Phytochemical Screening and Antibacterial Effect of *Boerhaavia diffusa*

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ABSTRACT

UTI is one of the serious health problem throughout the world and development of antibiotic resistance is a worldwide problem that has led to the need for development of novel antimicrobials and use of natural products which is safe and effective. Bioactive compounds of Medicinal plants have proven to be a potential and effective cure against many diseases since centuries. The objective of this study is to analyze phytoconstituents and antibacterial property of ethanol extract of *Boerhaavia diffusa* whole plant against multi-drug resistant UTI pathogens isolated from urine samples collected from patients suffering from UTI. Antimicrobial susceptibility test of 515 urinary isolates against 12 different antibiotics was performed to select multi drug resistant bacterial species and 7 different highly resistant bacterial species were selected for this study. Preliminary phytochemical analysis and *in-vitro* antibacterial activity was screened by well diffusion method against selected MDR bacterial species. MIC values were also determined to find minimum effective concentration. The ethanol extract showed good antibacterial activity against all selected MDR uropathogens. Qualitative phytochemical test showed presence of alkaloid, tannin, terpenoids and cardiac glycosides. Results of the present study showed that *B. diffusa* can be used as a potential source for drug development for the treatment of Urinary Tract Infection caused by multi drug resistant bacteria.

Key words: **UTI, Antibacterial activity, Phytochemical analysis, *Boerhaavia diffusa*, MIC**

I. INTRODUCTION

Plant's secondary metabolites have already demonstrated their potential as antimicrobials when used alone as synergists of other antimicrobial agents. Natural products, either as pure compounds or as standardized plant extracts, provide unlimited opportunities for new drug leads because of the unmatched availability of chemical diversity (Rojas *et. al*., 2003). Therefore researchers are increasingly turning their attention to folk medicine (Benkeblia, 2004). Even the World Health Organization (WHO) supports the use of medicinal plants, provided it is proven to be efficacious and safe (WHO 1995). The increasing failure of chemotherapeutics and antibiotic resistance exhibited by pathogenic microbial infectious agents has led to the screening of several medicinal plants for their potential antimicrobial activity (Colombo and Bosisio, 1996). A large number of plants are being used as medicinal agent all over the world (Choudhury *et. al.,* 2012).

Genus *Boerhaavia*, consisting of 40 species, is distributed in tropical and subtropical regions and warm climate. Among 40 species of *Boerhaavia*, 6 species are found in India, namely *B. diffusa, B. erecta, B. rependa, B. chinensis, B. hirsute* and *B. rubicunda*. *B. diffusa*, in India is found in warmer parts of the country and throughout up to 2,000 m altitude in the Himalayan region. (Mahesh *et. al*., 2012). Common name of *B. diffusa* is Hog weed, Pig wid, Punar-nava, Punerva, Punnarnava, Purnoi, Satodi, San, Santh, Santi, Satadi thikedi, Thikri. Common nameof B. *diffusa* are Hog weed, Pig wid, Punar-nava, Punerva, Punnarnava, Purnoi, Satodi, San, Santh, Santi, Satadi thikedi, Thikri.

Roots, leaves, seeds and whole plant is used for medicinal purpose (Pankaj, 2011). *Boerhaavia diffusa* is one of the renowned medicinal plants used to treat large number of human ailments as mentioned in Ayurveda, Charaka Samhita, and Sushrita Samhita. Plant in whole or its peculiar parts (Aerial parts and Roots) have a numerous medicinal properties and are used by endemic and tribal people in India and Unani medicine in Arab countries to show anti-bacterial, antinociceptive, hepato-protective, hypoglycemic, antiproliferative, antiestrogenic, antiinflammatory, anticonvulsant, antistress and antimetastatic activities and also in treatment of stress, dyspepsia, abdominal pain, inflammation, jaundice. Generally whole plant contains Punarnavine (Alkaloids), ß sitosterol (Phytosterols), Liriodendrin (lignans), Punarnavoside (Rotenoids), Boerhavine (Xanthones) and Potassium nitrate (Salts) phytochemical constituents (Mahesh *et. al*., 2012).

