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PHYTOCHEMICAL INVESTIGATION AND *IN-VITRO* ANTI BACTERIAL ACTIVITY OF DRIED LEAVES OF *AERVA LANATA*

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Abstract:
The study was carried out to ascertain the anti bacterial properties present in different extracts of dried scale leaves of Aerva lanata. The Anti bacterial testing of leaves extract Aerva lanata was evaluated by Agar well diffusion method using gram positive bacteria like Staphylococcus aureus, Bacillus subtilis, gram negative bacteria like Escherichia coli, Klebsiella pneumoniae. Amongst the test extracts, the results suggested that, Chloroform, Ethanol extracts of leaves showed significant antibacterial activity compared with standard drug.

Keywords: *Aerva lanata, Gentamycin, Flavonoids, Anthraquinons.*

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INTRODUCTION:

Traditionally plants are used as drugs and have genuine utility because they contain some components which have healing and pain relieving properties. For the primary health care about 80% of rural population depends on these medicinal plants. Usage of plants for the treatment of diseases is as old as human species which produces various secondary metabolites like alkaloids, terpenoids, steroids, phenols, tannins, flavonoids, and other metabolites and which have antimicrobial and antioxidant types of properties. Plants are the main source of food and rich nutrients content. Traditional societies around the world had deep knowledge of various plants and their medicinal value, though they did not possess knowledge on components present and their mode of action. Medicinal properties attributed to various herbs have paved way to the discovery of new drugs, as they are the reservoirs of potential chemical compounds. For the benefit of mankind it is necessary to prefer herbal usages to avoid chronic stress and synthetic drugs.

Herb is an immeasurable wealth of nature not only from the global environmental perspective but also from the medicinal point of view. It plays a significant role in ameliorating the disease resistant ability and combating against various unfavorable metabolic activities within the living system. Herbal medicine is the mainstay of about 75 – 80% of the world population, mainly in the developing countries, for primary health care because of better cultural acceptability, better compatibility with the human body and lesser side effects. The chemical constituents present in them are a part of the physiological functions of living flora and hence they are believed to have better compatibility with the human body. The clinical efficacy of many existing antibiotics is being threatened by the emergence of multidrug-resistant pathogens. There is a continuous and urgent need to discover new antimicrobial compounds with diverse chemical structures and novel mechanisms of action for new and re emerging infectious diseases. Therefore, researchers are increasingly turning their attention to folk medicine, looking for new leads to develop better drugs against microbial infections. *Aerva lanata* linn. belonging to the family Amaranthaceae. Herbs are perennial, 5–50 cm tall. Stem branched from base; branches ascending or stoloniferous, white lanose. Leaves opposite or nearly whorled, sessile, grayish green, subulate, linear, 1–2.5 cm × ca. 1 mm, abaxially white lanose, adaxially glabrous, base attenuate, sometimes vaginate. Spikes terminal, narrowly ovate or terete, 0.5–2.5 cm, 3–5 mm in diam., white lanose; rachis very short or absent. Bracts and bracteoles lanceolate, 1–2 mm, abaxially white lanose. The Phytoconstituents reported from stem are flavonoids, tannins and anthraquinones. However, from the above account, it is obvious that there is no information available about the anti bacterial activity of stem and leaves of *Aerva lanata*. The present investigation was to explore the anti bacterial activity of dried leaves of *Aerva lanata*.

MATERIALS AND METHODS:

Collection of plant material

The leaves of *Aerva lanata* were collected from surrounding places of Rangareddy Dist.

Phytochemical Evaluation

The different chemical tests were performed for establishing profile of the extract for its chemical composition; the following chemical tests for various phytoconstituents in the petroleum ether, chloroform, ethyl acetate, alcohol and water extracts were carried out as described below.

(A) Test for alkaloids:

i) Dragendroff's Test: In a test tube containing 1ml of extract, few drops of Dragendroff's reagent was added and the color developed was noticed. Appearance of orange color indicates the presence of alkaloids.

ii) Wagner's Test: To the extract, 2 ml of Wagner's reagent was added; the formation of a reddish brown precipitate indicates the presence of alkaloids.

ii) Mayer's Test: To the extract, 2 ml of Mayer's reagent was added, a dull white precipitate revealed the presence of alkaloids.

iv) Hager's Test: To the extract, 2 ml of Hager's reagent was added; the formation of yellow precipitate confirmed the presence of alkaloids.

