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Phytochemical, FT-IR and antibacterial activity of whole plant extract of *Aerva lanata* (L.) Juss. Ex. Schult.

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The present study deals with the phytochemical examination of therapeutic importance of whole plant extract of *Aerva lanata* (L.) Juss. Ex. Schult. an important medicinal plant. Qualitative phytochemical analysis of the methanol and ethanol extracts prepared from *Aerva lanata* whole plant revealed the presence of alkaloids, anthraquinones, catechins, coumarins, flavonoids, phenols, quinones, saponins, steroids, sugar, glycosides, tannins and xanthoproteins. The FT-IR spectrum confirmed the presence of alkyl group, methyl group, alcohol group, ethers, esters, carboxylic acid and anhydrides. The petroleum ether, benzene, ethyl acetate, methanol and ethanol extracts of whole plant of *Aerva lanata* were tested against *Bacillus thuringiensis*, *Bacillus subtilis*, *Streptococcus faecalis*, *S. pyogenes*, *Staphylococcus aureus*, *Enterococcus faecalis*, *Salmonella paratyphi* A and B, *Salmonella paratyphi*, *Proteus mirabilis*, *Proteus vulgaris*, *Escherichia coli*, *E. coli* (ESBL), *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and *P. aeruginosa* (ESBL) by the agar disc diffusion method.

Keyword: *Aerva lanata*, whole plant, Phytochemical screening, FT-IR, antibacterial, *P. mirabilis* and *S. pyogenes*.

1. Introduction

Almost all the medicinal plants available in the world have great potential sources for discovery as well as protection of new drugs of benefit to mankind. Presently, there is lot of approaches available to reach for new biologically active ingredients in the medicinal plants for the preparation of safe drugs. Scientifically many works have been expended to evaluate and discover new antioxidant, antimicrobial and antifungal ingredients from different kinds of natural sources like soil, microorganisms, animals and plants. Different types of folk medicine or herbal medicine are among the most important resources. Check and need to check or systematic screening of these available traditional herbs may result in the discovery of novel effective bioactive compounds for the formulation of drugs [1].

The increasing prevalence of multidrug resistant strains of bacteria and the recent appearance of strains with reduced susceptibility to antibiotics

raise serious concern of health delivery and accessibility due to untreatable bacterial infections. There is therefore the needed urgently to the search for new antimicrobial drug. Plants are important source of potentially useful structures for the development of new chemotherapeutic agents [2]. To the best of our knowledge, there is no record of work on the phytochemical screening, FT-IR analysis and antibacterial activity of the whole plant of *A. lanata*. Therefore, the present study was carried out to evaluate the phytochemical screening, FT-IR analysis and antibacterial activity of the whole plant of *A. lanata*.

2. Materials and Methods

The whole plant of *Aerva lanata* was collected from Sendranakkanoor, Krishnagiri District, Tamil Nadu, India. The plant was identified with help of local flora and authenticated in Botanical Survey of India, Southern circle, Coimbatore, Tamil Nadu. A voucher specimen (VOCB 5414)

was deposited in Ethnopharmacology Unit, Research Department of Botany, V.O.Chidambaram College, Tuticorin, Tamil Nadu.

2.1 Preparation of extracts for phytochemical screening

Freshly collected whole plant samples of *Aerva lanata* were dried in shade, and then coarsely powdered separately in a Willy mill. The coarse powder (100 g) was extracted successively with petroleum ether, benzene, ethyl acetate, methanol and ethanol, each 250 ml in a Soxhlet apparatus for 24 hrs. All the extracts were filtered through Whatman No.41 filter paper. All the extracts (petroleum ether, benzene, ethyl acetate, methanol and ethanol) were subjected to qualitative tests for the identification of various phytochemical constituents as per standard procedures [3-5].

2.2 FT-IR analysis

A little powder of plant specimen was mixed with KBr salt, using a mortar and pestle, and compressed into a thin pellet. Infrared spectra were recorded as KBr pellets on a Thermoscientific Nicot iS5 iD1 transmission, between 4000 – 400 cm^{-1} [6].

