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IN VITRO ANTIMICROBIAL POTENTIAL OF *BOERHAVIA DIFFUSA* L. ROOT EXTRACT ON PATHOGENIC ORGANISMS

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ABSTRACT

The increasing prevalence of drug-resistant pathogens has gained the attention of pharmaceutical and scientific communities towards potential antimicrobial agents from plant derived sources. The present research work has been undertaken to study the antimicrobial activity of the methanolic extract of *Boerhavia diffusa* L. roots against some human pathogens like *Escherichia coli* MTCC 43, *Pseudomonas aeruginosa* MTCC 424, *Salmonella typhimurium* MTCC 98, *Staphylococcus aureus* MTCC 96, *Shigella flexneri* MTCC 1457, *Streptococcus pneumoniae* MTCC 655, *Klebsiella pneumoniae* MTCC 432 and fungi *Aspergillus niger* MTCC No.282 by using agar well diffusion method. Inhibition zones ranged between 4.26 ± 0.12 - 16.61± 0.24mm. Roots extract inhibited the growth of all tested microorganisms with large zones of inhibition. The standard antibiotics chloramphenicol and miconazole nitrate were found to have zone of inhibitions 10.40 ± 0.26 - 24.80 ± 0.37mm at the concentration of 30 µg/ml. In contrast, the inhibition zone of methanol (negative control) was almost zero for all the tested microorganisms. The spectrum activity of methanolic extract of this plant could be a possible source to obtain new and effective herbal medicines to treat various infectious diseases.

KEYWORDS: Antimicrobial activity, *Boerhavia diffusa* L, methanolic extract, human pathogens, zones of inhibition.

INTRODUCTION

The use of plant and its products has a long history that began with folk medicine and through the years has been incorporated into traditional and allopathic medicine [1]. Since antiquity, many plants species reported to have pharmacological properties as they are known to possess various secondary metabolites like glycosides, saponins, flavonoids, steroids, tannins, alkaloids and terpenes which are utilized to combat the disease causing pathogens [24]. With the advancement in Science and Technology, remarkable progress has been made in the field of medicine with the discoveries of many natural and synthetic drugs [5]. Antibiotics are indisputably one of the most important therapeutic discoveries of the 20th century that had effectiveness against serious bacterial infections [6]. Despite the huge number of antimicrobial agents for various purposes that already exist, the search for new drugs is a continuous task since the target microorganisms often develop new genetic variants which subsequently become resistant to available antimicrobial agents [7-8]. The world's attention is now increasingly directed towards plant sources for developing antimicrobial drugs, since natural products are considered safer than synthetic ones [9-10]. According to the World Health Organization, medicinal plants would be the best source to obtain a variety of drugs [11]. Therefore, such plants should be investigated to better understand their properties, safety and efficacy [12]. There are several published reports describing the antimicrobial activity of various crude plant extracts [13-14]. It is estimated that there are about 2.5 million species of higher plants and the majority of these have not yet been examined for their pharmacological activities [15].

The different herbal plant extracts are traditionally has been used as anticancer antioxidant, antiulcer, analgesic and antidiabetic [16], and they also having the antiparasitic, antifungal, antibacterial, antimalarial activity, analgesic and anti-inflammatory activity [17]. Different species of *Boerhavia* are used as a folk medicine for the treatment of various ailments such as skin diseases. It has been reported that *Boerhavia* possesses anti-nociceptive, Hepato protective, Hypo glycemic, anti-proliferative, anti-estrogenic, anti-inflammatory, anticonvulsant, anti-stress, adaptogenic, immune modulatoryand anti-metastatic activities [18-23]. *Boerhavia diffusa* L. (*Nyctaginaceae*) commonly known as Raktapunarnava, Shothaghni, Kathillaka, Kshudra, Varshabhu, Raktapushpa, Varshaketu, Shilatika, is a perennial

