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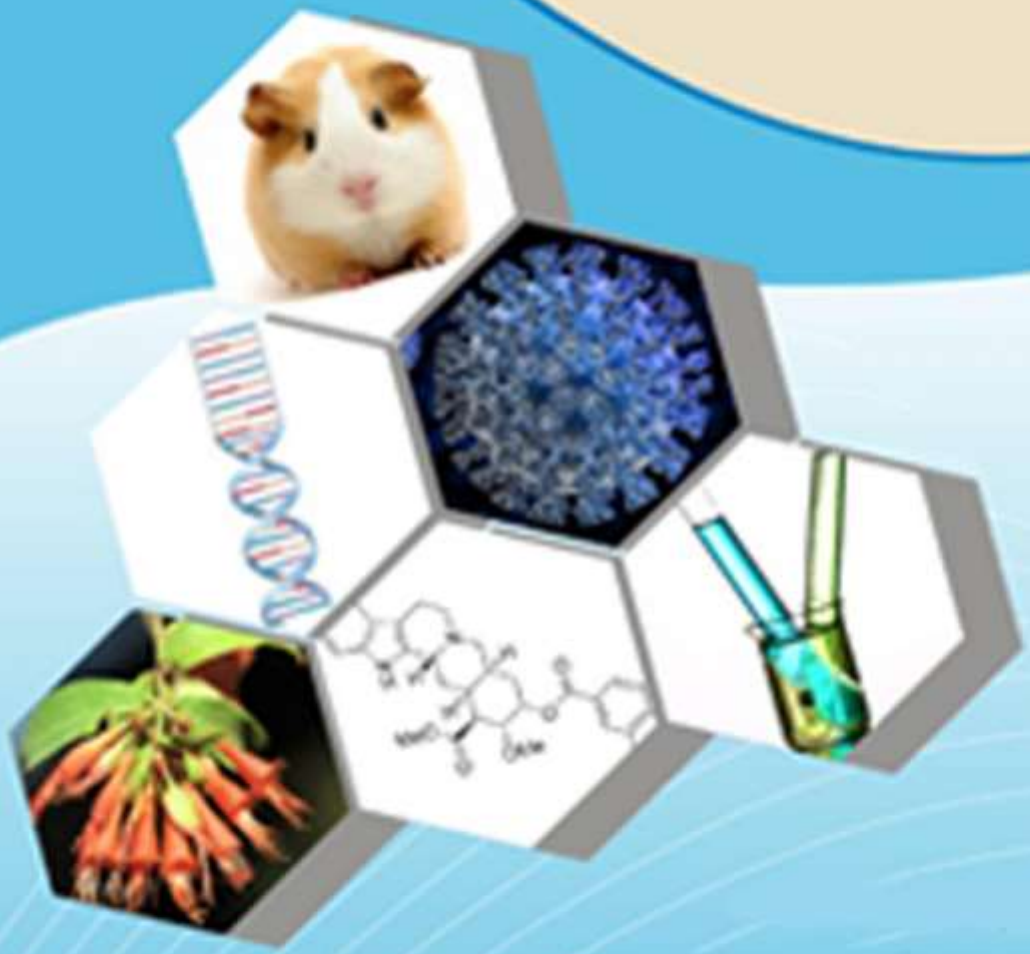

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A PHYTOCHEMICAL INQUIRY INTO THE DRIED LEAF OF AERVA LANATA AND AN EXAMINATION OF ITS IN-VITRO ANTIBACTERIAL ACTIVITY

G. Kamlesh Goud, P. Anil Kumar Yadav, Dr. C. H. Naveen Kumar

Abstract:
The study was carried out to ascertain the anti bacterial properties present in different extracts of dried scale leavesof 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.*

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 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 thehuman 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.*

Pharmaceutical Analysis
Dr.K.V. Subba Reddy Institute of Pharmacy
(Approved by AICTE,P.C.l New Delhi& Permanently Affiliated to JNTUA Anantapuramu
MOU with Government General Hospital &KMC, K urnool

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) Alkaloid detection test: I Dragendroff's reagent color development in 1ml extract in test tube after adding a few drops of Dragendroff's reagent. When alkaloids are present, they cause the color to become orange.

The presence of alkaloids was confirmed by adding 2 ml of Wagner's reagent to the extract, and the resulting reddish brown precipitate.

Extract was mixed with 2 ml of Mayer's reagent, and the presence of alkaloids was indicated by the formation of a dingy white precipitate.

iv) Hager's Test: 2 ml of Hager's reagent was added to the extract, and the presence of alkaloids was verified by the production of a yellow precipitate.

Salkowski test I 1 ml of extract was mixed with 1 ppm tin and 0.1 ml of thionyl chloride. Terpenoids are present when a pink hue is seen.

