IN VITRO-ANTIBACTERIAL ACTIVITY OF BOERHAVIA DIFFUSA (L) LEAF EXTRACT AGAINST SELECTED GRAM POSITIVE AND GRAM NEGATIVE BACTERIA

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ABSTRACT: This study was carried out with an aim to investigate the antibacterial activity of leaf extract of boerhavia diffusa. In this study we compared the antimicrobial efficacy of Methanolic and aqueous extracts of Boerhavia diffusa against E.coli ATCC 25922, Klebsiella pneumonia MTCC 3384, Staphylococcus aureus ATCC 25923, Pseudomonas aeruginosa ATCC 27853. The results of study suggests that Both Methanolic and aqueous extracts showed moderate antibacterial activity, which is clearly visible in the form of (ZOI) Zone of Inhibition. Pattern of Minimum Inhibitory concentration (MIC) of both extracts against all the pathogens were different which is possibly due to the presence of different phytoconstituents attribute to their antimicrobial activity.

Keywords:-Boerhavia diffusa, ZOI, MIC.

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1. Introduction

In many developing countries, there has been an increased interest in the study of different extracts obtained from traditionally used medicinal plants as potential sources of new antimicrobial agents. Naturally obtained antimicrobials are major area of interest among researchers as they are safe and inexpensive to use[1]. The genus Boerhaavia L. (Family: Nyctaginaceae) consists of 40 tropical and sub-tropical species found growing wild in different terrestrial habitats, ranging from managed grasslands, wastelands, agroecosystems to large forest gaps[2]. The plant was named in honour of Hermann Boerhaave, a famous Dutch physician of the 18th century[3]. The whole plant and preferably the roots are effectively used to cure several diseases including Jaundice and several other ailments related to liver as hepatoprotective potential of the root is well established[4]. Several other pharmacological and therapeutic uses of plant include Antiproliferative and antiestrogenic properties[5], Hypoglycemic[6] and Adaptogenic[7]properties etc . Present study is an effort to explore antibacterial potential of plant to provide a scope to develop newer chemotherapeutic agent.

2. Material and Methods.

2.1 Plant Material: Leaves of boerhavia diffusa was collected from Amer forest region, Jaipur. And Authenticated by Dept. of botany, Rajasthan University, Jaipur.

2.2 Culture of microorganisms

Pure cultures of all experimental bacteria were obtained from the Microbial Culture collection Division established at CIRD (Centre for innovation, research and development) of Dr. B. L Institute of Biotechnology, Jaipur.

2.3 Extraction Procedure

The collected material was dried in the shade for 5 weeks away from sunlight. The dried material was ground to a coarse powder and extracted (100 g) successively with 600 ml methanol in a Soxhlet extractor at 130^0C for 24-30 hrs. The extract was concentrated by using rotary evaporator to yield a light brown solid. The extract was preserved in a desiccator till further use.

For aqueous extract preparation, 100grams of leaves powder were added to 100 milliliters of boiling distilled water. The resulting mixture was filtered twice on White cotton and once on Whatman filter paper N0-4. The Filtrate obtained is preserved at temperature of 40°C in an oven for drying. The concentrated filtrates were used for further studies at different concentrations[8].

2.4 Preparation of test/stock solution

Test compounds were prepared by taking two different concentrations viz. 50 mg/ml and 100 mg/ml.

2.5. Preparation of Sub-culture:

One day prior to these testing inoculations of the above bacterial cultures were Made in the nutrient agar and incubated at 37˚C to 24 hours.

2.6. Preparation of inoculums:

A loopful of the organisms was emulsified in 100 ml sterile growth media under proper sterile conditions and incubated for 72 hrs at 37˚C.

2.7. Preparation of Agar plates:

The Petri dishes which measures around 90 mm in diameter, 4mm thickness were selected after sterilizing by dry heat in an oven. Base layer was obtained by pouring around 30 ml of Brain Heart Infusion Agar to obtain a approximately Thickness of 4mm(Total 360 ml agar is used for the study). The Petri plates then Kept aside for solidification. Overnight grown sub culture was taken into definite volumes of

peptone water and incubated at 37˚C at least for 4 hours prior to plating. After incubation, with the help of cotton swab the organisms were streaked on Petri dish containing base layer medium.

2.8 CUP PLATE METHOD[9, 10]:-

The sterile borer was used to prepare 4 cups of 8mm in diameter. One cup for standard two for 50 mg/ml and 100mg/ml conc. of test compound and one for negative control. All the plates were kept at room temperature for effecting diffusion of the test drug and standard. Later, they were incubated at 37˚C for 24 hours. The presence of definite zones around the cup of any size indicated antibacterial activity. The diameter of the zone of inhibition was measured and recorded.

