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Research Article

IN VITRO ANTI BACTERIAL ACTIVITY OF PHYTOSTEROL ISOLATED FROM *AERVA LANATA* ROOTS

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ABSTRACT

Background: Traditionally, root of *Aerva lanata* are used as sap for eye-complaints, an infusion is given to cure diarrhoea, kidney stone and in snake bite treatment.
***Aim*:** The aim of this study was to evaluate in-vitro anti-bacterial activities of solvent extract of the root of Aerva lanta.
Methods: Aerva lanata Plant was screened using the agar well diffusion and broth micro-dilution assay. The purity of isolated phytosterol was checked by TLC, column and qualitative phytochemical analysis and total phytosterolwere quantified.
Results: In the current study, the inhibitory action of the phytosterol was found to increase with an increase in concentration against all bacterial strains. The maximum zone of inhibition was observed at the concentration of 500 µg/ml against all the bacteria. In this study, the *S. aureus* and *E. coli* are the more susceptible than the other selected human pathogenic bacteria.
Conclusion: This study shows that *Aerva lanata*, could be a valuable source for antimicrobial properties and helps to produce antimicrobial agents to treat human pathogenic infections.

INTRODUCTION

India has one of the oldest, richest and most varied ethnic traditions related with the use of medicinal plants. Medicinal plants are excessive importance to the health of individuals and communities in general. The therapeutic value of plants lies in particular chemical substances that yield a definite physiological action on the human body. The most significant of these bioactive constituents of plants are alkaloids, tannins, flavonoids and phenolic compounds. Several of the indigenous medicinal plants are used as spices and food plants. Plant based antimicrobials characterise a vast untapped. Traditional therapeutic system is essential as a number of significant modern pharmaceuticals have been obtained from plants used by native people (Swapna Gurrapu and Estari Mamidala 2016).They also occasionally added to foods intended for pregnant women and harbouring mothers for therapeutic purposes as reported by Okwu, D. Elands Hill A.F. (Okwu, D. E., 1999, Okwu, D.E., 2001, Hill A.F., 1952). Herbs being effortlessly available to human beings have been explored to the extreme for their therapeutic properties. Perumal Samy and Gopala Krishnak (2007) reported that, the diverse parts of the plants resembling bark, roots, leaves, etc. are used as per therapeutic properties.

Aerva lanata is an essential therapeutic plant, found throughout tropical India as a common weed in fields and wasteland (Krishnamurthy, 2003). Because of its reputation in folk treatment, *Aerva lanata* has become the subject of intense pharmacological and chemical studies for the last 30 years. In addition to the traditional uses, the plant is reported for a numeral of pharmacological activities viz., anthelminthic, demulcent, anti-inflammatory (Vertichelvan *et al*., 2000), diuretic (M. Udupihille., and M.T.M. Jiffry, 1986), expectorant, hepatoprotective (S. Manokaran *et al*., 2008) and nephroprotective (A. Shirwaikar, D. Issac., and S. Malini, 2004) anti-diabetic (T. Vetrichelvan and M. Jegadeesan,2002), anti-hyperglycaemic, anti-microbial, Cytotoxicity(Ma.nokaran *et al*., 2008), urolithiatic, hypoglycaemic, anti-hyperlipidemic, anti-parasitic and anti-helmenthic activities. In order to identify the bioactive compounds accountable for the above pharmacological actions, phytochemical studies have been carried out by numerous researchers with the report of phenolic compounds in (J.B. Harbone, 1998). *Aerva lanata* plant root was selected for this studies based on its traditional medicinal use in (Rajendra Prasad Gujjeti and Estari Mamidala 2012).

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Antimicrobial activity was not studied for this compound till today. Therefore the objective of this present study is to study the antimicrobial activity of isolated from the *Aerva lanata* root.

MATERIALS AND METHODS

Collection of plant

Aerva lanata Plant roots were collected from the Chintoor mandal, Khammam district of Andhra Pradesh, India in the month of September and October 2012. The plant voucher specimens identification was done with the help of Prof.Vastsavaya. S.Raju Department of Botany, Kakatiya University, Warangal and the same was deposited at Infectious Diseases & Metabolic Disorders Research Lab, Department of Zoology, Kakatiya University, and Warangal.

Preparation of plant extract

Roots were collected in bulk, washed, shade dried, macerated and extracted with hexane, chloroform, ethyl acetate, acetone, and methanol. The extract was filtered and it was finally dried at low room temperature under pressure in a rotary vacuum evaporator (Thermotech, buchi type model th-012). The extracts were concentrated, percentage yield calculated and then subjected to phytochemical screening and TLC profiling studies. The dried extract was properly stored in the desiccators for further experiment and analysis.