Figure 1.1: Photographs of leaves, flower and root of *Boerhaavia diffusa*.

Urinary Tract Infection (UTI) is one of the serious health problem affecting millions of people every year. It is the second most common infection. UTIs are more common in persons aged 20-50 years (Griffiths, 2003). Women are more likely to get UTI than men because of their urinary tract's design. Nearly half of all women will have a UTI at some point in their lives (Foxman, 2003). Several potent antibiotics are available for treatment of UTI, but increasing drug resistance among bacteria has made therapy of UTI difficult. Bacteria have the genetic ability to transmit and acquire resistance to drugs (Soulsby, 2005). One major drawback to the use of antibiotic is the potential for development of antibiotic resistance among uropathogens (Head, 2008). The increasing prevalence of antibiotic resistant bacteria, escalating costs of antibiotic therapy and unsatisfactory therapeutic alternatives in recurrent UTIs have developed an interest in novel, non antibiotic based methods for preventing and controlling UTIs (Vaughan, 2007).

Despite the wide therapeutical use of *B. diffusa*, there are still not enough Scientific data in the literature which clearly demonstrate the existance of an antimicrobial activity and explaination for mechanism of its action (Ramchandra *et. al*., 2012). This study was conducted to investigate bioactive potential and phytochemical screening of *B. didffusa* whole plant ethanol extracts against multi-drug resistant (MDR) uropathogens isolated from urine sample of patients suffering from UTI to develop alternative drug development to treat UTI..

II. MATERIALS AND METHODS

2.1. Collection of urine samples and Selection of MDR bacterial strain from urine:

Urine samples from the patients suffering from UTI were collected from various laboratories: Bhanumati laboratory and Parsi Hospital, Navsari and Advanced Diagnostic Laboratory, Surat. The isolated bacterial UTI pathogens were identified on the basis of gram staining, morphological and biochemical characteristics (Holt *et. al.*, 1994).

From the identified bacterial isolates, MDR bacterial strain was selected by performing antibiotic susceptibility test (Baris *et. al.*, 2005) using Pathoteq 'Bio-Disc-12' (Pathoteq Biological Laboratories, India), which includes 12 antibiotics (Ampicillin/Sulbactam, Co-trimoxazole, Ceftizoxime, Chloramphenicol, Cephalexin, Tetracycline, Ciprofloxacin, Nitrofurantoin, Sparfloxacin, Gatifloxacin, Norfloxacin and Ofloxacin) used for treatment of UTI. The zones of growth inhibition were then measured. The diameter of the zone is related to the susceptibility of the test microorganism and to the diffusion rate of the drug through the agar medium. The results were interpreted using the criteria of the Clinical and Laboratory Standards Institute (CLSI, 2007).

2.2. Plant Extraction:

Boerhaavia diffusa was collected from Vaghai Botanical Garden, Vaghai, India. Taxonomic identification of the plant was confirmed by Dr. B. K. Dhaduk, Horticulture Department, Agriculture University, Navsari. The plant material used for the study was washed under running tap water thoroughly, air dried and homogenized to fine powder and 10 g of powdered plant material was extracted with 150 ml ethanol using Soxhlet extraction apparatus (Superfit Continental Pvt. Ltd.) for 8 h. The extract was concentrated and extractive % yield was calculated using following formula (Kepam, 1986):

Extractive %yield = Weight of final extract / Weight of Powdered sample x 100.

The dried extracts were re-dissolved in minimum volume of DMSO and then preserved in refrigerator for further studies.

2.3. Preliminary phytochemical analysis:

The plant extract was subjected to preliminary phytochemical analysis to study the presence of phytoconstituents *viz*., alkaloids, tannins, saponin, anthocyanide, phenolic flavonoids, flavonoids, carbohydrate, protein, terpenoids, cardiac glycosides, oil by standard methods described by Trease and Evans (1989).

