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Evaluation of Anti-microbial activity of *Boerhavia* species against important Bacterial Pathogens

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Abstract

The rapid emergence of antibiotic-resistant bacteria has reduced the effectiveness of many conventional antimicrobial drugs, highlighting the need to identify new antimicrobial agents from plant sources. Species of the genus *Boerhavia* are widely recognized in traditional medicine for their therapeutic properties and contain a variety of secondary metabolites with potential biological activities. The present study aimed to evaluate and compare the antimicrobial activity of leaves, stems, and roots of *Boerhavia diffusa* L. and *Boerhavia erecta* L. against selected human pathogenic bacteria. Antibacterial activity was determined against *Streptococcus pneumoniae*, *Bacillus subtilis*, *Salmonella typhi*, and *Escherichia coli* using the agar well diffusion method at extract concentrations of 50 and 100 mg/mL. The antimicrobial potential was assessed by measuring the diameter of the inhibition zones and comparing the activity of aqueous and ethanolic extracts with appropriate positive and negative controls. The results demonstrated that both *Boerhavia* species exhibited antibacterial activity against all tested microorganisms, although the magnitude of inhibition varied according to plant species, plant part, extraction solvent, bacterial strain, and extract concentration. Root extracts generally exhibited stronger antibacterial activity than the corresponding leaf and stem extracts, while ethanolic extracts showed greater inhibitory effects than aqueous extracts in several treatments. An increase in extract concentration generally enhanced antibacterial activity, indicating a concentration-dependent response. The observed antibacterial properties may be attributed to the presence of bioactive secondary metabolites such as flavonoids, phenolic compounds, alkaloids, tannins, and terpenoids. The findings suggest that *B. diffusa* L. and *B. erecta* L. represent promising natural sources of antimicrobial agents and provide scientific support for their traditional medicinal applications.

Key words : *Boerhavia diffusa* L., *Boerhavia erecta* L., antimicrobial activity, agar well diffusion.

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The rapid emergence and dissemination of antimicrobial resistance (AMR) have become one of the most serious public health concerns worldwide. The excessive and indiscriminate use of antibiotics in human medicine, veterinary practice, and agriculture has accelerated the evolution of multidrug-resistant microorganisms, thereby reducing the effectiveness of conventional antimicrobial therapies. According to the World Health Organization (WHO), antimicrobial resistance threatens the successful prevention and treatment of infectious diseases and has become a major obstacle to global healthcare systems (World Health Organization[14]. Consequently, there is an urgent need to discover alternative antimicrobial agents from natural sources that are effective, safe, affordable, and less likely to induce microbial resistance.

Medicinal plants have served as an indispensable source of therapeutic agents since ancient times and continue to play a significant role in primary healthcare throughout the world. Approximately 80% of the population in developing countries depends directly or indirectly on traditional herbal medicines for the treatment of various diseases[13]. Plants synthesize numerous secondary metabolites that contribute to their medicinal properties and protect them against microbial pathogens and environmental stress. These metabolites include alkaloids, flavonoids, phenolic compounds, tannins, terpenoids, glycosides, steroids, and saponins, many of which exhibit potent antimicrobial, antioxidant, anti-inflammatory, anticancer, antiviral, and immunomodulatory activities[7,8]. Owing to the increasing prevalence of antibiotic resistance and the adverse effects associated with synthetic drugs, medicinal plants are receiving considerable scientific attention as potential sources of novel antimicrobial compounds.

Among medicinal plants, the genus *Boerhavia* (Family: Nyctaginaceae) has attracted increasing interest because of its extensive use in traditional medicine. The genus comprises nearly 40 species distributed throughout tropical and subtropical regions of the world. Several species have been employed in Ayurvedic, Unani, and folk medicine for the treatment of liver disorders, kidney diseases, inflammation, fever, microbial infections, gastrointestinal disorders, and respiratory ailments[10]. The therapeutic potential of *Boerhavia* species is attributed to their rich diversity of bioactive phytochemicals, which have been extensively investigated in recent years.