Phytochemical Screening

Chemical tests for the screening and identification of bioactive chemical constituents like alkaloids, carbohydrates, glycosides, saponins, phenolic compounds, phytosterols, proteins, amino acids, flavonoids, and tannins, in the medicinal plants under study were carried out in extracts by using standard procedure (Shirsat, 2008).

Thin layer chromatographic studies

Each solvent extract was subjected to thin layer chromatography (TLC) as per conventional one dimensional ascending method using silica gel 60F254, 7X6 cm (Merck) were cut with normal household scissors. Plate markings were made with soft pencil. Glass capillaries were used to spot the sample for TLC applied sample volume 1-micro litre by using capillary at distance of 1 cm at 5 tracks. In the twin trough chamber with different solvent system Hexane: Acetic acid (9:1) solvent system I, in solvent system II Hexane: Ethyl acetate: Acetic acid (5:4:1), in solvent system III Hexane: Ethyl acetate: Acetic acid (4:4:2), in solvent system IV Hexane: Ethyl acetate: Acetic acid (3:6:1), in solvent system Hexane: Ethyl acetate: Acetic acid (2:7:1) used. After the run plates are dried and sprayed freshly prepared iodine reagents were used to detect the bands on the TLC plates. The movement of the active compound was expressed by its retention factor (Rf), Values were calculated for different samples by the following equation:

Rf = Distance travelled by the solute / Distance travelled by the solvent front TLC plates

Bacterial Cultures

Clinical isolates of *Escherichia coli*, *Pseudomonas aeruginosa*, *Shigella boydii*, *Staphylococcus aureus* and *Streptococcus faecalis* were obtained from the Department of Microbiology, Kakatiya University, and Telangana State, India. All the test strains were preserved on nutrient agar slopes (Hi-Media) and were sub-cultured once in every two-week. These bacteria functioned as test pathogens for antibacterial activity assay. (Estari Mamidala 2013b).

Antibacterial assays

Agar-well diffusion

The assay was conducted as described by Perez *et al*. (1990). Briefly, microorganisms from growth on nutrient agar incubated at 37^0C for 18 h were suspended in saline solution 0.85% NaCl. The suspension was used to inoculate 90 mm diameter Petri plates with a sterile non-toxic cotton swab on a wooden applicator. Six millimetres diameter wells were punched in the agar and filled with 50 µl of different concentration (125, 250 and 500 µg/ml) of alkaloids. The dissolution of the alkaloids was aided by 1% (v/v) DMSO which did not affect microorganism's growth, according to our control experiments. Commercial antibiotic (Ciprofloxacin) was used as positive reference standard to determine the sensitivity of the strains. Discs were directly placed onto the bacterial culture. Plates were incubated in air at 37^0C for 24 h. Antibacterial activities were evaluated by measuring inhibition zone diameters. The experiments were conducted twice.

Broth micro dilution assay

Broth micro dilution method was used to determine minimal inhibitory concentrations (MIC) of alkaloids against the test microorganisms as recommended by the National Committee for Clinical Laboratory Standards (NCCLS, 2002). The tests were performed in 96 well-plates. Alkaloids dissolved in 1% DMSO were transferred in plates to obtain a twofold serial dilutions ranging from 10 to 640 µg/ml. Then plates were inoculated with microbial suspensions diluted to have had 10^5 cfu/ml in each well. The final volumes in wells were 200 µl. After 24 h incubation in air at 37^0C, MIC was recorded as a lowest extract concentration demonstrating no visible growth in the broth.

Statistical analysis

Values are expressed as mean ± SE. Statistical significance was determined using one-way analysis of variance (ANOVA) and values with $p < 0.05$ were considered significant.

RESULTS AND DISCUSSION

Percentage of yield extract

The yield of sequential extracts (g) is shown in [Table 1]. The amount obtained from hexane, chloroform, ethyl acetate, Acetoneand methanol extracts are 5.020 gm, 4.080 gm, 2.750 gm, 1.720 gm, and 3.750 gm respectively.

Table 1 The percentage yield of different extracts of *Aerva lanata root*

S.No	Solvent	Colour of extract	Yield of the extract (in gm)	Percentage yield(%w/w)
1	Hexane	White	5.020	2.51%
2	Chloroform	Light brown	4.080	2.04%
3	Ethyl acetate	Light brown	2.750	1.37%
4	Acetone	Light brown	1.720	0.86%
5	Methanol	Dark brown	3.750	1.85%

Phytochemical Screening

The present study carried out in the *Aerva lanata* shown the presence of therapeutic active constituents. The phytochemical active compounds of *Aerva lanata* were qualitatively examined for roots and the results are presented in Table- 2.