2.4. Determination of antibacterial activity and MIC:

The ethanol extract of *B. diffusa* was subjected to antibacterial screening test by well diffusion assay (Magaldi *et. al*., 2004). The plates containing Muller Hinton Agar medium were inoculated with the selected MDR UTI pathogens and 8 mm wells were prepared. The test well was filled with 100 µl of *B. diffusa* ethanol extract and control well with methanol respectively. Plates were then incubated at 37 ^{0}C for 24 h. Antibacterial activity was evaluated by measuring the zone of inhibition against the selected MDR UTI pathogens. The experiment was performed in triplicate and the mean of zone diameter was calculated. MIC was also determined by well diffusion assay to find the minimum concentration required to serve as an antibacterial agent.

III. RESULTS AND DISCUSSION

3.1 Selection of MDR bacterial strain from urine:

In this study, 550 mid-stream urine samples were collected. Out of which, 543 uropathogens were recovered. Out of 543 isolates, 515 isolates were bacteria and 28 isolates were found to be *Candida albicans*. As the aim of the study was focused to check the antibacterial activity of medicinal plants, only bacterial isolates were used for further studies. The urinary isolates were identified based on morphological and biochemical characteristics. According to the current study, gram negative bacteria were responsible for 100% of UTIs.

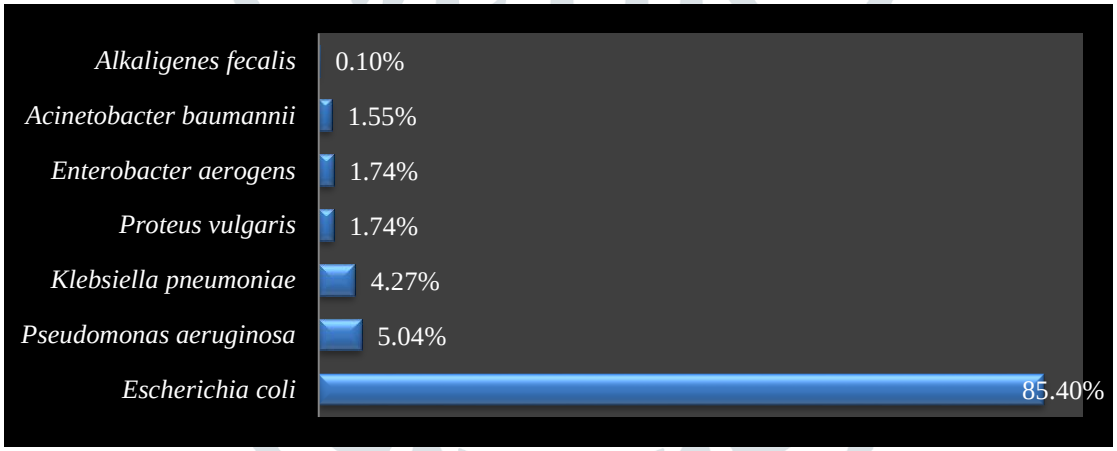

Figure 3.1: Percentage occurrence of bacterial pathogens in UTI

This study reported *Escherichia coli* was the most predominant uropathogen with 85.40%, followed by *Pseudomonas aeruginosa* 5.04%, *Klebsiella pneumonia* 4.27%, *Proteus vulgaris* and *Enterobacter aerogens* 1.74%, *Acinetobacter baumannii* 1.55% and *Alkaligenes fecalis* 0.10% (Figure-3.1). Studies carried out by the University of Florida, USA, with 81 patients having UTI. It was found that 89% of infection was due to *Escherichia coli*, 3.7% due to *Klebsiella*, 1.2% due to *Proteus*, 1.2% due to *Citrobacter*, 1.2% due to *Staphylococcus* and *Enterococcus* 3.7%.

