

PHYTOCHEMICAL SCREENING AND ANTIBACTERIAL ACTIVITY OF *Aerva Lanata* IN POLAR AND NON POLAR SOLVENTS

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ABSTRACT

Aerva lanata is a medicinal plant has been used as a folk medicine to urinary problems, diarrhea, and toothache and erect or prostate. The aim of this study deals with the investigation of antibacterial activity present in the leaves, flower and stem extract obtained by analytical standard polar and non- polar solvents. The antibacterial properties against some pathogenic gram positive and gram negative bacteria. The minimum zone of inhibition was found in *Bacillus subtilis*, *E.coli* and *Pseudomonas aeruginosa*. The maximum zone of inhibition was found in *Proteus mirabilis* and *S.aureus*. The leaf, flower and stem extract of *Aerva lanata* were evaluated for phytochemical screening to identify the active compounds.

KEYWORDS: *Aerva lanata*, Phytochemical screening, Antibacterial activity.

INTRODUCTION

Herbal plants have high medicinal values as they have in rich source of bioactive compound and have no side effects. The bioactive compound from the herbal plant reduces the virulence of the microorganisms, thereby preventing and protecting from the infection. *Aerva lanata* is a traditional medicinal plant, distributed throughout India. It has been used in folk medicine for the treatment of urinary infection, toothache, erect or prostrate, diarrhea.^[1] *Aerva lanata* family is *Amaranthaceae*. The antimicrobial compound from plant may inhibit bacterial growth by different mechanisms than those presently used. Plant have been known to synthesis active secondary metabolites such as tannins, steroids, phenolic compound etc., Phytochemical screening is a may result in the discovery of effective compounds.^[2] It is used

by ayurvedic practitioners for many pathological conditions. A variety of pharmacological activities of the *Aerva lanata* plant such as anthelmintic, demulcent, anti-inflammatory, diuretic, expectorant, hepato-protective and nepron-protective.^[3]

TAXANOMICAL CLASSIFICATION OF *Aerva lanata*

Kingdom	Plantae
Sub kingdom	Tracheobionta
Super division	Spermatophyta
Division	magnoliophyta
Class	magnoliopsida
Sub class	Cargophylidae
Order	Cargophyllales
Family	Amaranthaceae
Sub family	Amaranthoideae
Genus	<i>Aerva</i>
species	<i>lanata</i>

MATERIALS AND METHODS

Plant sample collection

Aerva lanata leaf, flower and stem were collected from the Thuraiyur, Trichy district, Tamil Nadu, India. The *Aerva lanata* were identified by the Rapinat Herbarium St. josephs college, Trichirapalli, Tamil Nadu, India. The plant parts were separated from the plant and dried under shade. After drying, it was powdered and used for our studies (Figure-1).

Preparation of extract

The leaves, Flower and Stem of *Aerva lanata* were dried under shade and powdered. The powdered material was subjected to successive solvents extraction with water, ethyl acetate and chloroform using Soxhlet apparatus. The extracts were concentrated to dryness under vacuum.

Antibacterial screening

Microbial strains used

Microorganisms

The test organisms included for our study is *Bacillus Sp.*, *Staphylococcus aureus* (MTCC – 96), *Esherchia coli* (MTCC – 78), *Pseudomonas aeruginosa* (MTCC – 2488), *Proteus*

mirabilis (MTCC – 425). The bacteria were grown in nutrient broth at 37°C and maintained on nutrient agar slants at 37°C.

Disc diffusion method

Disc diffusion method was carried out for antibacterial susceptibility testing according to the standard method to assess the presence of antibacterial activities of the plant extract. Antibacterial activities of the leaf, flower and stem extract from *Aerva lanata* was assessed using the disc diffusion method with help of Muller Hinton Agar medium was prepared and sterilized using autoclave. The medium was sterilized, poured over on sterile petriplate and it allowed for solidification. After solidification the respective organisms are swabbed uniformly. Different concentration of the diluted sample was loaded on the disc with each petriplate containing swabbed organisms and the plates were incubated at 37°C for 24 hours. After incubation the zone of inhibition were measured and tabulated.