2.3 Microorganisms

Bacterial strains of *Bacillus thuringiensis*(+), *Bacillus subtilis*(+), *Streptococcus faecalis*(+), *Staphylococcus aureus*(+), *Streptococcus pyogenes*(+), *Enterococcus faecalis* (+), *Mycobacterium smegmatis* (+), *Salmonella paratyphi-A & B* (-), *Salmonella paratyphi* (-), *Proteus mirabilis*(-), *Escherichia coli*(-), *Escherichia coli* (ESBL) (-), *Proteus vulgaris*(-), *Klebsiella pneumoniae*(-), *Serratia marcescens* (-), *Pseudomonas aeruginosa*(-), and *Pseudomonas aeruginosa* (ESBL) (-) were obtained from Department of Microbiology, Bharathidasan University, Trichy, Tamil Nadu, India. The bacteria were incubated on a nutrient agar-slant (Stationary cultures) for 48 h at 37 °C, followed by inoculation in Muller Hinton Agar (MHA) medium.

2.4 Antibacterial assay

Antimicrobial study was carried out by disc diffusion method [7] against the pathogens. A loopful of bacteria was taken from the stock culture and dissolved in 0.1 ml of saline. All the tests were done by placing the disc (6 mm diameter) impregnated with (20 mcg) respective different extracts on the Muller Hinton Agar surface previously inoculated with 10 ml of MHA liquid medium with Gram Positive and Gram Negative bacteria. Respective solvents without plant extract served as negative control. Standard antibiotic of tetracycline (30 mcg/disc) was used as reference or positive control. Plates were incubated at 37 °C for 24 hours. After the incubation period, the diameter of the inhibition zone around the plant extracts saturated discs were measured and also compared with the diameter of inhibition zone of commercial standard antibiotic discs. The inhibition zone and antibacterial activity against the pathogenic bacteria were recorded. The experiments were repeated in triplicate and the results were documented.

3. Results

Petroleum ether, benzene, ethyl acetate, methanol and ethanol extracts of whole plant of *Aerva lanata* were examined for the phytoconstituents presence and antibacterial activity against the human pathogens and the results are given in the table 1 and 3. The results of the phytochemical screening revealed that alkaloid, anthraquinone, catechin, coumarin, flavonoid, phenol, quinone, saponin, steroid, tannin, terpenoid and sugar presence in the methanol and ethanol extracts of *A. lanata* whole plant. The FT-IR spectroscopy studies of whole plant of *Aerva lanata* gave the following characteristic absorption peaks as shown in table 2. It is also given in fig. 1. From the FT-IR spectral data, C=O, C-H, C=C, O-H, C-CHO, C-N and C-Cl were identified.

Table 1: Preliminary phytochemical screening of whole plant of *A. lanata*

Bioactive components	Nature of extract				
	Petroleum ether	Benzene	Ethyl acetate	Methanol	Ethanol
Alkaloids	+	+	+	+	+
Anthroquinones	-	+	-	+	+
Catachin	-	+	+	+	+
Coumarin	-	+	+	+	+
Flavonoids	-	+	+	+	+
Phenols	-	-	-	+	+
Quinones	+	+	+	+	+
Saponins	-	-	-	+	+
Steroids	-	+	+	-	+
Tannins	-	-	-	+	+
Terpenoids	-	+	+	+	+
Glycosides	-	-	-	-	-
Xanthoprotein	-	+	+	-	+
Sugar	+	+	+	+	+
Fixed oil	-	-	-	-	-

Table 2: IR Spectroscopic data of whole plant of *A. lanata*

S.No	Group	Stretching Frequency (cm ⁻¹)
1	O-H	3319.26
2	C-H Stretching (carbonyl)	2921.96
3	C=C Stretching (Aromatic)	1612.38
4	C=O stretching (carbonyl)	1735.81
5	C-CHO Skeletal	1400.22
6	C-H Stretching (carbonyl compounds)	2879.89
7	C-N Stretching	1319.22
8	C-Cl Stretching	752.19,696.25

