



MICROSCOPIC EVALUATION, ISOLATION AND ANTIMICROBIAL ACTIVITY OF PHYTOCONSTITUENTS FROM THE LEAVES OF *BLECHNUM ORIENTALE* LINN

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ABSTRACT

Blechnum orientale Linn belongs to family Blechnaceae, found throughout India. The methanol extract of the plant was subjected to column chromatography for the isolation of phytoconstituent. saponins, tannins, terpenoids, flavonoids and alkaloids. Total two constituent has been isolated and characterized as Trimethyl ellagic acid and 11, 12 dimethoxy, 2-rhamnosyl, 4-ol, 5- keto flavone. Their structures were elucidated by spectroscopic methods such as UV, IR,

NMR and LCMS. The antimicrobial activity of the extracts was determined by disc diffusion method (NCCLS, 1997) in petriplates containing NA medium (20 mL media/plate), respectively.

KEYWORDS: *Blechnum orientale* Linn, Trimethyl ellagic acid, 11, 12 dimethoxy, 2-rhamnosyl, 4-ol, 5- keto flavone, spectroscopic method, disc diffusion method.

INTRODUCTION

Blechnum orientale Linn. belongs to family Blechnaceae,^[1] found throughout India. It is commonly cultivated in tropical regions.^[1] The major phytoconstituents of the drug under study are alkaloids. Alkaloids frequently occur as salts of plant acids such as malic acid, meconic acid, and quinic acid.^[9] There are many applications of the leaves of custard apple are used to overcome stomach troubles, skin infections, mucosae, laxatives, diarrhoea,^[3] dysentery,^[4] pregnancy, antiaborifacients,^[6] tumours,^[8] cancers, astringents, hydrogen cyanide,^[7] fish-poisons, insecticides, arachnicides, antibiotic, bacteristatic, fungistatic^[5] etc.

The present work was carried out on fresh leaves of *Blechnum orientale* for the isolation of phytoconstituents. The authenticated chopped fresh leaves of *Blechnum orientale* was extracted successively with increasing polarity in soxhlet extractor and methanol extract of *Blechnum orientale* was subjected to Column chromatography. Total two compounds were isolated. Their structures were elucidated by spectroscopic methods such as UV, IR, NMR and LCMS. After characterization the isolated compound I and II was found to be Trimethyl ellagic acid and 11, 12 dimethoxy, 2-rhamnosyl, 4-ol, 5- keto flavone respectively.

MATERIAL AND METHODS

Plant material

Blechnum orientale Linn leaves were collected and authenticated by Central Council for Research in Ayurveda and Siddha, Bangalore. A voucher specimen has been preserved in our Department of Pharmacognosy, Sagar Institute of Research, Technology & Science-Pharmacy (SIRTS-P), Ayodhya Bypass Road, Bhopal, M.P



General instrument details

UV: Shimadzu UV VIS-1700, Projection microscope, Columns, Soxhlet apparatus. IR: JASCO FTIR 5300; LCMS: Agilent 1100 LC-MSD APCI; ¹H-NMR (500 MHz): Bruker Avance 500.

Microscopic studies

The pieces of leaves were boiled in a test tube with chloral hydrate for several minutes until complete removal of chlorophyll. Microscopic studies were carried out by preparing thin section of leaf. Transverse section was obtained by cutting the leaf portion including mid rib

with the help of sharp blade The thin section were collected in watch glass and bleach with bleaching agent with little boiling, after that thin section were washed with water. Stained with Phloroglucinol HCL / safrannin and mounted in glycerin for observation. Thin sections were observed under binocular and projection microscope. Photograph at different magnification were taken by using Nikon digital camera 12 megapixels.

Determination of total flavonoid content

The total flavonoid content of methanol extracts was estimated according to a previously described method.^[24] The absorbance was measured against a blank at 510 nm. Results were expressed as mg of (+)-catechin equivalents per gram of dried extract. Different concentrations of (+)-catechin as standard (1, 10, 20, 40, 80 µg/mL) were used to construct a calibration curve. The total flavonoid contents were calculated by the linear equation derived from calibration curve: “ $y = 0.003x + 0.1307$ ” where y is absorbance and x is the flavonoid content in milligram of (+)-catechin equivalents per gram of dry extract (mg CE/g of extract). All measurements were carried out in triplicates.

Qualitative Phytochemical Screening

Phytochemical tests were done and the secondary metabolites were qualitatively tested according to the standard methods. Phytochemical tests for saponins, tannins, terpenoids, flavonoids and alkaloids were performed. Dragendorff reagent was used for alkaloids, foam test for saponins, Mg-HCl and Zn-HCl for flavonoids, Salkowski test for terpenoids, and ferric chloride and gelatin for tannins.

Extraction and isolation procedure

Coarsely powdered leaves (750 gm) were extracted with petroleum ether followed by chloroform and methanol by the process of continuous extraction (soxhlation). The crude extract was evaporated to dryness in a rotary film evaporator. Methanol extract was subjected to column chromatography over silica gel (60-120 mesh) using petroleum ether, taking 100 ml fraction each time.

Disc Diffusion Method

The antimicrobial activity of the extracts was determined by disc diffusion method (NCCLS, 1997) in petriplates containing NA medium (20 mL media/plate), respectively. The paper discs (6 mm in diameter) were separately impregnated with 15 µL of extracts placed on the agar which had previously been inoculated with the selected test microorganism.

