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Research Article

# EVALUATION OF ANTIMICROBIAL ACTIVITY OF *AERVA LANATA* ALONG WITH PRELIMINARY PHYTOCHEMICAL SCREENING

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**ABSTRACT**

The aim of the present study was to investigate the antibacterial properties and phytochemical evaluation of of *Aerva lanata*. The organic solvent ( Ethanol, Methanol, Hexane) and water extracts from the whole plant of *Aerva lanata* (Amaranthaceae) were tested against, *Salmonella typhimurium*, *Proteus vulagaris*, *Shigella dysenteriae* and a fungal pathogen *Candida albicans* by Agar disc diffusion method. The results showed prominent antimicrobial activity against the tested microbial pathogens. Of all those, Methanol extract was found to give a strong antimicrobial effect when compared to the other extracts (Ethanol, Hexane and water). Phytochemicals like tannins, flavonoids, alkaloids, steroids, antraquinones, saponins and terpenoids are found in the tested samples.

**Keywords**: *Aerva lanata*, Agar Disc Diffusion Method, Phytochemicals, Antimicrobial Properties

## INTRODUCTION

*Aerva lanata* (Amaranthaceae) is a herb distributed throughout India and other topical regions of the world. The various parts of the plant (leaves, roots, seeds and seed and seed oil) are widely used in variety of ailments in traditional system of medicine such as Ayurveda and Siddha.. The aim of present research is, to determine the preliminary phytochemical constituents, antimicrobial activity of Ethanol, Methanol, Hexane and water extracts of the leaves and stems of *Aerva lanata.*

Traditional medicines derived from medicinal plants are used by about 60% of the world's population. Though there are various approaches to control diseases and their secondary complications, herbal formulations are preferred due to lesser side effects and low cost. The use of and search for drugs and dietary supplements derived from plants has been increased in recent years. Botanists, Ethno pharmacologists, microbiologists, and chemists are combing the earth for phytochemicals and drugs which could be developed for treatment of highly infectious diseases in a natural way.. While 30 to 50% of current pharmaceuticals are derived from plants, only few of them are used as antimicrobials. Traditional healers have long used plants to prevent or cure infectious conditions. Plants are rich in a wide variety of secondary metabolites, such as Terpenoids, Tannins, Alkaloids, Flavonoids, saponins and Anthraquinones which have been found in vitro to have antimicrobial properties.

*Aerva lanata* is a prostrate dioecious herb having a tap root which is cylindrical, branched, 7-12 cm long, 2-8 mm thick, straight or slightly twisted with many slender, fibrous lateral roots, pale yellowish brown externally, whitish internally, camphoraceous odorous, it has many branches, branched from the root base; pubescent/ woolly-tomentose, striate. Leaves are simple, alternate, entire margin, lamina is elliptic or obovate or sub orbicular, obtuse or acute apex, tapering base, hairy above and more/ less white cottony beneath, short petiole, ex-stipulate. Spikate inflorescence, forms subglobose clusters bearing numerous flowers. Flowers are very small, sessile, usually bisexual, greenish/ hoary white. Stamens & perianth are five lobed, ovoid/ subglobous ovary. Fruits are greenish, round, compressed membranous utricle capsule with a coriaceous upper part/ lid containing a single seed. Seed are Reniform, shining black coriaceous testa [1-3].

*Aerva lanata*, commonly called mountain knotgrass, is a woody, prostrate or succulent, perennial herb in the Amaranthaceae family of the genus Aerva, the root has a camphor-like aroma. The dried flowers which look like soft spikes are sold under the commercial names as Buikallan or Boor. This plant is used for food for people and animals. The whole plant, especially the leaves, is edible. The leaves are put into soup or eaten as spinach or as a vegetable. The plant provides grazing for stock, game and chickens. The plant is used for the treatment of snakebite. The plant is also used as talisman against evil spirits, a good-luck talisman for hunters, and a talisman for the well-being of widows[5].

**Figure 1: *Aerva lanata* with flowers**

## MATERIALS AND METHODS

*Aerva lanata* plants were collected from various places in and around the areas of Kurnool. Whole Plants of were collected and identified by comparing with herbarium specimens. The stems along with leaves of plants were air- dried and powdered. The dry powder was extracted by reflexed in 100 mL methanol for 24 h, using a

Soxhlet apparatus (Khan *et al*., 1988). The extract was filtered using Whatman filter paper, No. 1. The filtrate was then evaporated using rotatory evaporator and dried at 55°C. Ethanol, methanol, hexane and distilled water extracts are obtained and all the extracts are preserved. Dried extract was stored at 20°C in labelled, sterile capped bottles. Stock cultures of microbes are maintained at a temperature of 4 degrees centigrade, active cultures are prepared by growing in tubes of Muller-Hinton (MHB) / Potato dextrose agar (PDA) for bacteria and Sabouraud dextrose broth (SDB) for fungi.

**Microorganisms**

The bacterial colonies were isolated from hospital samples at Kurnool, their pure cultures were maintained in nutrient agar and stored at 4°C.Three gram negative bacterial species were grown, namely *Salmonella typhimurium, Proteus vulagaris*, *Shigella dysenteria* and the fungus *Candida albicans*.