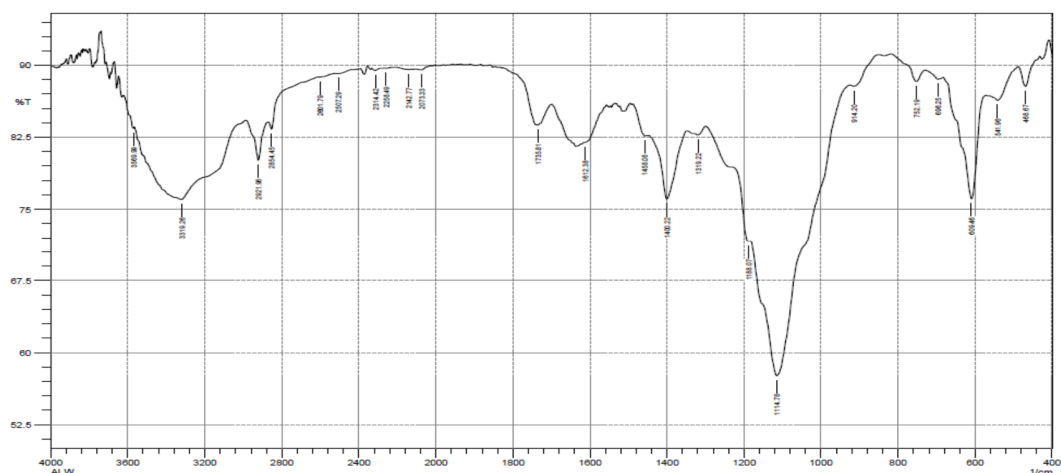


Fig 1: FT-IR Spectrum of whole plant of *A. lanata*

Table 3: Antibacterial activity of whole plant extracts of *A. lanata*

S. No	Microorganisms	Name of the extract / Zone of Inhibition (mm)					
		Petroleum ether	Benzene	Ethyl acetate	Methanol	Ethanol	AB
1.	<i>Bacillus thuringiensis</i>	-	07	08	09	10	20
2.	<i>Bacillus subtilis</i>	07	07	-	-	12	22
3.	<i>Streptococcus faecalis</i>	-	10	07	11	10	20
4.	<i>Staphylococcus aureus</i>	07	07	10	15	12	20
5.	<i>Streptococcus pyogenes</i>	-	-	-	-	06	24
6.	<i>Enterococcus faecalis</i>	-	-	-	-	12	24
7.	<i>Mycobacterium smegmatis</i>	07	06	06	-	13	22
8.	<i>Salmonella paratyphi-A</i>	12	08	09	07	-	21
9.	<i>Salmonella paratyphi-B</i>	-	-	08	06	12	22
10.	<i>Salmonella paratyphi</i>	09	10	11	-	10	26
11.	<i>Proteus mirabilis</i>	10	09	07	-	14	24
12.	<i>Escherichia coli</i>	14	08	09	-	07	28
13.	<i>Escherichia coli</i> (ESBL)	06	10	14	12	11	23
14.	<i>Proteus vulgaris</i>	-	-	-	-	-	28
15.	<i>Klebsiella pneumonia</i>	-	-	-	-	15	24
16.	<i>Serratia marcescens</i>	13	07	11	20	05	24
17.	<i>Pseudomonas aeruginosa</i>	10	12	10	14	09	25
18.	<i>Pseudomonas aeruginosa</i> (ESBL)	-	-	-	09	14	26

Among the five extracts tested against eighteen pathogens (Fig.2), ethanol extract was found to

be the most effective against the microorganisms.