2.9 MIC (MINIMUM INHIBITORY CONCENTRATION) Method[11]:

Ten borosilicate sterile glass test tubes were taken and placed in test tube stand. 200 µl of Brain Heart Infusion Broth solution were taken in test tubes serially numbered as 1-10. To the first test tube, 200 µl of stock solution was added; it must be pipette in and out to ensure proper mixing. From the first test tube, 200 µl of mixture was pipette out, mixed in second test tube, like wise from each test tube 200µ l of mixture taken out and added to next test tube in order till the end test tube. After mixing in 10th test tube, 200 µl of solution was pipette out and discarded. The suspension of the organisms of 105 CFU/ml in Brain Heart Infusion Broth was prepared and then 200 µl was inoculated to each test tube from 1st to 10th. Same procedure was followed for all test tubes. All test tubes were incubated at 37˚C for 24 hours, without shaking or agitation. After 24 hours of incubation all test tubes were read for MIC, turbidity indicates that the bacterial growth has not been inhibited by the concentration of the preparation contained in the medium. Total volume in each tube is 400 µl. Final concentration ranges between 125 mg/ml, 62.5 mg/ml, 31.25 mg/ml, 16 mg/ml, 8 mg/ml, 4 mg/ml, 2.0 mg/ml, 1.0 mg/ml, 0.5 mg/ml, and .25 mg/ml.

Clear solution indicates – Sensitive

Turbidity indicates - - Resistance

Statistical analysis

The values of antimicrobial activity of *Boerhavia diffusa* leaves extracts were expressed as mean ± standard error mean (n= 3) for each sample.

3 RESULTS AND DISCUSSION

Both Methanolic and aqueous extract of the plant showed a considerable antibacterial activity against all the selected pathogens, The highest zone of inhibition of Methanolic extract was observed against **Pseudomonas aeruginosa (24.33 mm)** and lowest inhibitory zone was **10.33** against **E .coli**, But in the case of Aqueous extract ZOI against both pathogens **Pseudomonas aeruginosa** and **E.coli** were almost same. The lowest inhibitory zone in the case of aqueous extract was observed against **Klebsiella pneumoniae (19.33)** and highest against **Staphylococcus aureus (22 mm).** Highest MIC value in MEBD was observed against **Staphylococcus aureus 31.25 mg/ml** and lowest MIC value was observed against **Pseudomonas aeruginosa 2 mg/ml** which is remarkably same against **Staphylococcus aureus** in case of Aqueous extract.

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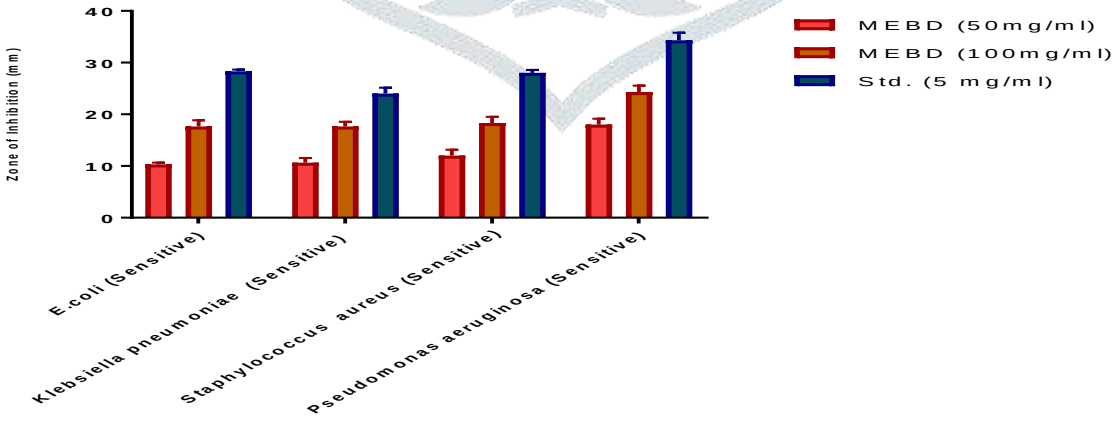
NAME OF ORGANISM	ZOI (mm)		
	TEST (B.D)		STD
	50mg/ml	100mg/ml	5mg/ml
E.coli (Sensitive)	10.33±.33	17.66±1.20	28.33±.33
Klebsiella pneumoniae (Sensitive)	10.66±0.88	17.66±.88	24±1.15
Staphylococcus aureus (Sensitive)	12±1.15	18.33±1.20	28±0.57
Pseudomonas aeruginosa (Sensitive)	18±1.15	24.33±1.21	34.33±1.45