herbaceous plant growing in tropical regions such as the Antilles, South America, India and Africa [24-26]. It is used in ayurvedic medicine system to treat various health problems. One of the most typical exemplary plants of the ayurvedic medicine is *Boerhavia diffusa Linn* [27]. The plant is mentioned in the Atharvaveda with the name 'Punarnava', because the top of the plant dries up during the summer season and regenerates again during the rainy season [28]. The plant was named in honor of Hermann Boer have, a famous Dutch physician of the 18th century [29]. *Boerhavia diffusa* is up to 1m long or more, having spreading branches. The stem is prostrate, woody or succulent, cylindrical, often purplish, hairy, and thickened at its nodes. The leaves are simple, thick, fleshy, and hairy, arranged in unequal pairs, green and glabrous above and usually white underneath. The shape of the leaves varies considerably ovate - oblong, round, or subcordate at the base and smooth above [30].The present research was set up to determine the antimicrobial activity of *Boerhavia diffusa* L. plant extraction against some pathogenic bacteria and fungi.

Fig: 1. *Boerhavia diffusa* L.

MATERIAL AND METHODS

Chemicals and Plant Collection

The following ingredients were used for the preparation of nutrient agar media and Potato dextrose media: Agar, Peptone, Sodium chloride, Beef extract, Potato, dextrose, water. All other chemicals and analytical reagents were purchased from Hi-media, India, unless stated otherwise. Mature plants of *Boerhavia diffusa* L. used for this study was collected from Acharya & BM Reddy College of Pharmacy medicinal garden, Bangalore, India.

Preparation of the Plant Extract

The fresh plant roots of *Boerhavia diffusa* L. were collected in November 2013 from medicinal garden of Acharya & BM Reddy College of Pharmacy, Bangalore, India and authenticated at Institute of Herbal Botany, Plant Anatomy Research Centre, Chennai by Proff. P. Jayaraman with accession no PARE/2013/2159. The plant roots were washed for 2-3 times with tap water and finally with distilled water and air dried in shade for ten days and then dry in an oven at 60ºC for one to two days, and finally milled to a coarse powder (Sieve no 80). 100 grams of powdered material was extracted by maceration in methanol (400 mL) for 14 days with frequent agitation [31-33]. The mixture was filtered through clean muslin cloth followed by double filtration with What man No.1 filter paper and the filtrate was concentrated by rotary evaporation under vacuum (vacuum pressure: 500 N/m^2) at 40ºC until a volume of about 15 mL waste reached. Next the concentrate was poured into glass petri dishes and brought to dryness in an oven at 60ºC. The obtained paste like mass was then stored in parafilm sealed petri dishes in a dark cabinet. The extracts were reconstituted by dissolving in methanol to the required concentrations. The reconstituted extracts were maintained at 2-8 ºC.

Test Microorganisms and Growth Media

Pure cultures of all experimental bacteria; *Escherichia coli* MTCC 43, *Pseudomonas aeruginosa* MTCC 424, *Salmonella typhimurium* MTCC 98, *Staphylococcus aureus* MTCC 96, *Shigella flexneri* MTCC 1457, *Streptococcus pneumoniae* MTCC 655, *Klebsiella pneumoniae* MTCC 432 and fungi *Aspergillus niger* MTCC No.282 were obtained from the Microbial Type Culture Collection and Gene Bank (MTCC), Institute of Microbial Technology (IMTECH), Chandigarh. The pure bacterial cultures were maintained on nutrient agar medium and fungal culture on potato dextrose agar (PDA) medium. Each bacterial and fungal culture was further maintained by sub culturing regularly on the same medium and stored at 4ºC before use in experiments.

Determination of the Antimicrobial Activity

Agar well-diffusion method was followed to determine the antimicrobial activity[34-36]. Nutrient agar (gm/l: beef extract, 3g; peptone, 5g; sodium chloride, 5g; agar, 20g) and Potato Dextrose Agar (39 gm/l) plates were swabbed (sterile cotton swabs) with 24h old-broth culture (10^6-10^8 bacteria CFU ml^{-l}) of respective bacteria and fungi. Wells (10mm diameter and about 2 cm a part) were made in each of these plates using sterile cork borer. Stock

solution of root extract was prepared at a concentration of 100 mg/ml. About 100 µl of root extract was added with sterile syringe into the wells and allowed to diffuse at room temperature for 2h. Control experiments comprising inoculums without root extract.30µg/ml chloramphenicol, and 30µg/ml miconazole nitrate were also used as positive controls for bacteria and fungi, respectively. The plates were incubated at 37°C for 24h for bacterial pathogens and 37°C for 48h fungal pathogens. The diameter of the inhibition zone (mm) around each well was measured and express as antimicrobial activity. Triplicates were maintained and the experiment was repeated thrice, for each replicates the readings were taken in three different fixed directions and the average values were recorded.

