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Antioxidant and Antibacterial Activity of *Boerhavia diffusa* against Gram Negative UTI causing Bacteria

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Abstract: Due to the use of Boerhavia diffusa in traditional medicine against kidney diseases and infectious processes, this study evaluated the in vitro antimicrobial potential, phytochemical composition and antioxidant activity of extracts from its stem and leaves. From leaves and stems of Boerhavia diffusa different extracts were obtained (ethanol: BDE , n-butyl alcohol: BDB). The antimicrobial activity was evaluated by the disc diffusion method using different concentrations against bacteria clinically isolated samples from UTI patients. Preliminary phytochemical screening of ethanol extracts of Boerhavia diffusa showed the presence of Flavanoids, Terpenoids, Saponins, absence of alkaloids, Tannins, Phenol, Anthocyanin, Carbohydrates. In the DPPH radical scavenging assay, the result shows as different concentration 12.5µg/mL, 25µg/mL,100µg/mL, 200µg/mL in both plant extracts with percentage of inhibition. These results encourage the identification of active substances which could be used as lead molecules in the development of new antimicrobial drugs for the treatment of UTI causing bacteria.
Keywords: Boerhavia diffusa , Antimicrobial, UTI, Phytochemicals, antioxidant.

I. INTRODUCTION

Medicinal plants have been a source for healing and medicine in local communities around the world for thousands of years ago. According to the World Health Organization 80% of the world's people depend on traditional medicine for their primary healthcare (Newman, Cragg, and Snader 2003, Bamola et al.,2017). Ethno-medicinal plant products with phytochemicals like flavonoids, tannins, terpenoids, polyphenols, steroids,alkaloids, chlorophyll, carotenoids, proteins, minerals, vitamins have strong antioxidant properties, cost effective and have minimal side effect or toxicity (Poddar *et al.*, 2020). Medicinal plants are considered a repository of numerous types of bioactive compounds (Hemalatha *et al.*, 2016).

UTI or urinary tract infection is an infection in any part of the urinary system. The urinary system includes the kidneys, ureters, bladder and urethra. Most infections involve the lower urinary tract such as the bladder and the urethra. Women are at greater risk of developing a UTI than men. Nowadays antibiotics are the typical treatment for UTI infections. Most infection arise from *E. coli, Proteus mirabilis, P. aeruginosa, Streptococcus* faecalis, S.aureus,*Klebsiella pneumonia, M.tuberculosis,* Candida etc. Patients with catheters or those who undergo urinary surgery and men with enlarged prostates are at higher risk for UTIs (M. komala *et al.*, 2013).

Boerhavia diffusa is a herbaceous plant belongs to Nyctaginaceae family commonly known as Punarnava. It is commonly seen in moist, wet places, grasslands, agricultural fields,waste lands, residential compounds ,ditches and marshy areas. The plant consists of 40 species distributed in tropical and subtropical regions and warm climate .The macroscopic character of the stem of the plant is greenish purple, slender, cylindrical and swollen at nodes. Branches from common stalks are about 1 m in length. Roots are elongated, fusiform.

They are cream or light brownish yellow with soft skin. Leaves are ovate, apex round ,unequal pairs, greenish color, thick in texture. Leaves size is larger one 25-37 nm long, smaller 12-18 mm long. Flowers are very small, pink in color, funnel shaped, arranged on slender, long stalks. Whole plant of *Boerhavia diffusa* is devoid of fragrance and taste is bitter, Glycosides, steroids, flavonoids, polyphenolic compounds are abundant in the plant (Nayak P *et al* 2016,Agrawal A *et al.*, 2004).

II. MATERIALS AND METHODS

A. Collection Of Plant Material

The leaves and stem of *Boerhavia diffusa* were collected from Sree Narayana Guru College,KG Chavadi, Coimbatore, Tamil Nadu. The collected leaves were washed with tap water and shade dried for one to two weeks. The dried leaves are powdered and stored in airtight containers.

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B. Preparation of Plant extract

30gm of *Boerhavia diffusa* plant extract were weighed separately in two conical flasks with 200 mL ethanol and n-butyl alcohol and was allowed to stand for 72 hours at the rotary shaker. Afterwards, each extract was filtered using Whatman No.1 filter paper. The extracts were dried and stored in eppendorf tubes at 4°C for further studies (Handa et al. 2008).

C. Phytochemical Screening

The phytochemical analysis of the leaf extracts were carried out to determine the presence of alkaloids, flavonoids, terpenoids, saponins, phenols, tannins, anthocyanin, carbohydrates. (Raaman et al.,2006, Apurba et al., 2012,Wajid et al., 2017).

D. Collection And Characterization Of Clinical Samples

The clinical samples of Urinary tract infected patients were collected from the hospital. The cultures were then subjected to gram staining and biochemical tests were carried out for further identification.