All the identified isolates were studied for antibiotic resistance profile to select MDR UTI pathogens. The results are shown in Table-3.1. *K. pneumoniae* showed maximum resistance, was sensitive to only 2 antibiotics, Nitrofurantoine and Gatifloxacin. *E. coli* showed sensitivity against three antibiotics i.e. Nitrofurantoin, Gatifloxacin and Chloramphenicol. *P. vulgaris* was also sensitive to three antibiotics; Nitrofurantoin, Sparfloxacine and Gatifloxacin while *A. baumannii* was sensitive to Co-trimoxazole, Chloramphenicol, Sparfloxacin and Gatifloxacin. Minimum resistance was observed with *E. aerogens* which showed sensitivity to all tested antibiotics except Co-trimoxazole and Norfloxacin followed by *A. fecalis* which was resistant to only four antibiotics i.e. Ampicillin/Sulbactam, Co-trimoxazole, Ceftizoxime and Tetracycline. Comparing the effect of all antibiotics on the tested seven bacterial isolates, Gatifloxacin was the only drug which was effective on all the selected UTI pathogens followed by Nitrofurantoin which was effective on all bacterial isolates except *A. baumannii* and Chloramphenicol which showed effectivity on all except *P. vulgaris* and *K. pneumoniae.*

Table 3.1: Antibiotic resistance profile of urinary isolates

Sr. No.	Name of antibiotic	Diameter of zone of inhibition (mm)						
		A.b.	***A.f.***	***E.a.***	***P.v.***	***K.p.***	***E.c.***	***P.a***
1.	**Ampicillin/ Sulbactam**	00	00	27	00	00	00	00
2.	**Co-trimoxazole**	30	00	10	00	00	00	00
3.	**Ceftizoxime**	00	00	00	00	00	00	00
4.	**Chloramphenicol**	11	14	20	00	00	11	21
5.	**Cephalexin**	00	20	16	00	00	00	00
6.	**Tetracycline**	00	00	16	00	00	00	17
7.	**Ciprofloxacin**	00	19	10	00	00	00	00
8.	**Nitrofurantoin**	00	10	15	19	10	17	18
9.	**Sparfloxacin**	15	21	10	10	00	00	12
10.	**Gatifloxacin**	11	22	15	11	11	11	14
11.	**Norfloxacin**	00	17	00	00	00	00	00
12.	**Ofloxacin**	00	18	12	00	00	00	11

A.b.-Acinetobacter baumannii, A.f.-Alkaligenes fecalis, E.a.-Enterobacter aerogens, P.v.-Proteus vulgaris, K.p.-Klebsiella pneumoniae, E.c.-Escherichia coli, P.a.-Pseudomonas aeruginosa.

3.2 Extractive yield:

The quantity and composition of metabolite of an extract depends on type of extraction, time of extraction, temperature and nature, concentration and polarity of the solvent (Ncube *et. al.*, 2008). In the present study, powder of whole plant *B. diffusa* was extracted with ethanol by Soxhlet extraction method showing extractive yield of 3.38%. The extractive %yield depends on the phytoconstituents present in plant and their solubility in a particular solvent.

3.3. Preliminary phytochemical analysis:

Phytochemical screening of ethanol extract of the *B. diffusa* showed presence of phytoconstituents such as alkaloid, tannin, terpenoids and cardiac glycosides. Flavonoids, carbohydrates, protein, saponin, anthocyanide, phenolic flavonoids and oil were found absent. The results are shown in Table-3.2.

Table 3.2: Preliminary phytochemical analysis of *B. diffusa* ethanol extracts

Sr. No.	Phytochemicals	***B. diffusa*** **Ethanol Extract**
1.	Alkaloid	+
2.	Tannin	+
3.	Saponin	-
4.	Anthocyanide	-
5.	Phenolic flavonoids	-
6.	Flavonoids	-
7.	Carbohydrate	-
8.	Protein	-
9.	Terpenoids	+
10.	Cardiac glycoside	+
11.	Oil	-

"+" = Present and "-" = Absent.