Phytochemical Analysis

A phytochemical test to detect the presence of Alkaloids, flavonoids, saponins, tannins, steroids, glycosides, oils and fats, phenolic compounds, protein and aminoacids, gums and mucilage and carbohydrates were carried out using standard procedure.^[4]

Mayer's Test

1ml of plant extract was mixed with small amount of dilute hydrochloric acid and 1 ml of Mayer's reagent formation of precipitate indicates the presence of alkaloid.

Mayer's reagent

Mercuric chloride (1.358 gm) was dissolved in 60 ml of distilled water and potassium iodine (5gm) was dissolved in 10ml of distilled water. The two solutions were making up to 30ml of distilled water.

Test for flavonoids

Ferric chloride test

One ml of extract was taken and a few drop of dilute ferric chloride solution were added. The colour changed into pale green or red brown colour, which indicates in the presence of flavonoids.

Test for saponins**Foam test**

One ml of extract was diluted with 20 ml of distilled water and shaken with graduated cylinder for 15 minutes, formation of air bubbles indicates the presence of saponins.

Test for carbohydrates**Molisch's test**

2 ml of plant extract was dissolved separately in 4 ml of distilled water and filtered. The filtrate was subjected to Molisch's test. Formation of reddish brown ring indicates the presence of carbohydrates.

Test for Tannins**Lead acetate test**

5ml of extract was added with 1ml of lead acetate solution. Flocculant brown precipitate indicates the presence of tannins.

Test for Sterols**Liebermann burchard reaction**

2ml of plant extract and a few crystal of sodium nitrate were taken in a dry test tube and a heated gently for a minute. It was cooled and 0.5 ml of concentrated H_2SO_4 was added. Formation of orange or pale orange indicates the presence of sterol.

Test for glycosides

2 ml of plant extract was hydrolysed with hydrochloric acid for few hours on a water bath and the hydrolysate was subjected to legal's test to detect the presence of different glycosides.

Legal's test

To the hydrolysate 1ml of sodium nitroprusside solution was added and then it was made alkaline with sodium hydroxide solution. If the extract produced pink to red colour, it indicates the presence of glycosides.

Test for oil and fats

Few drops of 0.5N alcoholic potassium hydroxide were added to small quantity of Various extract along with a drop of phenolphthalein. The mixture was heated on a water

bath for 1-2 hours. Formation of soaps or particle neutralization of alkali indicates the presence of Fixed oil and fats.

Test for phenolic compound

2ml of plant extracts were taken for the presence of phenolic compounds with dilute ferric chloride solution (5%) which gives violet color.

Test for protein and amino acids

Biuret test

2ml of extract was dissolved in few ml of water and treated with ninhydrin (1%) reagent gives purple colour.

Test for gums and mucilage

About 10 ml of extract was added to 25 ml of absolute alcohol with stirring and filtered. The precipitate was dried in air and examined for its swelling properties and for the presence of carbohydrates.

RESULT AND DISCUSSION

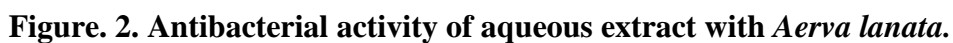
Phytochemical screening

The results of the preliminary phytochemical screening of crude extract and its fractions of *Aerva lanata* have been presented in Table 1. Compared with other solvents Aqueous extract having high phytochemicals than other solvents. The earlier study reported that alkaloids, saponins, tannins, aminoacids, flavanoids and terpenoides were present in all solvent extracts whereas phytosterols are present all extracts except methanol, phenolic compounds are in ethyl acetate and methanol extracts, proteins and carbohydrates were present in ethyl acetate and methanol extracts, Quinines were found in hexane, acetone and methanol extracts glycosides are absent in all solvent. [5,6,7,8]