(B) Test for terpenoids:

i) Salkowski test: To 1 ml of extract, tin (one bit) and thionyl chloride were added. Appearance of pink color indicates the presence of terpenoids.

ii) Hirshonn reaction: When the substance was heated with trichloroacetic acid, red to purple colour was observed.

(C) Test for steroids:

i) Liebermann Burchard Test: To 1ml of extract, 1ml of glacial acetic acid and 1ml of acetic anhydride and two drops of concentrated sulphuric acid were added. The solution become red, then blue and finally bluish green indicates the presence of steroids.

(D) Test for coumarins:

To 1 ml of extract, 1 ml of 10% sodium hydroxide was added. The presence of coumarins is indicated by the formation of yellow color.

(E) Test for tannins:

i) To few mg of extract, ferric chloride was added, formation of a dark blue or greenish black color showed the presence of tannins.

ii) The extract was mixed with basic lead acetate solution; formation of white precipitate indicated the presence of tannins.

(F) Test for saponins:

To 1 ml of the extract, 5 ml of water was added and the tube was shaken vigorously. Copious lather formation indicates the presence of saponins.

(G) Test for flavones:

i) Shinoda Test: To the extract, a few magnesium turnings and 2 drops of concentrated hydrochloric acid were added, formation of red color showed the presence of flavones.

ii) To the extract, 10% sodium hydroxide or ammonia was added; dark yellow color shows the presence of flavones.

(H) Test for quinones:

To 1 ml of the extract 1 ml of concentrated sulphuric acid was added. Formation of red color shows the presence of quinones.

(I) Test for flavanones:

i) To the extract, 10% sodium hydroxide was added and the colour changes from yellow to orange, which indicates the presence of flavanones.

ii) To the extract, conc. sulphuric acid was added, and the colour changes from orange to crimson red, which indicates the presence of flavanones.

(J) Test for anthocyanins:

i) To the extract, 10% sodium hydroxide was added, and the blue color shows the presence of anthocyanins.

ii) To the extract, conc. sulphuric acid was added, and the yellowish orange color confirms the presence of anthocyanins.

(K) Test for anthraquinones:

Borntrager's test: The extract was macerated with ether and after filtration; aqueous ammonia or caustic soda was added. Pink red or violet color in the aqueous layer after shaking indicates the presence of anthraquinones.

(L) Test for phenols:

Ferric chloride test: To the extract, few drops of 10 % aqueous ferric chloride were added. Appearance of blue or green color indicates the presence of phenols.

(M) Test for proteins:

i) Biuret Test: To the extract, 1 ml of 40% sodium hydroxide solution and two drops of one percent copper sulphate solution were added. Formation of violet color indicates the presence of proteins.

ii) Xanthoprotein Test: To the extract, 1 ml of concentrated nitric acid was added. A white precipitate was formed, it is then boiled and cooled. Then, 20% sodium hydroxide or ammonia was added. Orange color indicates the presence of aromatic amino acids.

iii) Tannic Acid Test: To the extract, 10% tannic acid was added. Formation of white precipitate indicates the presence of proteins.

(N) Test for carbohydrates:

i) Molisch's Test: To the extract, 1 ml of alpha-naphthol solution, and concentrated sulphuric acid through the sides of test tube were added. Purple or reddish violet color at the junction of the two liquids revealed the presence of carbohydrates.

ii) Fehling's Test: To the extract, equal quantities of fehling's solution A and B were added and on heating, formation of a brick red precipitate indicates the presence of carbohydrates.

iii) Benedict's Test: To 5 ml of Benedict's reagent, extract was added and boiled for two minutes and cooled. Formation of red precipitate showed the presence of carbohydrates.

(O) Test for amino acids:

Ninhydrin test: Two drops of ninhydrin solution were added to the extract, a characteristic purple color indicates the presence of amino acids.

