Analysis of phytochemicals, antimicrobial activity and ethnomedicinal uses of *Aerva lanata* from Western Ghats, India

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Abstract : In this study, phytochemical screening was carried out to find the presence of the active chemical constituents in the solvent extract (ethanol, ethyl acetate, chloroform, methanol and acetone) of *Aerva lanata*. The phytochemicals such as, flavonoids, alkaloids, phenolic compounds, tannins, steroids, saponins, terpenoids and glycosides were studied. The leaf of *A. lanata* was extracted with various solvents and antimicrobial activity was studied. The leaf extract showed moderate activity against *E. coli*, *S. epidermis*, *E. aerogenes* and *B. cereus*. The leaf extract of *A. lanata* showed 9 mm zone (chloroform extract), 11 mm zone (methanol extract) and 13 mm zone (acetone extract) against *E. coli*. The zone of inhibition was 11 mm (ethanol extract), 10 mm (ethyl acetate extract), 11 mm (chloroform extract), 10 mm (methanol extract) and 13 mm (acetone extract) against *S. epidermis*. The leaf of *A. lanata* was effective against *E. aerogenes*. The zone of inhibition was 9 mm in ethanol and ethyl acetate extract, 10 mm in methanol extract and 11 mm in acetone extract. The chloroform extract of leaf showed moderate activity (9 mm zone) against *B. cereus*, however, other extracts did not show any activity. The MIC concentrations ranged from 40 mg/ml to 80 mg/ml. The methanolic extract of *A. lanata* was highly effective against *E. aerogenes* and the MIC was 40 mg/ml. The result of present finding showed the presence of alcohol, alkenes, aromatic, amine, phenyl, ether, methylene, primary amines, 1° and 2° amine and aliphatic chloro compound. The peaks were observed at 3308.3, 2853.3, 2701.6, 1660.75, 1500.2, 1385.8, 1225.05, 1160.75, 1096.45, 982.25, 821.5, 757.2 and 660.7 cm^{-1}. The major band was observed at 3308 cm^{-1} that mainly could be O-H stretching vibrations of alcohol group.

Keywords: Medicinal plants, phytochemical, antimicrobial, medicine

I. INTRODUCTION

More than 1,500 species of medicinal plants are known and were widely used in the traditional system of medicine such as Siddha, Ayurveda and Unani. More than 3000 plant species are available from the empirical knowledge and are applied as ethnomedicine. More than 700 medicinal plant species have been reported chemically and are widely used in modern medicine (Vedprakash, 1998). However, knowledge about the magnitude of medicinal plant species diversity is highly critical for monitoring its utilization for pharmaceutical purpose, management and conservation strategies. These species diversity data may provide sustainable utilization of medicinal plants in a given ecosystem. Species diversity is the important scale to measure the magnitude of diversity in an ecosystem. In India, about 141 endemic genera and more than 2500 endemic species of medicinal plants are widely distributed in Himalayan region, 1788 species were observed in peninsular region and 185 species were identified in the Andaman and Nicobar islands. However, an accurate number of medicinal plants distributed in the particular ecosystem are very difficult to validate and correlate because of the risk in correlating the vernacular names used in traditional systems of medicine with respective scientific names. This is a very common fact in multi linguistic country like India (Vedprakash, 1993). It is estimated that more than 27 per cent of plant species were occupied in Western Ghats. The diversity of medicinal plant species were reported at Sothern Western Ghats of Kerala by various research groups (Vajaravelu, 1990). In Western Ghats, mainly the tribal populations use these medicinal plants till date and these medicinal plants are also useful to improve the health of rural population. The ethnic peoples and tribals are mainly depending on traditional medical system. The tribal groups in this area use many number of herbs for treating various diseases. In Tamil Nadu the ethnomedical value of medicinal plants in their habitat of tribal population and rural communities for treating various disorders and diseases has not been well documented. The information about traditional medicine provides novel information about the medicinal plants from tribal groups.

II. MATERIALS AND METHODS

Collection of medicinal plants

In this study documentation of medicinal plants was carried out by intensive exploration trips for a period of twelve months from Jan 2012 to Dec 2012 at Western Ghats, Tamilnadu, India. The medicinal plant (*Aerva lanata*) was collected based on the traditional knowledge provided by the Kanikkars healers of this area.