Boerhavia diffusa L., commonly known as Punarnava, is one of the most extensively studied medicinal plants in India. It has been traditionally used as a diuretic, hepatoprotective, nephroprotective, anti-inflammatory, and rejuvenating herb in Ayurvedic medicine. Modern pharmacological investigations have demonstrated that *B. diffusa* possesses antimicrobial, antioxidant, antidiabetic, anticancer, antiviral, immunomodulatory, and hepatoprotective activities[6]. These biological activities have been associated with the presence of numerous phytochemicals, including flavonoids, rotenoids, alkaloids, lignans, phenolic acids, glycosides, steroids, and terpenoids[11]. Such compounds have been reported to inhibit microbial growth by disrupting bacterial cell walls, altering membrane permeability, interfering with nucleic acid

synthesis, and inhibiting essential metabolic enzymes.

Compared with *B. diffusa*, *Boerhavia erecta* L. has received relatively limited scientific attention despite its widespread distribution and traditional medicinal importance. Ethnobotanical studies have reported its use in the treatment of fever, skin diseases, wounds, digestive disorders, inflammation, and microbial infections. Preliminary phytochemical investigations have confirmed the presence of alkaloids, flavonoids, phenolic compounds, tannins, saponins, steroids, and terpenoids in different plant parts, suggesting that the species possesses significant pharmacological potential[12]. However, comprehensive comparative studies evaluating the antimicrobial efficacy of *B. erecta* alongside *B. diffusa* remain scarce.

The antimicrobial activity of medicinal plants is largely attributed to their secondary metabolites. Phenolic compounds and flavonoids exert antibacterial effects by damaging bacterial cell membranes, inhibiting nucleic acid synthesis, and reducing microbial enzyme activity. Tannins form complexes with microbial proteins and enzymes, thereby suppressing bacterial growth. Alkaloids interfere with DNA replication and protein synthesis, while terpenoids and saponins increase membrane permeability, resulting in leakage of intracellular constituents and eventual cell death[4,5]. The combined action of these phytochemicals often produces synergistic antimicrobial effects, making medicinal plant extracts valuable candidates for the development of new antimicrobial agents.

The present investigation evaluated the antibacterial activity of plant extracts against four clinically important bacterial pathogens, namely *Streptococcus pneumoniae*, *Bacillus subtilis*, *Salmonella typhi*, and *Escherichia coli*. These microorganisms represent both Gram-positive and Gram-negative bacteria commonly associated with human infections.

Streptococcus pneumoniae is a Gram-positive bacterium responsible for pneumonia, meningitis, otitis media, and septicemia. Increasing resistance of pneumococcal strains to β-lactam antibiotics and macrolides has become a major public health concern, emphasizing the need for alternative therapeutic agents (Centers for Disease Control and Prevention[2]).

Bacillus subtilis is a Gram-positive bacterium widely used as a model organism in microbiological research. Although generally regarded as non-pathogenic, it serves as an important indicator organism for evaluating antibacterial activity because of its well-characterized physiology and susceptibility profile[9].

Salmonella typhi, Gram-negative bacterium, causative agent of typhoid fever remains a major public health problem in many developing countries. The emergence of multidrug-resistant and extensively drug-resistant strains has reduced the effectiveness of conventional antibiotics and increased the importance of identifying alternative antimicrobial compounds[14].

Escherichia coli is a Gram-negative bacterium commonly associated with urinary tract infections, gastroenteritis, and wound infections. The widespread occurrence of

extended-spectrum β-lactamase (ESBL)-producing *E. coli* strains has significantly limited available treatment options and stimulated research into plant-derived antimicrobial agents[3].

Among the various methods employed for antimicrobial screening, the agar well diffusion method is one of the most widely accepted techniques because it is simple, rapid, reproducible, and suitable for evaluating crude plant extracts. The method allows direct comparison of inhibition zones produced by different extracts against selected microorganisms and provides preliminary information regarding their antibacterial potential[1].

Although several reports have described the pharmacological properties of *Boerhavia diffusa*, comparative investigations involving different plant parts of both *B. diffusa* and *B. erecta* are limited. Comparative evaluation of leaves, stems, and roots may provide valuable insight into the distribution of antimicrobial phytochemicals within these medicinal species and facilitate the identification of plant parts with superior therapeutic potential.