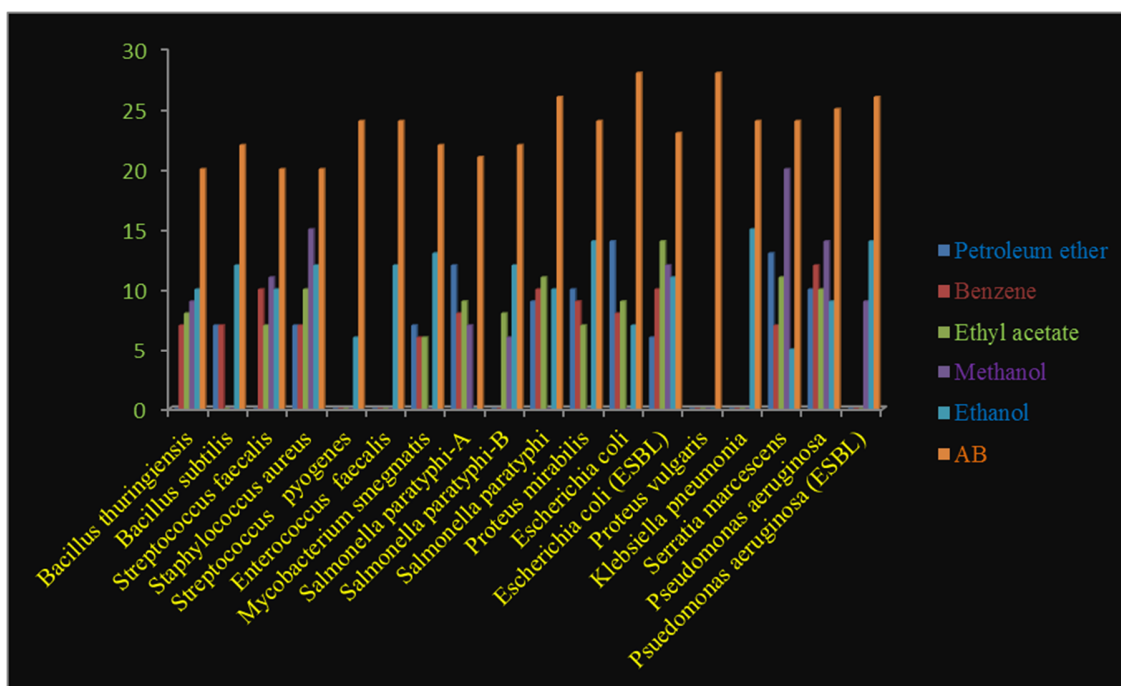


Fig 2: Antibacterial activity of whole plant extract of *A. lanata*

The commercially available antibiotic tetracycline was used as control to compare the efficacy of plant extracts against the microorganism studied.

The maximum zone of inhibition 20 mm in methanol extract was obtained against *Serratia marcescens*, followed by 15 mm against *Staphylococcus aureus* in the same extract. Fifteen millimeter of inhibition zone was observed in ethanol extract against the pathogenic organism *Klebsiella pneumoniae* followed by 14 millimeter against *Proteus mirabilis* and *Pseudomonas aeruginosa* (ESBL) respectively. Ethanol extract was ineffective against only two pathogenic viz; *Salmonella paratyphi-A* and *Proteus vulgaris*; whereas methanol extract was ineffective against nine pathogenic viz; *Bacillus subtilis*, *Streptococcus pyogenes*, *Enterococcus faecalis*, *Salmonella paratyphi*, *Proteus mirabilis*, *Escherichia coli*, *Proteus vulgaris*, *Klebsiella pneumoniae* and *Mycobacterium smegmatis*. Benzene and ethyl acetate extracts were ineffective against only four pathogens and

petroleum ether was ineffective against only eight pathogens.