Preparation of plant extract

10 g air dried powder was weighed and transferred in 100 ml solvent such as, ethanol, ethyl acetate, chloroform, methanol and acetone for a day. The Erlenmeyer flask was kept on an orbital shaker at 150 – 200 rpm for 24 h. Then the sample

was filtered using whatman's no 1 filter paper. The filtrates were further evaporated under reduced pressure and gummy residue was obtained with the help of rotary evaporator. **Phytochemical screening**

In this study, phytochemical screening was carried out to find the presence of the active chemical constituents in the solvent extract (ethanol, ethyl acetate, chloroform, methanol and acetone) of medicinal plant. The following phytochemicals such as, flavonoids, alkaloids, phenolic compounds, tannins, steroids, saponins, terpenoids and glycosides were studied.

Antibiotics

Antibiotics were used as positive control during antimicrobial activity studies. These were purchased from Himedia, Mumbai, India. Amikacin (30 µg) and Nystatin (30 µg) were used as the positive control.

Antibacterial screening

The different solvent fractions (ethanol, ethyl acetate, chloroform, methanol and acetone) were evaluated for antibacterial activity against eight bacterial strains such as *Staphylococcus epidermidis*, *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Proteus vulgaris*, *Bacillus cereus*, *Enterobacter aerogenes* and *Enterococcus faecalis.* Mueller Hinton Agar was prepared in Erlenmeyer flask according to the manufactures instructions. The culture media was finally poured into Petri dishes under sterile condition. All solvent extracts were dissolved in dimethyl sulfoxide (DMSO) (100%) to achieve a final concentration of 20 mg/ml. The sample well was made and 20 µl of sample was loaded into the well. Amikacin (30 µg) was used as the positive control and DMSO was used as the negative control. All plates were incubated for 24 h at 37°C. The diameter of the zone of inhibition was measured in millimetre (mm).

Antifungal screening

Antifungal activity of the solvent extract of the sample was carried out as described previously. *Candida albicans* and *Aspergillus niger* were grown on Mueller Hinton Agar plates. All plant extracts were dissolved in DMSO to achieve a final concentration of 20 mg/ml. Nystatin (30 µg) was used as the positive control and DMSO was used as the negative control. All plates were incubated for 72 h at 30°C. The diameter of the zone of inhibition was measured in millimetres (mm).

Minimum Inhibitory Concentration (MIC)

Broth dilution test

The plant extract-containing sterilized tubes were inoculated with a standardized bacterial suspension (1–5×10^5 CFU/ml). Following 18 h incubation at 37°C, the test tubes were examined carefully for visible bacterial growth as evidenced by turbidity. To determine the MIC for the fungal isolates, the fungal spore suspension was adjusted as 2 × 10^6 spores/ml and was taken in sterile test tubes with nutrient broth supplemented with five different concentrations of plant extracts (5 to 80 mg/ml). Three control tubes were maintained for each test group; sterility control (Mueller-Hinton broth and DMSO), the positive control (antibiotic, Mueller-Hinton broth and the test organism) and negative control (Mueller-Hinton broth, test organism and DMSO). MIC value was defined as the lowest concentration of each plant extract, which completely inhibited microbial growth. The results were expressed in milligrams per millilitres.

Fourier Transform Infrared Spectroscopy (FT-IR)

10 mg of the dried extract powder was encapsulated in 100 mg of KBr pellet, in order to prepare translucent sample discs. All the nine powdered samples were loaded in FTIR spectroscope (Shimadzu, Japan), with a scan range from 400 to 4000 cm^1.

II. RESULTS

Phytochemicals of *Aerva lanata*

The phytochemical analysis of the leaf of *A. lanata* showed less number of phytochemicals than other medicinal plants. Methanol extract showed the presence of phenoilc compounds, flavonoids, tannins, saponins and glycosides. Ho wever, terpenoids was not detected in any solvent extracts (Table 1).

Table 1. Phytochemical analysis of the Leaves Extracts of *Aerva lanata*

Phytochemicals	Solvents				
	Ethanol	**Ethyl acetate**	**Chloroform**	**Methanol**	**Acetone**
Flavonoids	--	+	+	+	--
Alkaloids	+	--	--	--	--
Phenolic compounds	--	--	--	+	--
Tannins	+	--	--	+	--
Steroids	--	--	--	--	--
Saponins	--	+	+	+	+
Terpenoids	--	--	--	--	--
Glycosides	--	--	--	+	+

