

RESEARCH ARTICLE

PHARMACOGNOSY

PHYSICAL, PHYTOCHEMICAL SCREENING AND ANTIBACTERIAL ACTIVITIES OF CLERODENDRON INFORTUNATUM LINN ROOT**Dr. N.A. ALEY KUTTY, SANTHOSH M. MATHEWS, LEENA P.N.***

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ABSTRACT

Plant materials are used throughout developed and developing countries as home remedies, over the counter drug products and raw materials for the pharmaceutical industry and represent a substantial proportion of the global drug market. It is therefore essential to establish internationally recognized guidelines for assessing their quality. Some of quality control parameters of the roots of *Clerodendron infortunatum* belonging to Verbenaceae family were analyzed. It includes root powder characters, moisture content determination by LOD method, FOM determination, Rf value detection by TLC, using different solvents, Ashvalues, extractive values. The root portions of *Clerodendron infortunatum* Linn extracted with ethanol, chloroform by cold maceration process. The extracts were vacuum dried and subjected to antibacterial (*Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli* and *Klebsiella pneumoniae*) screening by Agar disc diffusion method. Minimum Inhibitory Concentration of microbial growth was also evaluated. The phytochemical screening was performed and different phytoconstituents present in the extracts were identified. The extracts exhibited potent activity against *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli* and *Klebsiella pneumoniae*.

KEYWORDS

Clerodendron infortunatum Linn, Physical method of evaluation, antibacterial, Phytoconstituents, Minimum Inhibitory Concentration (MIC)

INTRODUCTION

Clerodendron infortunatum Linn, (Family :Verbenaceae) ¹ is a species found in India, It is reported as folk remedy for tumours, leprosy, fever, infection, inflammation. The roots have been reported to possess laxative, diuretic, analgesic, anti-inflammatory, anti-tumour and antibacterial activities¹. To our knowledge there were no scientific reports on the antibacterial activities of *Clerodendron infortunatum* Linn root. In the present study the root portions of *Clerodendron infortunatum* Linn evaluated by their physical method, phytochemical method and pharmacological activities. First the root powder was extracted with ethanol, chloroform by cold extraction. The vacuum dried extracts (25, 50 and 100mcg/mL) were screened for antibacterial activities^{3,4}. Minimum Inhibitory Concentration was also determined⁵.

MATERIALS AND METHODS

Plant material and Extraction Procedure

The plant *C.infortunatum* was collected from Pathanamthitta district of Kerala and identified by Thomas Mathew, HOD of Botany, Marthoma College Tiruvalla, Kerala. Voucher no. VSCI-13 was deposited in the Pharmacognosy department, Pushpagiri College of pharmacy, Tiruvalla. The root portion of the plant was washed with running water to remove soil and other matter and dried in shade for 20 days, powdered. The physical method of evaluation include root powder characters, moisture content determination by LOD method, FOM determination, Rf value detection by TLC, using different solvents, Ash values, extractive values ₆. The root powder extracted 500gm with chloroform (CECI) ethanol (EECI) by cold extraction to yield the respective extracts. The

extracts were reduced to molten mass by rotary vacuum evaporator and the yield was 18%, 21% respectively.

Preliminary phytochemical screening was performed as per standard procedure and various phytochemical constituents were identified^{6,7}.

Antibacterial activity

The extracts (CECI,EECI) were subjected to antibacterial (*Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli* and *Klebsiella pneumoniae*) screening. The antibacterial screening was done by Agar diffusion method using a paper disc^{3,4}. Nutrient Agar and Sabouraud dextrose Agar media were used for antibacterial screening. The sterilized (autoclaved at 120° for 30 mins) medium (40°-45°) was inoculated (1mL /100mL of medium) with suspension of the micro organism (match with McFarland barium sulphate standard).

The paper impregnated with the extract (25,50,100mcg/ml in dimethyl sulphoxide) was placed on the solidified medium. The plates were pre inoculated for 1 h at room temperature and incubated at 37° for 24 and 48h for antibacterial activity determination.

Amikacin (5mcg/disc) was used as standard for antibacterial activity. The observed zones of inhibition are presented in table 1 and fig no 1 and 11. The MIC for the above organism was found by Agar streak dilution method⁵. About 20ml of the media containing different concentrations of the extract was poured into each sterile petridish and allowed to solidify. Microorganisms were then streaked one by one on the agar plate aseptically. After streaking all the plates were incubated at 37°C for 24h. Then the plates

were observed for the growth of micro organisms. The lowest concentration of the plant extract required for inhibiting the growth was considered as the MIC of the extracts against

bacterial strains. The MIC values of each extract against the tested micro organism were vide Table 11.

