Antimicrobial Activity & Minimum Inhibitory concentration study of Leaf Extracts of *Laportea interrupta L.*

Das Biswajit* [1], Dash Suvakanta[1], Deb Roy Saumendu[1], Raul Sarojkumar[2]

1. Girijananda Chowdhury Institute of Pharmaceutical Science, Guwahati, Assam.
2. Department of Chemistry Maharajah's college of pharmacy, Vizianagaram, Andhrapradesh.

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ABSTRACT

Plants are the oldest source of pharmacologically active compounds, and have provided mankind with many medically useful compounds for centuries. In this study aqueous and methanolic extract of *Laportea interrupta L.* leaf was Studied against Gram negative *Escherichia coli* (ATCC25923) and Gram positive *Staphylococcus aureus* (ATCC25923) organisms and Gentamycin, was used as standard antibacterial drug. The antimicrobial assay and MIC studies were done by disc diffusion and serial dilution methods. Methanolic extract of *Laportea interrupta L* has shown highest activity against *Escherichia coli &.Staphylococcus aureus*. The inhibitory effect was less in magnitude than that of standard antibiotics used.

KEYWORDS: *Laportea interrupta L*, MIC, Disc diffusion, Serial Dillution.

Corresponding Author:
Biswajit Das,
E. Mail: bapipm@gmail.com,
Cell No.:9435902134

INTRODUCTION

Traditional medicine cures diseases and infections, by using various plants, minerals and the like. A huge percentage of world's population partially or entirely still depends on botanicals to treat human diseases and infections .[1] The use of plants whether herbs, shrubs or trees in parts or in whole for the treatment and management of diseases and disorders date back to pre-historic days.[2] Plant extracts have been used in folk medical practices for the treatment of various ailments since antiquity.[3]The medicinal properties of various plant material and extracts have been recognized since the beginning of the 5th century. In the era of modernization and changed environmental conditions, man frequently encounters pathogenic microorganisms causing infectious diseases. The indiscriminate use of commercially available antibiotics for the treatment of infectious diseases will results in multiple drug resistance in the microorganisms, putting new challenge before the drug industries for identification of new efficient antimicrobial compounds. Herbal drugs therapy is regarded as an important alternate, leading the researchers to focus and evaluate the traditionally recommended medicinal plants for their efficacy in various diseases. [4] As reported by World Health Organization (WHO), traditional medicinal plants are the best reservoirs to develop newer medicines.[5] Medicinal plants are renewable sources, therefore farmers get encouraged to include them in traditional agriculture .[6] Medicinal plants are known to owe their curative potentials to certain biological active substances, which exist in parts of the plants. The traditional use of plant derived medicinal compounds received much attention against the multifactorial antibiotic resistance, as they are well tested for their efficacy and are being used to treat various microbial diseases since long. *Laportea interrupta L* commonly known as Bichhuati (odiya) or wood nettle is a plant belonging to the family Urticaceae. The leaf extract is used in fevers, rheumatic pain and headache.[6] The plant is distributed in India from sea level to high hills. It is a prostrate herb with medicinal values.[7]

MATERIALS AND METHODS

- **Collection of Plant Material**

Fresh plant material leaves of *Laportea interrupta L*. was collected from its natural habitat, from the grassland and field of Cuttack district Odisha and was identified by Dr. S. S. Dash, BSI.

The collected fresh plant materials (leaves) were washed with water, shade dried and then homogenized to fine powder of 40 mesh sizes and stored in airtight bottles at 4°C.

- **Preparation of Sample**[8]

About 100gm of leaf powder was subjected to extraction by a hot percolation method with 150ml of solvent in their increasing polarity (methanol and water respectively), in soxhlet apparatus. Each solvent extraction step was carried out for 24 hrs. After extraction, the extracts were concentrated and stored at 4°C for further study. For each plant material, 1% stock solution was prepared with 0.1% Dimethyl sulphoxide solution. The extract was filtered using membrane filter. The final extracts obtained were stored in a refrigerator at 4°C until required for use.

- **Drugs and chemicals used**

Drug: Gentamycin as Standard Drug was obtained as Gift Sample from Hetero Labs, Baddi.

Chemicals: Iso-propyl alcohol, ethanol, glacial acetic acid, Dimethyl sulphoxide, methanol, water, Nutrient Agar, peptone and beef extract used were that of Analytical Grade.

Micro-organisms: The organisms used in this studywere three Gram-negative and gram positive bacteria.