**Antimicrobial assay**

Sensitivity tests were performed by disc diffusion with standard antibiotics, following Kirby-Bauer method (Bauer *et al*., 1966). The assessment of antimicrobial activity was done based on measurements of the diameter of inhibition zones (NCCLS, 1998) .Of the four extracts ,Methanolic extract has given interesting results and the aqueous extract showed no response.

**Phytochemical screening**

Phytochemical testing is done for the methanolic extracts as it has shown the interesting activity. The details of the tests are as follows:

1. Braemer's test for Tannins : To a 2–3 ml of methanolic extract, 10% alcoholic ferric chloride solution was added. (Dark blue or greenish grey coloration of the solution indicate the presence of tannins in the drug).
2. Liebermann-burchardt test for Steroids : To 1 ml of methanolic extract of drug, 1 ml of chloroform, 2–3 ml of acetic anhydride and 1 to 2 drops of concentrated sulfuric acid were added. (Dark green coloration of the solution indicate the presence of Steroids)
3. Liebermann-burchardt test for Terpinoids: To 1 ml of methanolic extract of drug, 1 ml of chloroform, 2–3 ml of acetic anhydride and 1 to 2 drops of concentrated sulfuric acid were added. (dark pink or red coloration of the solution indicate the presence of terpenoids).
   a) Salkowski Test for Terpenoids: The extract was mixed with 2ml of chloroform and concentrate H2SO4 (3ml) is carefully added to form a layer. A reddish brown coloration of the interface is formed to show positive result of the presence of terprnoids.
4. Dragendroff's reagent test for Alkaloids :A drop of methanolic extract was spotted on a small piece of precoated TLC plate and the plate was sprayed with modified Dragendorff's reagent. (Orange coloration of the spot indicates the presence of alkaloids)
5. Shinoda test for Flavanoids: To 2–3 ml of methanolic extract, a piece of magnesium ribbon and 1 ml of concentrated hydrochloric acid were added. (Pink red or red coloration of the solution indicate the presence of flavonoids in the drug).
6. Bornträger's test for anthraquinones: About 50 mg of methanolic extract was heated with 10% ferric chloride solution and 1 ml of concentrated hydrochloric acid. The extract was cooled, filtered and the filtrate was shaken with diethyl ether. The ether extract was further extracted with strong ammonia. (Pink or deep red coloration of aqueous layer indicate the presence of anthraquinones).
7. Keller-Kilianii test for Cardiac glycosides: Methanol extract was obtained and the extract reduced to dryness. 50 mg of this was dissolved in 2 ml chloroform. H2SO4 was added to form a layer and the colour at interphase recorded. Brown ring at interphase is characteristic of deoxysugars in cardenolides.
8. Frothing test for saponins : A small amount of extract was shaken with water and observed for the formation of persistent foam.

**Antimicrobial disc diffusion assay**

Antibacterial and antifungal activities of the four plant extracts were investigated by the disc diffusion method[4] . The MHA plates, containing an innoculum size of 106 colony-forming units (CFU)/mL of bacteria or 2x105 CFU/mL yeast cells on SDA were spread on the solid plates with a glass rod. Then discs (4.0-mm diam.) impregnated with 50 µL of each extract at a concentration of 100.0mg/mL were placed on the inoculated plates. Similarly, each plate carried a blank disk by adding solvent control alone in the centre, and antibiotic discs (6.0-mm diam) of (20 µg/ml, Streptomycin sulphate for bacteria) and Nystatin (20 µg/ml, for fungal) were also used as a positive control. All of the plates were incubated at 37°C for 18 hours for bacteria and at 28°C for 48 hours for fungi. The zones of growth inhibition around the discs were measured after 18 hours of in incubation at 37°C for bacteria and 48 hours for fungi at 28°C, respectively. The sensitivity of the microorganism species to the plant extracts was determined by measuring the sizes of inhibitory zones on the agar surface around the discs.

**RESULTS**

The aqueous extracts of plant has shown negligible antimicrobial activity on tested pathogens, whereas the Methanol extract of plant has shown shown maximum inhibition on *Salmonella typhimurium (13±0.2),*minimum on *candida albicans(9.7) and* it has no effect on *Proteus vulgaris*. Ethanol extract of plant has shown maximum inhibition on *Salmonella typhimurium (12±0.8),* minimum on *Candida albican (10.46)* and it also has no effect on *Proteus vulgaris* .Hexane extract of plant has shown maximum inhibition on *Salmonella typhimurium* (10.03) and it has no effect on *Proteus vulgaris* and *Candida albicans.* Of all the extracts Methanolic extracts have shown maximum inhibition, so it is used for phytochemical screening of secondary metabolites.

