great medicinal relevance with the recent years, infections have increased to a great extent and resistant against antibiotics becomes an ever in-creasing therapeutic problem. The presence of antifungal and antimicrobial substances in the higher plants are well established as they have provided a source of inspiration for novel drug compounds as plants derived medicines have made significant contribution towards human health.

Knowledge of the phytochemical constituents of plants is desirable, not only for the discovery of therapeutic agents, but also because such information may be of value in disclosing new sources of economic materials such as tannins, oils, gums, flavonoids, saponins, essential oils precursors for the synthesis of complex chemical substances (Akrout et al., 2010).

The results of phytochemical screening of *A. lanata* leaves clearly imply that the strength of active principle depends upon the use of solvent besides the type of plant species to achieve the positive results. The identified phytochemical compounds have many biological properties. For instance, hexadecanoic acid, methyl ester which is a palmitic acid compound found to be an anti-oxidant, hypocholesterolemic, nematicide, pesticide, lubricant activities and hemolytic 5-alpha is a reductase inhibitors. These results are strengthened by the findings of Sermakkani and Thangapandian (2012) who observed the presence of this compound in methanol extract of *Cassia italica* leaves.

9-Octadecenoic acid is a linoleic acid compound and reported to have an anti- inflammatory, nematicide, insectifuge, hypocholesterolemic, anti-cancer, hepatoprotective, anti-histaminic, anti-acne, anti-arthritic and anti-eczemic properties. Similarly, the presence of 9-octadecenoic acid was observed in the ethanolic root of *Plumbago zeylanica* by Ajayi et al. (2011). 2-Hexadecen-1-ol,

Table 1. Preliminary phytochemical screening of different solvent extracts of *A. lanata* (L.) leaves.

Active component /Test	Acetone	Ethyl acetate	Ethanol
Alkaloids			
Mayer's test	+	-	+
Dragendorff 's test	+	-	+
Hager's test	+	-	+
Wager's test	+	-	+
Protein and amino acid			
Millon's test	+	+	+
Ninhydrin test	+	+	+
Biuret test	+	+	+
Anthraquinone glycosides			
Borntrager's test	+	-	+
Flavonoids			
Shinoda 's test	+	+	+
Tannins and phenolic compounds			
Ferric chloride test	+	+	+
Lead acetate test	+	+	+
Gelatin test	+	+	+
Carbohydrates			
Molisch's test	+	-	-
Barfoed's test	+	-	-
Fehling's test	+	-	-
Saponins			
Frothing test	+	-	-
Terpenes			
Chloroform + Conc. H_2SO_4	+	-	+
Coumarin			
10% NaOH Solution	+	+	+
Quinone			
Conc. H_2SO_4	+	+	+

+ = Positive; - = Negative.

3, 7, 11, 15-tetramethyl-[R-[R*, R*-(E)]]- phytol is a diterpene compound which is reported to possess anti-microbial, anti-cancer, anti-inflammatory and diuretic agent (Praveen Kumar et al., 2010).

Similarly, the presence of phytol was observed in the leaves of *Lantana camara* (Mariai et al., 2011; Sathish and Manimegalai, 2008) and *Mimosa pudica* (Sridharan et al., 2011). Phytol was observed to have anti-bacterial activities against *S. aureus* by damaging the cell membranes which in turn causes leakage of potassium ions from bacterial cells. Phytol is a key acyclic diterpene alcohol that is a precursor for vitamins E and K_1. It is used along with simple sugar or corn syrup as a hardener in candies (Inoue et al., 2005). Plants are important source

Table 2. Phytochemical constituents of acetone extract of leaves of *A. lanata* (L.) by GC-MS spectra.