E. Antibacterial assay

Antibacterial activity of ethanol and n -butyl alcohol plant extracts of *Boerhavia diffusa* was determined by using agar disc diffusion method. Different concentrations of plant extract were loaded into the sterile discs (40µL, 60µL, 80µL,100µL,120µL)and placed onto the swabbed plate. All plates were incubated at 37°c for 48 hrs. Clearance zones around the discs were noted and measured in millimeters (Girish HV 2008).

F. Antioxidant Assay

The radical scavenging activity of different extracts was determined by using DPPH assay according to Chang et al., (2001).The decrease in the absorption of the DPPH solution after the addition of an antioxidant was measured at 517 nm. Ascorbic acid (10mg/ml DMSO) was used as reference. Different concentrations of sample such as 12.5µg/mL- 200µg/mL from stock solution were made up to a final volume of 20µl with DMSO and 1.48ml DPPH (0.1mM) solution was added. The absorbance of the mixture was read at 517 nm (Bhuvaneswari et al. 2014). 3 ml of DPPH was taken as control (Braca et al. 2001).

$$(\%)\ \text{Percentage of inhibition} = \frac{\text{Control} - \text{Test Sample}}{\text{Control}} \times 100$$

III. RESULTS AND DISCUSSION

A. Collection And Extraction Of Plant Material

The whole plant material of *Boerhavia diffusa* was powdered and extracted using the shaker flask method.

B. Qualitative Phytochemical analysis

Phytochemical constituents present in BDE and BDB extract of *Boerhavia diffusa* were tabulated in Table 1. Preliminary phytochemical screening of both plant extracts of *Boerhavia diffusa* showed the presence of various bioactive compounds.

Table 1: Phytochemical Analysis of plant extract of *Boerhavia diffusa*

S.No	Constituents	BDE	BDB
1	Alkaloids	-ve	-ve
2	Flavonoids	+ ve	-ve
3	Terpenoids	+ ve	-ve
4	Saponins	+ ve	+ ve
5	Phenol	+ ve	-ve
6	Tannins	+ ve	-ve
7	Anthocyanin	-ve	+ ve
8	Carbohydrates	-ve	+ ve

-ve Negative , +ve Positive

C. Collection and characterization of clinical sample

The bacterial samples were cultured and subjected for various biochemical tests. The results were tabulated in Table 2.

Table 2: Biochemical characterization of clinical isolates.

Biochemical tests	*Serratia spp*	*Acinetobacter spp*	*E.coli*	*Klebsiella spp*
Gram stain	Gram negative	Gram negative	Gram negative	Gram negative
Indole	- ve	- ve	+ve	- ve
Methyl red	- ve	- ve	+ve	- ve
Voges proskauer	+ve	- ve	- ve	+ve
Citrate	+ve	+ve	- ve	+ve
Urease	+ve	- ve	- ve	+ve
TSI	Acid/alk/gas	Alk/alk	A/A/gas	A/A
Catalase	+ve	+ve	+ve	+ve
Oxidase	- ve	- ve	- ve	- ve

+ve Positive -ve Negative

D. Antibacterial Activity

Antibacterial activity was carried out using disc diffusion method against gram negative UTI causing bacteria and the results were shown in Table 3. The present study showed that ethanol and n-butyl alcohol extracts *Boerhavia diffusa* with stem and leaves and has high antimicrobial activity. Among the exacts tested the ethanol extracts exerted highest activity on bacterial strain when compared to n-butyl-alcohol extract. This is because different solvent have diverse solubility capacities for different constituents.

Table 3: Antibacterial screening of plant extracts against some UTI causing bacteria

Sl no	Organism	Sample Name	40µL	60µL	80µL	100µL	120µL
1	*Serratia* spp	BDE	2mm	2mm	6mm	10mm	9mm
		BDB	1mm	3mm	5mm	8mm	3mm
2	*Acinetobacter* spp	BDE	3mm	3mm	5mm	7mm	7mm
		BDB	2mm	2mm	2mm	2mm	3mm
3	*E .coli*	BDE	3mm	3mm	5mm	9mm	8mm
		BDB	2mm	2mm	3mm	5mm	4mm
4	*Klebsiella* spp	BDE	2mm	4mm	4mm	6mm	6mm
		BDB	5mm	5mm	8mm	8mm	10mm

BDE-Ethanolic extract of *B.diffusa*, BDB- n Butyl alcoholic extract of *B.diffusa*

E. Antioxidant Assay

The radical scavenging activity of different extracts was determined by using DPPH assay. The results are shown in table 4 and figure 1.

Table 4:Antioxidant Activity of plant extract

Concentration mg/ mL	Inhibition % of BDE	Inhibition % of BDB	Inhibition % of Ascorbic Acid
12.5	8.08	4.91	26.82
25	19.67	17.62	51.88
50	30.23	24.04	65.92
100	48.24	44.02	81.96
200	69.87	62.25	94.04

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Fig 1: DPPH assay of BDE and BDB

The ethanol extract of *Boerhavia diffusa* shows maximum % of inhibition as compared to n- butyl alcohol extract of *Boerhavia diffusa* with standard of ascorbic acid.

