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IN VITRO ANTIBACTERIAL STUDY OF *BOERHAVIA DIFFUSA L.* ROOT EXTRACT ON SLAUGHTERHOUSE ISOLATE *BACILLUS CEREUS* GD55

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ABSTRACT

The predominance of drug-resistant pathogens have extended the devotion of pharmaceutical and scientific communities towards potential antimicrobial agents from plant derived sources. The present research work has commenced to study the antimicrobial activity of the methanolic extract of Boerhavia diffusa L. roots against slaughterhouse isolate Bacillus cereus GD55, by using the agar well diffusion method. Inhibition zones ranged between 17.68 ± 0.22 mm. The root extract inhibited the growth of Bacillus cereus GD55. The standard antibiotic chloramphenicol found to have a zone of inhibition 20.72 ± 0.26 mm at the concentration of 30 µg/ml. In divergence, the inhibition zone of methanol (negative control) was almost zero for testing microorganism. The spectrum activity of methanolic extract of this plant could be a possible source to obtain new and effective herbal medicines to treat various bacterial diseases.

Keywords: Antimicrobial, *Boerhavia diffusa L.*, Methanolic root extract, *Bacillus cereus* GD55, Zone of inhibition.

Contribution/ Originality

The paper's primary contribution is finding *in vitro* antimicrobial activity of the methanolic extract of *Boerhavia diffusa L.* roots against slaughterhouse isolate *Bacillus cereus GD55.* This study documents the antibacterial effect of *Boerhavia diffusa L.* Therefore more of such research should be encouraged in the area.

1. INTRODUCTION

The use of plant and its products has an extensive history that began with folk medicine and through the years has been merged into traditional and allopathic medicine (Dubey *et al.*, 2011). Since ancient times, many plant species conveyed to have pharmacological properties as they are known to hold various secondary metabolites like glycosides, saponins, flavonoids, steroids, tannins, alkaloids and terpenes which are utilized to combat the disease triggering pathogens (Kamali and Amir, 2010; Lalitha *et al.*, 2010; Hussain *et al.*, 2011). With the progression in Science

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and Technology, incredible progress has been made in the field of medicine with the discoveries of many natural and synthetic drugs (Preethi *et al*., 2010). Antibiotics are indubitably one of the most important therapeutic discovery of the 20th century that had effectiveness against serious bacterial infections (Sharma, 2011). 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 (Enne *et al*., 2001; Westh *et al*., 2004). The world's attention is now progressively directed towards plant sources for developing antimicrobial drugs, since natural products are considered safer than synthetic ones (Kim *et al*., 1999; Alagesa, 2011). According to the World Health Organization, medicinal plants would be the best source to acquire a variety of drugs (Ahmad and Beg, 2001). Therefore, such plants should be explored to better understand their properties, safety and efficacy (Nascimento *et al*., 2000). There are several published reports reporting the antimicrobial activity of various crude plant extracts (Alzoreky and Nakahara, 2003; Igoli *et al*., 2005). 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 (Ram *et al*., 2003). The different herbal plant extracts are traditionally having been used as anticancer, antioxidant, antiulcer, analgesic and antidiabetic (Pankaj and Kaushik, 2011) and they also have the antiparasitic, antifungal, antibacterial, antimalarial activity, analgesic and anti-inflammatory activity (Acharyya *et al*., 2011). Different species of *Boerhavia* are used as a folk medicine for the treatment of various illnesses such as skin diseases. It has been reported that *Boerhavia* possesses antinociceptive, Hepatoprotective, Hypoglycemic, antiproliferative, antiestrogenic, anti-inflammatory, anticonvulsant, antistress, adaptogenic, immune modulator and anti-metastatic activities (Rawat *et al*., 1997; Rajpoot and Mishra, 2006; Ahmad *et al*., 2008; Goyal *et al*., 2010; Krishna *et al*., 2010; Nwakanma and Okoli, 2010). *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 (Rendle, 1925; Yelne *et al*., 2000; Meena *et al*., 2010). It is used in the Ayurveda medicine system to treat various health problems. One of the most typical exemplary plants of the Ayurveda medicine is *Boerhavia diffusa Linn* (Akhilesh *et al*., 1999).