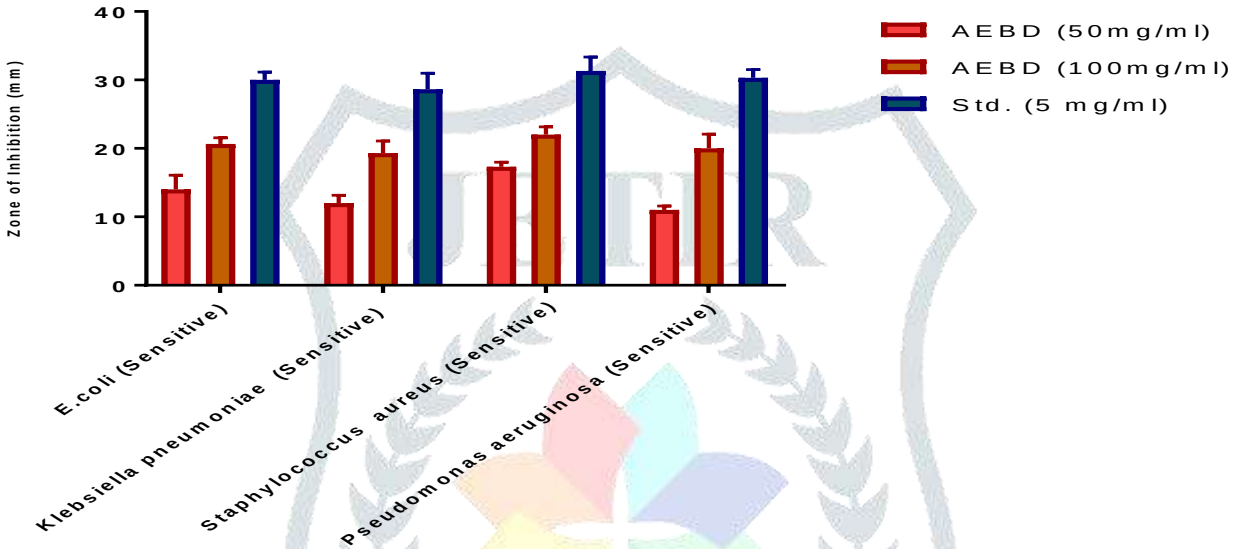
Table1. : - Antimicrobial activity of Methanolic extract of boerhavia diffusa. Values are expressed as mean ± sem, n=3. ZOI*=Zone of Inhibition.



Graph 1. Antimicrobial activity of Methanolic extracts of boerhavia diffusa.

NAME OF ORGANISM	ZOI (mm)		
	TEST (B.D)		STD
	50mg/ml	100mg/ml	5mg/ml
E.coli (Sensitive)	14±2.08	20.66±0.88	30±1.15
Klebsiella pneumoniae (Sensitive)	12±1.15	19.33±1.76	28.66±2.33
Staphylococcus aureus (Sensitive)	17.33±0.66	22±1.15	31.33±2.02
Pseudomonas aeruginosa (Sensitive)	11±0.57	20±2.08	30.33±1.20

Table2. : - Antimicrobial activity of Aqueous extract of boerhavia diffusa. Values are expressed as mean ± sem, n=3. ZOI*=Zone of Inhibition.



Graph 2. : - Antimicrobial activity of Aqueous extract of boerhavia diffusa.

S.NO	MICROBES	125 mg/ml	62.5 mg/ml	31.25 mg/ml	16.6 mg/ml	8.3 mg/ml	4.01 mg/ml	2.0 mg/ml	1.0 mg/ml	0.5 mg/ml	0.25 mg/ml
1	E. coli	S	S	S	S	R	R	R	R	R	R
2	Klebsiella pneumoniae	S	S	S	R	R	R	R	R	R	R
3	Staphylococcus aureus	S	S	S	S	S	S	R	R	R	R
4	Pseudomonas aeruginosa	S	S	S	S	S	S	S	R	R	R

Table 3: - Evaluatiom of Minimum Inhibitory concentration of Methanolic extract of boerhavia diffusa.

S.NO	MICROBES	125 mg/ml	62.5 mg/ml	31.25 mg/ml	16.6 mg/ml	8.3 mg/ml	4.01 mg/ml	2.0 mg/ml	1.0 mg/ml	0.5 mg/ml	0.25 mg/ml
1	E. coli	S	S	S	S	S	R	R	R	R	R
2	Klebsiella pneumoniae	S	S	S	R	R	R	R	R	R	R
3	Staphylococcus aureus	S	S	S	S	S	S	S	R	R	R
4	Pseudomonas aeruginosa	S	S	S	S	R	R	R	R	R	R

Table 4:- Evaluation of Minimum Inhibitory concentration of Aqueous Extract of boerhavia diffusa.

4. Conclusion:-

This study led to the conclusion that the antimicrobial activity of the Methanolic and aqueous extract of Plant boerhavia diffusa was satisfactory against the bacteria E. coli, Klebsiella pneumoniae, Staphylococcus aureus and Pseudomonas aeruginosa. It further elucidated that secondary metabolites from medicinal plant parts might be responsible for the antibacterial activity of the plant extracts.

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