Streptomycin was used as a positive reference for bacteria. Discs without samples were used as a negative control. Plates were kept at 4 °C for 1h. The plates were incubated at 37 °C for 24 h for bacteria. Antimicrobial activity was assessed by measuring the diameter of the growth-inhibition zone in millimeters (including disc diameter of 6 mm) for the test organisms comparing to the controls.

Determination of Antibacterial Activity

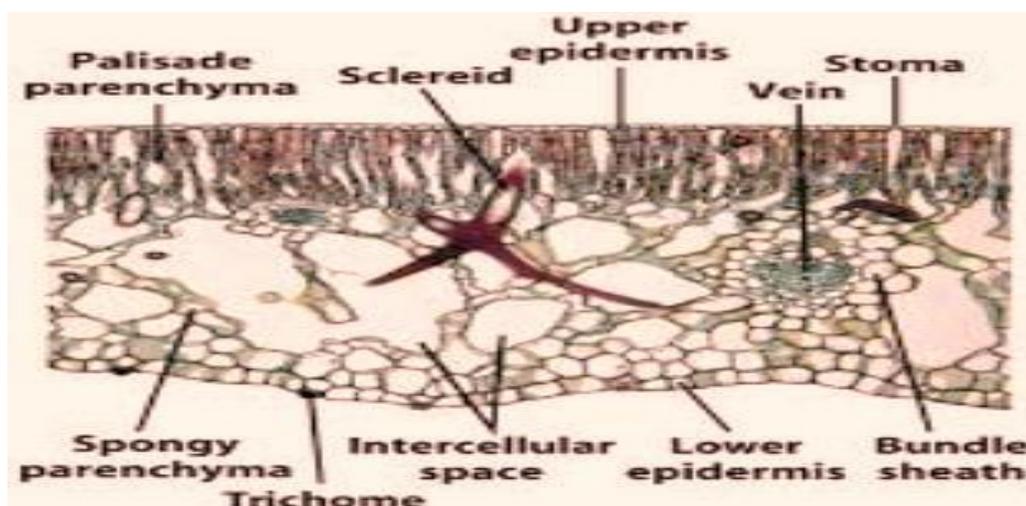
The extracts were individually tested against a panel of microorganisms selected. Bacterial strains were cultured overnight at 37 °C in nutrient agar (NA). The paper discs (6 mm in diameter) were separately impregnated with 15 µL of extracts placed on the agar which had previously been inoculated with the selected test microorganism. The diameters of zone of inhibition observed were measured.

Determination of MIC

The plant extracts were evaluated to determine MIC value. The broth dilution method was adopted by using N-saline for diluting the plant extracts and was incubated for 48 h. The minimum dilution of the plant extracts that kills microbial growth. The minimum dilution of plant extracts that inhibits the growth of the organism was taken as MIC.

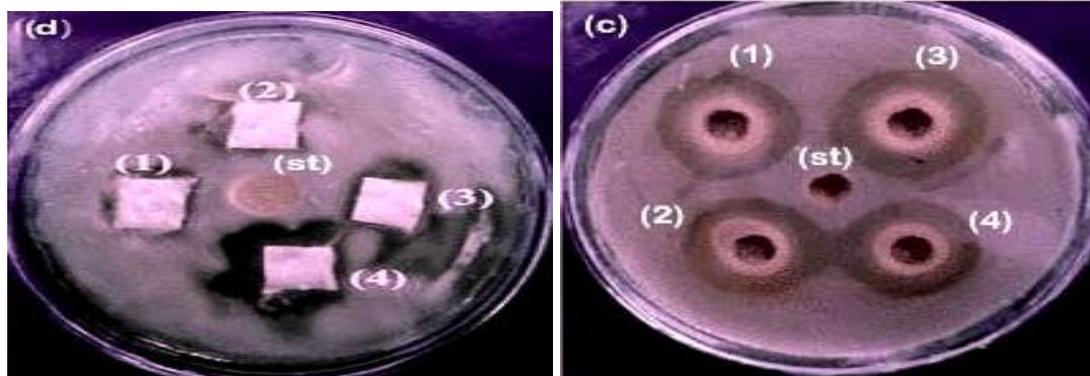
RESULTS AND DISCUSSION

Microscopic studies show the presence of palisade parenchyma, sclereid, stoma, vein, spongy parenchyma, lower epidermis, bundle sheath etc.



Phytochemical analysis revealed the presence of flavonoids, terpenoids and tannins. Total two compounds were isolated. Their structures were elucidated by spectroscopic methods

such as UV, IR, NMR and LCMS. After characterization the isolated compound I and II was found to be Trimethyl ellagic acid and 11, 12 dimethoxy, 2-rhamnosyl, 4-ol, 5- keto flavone respectively.

*E-coli**Salmonella typhi**Staphylococcus aureus**Bacillus pumilus*

S. No.	Test Organism	Inhibition Zone in diameter (mm)				
		Streptomycin	Aqueous	Ethanol	Acetone	Pet ether
1	<i>E-coli</i>	12	15	17	20	17
2	<i>Salmonella typhi</i>	10	16	18	15	21
3	<i>Staphylococcus aureus</i>	8	13	15	21	15
4	<i>Bacillus pumilus</i>	11	18	14	22	20

NA- Nutrient Agar

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