Phytochemical research has demonstrated the presence of alkaloids and amino acids in *B. diffusa* (Garg, 1978). The whole plant analysis of *B. diffusa* is known to contain numerous phytochemical constituents that include flavonoids, alkaloids, triterpenoids, steroids, lipids, lignins, tannins, phlobaphenes and ursolic acid (Jain and Singh, 1994).

3.4 Determination of antibacterial activity and MIC:

Antibacterial activity of *B. diffusa* ethanol extract is represented in Table-3.3 and figure 3.2. All the selected MDR urinary isolates were inhibited by *B. diffusa* ethanol extract and zone of inhibition was not observed with control.

Table 3.3: Antibacterial activity of *B. diffusa* ethanol extract

Sr. No.	Name of organism	Diameter of zone of inhibition in mm
1.	*Acinetobacter baumannii*	13
2.	*Alkaligenes fecalis*	12
3.	*Enterobacter aerogens*	13
4.	*Proteus vulgaris*	13
5.	*Klebsiella pneumoniae*	15
6.	*Escherichia coli*	12
7.	*Pseudomonas aeruginosa*	12
8.	Control	00

Among seven different MDR uropathogens, ethanol extracts of *B. diffusa* showed maximum inhibition zone against *Klebsiella pneumoniae* (15 mm) followed by *A. baumannii*, *Enterobacter aerogens* and *Proteus vulgaris* i.e. 13 mm, and minimum inhibition zone was obtained with *P. aeruginosa*, *A. fecalis* and *Escherichia coli*, i.e. 12 mm.

The zone of the inhibition noted with ethanol extract against *K. pneumoniae* (15 mm) was higher than inhibition zone obtained with Nitrofurantoine (10 mm) and Gatifloxacin (11 mm). Similarly, the zone of inhibition obtained with plant extract against *E. coli* (12 mm) was nearer to the inhibition zone obtained with Gatifloxacin (11 mm) and Chloramphenicol (11 mm) while lower than Nitrofurantoin (17 mm). This demonstrates remarkable antibacterial activity on MDR uropathogens compare to synthetic antibiotics.

Sharma *et. al*. reported in 2010 that potent antibacterial activity against gram positive and gram negative bacteria was shown by the leaves of *B. diffusa*. Ethanol extract showed an inhibitory effect on gram positive bacteria like *S. aureus, B. subtilis, S. faecalis, M. luteus* and all gram-negative bacteria selected for the study. Methanol extract showed inhibitory effect against all gram positive bacteria selected for the study except *M. luteus* and gram negative bacteria like *K. pneumoniae, P. vulgaris, S. marcescens* and *S. flexneri.*