- Gram negative *Escherichia coli* (ATCC25923)
- Gram positive *Staphylococcus aureus* (ATCC25923)

ASSAY METHODS

The antimicrobial activity was evaluated by disc diffusion method. Agar media was used for maintaining, culturing of microorganism and to carry out the antimicrobial susceptibility and was prepared by Dissolving 5 gm of peptone, 3 gm of beef extract, and 5gmof NaCl and 20 gm of agar in 0.5 L of distilled water. PH was adjusted to about 6.8-7.2 and final volume was adjusted to 1 L and autoclaved at 121°C and 15Lbs for 15 minutes.[9] The antimicrobial activity of leaf extracts were evaluated by growing the broth of each tested microorganism till the late log phase. Sterilized water was taken as negative control and Gentamycin was taken as positive control.

- **Disc Diffusion Method**

Fresh overnight cultures of inoculums (0.1ml) of each culture, was spread on agar plate. The plates were kept for 10 min and then the prepared sterilized discs (5 mm diameter sterilized Whattman filter paper) soaked with the concentrated extract were impregnated over the surface of the plate inoculated with the microorganisms and allowed to incubate at 37 °C for 24 hrs. The zone of inhibition in mm was determined after incubation period. The microbes were plated and average zone diameter was noted.[10]

- **Determination of Minimum InhibitoryConcentration (MIC)**

MIC is the lowest concentration of a drug that prevents growth of a particular pathogen. For determination of MIC, serial dilution susceptibility test was applied which was carried out by using the extract from 22µg/ml to 2 µg/ml applied on dics. The second lowest concentration of plant extract resulting in lowest inhibition, after required

incubation is determined as the MIC.[11] Some idea of the effectiveness of a chemotherapeutic agent against a pathogen can be obtained from the minimal inhibitory concentration.[12]

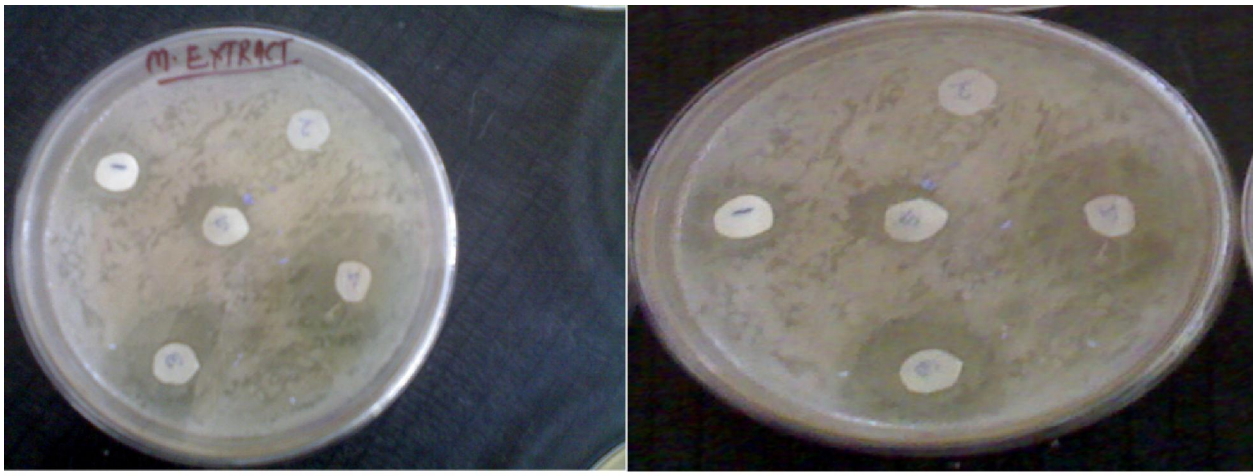
RESULT & DISCUSSION

- **Antimicrobial Activity**

The antimicrobial activity of *Laporteainterrupta L* plant leaf extract are shown in table 1 .The antimicrobial activity was determined in comparison with Gentamycin. Methanolic and aqueous extracts of leaves were taken at µg/ml concentrations against one concentration of the Standard, presented in table: 1. Methanolic extract showed the high activity 8.6 mm against *E. coli* at a dose of 60 µg/ml followed by 7.9mm for *S. aureus* at the same dose, while aqueous extract showed activity of 7.9mm for *E. coli* at a dose of 60 µg/ml followed by 6.8mm for *S. aureus* at the same dose. Gentamycin has shown 12.4 mm zone of inhibition for *E. coli* at 10 µg/ml concentration and 11.1 mm inhibition for *S. aureus* at the same concentration, as shown in Fig-1& 2.[13, 14]