**Table I: Antimicrobial activity** of ***Aerva lanata***

| Solvent extracts | µL | Zone of inhibition in mm | | | |
|---|---|---|---|---|---|
| | | *Salmonella typhimurium* | *Shigella dysenteriae* | *Proteus vulagaris* | *Candida albicans* |
| Aqueous | 50 | -N- | -N- | -N- | -N- |
| Methanol | 50 | 13±0.2 | 10±0.7 | -N- | 9.7 |
| Ethanol | 50 | 12±0.8 | 11.2 | -N- | 10.46 |
| Hexane | 50 | 10.03 | 9.78 | -N- | -N- |
| Streptomycin sulphate(µg/ml) | 20 | 26±0.8 | 24±0.7 | 20±0.9 | - |
| Nystatin(µg/ml) | 20 | - | - | - | 19±1.7 |

-N- --No Activity

**Table II: Phytochemical Screening of Secondary Metabolites from *Aerva lanata* Methanolic extract**

| S.NO | Secondary metabolites | Name of the test | Leaf | Stem | Flower | Root |
|---|---|---|---|---|---|---|
| 1. | Tannins | Braemer's test | + | -- | -- | -- |
| 2. | Flavonoids | Shinoda test | + | + | + | + |
| 3. | Anthraquinone | Bornträger's test | + | -- | -- | -- |
| 4. | Saponins | Frothing test | + | + | + | + |
| 5. | Cardiac glycosides | Keller-Kilianii test | + | + | + | + |
| 6. | Alkaloid | Dragendorff test | + | + | + | + |
| 7. | Steroids | Lieberman Burchardt test | -- | + | + | + |
| 8. | Terpenoids | LiebermannBurchardt test | + | + | + | + |
| | | Salkowski test | + | + | + | + |

'+' Present , '--' Absent

The results of the phytochemical screening to test the presence of tannin, antharaquinone, alkaloid, saponin, phlobatannin, flavonoid, cardiac glycosides, volatile oils, terpenoids and steroids in the extracts from various parts of *Aerva lanata* are shown in Table I.

The preliminary phytochemical screening study revealed that the leaf of *Aerva lanata* has presence of tannins, cardiac glycosides, alkaloids, flavonoid, terpenoid & saponins. Except steroids remaining tested secondary metabolites are present in leaf. The stems of *Aerva lanata* contain cardiac glycosides, alkaloids, flavonoid, steroids and terpenoids. Tannins and Antharaquinones are absent in the root and flowers. Antharaquinones were found to be absent in the stem, root and flowers.

## DISCUSSION

Treating Gram-negative bacterial infections can be difficult because of several unique features of these bacteria. For example, the unique nature of their cell wall makes them resistant to several classes of antibiotics. Infections have typically been treated with broad-spectrum antibiotics, such as beta-lactams followed by carbapenems. However, even these drugs have become ineffective against some bacteria, leaving researchers to go for natural resources, which are medicinal plants. New drugs to combat Gram-negative bacterial infections are needed. In addition, researchers are unraveling the molecular mechanisms of drug resistance in Gram-negative bacteria to identify novel strategies to combat these pathogens.

Background check reveals that the flavonoids (Rauha, 2000), sterols (Pereira et al., 2006) and phenolic compounds (Proestos, 2005) show antimicrobial activity against several pathogens (Eloff, 2004). The plant when screened was found to contain many trace elements which play important role in herbal formulations[6]. Preliminary phytochemical analysis of the plant extracts and its fractions shows the presence of tannins, antharaquinones, alkaloids, saponins, phlobatannins, flavonoids, cardiac glycosides, volatile oils, terpenoids and steroids in *Aerva lanata*. Hence the antimicrobial activity exhibited by the Methanolic extracts could be related with the concentrations of above said secondary metabolites present in these seleted plant extracts. The results obtained here with antimicrobial activity support the folkloric claims regarding the plant and its medicinal values. This paper helps in formulating natural principles to combat drug resistance of certain gram negative bacteria. It is therefore, implied the isolation and proper characterization of the active constituents from the extracts of this plant species as possible antimicrobial agents.

## CONCLUSION

In conclusion, all the extracts investigated except water possessed activity against at least one strain of bacteria and/or fungi. Further studies aimed at the isolation and identification of active substances from the Methanol extracts of *Aerva lanata* could also evolve compounds with effective natural medicinal values for the cure of microbial disorders. The plant is said to be a source of many bioactive compounds acting against some human diseases. The present study helps in herbal formulation of *Aerva lanata* for its fight against infectious microbes.

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## REFERENCES

1. Kirtikar KR, Basu BD. Indian Medicinal Plants, Edn 2, Vol. I, Dehradun 1994, 314-317.
2. Prajapati ND, Purohit SS, Sharma AK,Kumar TA. Handbook of Medicinal Plants, AGROBIOS, (India) Jodhpur,2003,3.
3. Nadkarni AK. Indian Materia Medica, Popular Prakashan (Pvt)Ltd, Bombay, 1995, 8-9.
4. Bauer AW, Kirby WMM, Sherris JC, Turck M. Antibiotic susceptibility testing by standardized single disc method, American Journal of Clinical Pathology, 1966; 36: 493–496.http://en.Wikipedia.org /wiki/*Aerva lanata*_, Accessed –13 October, 2014.
5. Ragavendran P, Arul Raj C,Sophia D,Starlin T,Gopalakrishnan V.K.,Elemental Analysis of *Aerva lanata* by EDX method, International Research Journal of Pharmacy, 2012;3(7):218-220

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