Table 2 The zone of inhibition produced by Phytosterol compounds isolated from Aerva Lanta root

Bacteria Strains	Inhibition zone (mm)				
	125 µg/ml	250 µg/ml	500 µg/ml	Standard (Ciprofloxacin, 25 µg/disc)	Control (DMSO)
Escherichia coli	10.5± 0.47	10.9± 0.47	14.2± 10.49*	19.8± 0.43	0
Pseudomonas aeruginosa	0.00 ± 0.00	8.5 ± 0.49	9.3 ± 0.45	10 ± 0.44	0
Shigella boydii	9.7 ± 0.44	10 ± 0.00	12.7 ± 0.48*	10.7 ± 0.43	0
Staphylococcus aureus	12 ±0.48	12.8 ± 0.49	16 ± 0.48*	23 ± 0.38	0
Streptococcus faecalis	9.2 ± 0.43	9.5± 0.48	12 ± 0.44*	20.1± 0.37	0

Values are expressed as mean ± SEM and analyzed by one-way analysis of variance (ANOVA) followed by Dennett's *t* test; $*P< 0.05$;

In these screening process alkaloids, glycosides, saponins, phenolic compounds, tannins, phytosterols, carbohydrates, proteins, amino acids, flavanoids, quinones and terpenoids displays different types of results in various solvents extracts. Among these phytochemical screening, alkaloids, saponins, tannins, amino acids, flavanoids and terpenoids were existing 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, quinones were found in hexane, acetone, and methanol extracts, glycosides are absent in all solvent extracts.

Thin layer chromatographic studies

A large number of solvent systems were tried to accomplish a good resolution. Finally, the solvents hexane: ethyl acetate: acetic acid was used. Thin layer chromatographic studies of the hexane extract of *Aerva lanata*. Solvent system I Hexane: Acetic acid (9:1), 3 spots were visible RF values 0.20, 0.36 and 0.52. In solvent system II Hexane: Ethyl acetate: Acetic acid (5:4:1), 1 spot detected RF value 0.90. In solvent system III Hexane: Ethyl acetate: Acetic acid (4:4:2), 1 spot detected RF value 0.90. In solvent system IV Hexane: Ethyl acetate: Acetic acid (3:6:1), 2 spots were visible RF values 0.07 and 0.81. In solvent system V Hexane: Ethyl acetate: Acetic acid (2:7:1), 3 spots were obtained having RF of 0.09, 0.81 and 0.94.

TLC studies of the Chloroform extract of *Aerva lanata*. Solvent system I Hexane: Acetic acid (9:1), 2 spots were visible Rf values 0.14 and 0.40. In solvent system II Hexane: Ethyl acetate: Acetic acid (5:4:1), 3 spots were detected Rfvalues 0.10, 0.82 and 0.90. In solvent system III Hexane: Ethyl acetate: Acetic acid (4:4:2), 2 spots were detected Rfvalues 0.05 and 0.90. In solvent system IV Hexane: Ethyl acetate: Acetic acid (3:6:1), 2 spots were visible Rf values0.09 and 0.78. In solvent system V Hexane: Ethyl acetate: Acetic acid (2:7:1), 2 spots were obtained having Rf of 0.18and 0.94.

TLC studies of the Ethyl acetate extract of *Aerva lanata*. Solvent system I Hexane: Acetic acid (9:1), 2 spots were visible Rf values 0.10 and 0.40. In solvent system II Hexane: Ethyl acetate: Acetic acid (5:4:1), 2 spots were detected RFvalues 0.82 and 0.90. In solvent system III Hexane: Ethyl acetate: Acetic acid (4:4:2), 1 spot detected RF value 0.85. Insolvent system IV Hexane: Ethyl acetate: Acetic acid (3:6:1), 2 spots were visible RF values 0.07 and 0.81. In solvent system V Hexane: Ethyl acetate: Acetic acid (2:7:1), 2 spots were obtained having Rf of 0.03 and 0.94.

TLC studies of the Acetone extract of *Aerva lanata*. Solvent system I Hexane: Acetic acid (9:1), 2 spots were visible Rfvalues 0.16 and 0.44. In solvent system II Hexane: Ethyl acetate: Acetic acid (5:4:1), 1 spot detected Rf f value 0.82. Insolvent system III Hexane: Ethyl acetate: Acetic acid (4:4:2), 2 spots were detected Rf values 0.85. In solvent system IV Hexane: Ethyl acetate: Acetic acid (3:6:1), 2 spots were visible Rf values 0.05 and 0.90. In solvent system VHexane: Ethyl acetate: Acetic acid (2:7:1), 2 spots were obtained having Rf of 0.09 and 0.80.