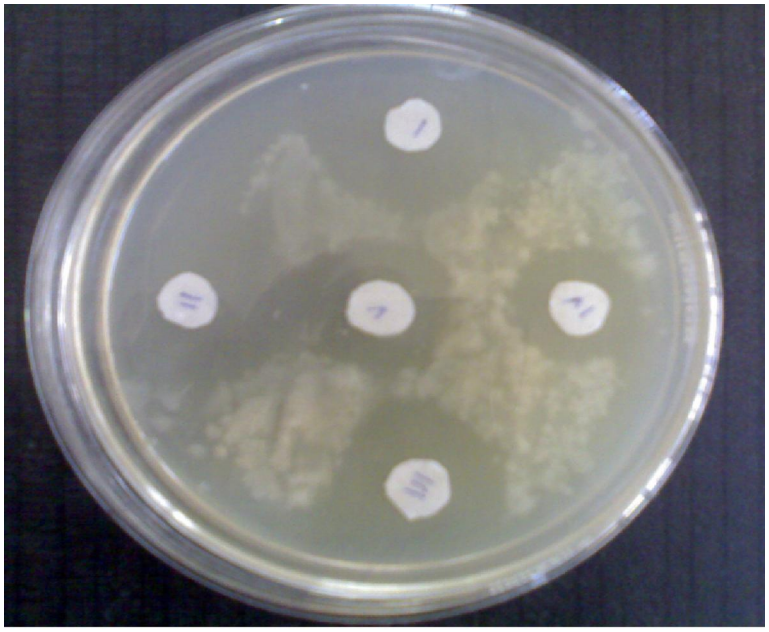
DRUGS/EXTRACT	Dose (µg/ml)	Zone of Inhibition (mm) E. Coli	Zone of Inhibition (mm) S. Aureus
Aqueous Extract	10	2	2.5
	20	3.5	3.9
	30	5.4	6.1
	60	7.9	6.8
Methanolic Extract	10	3.2	4.2
	20	4	4.5
	30	5.1	6.8
	60	8.6	7.9
Gentamycin	10	12.4	11.1

Table 1:- Both Methanolic and Aqueous extract of *Laporteainterrupta L.* showing zone of inhibition with respect to Gentamycin(Standard drug)



METHANOLIC EXTRACT OF *Laportea interrupta L.* (urticaceae) SHOWING ZONE OF INHIBITION

Fig 1:-Methanolic extract of *Laporteainterrupta L* showing zone of inhibition



Aqueous extract showing zone of inhibition

Fig 2:- Methanolic extract of *Laporteainterrupta L* showing zone of inhibition

Graphs were plotted against Zone of Inhibition Vs Conc. of Methanolic and Aqueous Extract of *Laportea interrupta L.* for each Microorganisms. in Fig: 3 which shows different conc. of extracts having significant antimicrobial activity.[15]

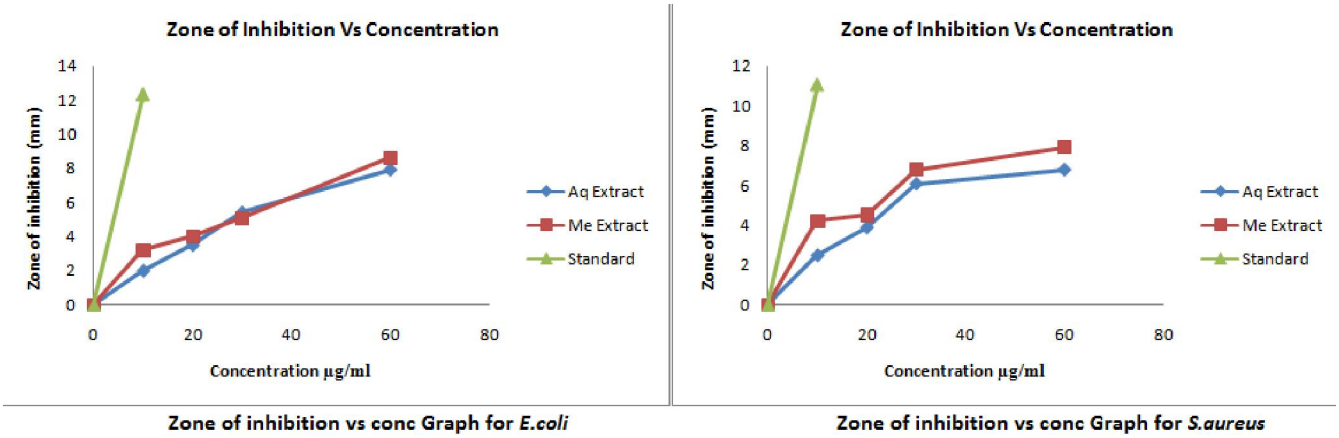


Fig 3:- Showing Zone of Inhibition Vs Conc. graph for both microorganisms.