4. Discussion

Mohan *et al.*^[8] used chloroform, benzene and methanol as a solvent source. In the present study we used the petroleum ether, benzene, ethyl acetate, methanol and ethanol as solvent sources for the extraction of the metabolites. Since the polarity of ethanol is higher, most of the secondary metabolites of *A. lanata* whole plant were dissolved. Out of fifteen qualitative tests screened for the presence of secondary metabolites thirteen showed positive results. Flavonoids have been referred to as nature's biological response modifiers because of strong experimental evidence of their inherent ability to modify the body's reaction to allergen, virus and carcinogens. They show antiallergic, antiinflammatory, antimicrobial and anticancer activity^[9, 10]. Tannins are known to possess general antimicrobial and antioxidant activities^[11]. Recent reports show that tannins may have potential value as cytotoxic and antineoplastic

agents^[12]. Saponins are a mild detergent used in intracellular histochemistry staining to allow antibody access to intercellular proteins. In medicine, it is used in hypercholesterolaemia, hyperglycemia, antioxidant, anticancer, anti-inflammatory and weight loss etc. It is also known to have anti fungal properties^[13]. Saponins have been implicated as bioactive antibacterial agents of plants^[14, 15]. Plant steroids are known to be important for their cardiotoxic activities, possess insecticidal and antimicrobial properties. Plant derived natural products such as flavonoids, terpenoids and steroids etc have received considerable attention in recent years due to their diverse pharmacological properties including antioxidant and antitumor activity. Phenolic phytochemicals have antioxidative, antidiabetic, anticarcinogenic, antimicrobial, antiallergic, antimutagenic and anti-inflammatory^[16, 17]. It suggests that the plants can be used as antimicrobial activity, antioxidant, antiallergic, antiinflammatory, antidiabetic, anticarcinogenic, anticancer agents in the future.

The FT-IR spectrum was used to identify the functional group of the active components based on the peak value in the region of infrared radiation. IR analysis of the whole plant powder of *A. lanata* revealed that the presence of different functional groups ranging from O-H stretching, hydroxyl (3319.26 cm^{-1}), C-H stretching, carbonyl (2921.96 cm^{-1}), C=C stretching, aromatic ring (1612.39 cm^{-1}), C=O stretching, carboxylic acid, carbonyl (1735.81 cm^{-1}), C-CHO skeletal, aldehyde (1400.22 cm^{-1}), C-N stretching, amine (1319.22 cm^{-1}) and C-Cl stretching, halogen compounds (752.19 & 696.25 cm^{-1}). Therefore, the FT-IR analysis on *A. lanata* displayed novel phytochemical markers as useful analytical tool to check out not only the quality of the powder but also to identify the medicinally important plant.

The ethanol extracted solvents showed high degree (16/18 pathogens) of antibacterial activity against the selected pathogens with varied rate of inhibition. Present study on ethanol extracts *A. lanata* revealed the high degree of antibacterial activity against *Klebsiella pneumonia* (15mm).

Klebsiella pneumoniae is an important cause of human infections and several diseases viz; urinary tract infections, nosocomial infections, pneumonia, septicemias and soft tissue infections. The disease caused by *Klebsiella pneumonia* can result in death of patients who are immunodeficient. In the present study ethanol extract of *A. lanata* displayed antibacterial activity against the plants can be used to treat urinary tract infection, nosocomial infection, pneumonia, septicemias and soft tissue.

In the present study methanol extract of *A. lanata* showed highest activity against *Serratia marcescens* (20 mm). *Serratia marcescens* can cause infection in several sites, including the urinary tract, respiratory tract, wounds and the eye, where it may cause conjunctivitis, keratitis, endophthalmitis and tear duct infections. It is also a rare cause of endocarditic, osteomyelitis pneumonia and meningitis^[13].

The presence of antimicrobial activity in a particular part of a particular species may be due to the presence of one or more bioactive compounds such as alkaloids, glycosides, flavonoids, steroids, saponins etc.,^[18]. Recently, a number of plants have been reported for antibacterial properties across the world^[19-21]. Based on the present study, it is concluded that the whole plants of *A. lanata* contains various bioactive components with high degree of antibacterial activity against various pathogens. It is hoped that this study would direct to the establishment of some compounds that could be used to invent new and more potent antibacterial drugs of natural origin. Further work will emphasize the isolation and characterization of active principles responsible for bio-efficacy and bioactivity.

5. References

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