TLC studies of the Methanol extract of *Aerva lanata*. Solvent system I Hexane: Acetic acid (9:1), 1 spot detected Rfvalue 0.10. In solvent system II Hexane: Ethyl acetate: Acetic acid (5:4:1), 1 spot detected RF value 0.92. In solventsystem III Hexane: Ethyl acetate: Acetic acid (4:4:2), 4 spots were detected RF values 0.05, 025, 080 and 0.90. Insolvent system IV Hexane: Ethyl acetate: Acetic acid (3:6:1), 2 spots were visible RF values 0.10 and 0.81. In solvent system V Hexane: Ethyl acetate: Acetic acid (2:7:1), 2 spots were obtained having Rf of 0.09 and 0.81.

Antibacterial study

The antibacterial activity of isolated phytosterol compound was determined by using agar well diffusing methods. The results in Table-2 show that the isolated compound has good antibacterial activity against selected human pathogenic bacteria. In the present study, the inhibitory action of the phytosterol was found to increase with an increase in concentration against all bacterial strains. The tested bacterial strains showed different patterns of inhibition. This was supported by an earlier study on an alcoholic extract that exhibited greater activity than the aqueous and hexane extracts against bacteria, with no cellular toxicity (Xiao Z, Storms R .al 2006). The broad spectrum of antibacterial activity was reported for *Physalis angulata* (Swapna Gurrapu and Estari Mamidala 2017).

The isolated Phytosterol compound at a concentration of 500 µg/disc showed maximum inhibition against *S. aureus* (16 mm), followed by *E. coli* (14.2 mm), *S. boydii* (12.7 mm), *S. faescalis* (12 mm) and *P. aeruginosa*.(9.3 mm) by broth dilution method. The maximum zone of inhibition was observed at the concentration of 500 µg/ml against all the bacteria.

(Shirsat,2008) reported that a *Aerva lanata* root methanolic extract showed maximum activity against gram-negative

bacteria and showed the highest inhibition zones against *P. aeruginosa* and *E. coli*. This study confirms the presence of the physagulin compounds may be responsible for the antibacterial activity against various bacterial strains.

The varying concentrations between 10 to 640 ug/ml of the isolated Phytosterol compound of *Aerva* lanata were tested in order to determine their MICs. The MICs of the isolated phytosterol compound against the five tested bacterial is presented in Table-2. The lowest MICs were obtained in the Phytosterol compound having 41.5 µg/ml against *E. coli,* 56.5 µg/ml against *S. aureus*, 60.5µg/ml against *S. boydii*, 81.5 ug/ml against *P. aeruginosa* and 88.5 µg/ml against *S. faescalis*. The MIC ranged from 10 to 640 µg/ml for all studied microorganisms while for ciprofloxacin it ranged from 0.1 to 10 µg/ml. These phytosterol known tobe biologically active as well as showing antimicrobial activities. In this study, the *S. aureus* and *E. coli* are the more susceptible than the other selected human pathogenic bacteria.

Table 3 Antibacterial activity of phytosterol compound isolated from *Aerva lanata* root against selected human pathogenic bacteria by minimum inhibitory concentration (MIC) method

Bacterial strains	**MICs (µg/ml) of isolated alkaloid compound**	**Standard (Ciprofloxacin)**	**Control (DMSO)**
Escherichia coli	41.5	0.5	0
Pseudomonas aeruginosa	81.5	0.3	0
Shigella boydii	60.5	0.5	0
Staphylococcus aureus	56.5	0.5	0
Streptococcus faecalis	88.5	0.8	0

In this study, this antimicrobial activity may be due to the presence of (OH) group in the structure isolated phytosterol which increased the activity to inhibit the bacterial growth by changing the nature of cell protein (denaturation), thus increasing the permeability of cell membranes (Rastogi and Mehrotra, 2002), either by increasing the permeability of the cell membrane of the bacteria. The cell membrane causes loss or leakage of the contents of a cell of bacteria to the outside or through a direct link membrane of cell bacteria, causing the demise of polar membrane of bacteria, which leads to the death of cell bacteria gradually (Venkanna L, Estari M, 2012).

CONCLUSION

The result of the present study showed that the isolated Phytosterol compound of the fruits of *Aerva lanata were effective* against the bacterial species tested. Traditionally, root of *Aerva lanata* are used as sap for eye-complaints, an infusion is given to cure diarrhoea, kidney stone and in snake bite treatment. The plant is used for curing diabetes, lithiasis, cough, sore throat and wounds and it possess anti-inflammatory and nephroprotective properties (Deshmukh TA *et al*., 2008). This investigation has opened up the possibility of the use of this plant for formulating a drug for human consumption possibly for the treatment of bacterial infections. These findings support the traditional knowledge of local users about their selection of this plant sample as antimicrobial agents and it is a preliminary scientific validation for the use of this plant for antibacterial activity. The results of the present study also support the medicinal usage of the phytosterolisolated from the root of Aerva lanata can be used as antimicrobial agents in new drugs for therapy and can be subjected to identification and isolation of the therapeutic antimicrobials and undergo further pharmacological screening that can be used as sources for new drugs.

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