+ present; -- absent

Antibacterial and Antifungal activity of *Aerva lanata*

The leaf of *A. lanata* was extracted with various solvents and antimicrobial activity was studied. The leaf extract showed moderate activity against *E. coli*, *S. epidermis*, *E. aerogenes* and *B. cereus*. This sample was not effective against *A. niger* and *C. albicans*. The leaf extract of *A. lanata* showed 9 mm zone (chloroform extract), 11 mm zone (methanol extract) and 13 mm zone (acetoneextract) against *E. coli*. The zone of inhibition was 11 mm (ethanol extract), 10 mm (ethyl acetate extract), 11 mm (chloroform extract), 10 mm (methanol extract) and 13 mm (acetone extract) against *S. epidermis*. The leaf of *A. lanata* was effective against *E. aerogenes*. The zone of inhibition was 9 mm in ethanol and ethyl acetate extract, 10 mm in methanol extract and 11 mm in acetone extract. The chloroform extract of leaf showed moderate activity (9 mm zone) against *B. cereus*, however, other extracts did not show any activity (Fig. 1).

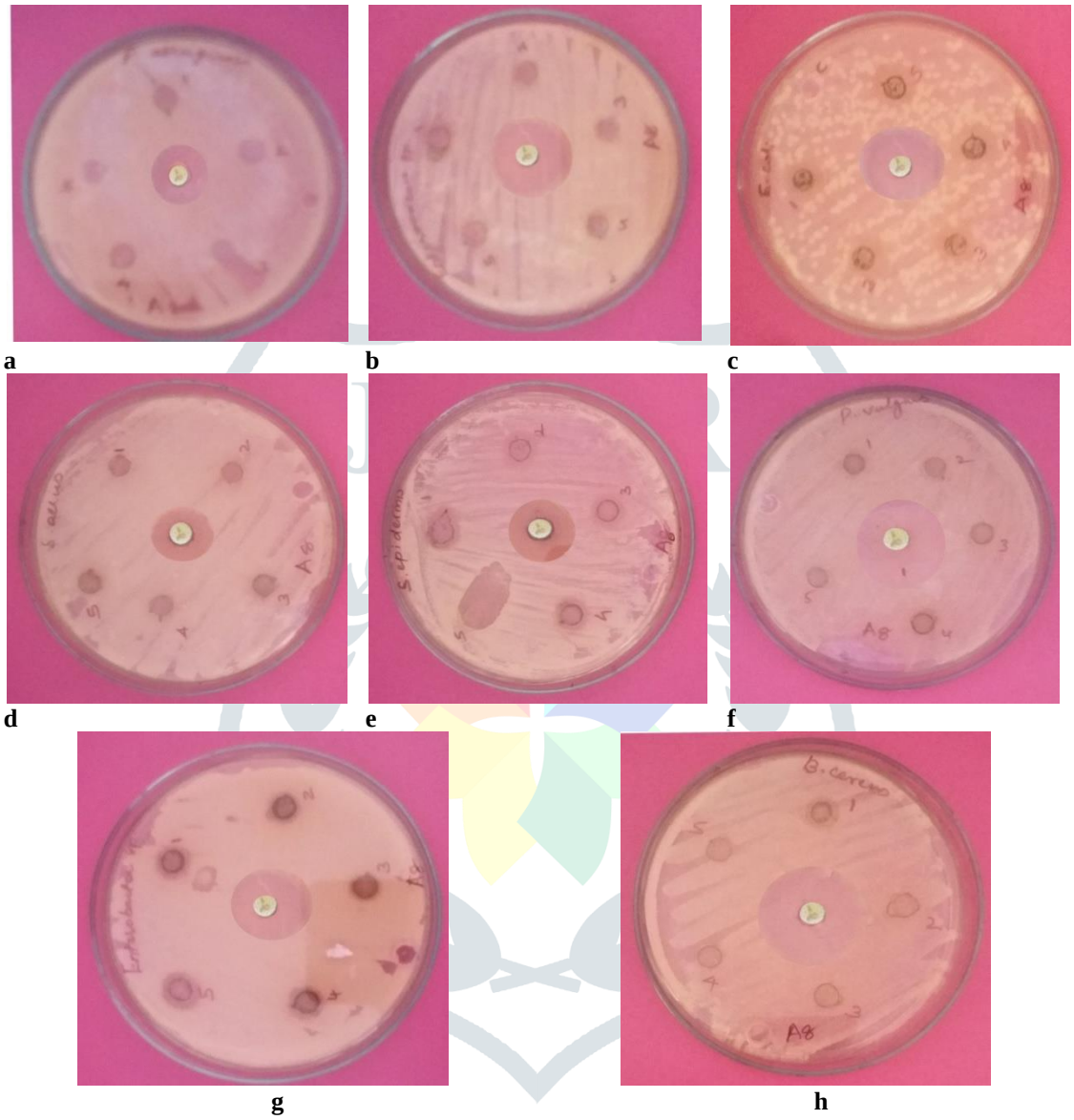

Fig. 1. Antibacterial activity of *A. lanata* against *Pseudomonas aeruginosa (a), Enterococcus faecalis (b), Escherichia coli (c), Staphylococcus aureus (d), Staphylococcus epidermidis (e), Enterobacter aerogenes (f), Proteus vulgaris (7)* and *Bacillus cereus.* 20 µl of crude extract was loaded into the well and antimicrobial activity was assayed from the five different solvent extracts. The selected solvents were *1*=ethanol, 2=ethyl acetate, 3=chloroform, 4=methanol and 5=acetone.

Minimum Inhibitory Concentration (MIC) of methanol extract of *A. lanata*

Methanolic extract of *A. lanata* showed the antimicrobial activity against the selected bacteria and fungi. The MIC concentrations ranged from 40 mg/ml to 80 mg/ml. The methanolic extract of *A. lanata* was highly effective against *E. aerogenes* and the MIC was 40 mg/ml (Fig. 2).

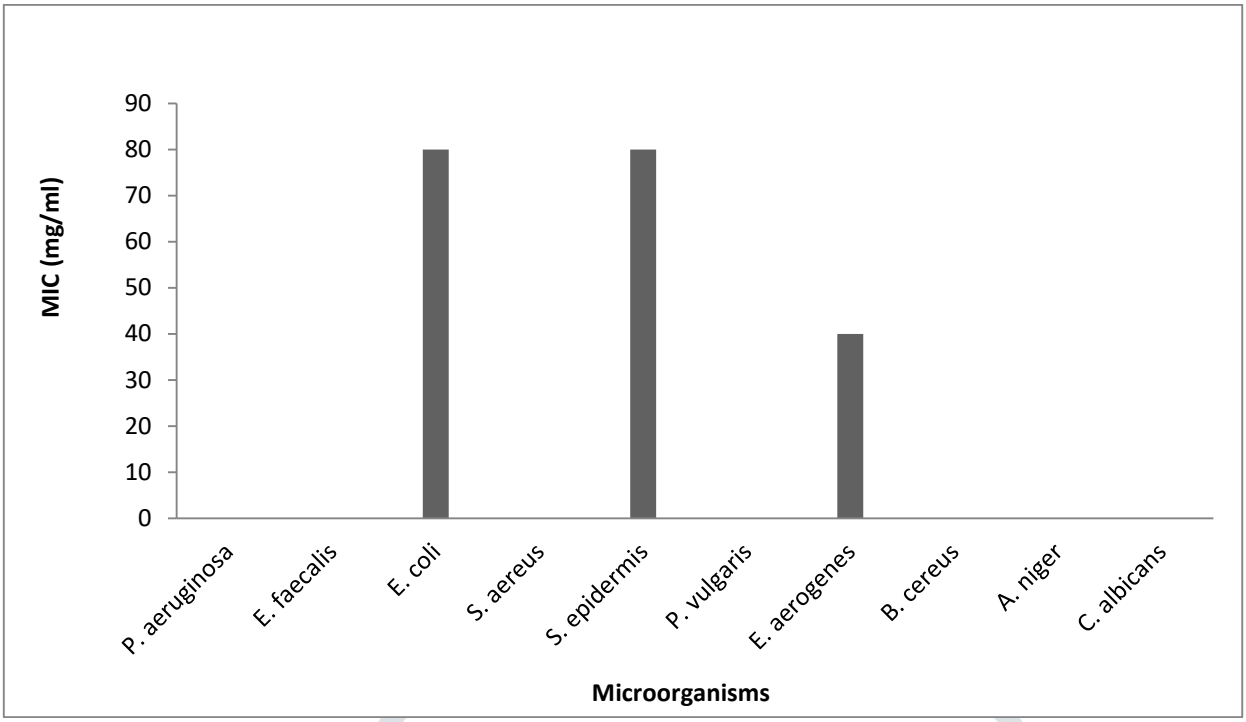

Fig. 2. Minimum Inhibitory Concentration (MIC) of *A. lanata* extracted with Methanol for antibacterial and antifungal activity (mg/ml).