- **MIC of Leaf Extracts**

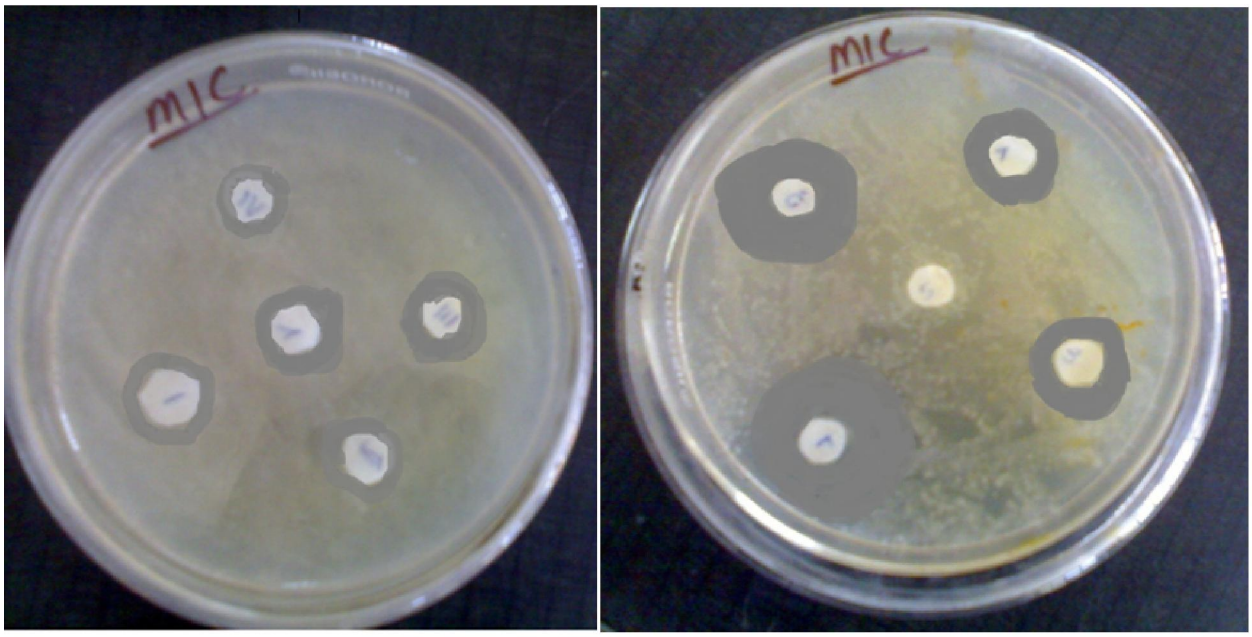
Methanolic and Aqueous extracts of leaf of *Laportea interrupta L* were screened for their antimicrobial potential. MIC values of different leaf extracts for pathogenic microorganisms are presented in table: 2 & 3 and the fig .4. Leaf extract of *Laportea interrupta L* shows antimicrobial activity, the Methanolic extract showed MIC against *S. aureus* at 04µg/ml followed by *E. coli* at 04µg/ml where as Aqueous extract shows MIC against *S. aureus* at 4µg/ml and for *E. coli* at 06µg/ml.

Serial Number	Concof Aqueous extract (µg/ml)	Zone of inhibition in (mm)	
		S.aureus	E.coli
01	22	4.1	3.9
02	20	3.9	3.4
03	18	3.4	3.4
04	15	3.5	2.8
05	12	2.9	2.6
06	10	2.6	2.1
07	08	1.4	1.5
08	06	0.9	0.8
09	04	0.6	0
10	02	0	0

Table 2:- MIC report of *Laportea interrupta L.* Aqueous extract for *S.aureus*

Serial no	Concof Methanol extract (µg/ml)	Zone of inhibition in (mm)	
		S.aureus	E.coli
01	22	5.1	4.9
02	20	4.7	4.2
03	18	4.4	3.9
04	15	4.2	3.7
05	12	4.0	3.5
06	10	3.9	3.2
07	08	2.9	2.8
08	06	1.8	1.3
09	04	0.9	0.7
10	02	0	0

Table 3:-MIC Report of *Laportea interrupta L.* Methanolic Extract for *S.aureus*



MIC STUDY OF *Laportea interrupta L.* (urticaceae)

Fig 4:- MIC Study of *Laportea interrupta L*

CONCLUSION:

Leaf extracts of *Laporteainterrupta L* in this study demonstrated some antimicrobial activity against both gram-positive and gram-negative bacteria. Bioactive substances from this plant can therefore be employed in the formulation of antimicrobial agents for the treatment of various bacterial infections .Isolation, identification and purification of phyto-constituents and determination of their respective antimicrobial potencies and toxicological evaluation with the view to formulating novel chemotherapeutic agents should be the future direction for investigation. Further detailed investigation of the active components of the plant for the exact mechanism of action will contribute greatly to the development new pharmaceuticals.

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