Anti bacterial assay of the water, Ethyl acetate and chloroform extract of *Aerva lanata* exhibited dose dependent antibacterial activity against the tested microorganisms. Such as *Escherichia coli*, *Staphylococcus aureus*, *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Proteus mirabilis*. The zone of inhibition shown by the water, Ethyl acetate and Chloroform (Table 2,3,4). The antibacterial activity was assayed by disc diffusion method against five strains. The antibacterial activity of the aqueous with plant extract of *Aerva lanata* was more effective against *Staphylococcus aureus*, *Escherichia coli*, *Proteus mirabilis*, *Bacillus subtilis*

and *Pseudomonas aeruginosa*. The bacterial strains were revealed the zone of inhibition. The antibacterial activity against polar solvents aqueous in leaf, flower and stem extract *Bacillus subtilis* (L-6mm, F-6mm, S-6mm), *Staphylococcus aureus* (L-11mm, F-10mm, S-6mm), *Proteus mirabilis* (L-9mm, F-8mm, S-10mm), *Escherichia coli* (L-6mm, F-6mm, S-10mm), *Pseudomonas aeruginosa* (L-6mm, S-6mm). The non-polar chloroform *Aerva lanata* extract gave the inhibition of *Bacilli* (L-6mm, F-10mm, S-10mm), *Staphylococcus aureus* (F-10mm, S-6mm), *Proteus* (L-6mm, F-6mm), *Escherichia coli* (F-6mm), *Pseudomonas aeruginosa* (L-10mm, F-6mm). The Ethyl acetate extract gave the inhibition of *Bacillus subtilis* (F-6mm), *Staphylococcus aureus* (F-10mm), *Proteus mirabilis* (L-, F-, S-), *Escherichia coli* (F-6mm), *Pseudomonas aeruginosa* (L-6mm, F-10mm, S-6mm). The minimum zone of inhibition showed in crude extract. (Figure 2 and Table 2). The maximum zone of inhibition showed in chloroform extract. (Figure 3 and Table 3). The best zone of inhibition was produced by aqueous leaf extract with against *Bacillus subtilis* (L-6mm, F-6mm, S-6mm), *Staphylococcus aureus* (L-11mm, F-10mm, S-6mm), *Proteus mirabilis* (L-9mm, F-8mm, S-10mm), *Escherichia coli* (L-6mm, F-6mm, S-10mm), *Pseudomonas mirabilis* (L-6mm, S-6mm). The least zone of inhibition was produced by ethyl acetate extract *Bacillus subtilis* (F-6mm), *Staphylococcus aureus* (F-10mm), *Proteus* (L-, F-, S-), *Escherichia coli* (F-6mm), *Pseudomonas mirabilis* (L-6mm, F-10mm, S-6mm).

Overall, the antibacterial activity of the *Aerva lanata* revealed that the best antibacterial activity was produced by leaf extract. The extract produced better zone of inhibition against *Staphylococcus aureus*, *Bacillus subtilis* and *pseudomonas aeruginosa*. The least zone of inhibition against *Escherichia coli* and *Proteus mirabilis* sp., in the present study to analyses the solvent extract using polar and non-polar extract. The results conclude the plant leaf gave the maximum zone of inhibition in the polar compounds when compared to non-polar compounds. Previous study reported that flavonoids, phenolic compounds are responsible for antioxidant activity. The ethanolic extract and its fractions of chloroform and hexane of *A.lanata* were found to possess concentration dependent scavenging activity on hydroxyl radicals. In other study also reported that *A.lanata* having high antimicrobial activity against the pathogens.^[9,10,11,12,13]



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1. *E.coli*, 2. *Pseudomonas aeruginosa*, 3. *Proteus mirabilis* 4. *Bacillus subtilis* 5. *S.aureus*