Hirshonn reaction (ii): The material turned from red to purple when heated with trichloroacetic acid.

As for the steroid test, which is option (C), here are the results:

"(D) Liebermann Burchard" For this test, we mixed 1 milliliter of extract with 1 milliliter of glacial acetic acid, 1 milliliter of acetic anhydride, and 2 drops of concentrated sulphuric acid. Steroids are present when the solution becomes red, then blue, and lastly bluish green. In order to detect coumarins, we mixed 1 milliliter of extract with 1 milliliter of 10% sodium hydroxide. The appearance of a bright yellow tint is a telltale sign that coumarins are present.

Tannins were detected using the following method (E): I after adding ferric chloride to a little amount (only a few mg) of extract, a dark blue or greenish black hue developed, indicating the presence of tannins.

When the extract was combined with a basic lead acetate solution, a white precipitate formed, proving the presence of tannins.

(F) Test for saponins: \sTo 1 ml of the extract, 5 ml of water was added and the tube was shaken briskly. The presence of saponins is shown by the production of a large amount of lather.

The flavone content is determined by the Shinoda The existence of flavones was determined by adding a few magnesium turnings and 2 drops of strong hydrochloric acid to the extract, which caused a crimson hue to appear.

ii) Ten percent sodium hydroxide or ammonia was added to the extract, which became a dark yellow hue due to the presence of flavones.

To conduct the quinones test (method H), 1 ml of the extract was mixed with 1 ml of concentrated sulphuric acid. The presence of quinones is indicated by the development of a red hue.

I. Sodium hydroxide test for flavanones: I adding 10% sodium hydroxide to the extract causes a change in color from yellow to orange, proving the presence of flavanones.

The presence of flavanones is indicated by a change in color from orange to blood red upon addition of concentrated sulphuric acid to the extract.

Anthocyanins may be detected using the following procedure (J): I Adding 10% sodium hydroxide to the extract causes a blue tint, which is indicative of the existence of anthocyanins.

The presence of anthocyanins in the extract was confirmed by adding concentrated sulphuric acid, which produced a yellowish orange tint.

The Borntrager test (K) for anthraquinones involves macerating the extract with ether and then adding aqueous ammonia or caustic soda after it has been filtered. In the presence of anthraquinones, the aqueous layer will become a pinkish red or violet hue following shaking.

Test for phenols (L): Ferric chloride test; a few drops of 10% aqueous ferric chloride were added to the extract. For phenols to be present, a blue or green hue must appear.

I added 1 ml of a 40% sodium hydroxide solution and 2 drops of a 1% copper sulphate solution to the extract to conduct the I Biuret Test for proteins. In the presence of proteins, a violet hue forms.

The Xanthoprotein ii) Test included adding 1 ml of strong nitric acid to the extract. There was a white precipitate that was cooked and chilled. Twenty percent ammonia or sodium hydroxide was then added. The presence of aromatic amino acids is shown by an orange hue.

iii) Tannic Acid Test: 10% tannic acid was added to the extract. When proteins are present, they tend to precipitate out into a white color.

For the (N) carbohydrate test, I Molisch's Test, 1 ml of alpha-naphthol solution and concentrated sulphuric acid were added to the extract through the test tube's sides. Carbohydrates were identified by the appearance of a purple or reddish violet tint at the interface of the two fluids.

After adding the same volume of fehling's solution A and B to the extract, we heated the mixture to see if any carbs would precipitate out, and sure enough, we got a nice brick red precipitate, so we know we had carbs.
Extract was added to 5 ml of Benedict's reagent, heated for 2 minutes, and then chilled for the iii) Benedict's Test. Carbohydrates were detected due to the formation of a crimson precipitate.
(O) Amino acid screening:
Amino acid content was confirmed by the ninhydrin test, in which two drops of ninhydrin solution were added to the extract to produce a distinctive purple hue.
Extraction Technique
A coarse powder was made by grinding dried Aerva lanata leaves. For the manufacture of various extracts, the powder was extracted with various solvents such Ethanol, Chloroform by soxhlation for 6 hours, and the resultant extracts were tested for antibacterial properties.

Microorganisms

The test organisms included for study were gram positive bacteria like *Staphylococcus aureus*, *Bacillussubtilis*, 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.

RESULTS AND DISCUSSION:

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*, gramnegative bacteria like *Escherichia coli*, *Klebsiellapneumoniae* 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.