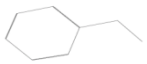
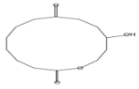
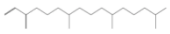
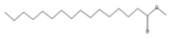
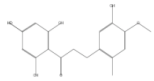
Retention time (min)	Name of the compound	Molecular formula	MW	Area (%)	Compound structure
4.71	(*R*)-(+)-ç-valerolactone	$C_5H_8O_2$	100	70.65	
5.26	2-Propynoic acid, methyl ester	$C_4H_4O_2$	84	5.46	
6.44	Trimethylamine borane	$C_3H_{12}BN$	73	0.72	
7.38	Ethyl 2-bromo-3-hydroxy-3-methylpentanoate	$C_8H_{15}BrO_3$	238	0.43	
22.05	1-Hexadecene	$C_{16}H_{32}$	224	0.43	
25.29	2,3-Trimethylsilyl-CC'-dimethyl-4,5-dicarbanido-hexaborane	$C_7H_{20}B_4Si$	176	1.67	
26.65	11-Hydroxy-8-oxo-13-tridecanolide	$C_{13}H_{22}O_4$	242	0.66	
27.61	Neophytadiene	$C_{20}H_{38}$	278	1.62	
29.49	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	270	0.28	
30.44	1-(2,4,6-Trihydroxyphenyl)-3-(3-hydroxy-4-methoxy-6 methylphenyl)propanone	$C_{17}H_{18}O_6$	318	4.24	
33.72	2-Hexadecen-1-ol, 3,7,11,15-tetramethyl-,[R-[R*,R*-(E)]]- phytol	$C_{20}H_{40}O$	296	1.28	
34.42	9-Octadecenoic acid	$C_{18}H_{34}O_2$	282	5.70	
37.47	n- Docosane	$C_{22}H_{46}$	310	0.32	
39.79	n- Heptacosane	$C_{27}H_{56}$	380	0.58	

Table 2. Contd.

42.22	n- Octacosane	$C_{28}H_{58}$	394	1.31	
44.83	5,14-Di (N-Butyl) octadecane	$C_{29}H_{60}$	408	1.47	

Table 3. Antibacterial activity of different solvent extracts of *A. lanata* (L.) leaves against bacterial (MTCC) strains by well diffusion method.

Bacterial strain	Zone of inhibition (mm)											
	Plant extracts concentration (µg/well)											
	Acetone				Ethyl acetate				Ethanol			
	300	600	900	1200	300	600	900	1200	300	600	900	1200
S. aureus	10	12	13	15	6	9	12	13	6	8	10	12
E. coli	6	7	8	9	6	8	10	11	8	10	12	14
K. pneumoniae	8	10	12	15	-	-	10	12	-	-	10	12
S. typhi	10	11	12	13	10	12	14	15	-	8	10	12
P. mirabilis	12	14	15	16	8	9	10	12	6	8	10	12
P. aeruginosa	8	10	12	14	-	-	8	10	6	8	10	11
B. subtilis	8	10	12	14	6	8	10	12	-	-	10	11

Table 4. Minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC) of acetone extracts of *A. lanata* (L.) leaves.

Bacterial strain	Minimal inhibitory concentration (MIC)									
	Plant extract concentration (mg/ml)									
	100	50	25	12.25	6.12	3.06	1.53	0.76	0.38	0.19
S. aureus	-	-	-	-	-	-	β	+	+	+
E. coli	-	-	-	-	-	β	+	+	+	+
K.pneumoniae	-	-	-	-	-	β	+	+	+	+
S. typhi	-	-	-	-	-	-	β	+	+	+
P. mirabilis	-	-	-	-	-	-	β	+	+	+
P.aeruginosa	-	-	-	-	-	β	+	+	+	+
B. subtilis	-	-	-	-	-	β	+	+	+	+
Minimal bactericidal concentration (MBC)										
S. aureus	-	-	-	-	**B**	+	+	+	+	+
E. coli	-	-	**B**	+	+	+	+	+	+	+
K. pneumoniae	-	-	**B**	+	+	+	+	+	+	+
S. typhi	-	-	-	-	**B**	+	+	+	+	+
P. mirabilis	-	-	-	-	**B**	+	+	+	+	+
P.aeruginosa	-	-	**B**	+	+	+	+	+	+	+
B. subtilis	-	-	-	**B**	+	+	+	+	+	+

Table 5. Minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC) of ethyl acetate extracts of *A. lanata* (L.) leaves.