RESULT AND DISCUSSION

The various physico-chemical constants observed for the roots of were summarized as FOM 1.9% w/w, Moisture content were 8.35% w/w, Total ash content 12.1%, which is due to the presence of inorganic matter present in these *Clerodendron* species. . Acid insoluble ash indicate the presence of more siliceous matter in the drug. ie 2.1% . The

alcohol soluble extractive reveals the presence of polar compounds like anthraquinone, alkaloids, glycoside of flavanoids, steroids materials. It was seen to be 16% . The water soluble extractive reveals the presence of water soluble matter such as Sugars, Carboxylic acid, Vitamins. Table 1.

TABLE : 1
PHYSICO-CHEMICAL VALUES *Ci* root .

Parameter	<i>C. infortunatan</i> (Ci)	
Powder characters of Root	UV	VISIBLE
i. Powder	Light Brown	Light brown
ii. In NaOH	Dark brown	brownYellow
iii. In HCl	Yellow Light	Yellow
iv. In HNO ₃	yellow	Yellow

2. Powder reaction with reagents

Parameter	Ci ROOT
(1) Phluroglucinol	Pink colour
(2) Iodine soln	Blue colour
(3) Acetic acid	Insoluble
(4) 60% H ₂ SO ₄	Soluble
(5) HNO ₃	Brown colour
(6) H ₂ SO ₄	Light purple
(7) 10% NaOH	Brown

(8) IN HCl	Yellow colour
(9) 5% FeCl ₃	Greenish colour
3. Foreign Organic matter	1.91% w/w
4. Moisture content detection by (LOD method)	8.35% w/w
5. Water solubility	Soluble
6. Alcohol solubility	Soluble
7. CHCl ₃ solubility	In soluble
8. Acetone solubility	Soluble
9. Ether solubility	Sp. Soluble
10. Water extractive value	12.8%
11. Alcohol extractive value	1.6%
12. CHCl ₃ extractive value	0.8%
13. Total ash	12.1%
14. Water soluble ash	6.5%
15. Alcohol soluble ash	2.1%

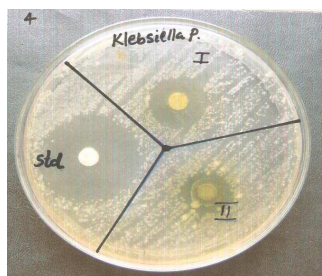
The preliminary phytochemical screening carried out on *Clerodendron infortunatum* Linn root indicated the presence of carbohydrates, starch, mucilage, saponins, flavanoids, tannins, phenolic compounds in the ethanol extract. The antibacterial activities of the extracts were determined by measuring the zone of inhibition produced by the extracts against various tested organisms at different concentration. All the extracts exhibited marked activities against the tested organisms. *E.coli* and *K.pneumonia*, *staphylococcus aureus* were more sensitive

towards the extract at 100mcg indicated by a greater degree of inhibition on comparison with standard. All the organisms exhibited moderate activity against the extract as evident from Table1. From the above results it is evident that the drug *Clerodendron infortunatum* Linn

acts to be a better antibacterial agent and can be developed into a potential antibacterial drug with a minimum of adverse effects.

Table2
Zone of Inhibition (in mm) and Minimum Inhibitory Concentration (MIC) of
***Clerodendron infortunatum* Linn root extract.**

ORGANISM	EXTRACT	STANDARD {Amikacin (5mcg/disc)}in mm	25mcg	50mcg	100mcg	MIC
Bacillus subtilis Strain no. NCIM-2067	CECI	24	16	18	20	20
	EECI	28	18	20	22	19
Staphylococcus aureus Strain no. NCIM-2079	CECI	46	16	21	30	21
	EECI	46	16	20	26	21
Escherichia coli Strain no. NCIM-2065	CECI	33	16	18	20	20
	EECI	33	15	18	21	21
Klebsiella pneumoniae Strain no. NCIM-2070	CECI	36	16	18	23	20
	EECI	36	17	19	23	21



CECI: Chloroform extract of *Clerodendron infortunatum* Linn root; EECI: Ethanol extract of *Clerodendron infortunatum* Linn root

CONCLUSION

The present study is evident that the drug *Clerodendron infortunatum* Linn root acts to be

a better antibacterial agent and can be developed into a potential antibacterial drug with a minimum of adverse effects.



ACKNOWLEDGEMENT

The authour Acknowledge Karpagam University,Coimbatore for providing the

suitable instructions for doing the work .Management of Pushpagiri group of institutions, Tiruvalla for providing the necessary facilities needed for the research .

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