Statistical Analysis

The results of the experiment are expressed as mean ± SE of three replicates in each test. The data were evaluated by one-way analysis of variance (ANOVA) followed by Tukey's multiple pair wise comparison tests to assess the statistical significance.

RESULTS AND DISCUSSION

The search for antimicrobials from natural sources has received much attention and efforts have been put in to identify compounds that can act as suitable antimicrobials agent to replace synthetic ones. Phytochemicals derived from plant products serve as a prototype to develop less toxic and more effective medicines in controlling the growth of microorganism [37-38]. These compounds have significant therapeutic application against human pathogens including bacteria, fungi or virus. Numerous studies have been conducted with the extracts of various plants, screening antimicrobial activity as well as for the discovery of new antimicrobial compounds [39-40]. Therefore, medicinal plants are finding their way into pharmaceuticals, neutralceuticals and food Supplements.

In the present investigation, the inhibitory effect of *Boerhavia diffusa* L roots methanolic extract was evaluated against both fungal and bacterial strains. The antimicrobial activity was determined by using agar well diffusion method and the results are summarized in Table 1.Methanolic extract (100.00 mg/ml) of the roots displayed good antibacterial activity against *Escherichia coli* MTCC 43, *Pseudomonas aeruginosa* MTCC 424, *Salmonella typhimurium* MTCC 98, *Staphylococcus aureus* MTCC 96, *Shigella flexneri* MTCC 1457, *Streptococcus pneumoniae* MTCC 655, *Klebsiella pneumoniae* MTCC 432 and fungi *Aspergillus niger* MTCC No.282. Methanolic extract inhibited the growth of all tested microorganisms with large zones of inhibition ranged from 4.26 ± 0.12 - 16.61± 0.24mm.

Table-1: Antimicrobial activity of *Boerhavia diffusa* L.expressed as zone of inhibition (mm).

Microorganism	Zone of Inhibition (mm)		
	***Boerhavia diffusa* L. roots**	**Chloramphenicol***	**Miconazolenitrate***
Staphylococcus aureus (MTCC 96)	10.95 ± 0.31	12.26 ± 0.50	ND
Escherichia coli (MTCC 43)	13.25 ± 0.44	20.22 ± 0.86	ND
Aspergillu sniger (MTCC No.282)	16. 61± 0.24	ND	24.80 ± 0.37
Pseudomonas aeruginosa (MTCC 424)	10.46 ± 0.14	14.82 ± 0.86	ND
Salmonella typhimurium(MTCC 98)	8.91 ± 0.37	10.82 ± 0.34	ND
Shigella flexneri(MTCC 1457)	4.26 ± 0.12	12.32 ± 0.27	ND
Streptococcus pneumoniae(MTCC 655)	8.01± 0.31	14.95 ± 0.37	ND
Klebsiella pneumoniae (MTCC 432)	6.83 ± 0.12	10.40 ± 0.26	ND

ND: Not determined. The inhibition zone diameter was taken as an average value of triplicate plates for each microorganism at 100 µL of 100 mg/m1 crude extract, 30 µg/ml of chloramphenicol and 30 µg/ml of miconazole nitrate. The values are the mean of three experiments ± S.E. *$p < 0.001$ vs. Standard antibiotic (Tukey's pairwise comparison test). The standard antibiotics chloramphenicol and miconazole nitrate were found to have zone of inhibitions 10.40 ± 0.26-24.80 ± 0.37mm at the concentration of 30 µg/ml. In contrast, the inhibition zone of methanol (negative control) was almost zero for all the tested microorganisms. The large inhibition zones exhibited by the extract against *Aspergillus niger* justified the plant use in the treatment of fungal infections.

CONCLUSION

Bacterial and fungal infections can be treated with the *Boerhavia diffusa* L.since it exhibited favorable antibacterial and antifungal activities. On the basis of the present study, further phytochemical and pharmacological studies will be needed to isolate the bioactive compound(s) and investigate the antimicrobial activities against a wider range of pathogenic microorganisms.

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