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 ofinhibition 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	--	--

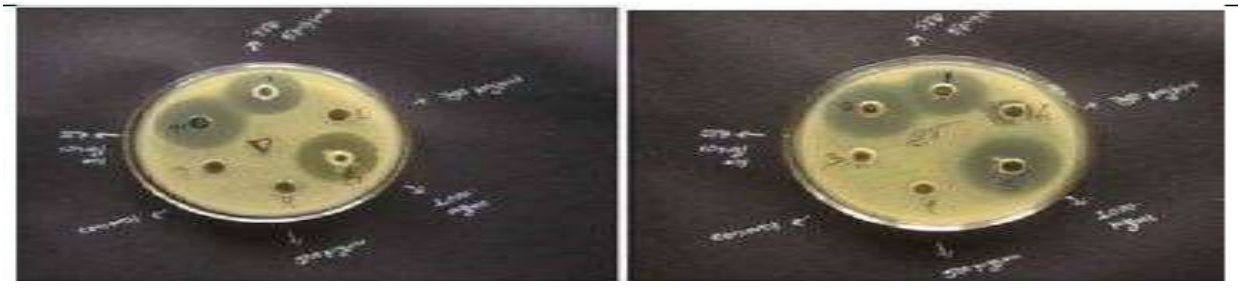

Fig 1: Zone of inhibition shown by the Ethanol and Chloroform extracts of leaves of *Aerva lanata* on *Bacillus subtilis* bacteria

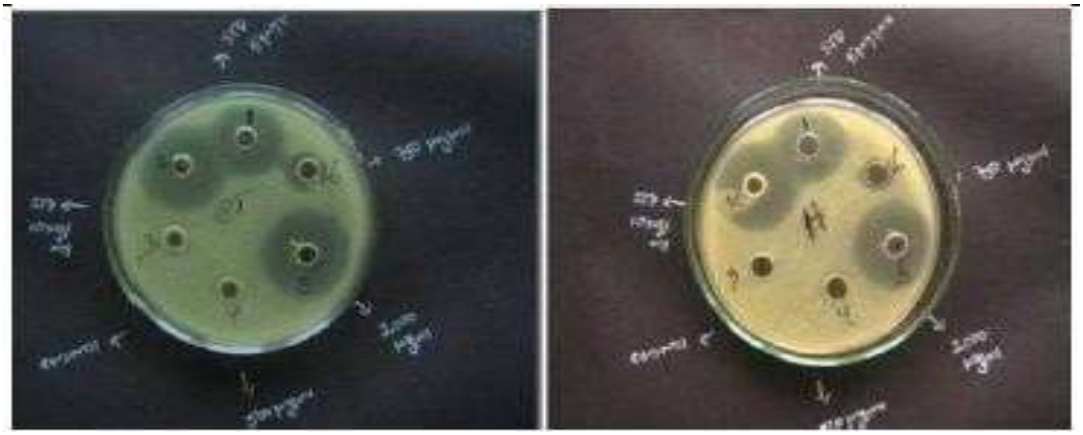

Fig 2: Zone of inhibition shown by the Ethanol and Chloroform extracts of leaves of *Aerva lanata* on *klebsiella pneumoniae* bacteria

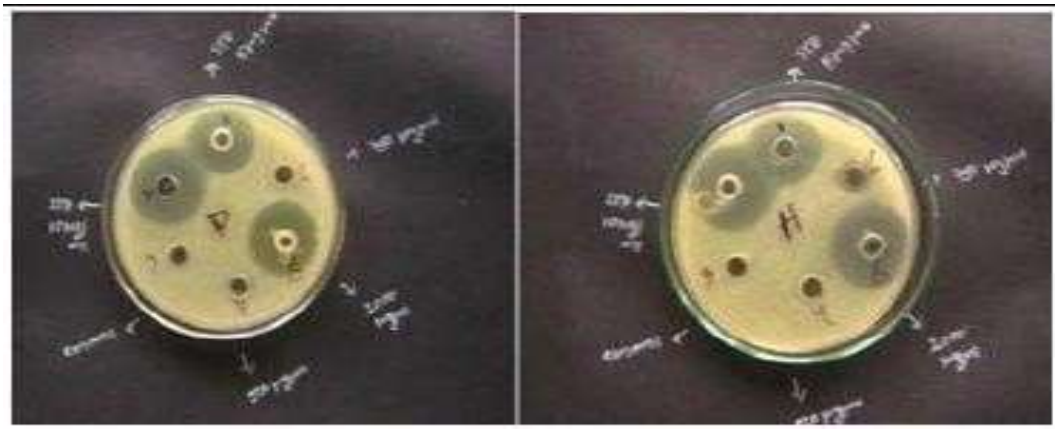

Fig 3: Zone of inhibition shown by the Ethanol and Chloroform extracts of leaves of *Aerva lanata* on *Staphylococcus* bacteria

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. Thisknowledge about the medicinal plants usage can also be extended to other fields like field of pharmacology.

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