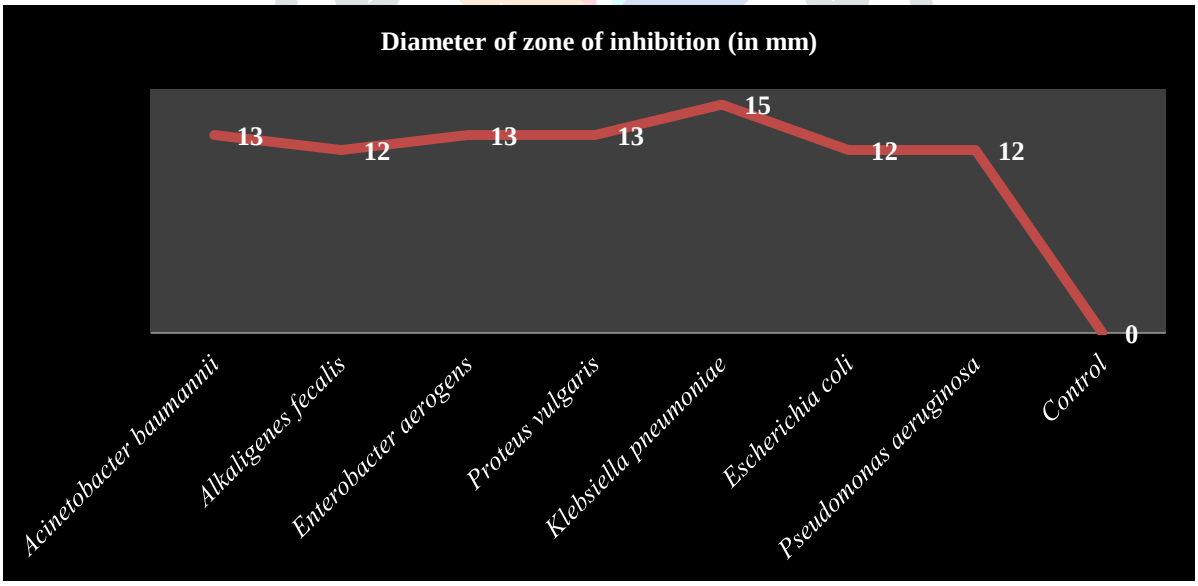

Figure 3.2: Diameter of zone of inhibition of *B. diffusa* ethanol extract and control

Results from the study done by Desai *et. al*., (2011), showed that the aqueous and ethanol extracts of *B. diffusa* had antibacterial activity on *E. coli*, *S. aureus* and *P. aeruginosa*. The four microorganisms namely *E. coli* strains 871, 2142 and C1, *B. subtilis* UC564, *S. aureus* 15, ML296 and ML329, *S. typhi* DI have been used for the study of the antimicrobial activity of the ethanolic extract of the whole parts of *B. diffusa* by Das, (2012). It was reported that the ethanolic extract possessed antimicrobial activity against most of the tested organisms. There is variation in the degrees of antibacterial activity of extracts on urinary isolates which is presumed to be due to difference in the response by isolates to different active compounds present in plant extracts.

Ethanol extract of *B. diffusa* showed MIC of 4.22 mg/ml against *K. pneumoniae* and *E. coli* was inhibited at concentration of 8.45 mg/ml (Table-3.4). The MIC of Nitrofurantoine, Ofloxacine and Chloramphenocol, the potent synthetic antibiotics used for treatment of UTI was also determined as a control against *E. coli* and *K. pneumoniae*. MIC of Nitrofurantoin and Ofloxacin against *K. pneumoniae* was 2.0 mg/ml and 1.25 mg/ml and against *E. coli* 0.25 mg/ml and 1.25 mg/ml respectively which is less then MIC obtained with plant extract. *K. pneumoniae* showed MIC of 0.25 mg/ml against *E. coli* and resistance to Chloramphenicol while it was inhibited by the studied plant extract.

Table 3.4: MIC of *B. diffusa* ethanol extract and synthetic antibiotics

Sr. No.	Name of plant / Antibiotic	MIC in mg/ml	
		K. pneumoniae	*E. coli*
1.	*Boerhaavia diffusa* ethanol extract	4.22	8.45
2.	Nitrofurantoin	2.0	0.25
3.	Ofloxacin	1.25	1.25
4.	Chloramphenicol	R	0.25

IV. CONCLUSION

This research concludes presence of many phytoconstituents in ethanol extract of *Boerhaavia diffusa* which can provide various useful biological activities to this medicinal plant. The results have clearly demonstrated remarkable antibacterial activity as compare to synthetic antibiotics. It may be considered as a potential source of new chemotherapeutic drugs because of their diverse phytochemicals against MDR Urinary Tract Pathogens and this has introduced the plant as a potential material for drug development for the treatment of Urinary Tract Infection caused by multi drug resistant bacteria. Being naturally and widely present along with its cost effectiveness can prove as an advantageous over therapeutic drugs. However there would be the need of further studies whether any single or combination of pure active metabolites would be better, safer and more efficient in treating UTI than the crude extract of whole plant.

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