Bacterial strain	Minimal inhibitory concentration (MIC)									
	Plant extract concentration (mg/ml)									
	100	50	25	12.25	6.12	3.06	1.53	0.76	0.38	0.19
S. aureus	-	-	-	-	-	β	+	+	+	+
E. coli	-	-	-	-	β	+	+	+	+	+
K.pneumoniae	-	-	-	-	β	+	+	+	+	+
S. typhi	-	-	-	-	-	-	β	+	+	+
P. mirabilis	-	-	-	-	-	β	+	+	+	+
P.aeruginosa	-	-	-	β	+	+	+	+	+	+
B. subtilis	-	-	-	-	-	β	+	+	+	+
Minimal bactericidal concentration (MBC)										
S. aureus	-	-	-	B	+	+	+	+	+	+
E. coli	-	B	+	+	+	+	+	+	+	+
K.pneumoniae	-	-	B	+	+	+	+	+	+	+
S. typhi	-	-	-	B	+	+	+	+	+	+
P. mirabilis	-	-	B	+	+	+	+	+	+	+
P.aeruginosa	-	B	+	+	+	+	+	+	+	+
B. subtilis	-	-	-	B	+	+	+	+	+	+

+ = Growth; - = no growth; β = MIC value and B = MBC value.

Table 6. Minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC) of ethanol extracts of *A. lanata* (L.) leaves.

Bacterial strain	Minimal inhibitory concentration (MIC)									
	Plant extract concentration (mg/ml)									
	100	50	25	12.25	6.12	3.06	1.53	0.76	0.38	0.19
S. aureus	-	-	-	-	-	-	β	+	+	+
E. coli	-	-	-	-	-	-	β	+	+	+
K.pneumoniae	-	-	-	-	β	+	+	+	+	+
S. typhi	-	-	-	-	-	β	+	+	+	+
P. mirabilis	-	-	-	-	-	β	+	+	+	+
P.aeruginosa	-	-	-	-	β	+	+	+	+	+
B. subtilis	-	-	-	-	-	β	+	+	+	+
Minimal bactericidal concentration (MBC)										
S. aureus	-	-	-	-	B	+	+	+	+	+
E. coli	-	-	-	B	+	+	+	+	+	+
K.pneumoniae	-	-	B	+	+	+	+	+	+	+
S. typhi	-	-	-	B	+	+	+	+	+	+
P. mirabilis	-	-	-	B	+	+	+	+	+	+
P.aeruginosa	-	-	B	+	+	+	+	+	+	+
B. subtilis	-	-	-	B	+	+	+	+	+	+

+ = Growth; - = no growth; β = MIC value and B = MBC value.

Table 7. Antibacterial activity of different solvent extracts of *A. lanata* (L.) in terms of MIC index (MBC/MIC).

Bacterial strain	MIC index		
	Acetone	Ethyl acetate	Ethanol
S. aureus	4.0	4.0	4.0
E. coli	8.16	8.16	4.0
K. pneumoniae	8.16	4.0	8.0
S. typhi	4.0	8.0	4.0
P. mirabilis	4.0	4.0	4.0
P.aeruginosa	8.0	4.0	4.0
B. subtilis	4.0	4.0	4.0

of potentially useful compounds for the development of new chemotherapeutic agents. *In vitro* evaluation of plants for anti-microbial property is the first step towards achieving the goal for developing eco-friendly management of infectious disease of humans by search for new biomolecules of plant origin (Samy et al., 2008; Mohana et al., 2008).

On comparison of three different *A. lanata* leaves extract, acetone extract showed the highest anti-bacterial activity against the bacterial strains. Senthilkumar et al. (2011) reported that the bark acetone, isopropanol and hexane extract of pomegranate possess anti-bacterial activity against *S. typhi* and *S. paratyphi* A isolates and also proved that among three solvents, acetone and hexane extracts showed better efficiency than isopropanol extract.

The increase of antibiotic resistance of microorganisms to conventional drugs has necessitated the search for new, efficient and cost effective ways for the control of infectious diseases. The results of different studies provide evidence that some medicinal plants might indeed be potential sources of new anti-bacterial agents (Kone et al., 2004). The use of medicinal plants is part of the Indian tradition. Many local regions all over India have a great variety of vegetation used by the local population to treat and prevent diseases (Bonyadi et al., 2009).

Conclusion

From this study it is concluded that leaves of *A. lanata* have a broad spectrum of anti-bacterial activity and supports the traditional use of these plants as medicines. This study demonstrated that herbal medicine can be as effective as modern medicine to combat pathogenic microorganisms and overwhelming the antibiotic resistance. Further studies are needed with this plant on its isolation, structural elucidation of bioactive compounds and about its *in vivo* toxic effects in experimental animals to formulate a new drug for regular practice.

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