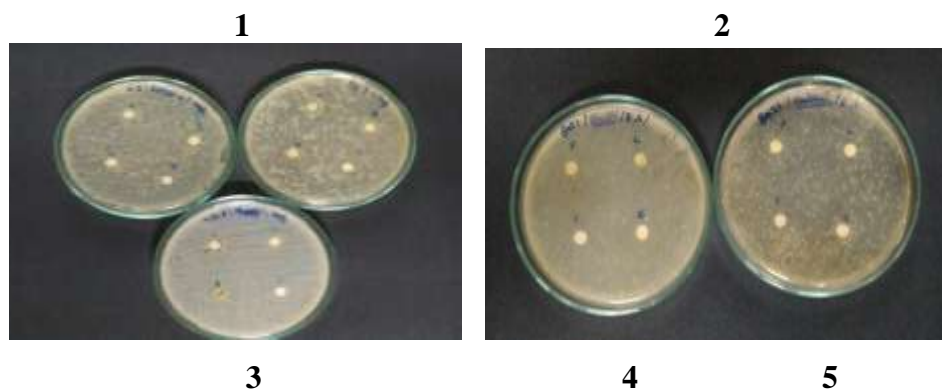


Figure. 4. Antibacterial activity of ethyl acetate with *Aerva lanata*.

1. *S. aureus* 2 *Pseudomonas aeruginosa* 3. *E.coli* 4. *Bacillus subtilis* 5. *Proteus mirabilis*

Table 1. Phytochemical screening of *Aerva lanata* in crude extracts.

S.NO	Phytochemical Constituents	Leaf extract	Stem Extract	Flower Extract
1	Alkaloids	+	+	+
2	Flavonoids	+	+	+
3	Saponins	+	+	+
4	Carbohydrates	+	+	+
5	Tanins	+	+	+
6	Sterols	+	+	+
7	Glycosides	+	+	+
8	Oil and fats	—	—	—
9	Phenolic compounds	—	—	—
10	Protein and amino acids	+	+	+
11	Gums and mucilage	-	-	-

(+): positive (-): Negative

Table. 2. Zone of inhibition formed by crude extract of *Aerva lanata* against bacterial strains.

S.No	Sample	Standard value	Bacterial strains		$\frac{\sum (O - E)^2}{2/E}$		$\frac{\sum (O - E)^2}{2/E}$		$\frac{\sum (O - E)^2}{2/E}$
	<i>Aerva lanata</i>			L	OV	F	OV	S	OV
1	(leaf, flower and stem extracts)	23	<i>E.coli</i>	6	5	6	5	10	9
2		23	<i>P.aeruginosa</i>	6	5	—	—	6	5
3		23	<i>S.aureus</i>	11	10	10	9	6	5
4		20	<i>Bacillus sp.</i> ,	6	5	6	5	6	5
5		19	<i>P.mirabilis</i>	8	8	8	9	10	9

Table value $\chi^2 (0.05) = 3.841$ Chi – square value significance at 5% level.

OV-Observed value, L-Leaf, F-Flower, S- Stem.

Table. 3. Zone of inhibition formed by Chloroform extract of *Aerva lanata* against bacterial strains.

S.No	Sample	Standard value	Bacterial strains		$\frac{\sum (O - E)^2}{E}$		$\frac{\sum (O - E)^2}{E}$		$\frac{\sum (O - E)^2}{E}$
	<i>Aerva lanata</i>			L	OV	F	OV	S	OV
1	leaf,	23	<i>E.coli</i>	—	—	6	5	—	—
2	flower and	23	<i>P.aeruginosa</i>	10	9	6	5	—	—
3	stem	23	<i>S.aureus</i>	—	—	10	9	—	—
4	extracts.	20	<i>Bacillus sp.</i>	6	5	10	9	10	9
5		19	<i>P.mirabilis</i>	6	5	6	5	6	5

Table value $\chi^2 (0.05) = 3.841$ Chi – square value significance at 5% level.

OV-Observed value, L-Leaf, F-Flower, S- Stem.

CONCLUSION

The water, chloroform, Ethyl acetate extract of *Aerva lanata* have various known antioxidants showed concentration dependent antioxidants activity. The plant extract contain a perceptible amount of phytochemical screening. These kinds of biological activities depending their therapeutic uses. This study conclude the significance of *Aerva lanata* plant leaves as valuable source of secondary metabolites like alkaloid, flavonoids, saponins, tannins, from aqueous extracts. This study would be a base for the future isolation of this plant material.

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