Procedure for extraction

Dried leaves of *Aerva lanata* were ground to coarse powder. The powder was extracted with different solvents like Ethanol, Chloroform by soxhlation for 6 hours for the preparation of different extracts and the obtained extracts were subjected to antibacterial screening.

Microorganisms

The test organisms included for study were gram positive bacteria like *Staphylococcus aureus, Bacillus subtilis,* gram negative bacteria like *Escherichia coli, Klebsiella pneumoniae*. All the bacterial strains were

procured from Osmania University, Hyderabad, Telangana. The bacteria were grown in the nutrient broth at 37 ^{0}C and maintained on nutrient agar slants at 4 ^{0}C.

Bacterial media

Muller Hinton Media was mixed with distilled water and then sterilized in autoclave at 15 lb pressure for 15 minutes. The sterilized media were poured into Petri dishes and allowed for solidification. The solidified plates were bored with 5mm diameter cork borer. The plates with wells were used for the antibacterial studies.

Antibacterial activity of the plant extracts

Different leaves extracts of *Aerva lanata* at a concentration of 500µg/ml, 750µg/ml, 1000µg/ml were tested against the gram positive bacteria like *Staphylococcus aureus, Bacillus subtilis,* gram negative bacteria like *Escherichia coli, Klebsiella pneumoniae* by Well Diffusion Method.

Well Diffusion Method

Antibacterial activity of the plant extract was tested using Well diffusion method. The prepared culture plates were inoculated with different selected strains of bacteria using streak plate method. Wells were made on the agar surface with 6mm cork borer. The dried extracts were dissolved in 95% of ethanol for preparation of different concentration ranges of extracts. The extracts were poured into the well using sterile syringe. The plates were incubated at 37 ^{0}C±2 ^{0}C for 24 hours for bacterial activity. The plates were observed for the zone clearance around the wells. The extracts of the dried scale leaves were used for the study. The extracts were dissolved in sterile distilled water to form dilution such as 500µg/ml, 750µg/ml and 1000µg/ml. Each concentration of the extract was tested against different bacterial pathogens. Gentamycin at a concentration of 5µg/ml and 10µg/ml was used as standard antibacterial drug. The zone of inhibition was calculated by measuring the diameter of the inhibition zone around the well (in mm) including the well diameter. The readings were taken in three different fixed directions in all three replicates and the average values were tabulated.

RESULTS AND DISCUSSION:

Table 1: Preliminary phytochemical screening of *Aerva lanata* leaves

Constituents	Pet ether Extract	Chloroform extract	Ethyl acetate extract	Alcohol extract	Water extract
Terpenoids	-	-	-	-	-
Saponins	+	+	-	+	-
Steroids	-	+	+	-	-
Phenols	-	-	+	-	-
Flavonoids	-	-	-	+	-
Coumarins	-	-	+	+	+
Reducing sugars	-	+	-	-	-
Alkaloids	-	-	+	+	-
Quinones	-	+	+	+	+
Tannins	-	+	+	-	-
Proteins	-	-	-	-	-
Amino acids	-	-	-	-	-
Anthraquinones	-	+	+	+	-

+ Present, - Absent

Antibacterial assay of the Ethanol, Chloroform extracts of dried leaves of *Aerva lanata* exhibited dose dependent antibacterial activity against the tested microorganisms at three different concentrations of 500, 750 and 1000µg/ml. The potential sensitivity of the extracts was obtained against all the tested micro organisms and the zone of inhibition was recorded and presented in the table given below (Table 2). From the above study the zone of inhibition obtained was dose dependent and the activity shown by the Chloroform, Ethanol extracts of leaves of *Aerva lanata* at a concentration of 1000µg/ml against gram positive bacteria like *Staphylococcus aureus*, *Bacillus subtilis*, and gram negative bacteria like *Escherichia coli*, *Klebsiella pneumoniae* strains involved in present study was more in comparison to Gentamycin at a concentration of 5µg/ml. The extracts prepared by solvents like water, isopropyl alcohol showed no zone of inhibition. The zone of inhibition shown by the water were tabulated in the below given below (Table 3). The antibacterial potential exhibited by leaves extracts may be contributed to the presence of tannins, flavonoids and anthraquinones in preliminary phytochemical investigations. Further study is needed to characterize the active principles.