The plant is stated in the Atharvaveda with the name 'Punarnava', because the top of the plant dries up during the summer and regenerates again during the rainy season (Singh, 2007). The plant was named in honor of Hermann Boerhave, a famous Dutch physician of the 18th century (Mahesh *et al*., 2012). *Boerhavia diffusa* is up to 1 m 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 (Kirtikar and

Basu, 1956). The present research was set up to determine the antimicrobial activity of *Boerhavia diffusa L.* plant extraction against slaughterhouse isolate *Bacillus cereus* GD55.

2. MATERIAL AND METHODS

2.1. Chemicals and Plant Collection

The following ingredients were used for the preparation of nutrient agar media: Agar, Peptone, Sodium chloride, Beef extract and 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.

2.2. Preparation of the Plant Extract

The fresh plant roots of *Boerhavia diffusa L.* were collected in November 2013 from the medicinal garden of Acharya & BM Reddy College of Pharmacy, Bangalore, India and authenticated at Institute of Herbal Botany, Plant Anatomy Research Centre, Chennai by Prof. 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 g of powdered material was extracted by maceration in methanol (400 ml) for 14 days with frequent agitation (Freitas *et al.*, 1991; Qaisar *et al.*, 2012; Venkatanagaraju and Divakar, 2014a). The mixture was filtered through a clean muslin cloth followed by double filtration with Whatman No.1 filter paper and the filtrate was concentrated by rotary evaporation under vacuum (pressure: 500 N/m^2) at 40°C until a volume of about 15 ml waste reached. Next the concentrate was poured into glass petri dishes and brought to dry in an oven at 60°C. 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°C.

2.3. Bacterial Strain and Culture Conditions

The strain of *Bacillus cereus* GD55 used in the experiment was previously screened from slaughterhouse soils of various regions in Bangalore, India, identified at Institute of Microbial Technology (IMTECH), Chandigarh, India. and used for fibrinolytic protease production by submerged fermentation and solid state fermentation (Venkatanagaraju and Divakar, 2013a; Venkatanagaraju and Divakar, 2013b; Venkatanagaraju and Divakar, 2013c; Venkatanagaraju and Divakar, 2013e). The isolate was grown in nutrient broth medium contained (g/l) of beef extract, 3; peptone, 5; sodium chloride, 5 with 70% glycerol. Cultures were preserved at -20°C (Richard and Murray, 2009). The inoculum was prepared by transferring a loopful of stock culture into 100

ml of sterile nutrient broth, stock medium, then incubated it overnight at 37°C on a rotary shaker with 200 rpm, before being used for inoculation (Gitishree and Prasad, 2010).

2.4. Determination of the Antimicrobial Activity

Agar well-diffusion method was followed to determine the antimicrobial activity (Didry *et al*., 1998; Esimone *et al*., 1998; Castello *et al*., 2002; Venkatanagaraju and Divakar, 2013d; Sandeep *et al*., 2014; Venkatanagaraju *et al*., 2014b). Nutrient agar (gm/l: beef extract, 3g; peptone, 5g; sodium chloride, 5g; agar, 20g) plates were swabbed (sterile cotton swabs) with 24h old-broth culture (10^6-10^8 bacteria CFU/ml) of *Bacillus cereus* GD55. Wells (10 mm diameter and about 2 cm a part) were made in each of these plates using sterile cork borer. A stock solution of root extract was prepared at a concentration of 100 mg/ml. About 100 µl of root extract was added with a sterile syringe into the wells and allowed to diffuse at room temperature for 2 h. Control experiments comprising inoculums without root extract. 30 µg/ml chloramphenicol was also used as positive controls for *Bacillus cereus* GD55 respectively. The plates were incubated at 37°C for 24 h. 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 replicate the readings were taken in three different fixed directions and the average values were recorded.

2.5. Statistical Data 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 pairwise comparison tests to assess the statistical significance.