Fourier Transform Infrared spectroscopy spectra analysis of *A.* lanata

The FT-IR spectrum of methanol extract of *A. lanata* is presented in Fig. 3. The peak values and the functional groups are presented in Table 2. The result of present finding showed the presence of alcohol, alkenes, aromatic, amine, phenyl, ether, methylene, primary amines, 1° and 2° amine and aliphatic chloro compound. The peaks were observed at 3308.3, 2853.3, 2701.6, 1660.75, 1500.2, 1385.8, 1225.05, 1160.75, 1096.45, 982.25, 821.5, 757.2 and 660.7 cm^{-1}. The major band was observed at 3308 cm^{-1} that mainly could be O-H stretching vibrations of alcohol group.

Table 2. Fourier Transform Infrared spectroscopy spectra analysis of the *Aerva lanata*

Wave number (cm^{-1})	Components (peak)	Functional groups
3208.3	OH	Alcohol group
2843.3	C-H	Alkenes
2700.6	O-H	Alkenes
1660.5	C-H	Aromatic
1501.2	NH	Amine
1382.8	OH	Phenyl
1224.05	C-O	Ether
1162.75	CH_2	Methylene group
1094.45	C-O-C	Aromatic cyclic ether
980.25	C-N	Primary amines
823.5	N-H	1° and 2° amines
758.2	C-Cl	Aliphatic chloro compound
665.7	OH	Alcohol

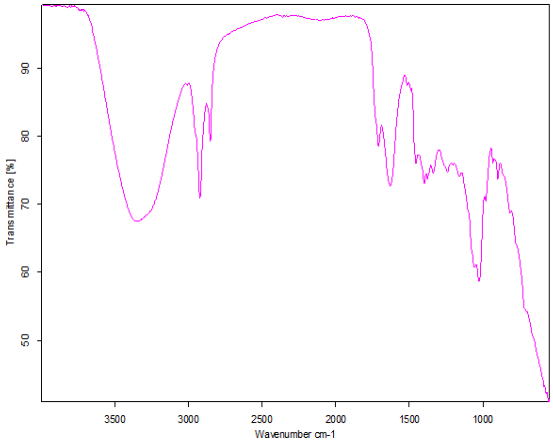

Fig. 3. Fourier Transform Infrared spectroscopy spectra analysis of the methanolic

DISCUSSION

A. lanata is an herb and has climbing branches. Leaves are alternate and opposite. Flowers are small, hermaphrodite and small. Traditionally this plant is used for its diuretic, anthelmintic, anti diabetic, hepatoprotective and anti-inflammatory activity. It is useful to treat boils and cough. The plant extract is proved for its nephroprotective activity, cytotoxicity, antioxidant, immunomodulatory effect, anti-inflammatory effect and anti hyperglycemic effect (Raja et al., 2011). The previous study also confirmed the traditional application for supplement treatment and pharmacological observations for health problems such as arthritis, allergic reactions and hormones regulation (Payal *et al.*, 2015). It could be noted that this medicinal plants also have notable pharmacological properties such as, nephron protective, hepato-protective, diuretic, anti-inflammatory, anthelmintic and demulcent properties (Yamunadevi *et al.*, 2010). Hence, it is not surprising to use this plant by the tribals for various traditional applications in the study area. The low antibacterial effect on the leaves of *A. lanta* was reported previously by various research groups. When compared with all selected nine medicinal plants *A. lanta* showed least antimicrobial activity. However, in FT-IR analysis many active principles were determined. These active principles could have other biological properties. The medicinal property of this plant was extensively studied by many research groups. Accordingly, *A lanata* is used to treat various diseases such as, cough, antidote, skin infections, emollient and anti diuretic **(**Athira and Nair, 2017). The FT-IR spectrum of methanol extract of *A. lanata* showed the presence of alcohol, alkenes, aromatic, amine, phenyl, ether, methylene, primary amines, 1°C and 2°C amine and aliphatic chloro compound. Recently, Ragavendran *et al.* (2011) analyzed the functional groups from *A. lanata* and detected carboxylic acids, amines, sulphur derivatives, amides, polysaccharides, halogens and organic hydrocarbons. In another study, Packialakshmi and Sudharsan (2016) analyzed the function groups of the leaf extract of *A. lanta* from the aqueous extract and determined the phytochemcials such as, amines, carboxylic acids, alkenes, phenols, ethers and halogens.

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