Table 2: Zone of inhibition shown by the Gentamycin and the Ethanol, Chloroform extracts of dried leaves of *Aerva lanata*

Micro organism	Zone of inhibition (mm)			
	GENTAMYCIN		EXTRACTS (1000µg/ml)	
	5µg/ml	10µg/ml	Ethanol extract	Chloroform extract
Bacillus subtilis	7.5 mm	9 mm	8 mm	7 mm
Escherichia coli	7 mm	9 mm	6.5 mm	6 mm
Klebsiella pneumoniae	7 mm	9 mm	8 mm	7 mm
Staphylococcus aureus	7.5 mm	9 mm	8 mm	8 mm

Table 3: Zone of inhibition shown by the Gentamycin and the Water, Isopropyl alcohol extracts of leaves of *Aerva lanata*

Micro organism	Zone of inhibition (mm)			
	GENTAMYCIN		EXTRACTS (1000µg/ml)	
	5µg/ml	10µg/ml	Water extract	Isopropyl alcohol extract
Bacillus subtilis	7.5 mm	9 mm	--	--
Escherichia coli	7 mm	9 mm	--	--
Klebsiella pneumoniae	7 mm	9 mm	--	--
Staphylococcus aureus	7.5 mm	9 mm	--	--

Fig 1: Zone of inhibition shown by the Ethanol and Chloroform extracts of leaves of *Aerva lanata* on *Bacillus subtilis* bacteria

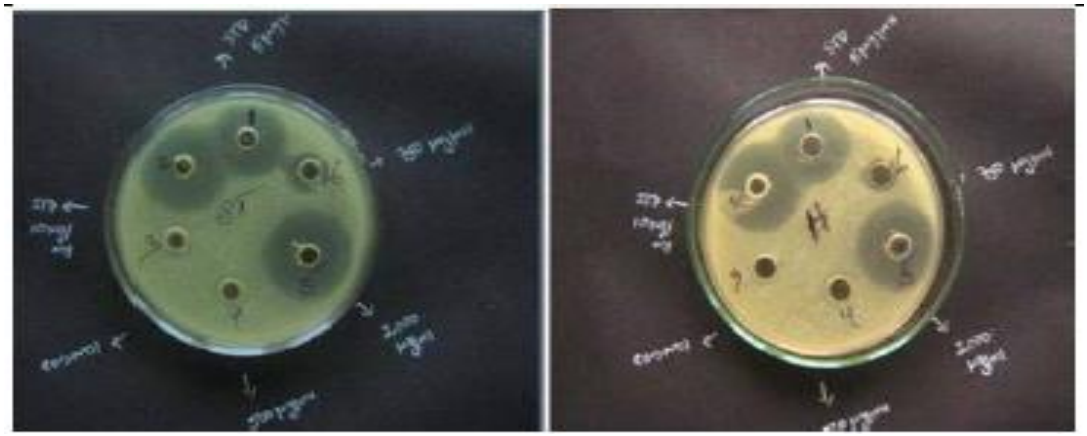

Fig 2: Zone of inhibition shown by the Ethanol and Chloroform extracts of leaves of *Aerva lanata* on *klebsiella pneumoniae* bacteria

Fig 3: Zone of inhibition shown by the Ethanol and Chloroform extracts of leaves of *Aerva lanata* on *Staphylococcus* bacteria

Fig 4: Zone of inhibition shown by the Ethanol and chloroform extracts of leaves of *Aerva lanata* on *E. coli* bacteria

CONCLUSION:

From the above study, it is concluded that the leaves of *Aerva lanata* may represent a new source of anti bacterial with stable, biologically active components that can establish a scientific base for the use of this in modern medicine. These local ethno medical preparations of plant sources should be scientifically evaluated and then disseminated properly. This knowledge about the medicinal plants usage can also be extended to other fields like field of pharmacology.

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