3. RESULTS AND DISCUSSION

The search for antimicrobial from natural sources has received much attention and efforts have been put in to identify compounds that can act as suitable antimicrobial 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 (Kelmanson *et al*., 2000; Ahmad and Beg, 2001). 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 (Guleria and Kumar, 2006; Zakaria *et al*., 2007). Therefore, medicinal plants are finding their way into pharmaceuticals, nutraceuticals and food supplements.

In the present investigation, the inhibitory effect of *Boerhavia diffusa L* root methanolic extract was evaluated against *Bacillus cereus* GD55. The antimicrobial activity was determined by using agar well diffusion method and the results are summarized in Figure 1 and Table 1. Methanolic extract (100.00 mg/ml) of the roots displayed good antibacterial activity against

Bacillus cereus GD55. Methanolic extract inhibited the growth of testing microorganism with large zones of inhibition 17.68 ± 0.22 mm. The standard antibiotic chloramphenicol was found to have a zone of inhibitions 20.72 ± 0.26 mm at the concentration of 30 µg/ml. In contrast, the inhibition zone of methanol (negative control) was almost zero for all the tested *Bacillus cereus* GD55.

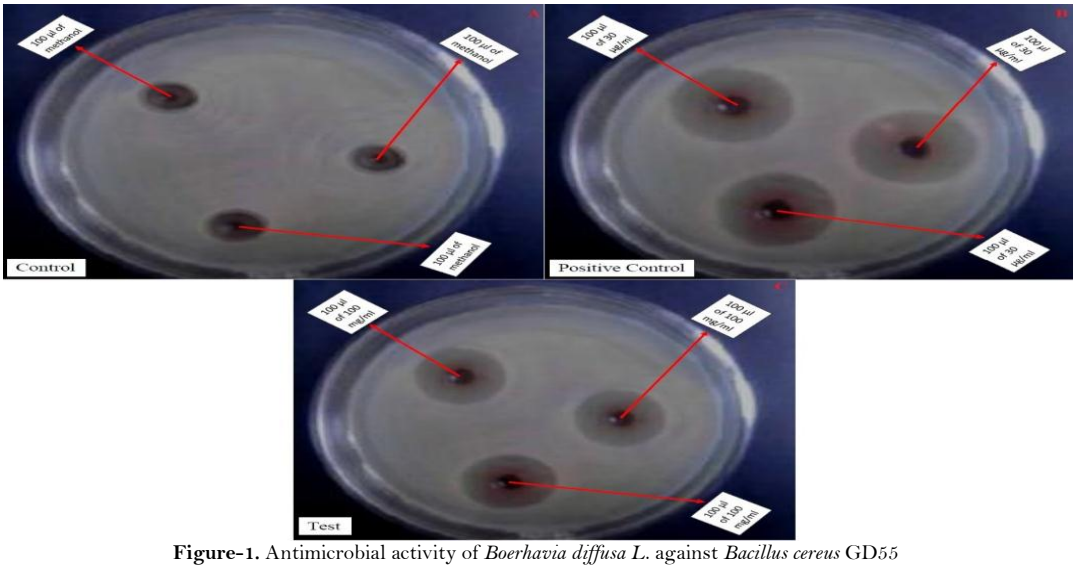

Figure-1. Antimicrobial activity of *Boerhavia diffusa L.* against *Bacillus cereus* GD55

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

Microorganism		**Zone of Inhibition (mm)**	
	Control	***Boerhavia diffusa L.* roots**	**Chloramphenicol***
Bacillus cereus GD55	0	17.68 ± 0.22	20.72 ± 0.26

The inhibition zone diameter was taken as an average value of triplicate plates for each microorganism at 100 µl of 100 mg/ml crude extract, 30 µg/ml of chloramphenicol. The values are the mean of three experiments ± S.E. *p< 0.001 vs. Standard antibiotic (Tukey's pairwise comparison test)

4. CONCLUSION

Bacterial infections can be treated with the *Boerhavia diffusa L.* since it exhibited favorable antibacterial. 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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