IV. CONCLUSION

The wide variety of microorganisms show resistance to antibiotics and multiple drug resistance are emerging and these organisms cause serious threat to the treatment of infectious disease. The stem and leaf extracts of *Boerhavia diffusa* are prepared using different solvent such as ethanol and n-butyl alcohol .The two plant extracts were studied for their antibacterial activity by disc diffusion method. In case of both gram negative and gram positive bacteria show maximum antibacterial activity. Both plant extracts show the presence of bioactive compounds which are responsible for antioxidant, anti-bacterial properties such as alkaloids, flavonoids etc .This is an indication that there are possibilities of alternative antibiotic substance in these plants for the development of newer antibacterial agents. These extracts show broad spectrum antimicrobial activity and thus can be used for the development of drugs. This can be used to control the variety of bacterial and fungal infections. Plants are valuable sources for new compounds and should receive special attention in research strategies to develop new antimicrobials urgently required in the future.

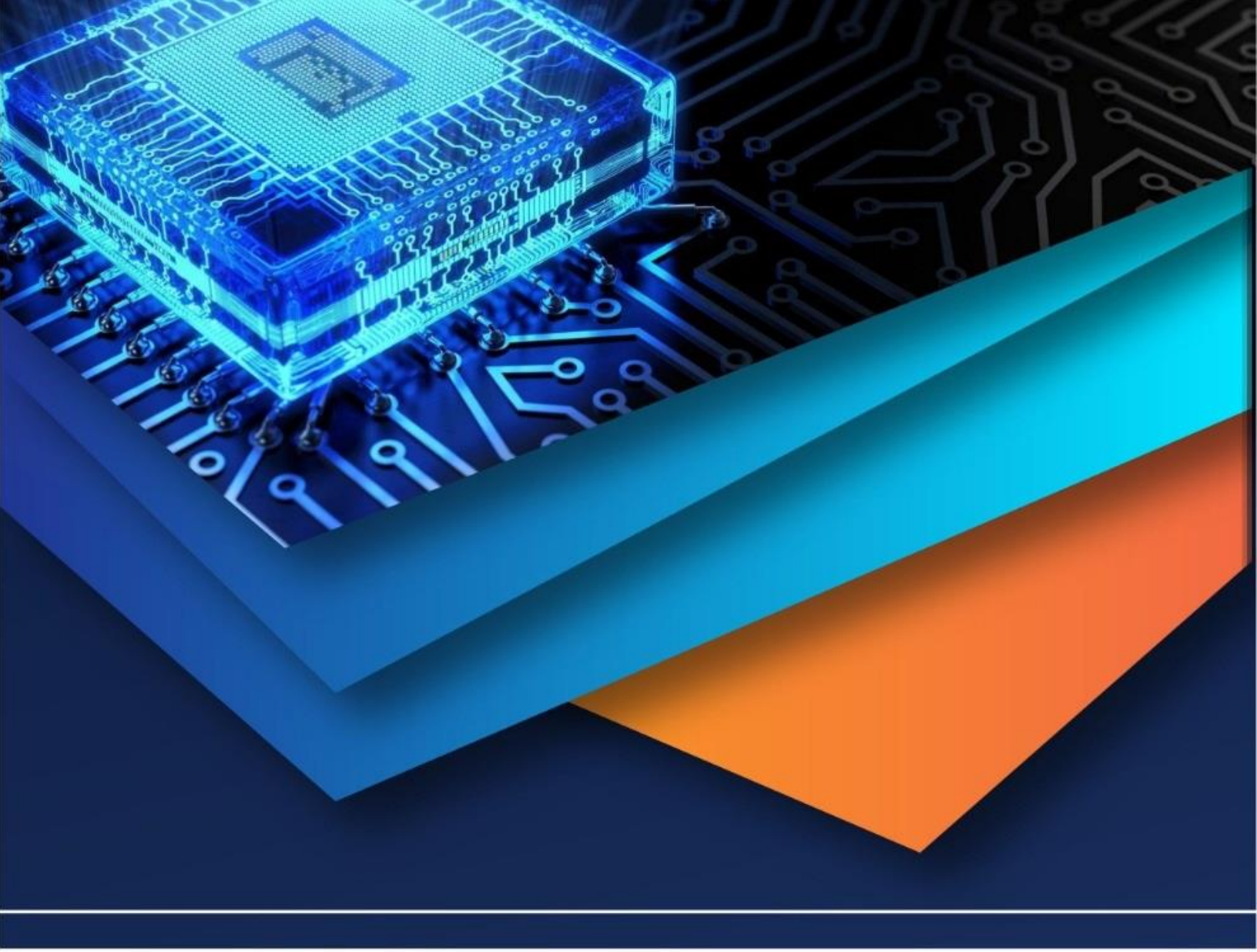
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