Preparation of Extract :

Plants were collected from Palanpur, Gujarat, India. Collected material was cleaned, naturally dried and root, stem, leaves were separated. Each plant part was finely pulverized. This powder form was used for extraction with methanol for 24 hours by Soxhlet equipment and filtered through membrane filter. This filtrate was evaporated and dried at 50ºC. For antimicrobial evaluation, the dried extracts were dissolved separately in sterile distilled water and ethanol to obtain working concentrations of 50 mg/mL and 100 mg/mL.

Selection of the Microorganism :

The antibacterial activity of the extracts was evaluated against four clinically important bacterial strains:

- *Streptococcus pneumoniae*
- *Bacillus subtilis*
- *Salmonella typhi*
- *Escherichia coli*

Pure cultures of the bacterial strains were maintained on nutrient agar slants and subcultured before antimicrobial analysis. The antibacterial activity of the extracts was determined using the agar well diffusion method following standard microbiological procedures[1]. Sterile Mueller–Hinton agar medium was prepared and poured into sterile Petri plates. After solidification, each bacterial suspension was uniformly spread over the agar surface using a sterile cotton swab to obtain a confluent bacterial lawn. Sterile cork borers were used to prepare wells of approximately 6 mm diameter in the agar medium. Each well was filled with the prepared plant extracts at concentrations of 50 mg/mL and 100 mg/mL. Azithromycin was used as the positive control, while sterile distilled water served as the negative control. All antimicrobial experiments were carried out in triplicate. The inhibition zones were expressed as mean ± standard deviation (Mean ± SD).

For both plants *Boerhavia diffusa* L. and *Boerhavia erecta* L. triplicate test were performed at 50 mg/ml and 100mg/ml for stem, root and leaf separately. Among both the plants

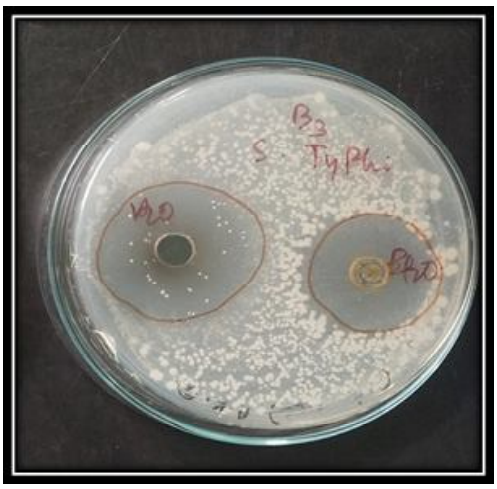
Table-1. Antimicrobial activity of *Boerhavia diffusa* L. and *Boerhavia erecta* L.

Sr No.	Name of Organism	Concen-tration (Mg/ml)	Extract	Zone of inhibition(mm)					
				Boerhavia diffusa L.			*Boerhavia erecta* L.		
				Leaf	Stem	Root	Leaf	Stem	Root
1.	*Streptococcus pneumoniae*	50mg/ml	H_2O	0	0	32.33±0.58	22±1	23.67±0.58	12.67±1.53
			EtOH	0	11±1	20±1	22.34±1.15	18±1	26.33±1.53
		+ve Control	44±2.65						
		-ve Control	0						
		100mg/ml	H_2O	15±1	0	27±1	18.33±0.58	13±1	36.33±3.79
			EtOH	12.33±1.15	16.67±1.53	23±1.73	21.67±0.58	13.67±1.15	32.33±2.52
		+ve Control	34.67±2.52						
		-ve Control	0						
2.	*Bacillus subtilis*	50mg/ml	H_2O	0	0	20.67±1.15	17±1	21.63±0.58	10.33±0.58
			EtOH	0	12±1.73	18.33±1.53	22±1	0	24.33±1.53
		+ve Control	27.67±2.31						
		-ve/ Control	0						
		100mg/ml	H_2O	0	0	17.67±1.53	19.33±3.06	18.67±1.15	24.67±0.58
			EtOH	13.67±0.58	13.67±1.15	20.33±0.58	26±1	22.67±0.58	0
		+ve Control	24±1						
		-ve Control	0						
3.	*Salmonella typhi*	50mg/ml	H_2O	14.67±0.58	11.3±0.5	37±1.73	17.67±0.58	18.33±1.53	0
			EtOH	15±1	15±2	30.67±0.58	27.33±0.58	23.67±0.58	28.33±1.53

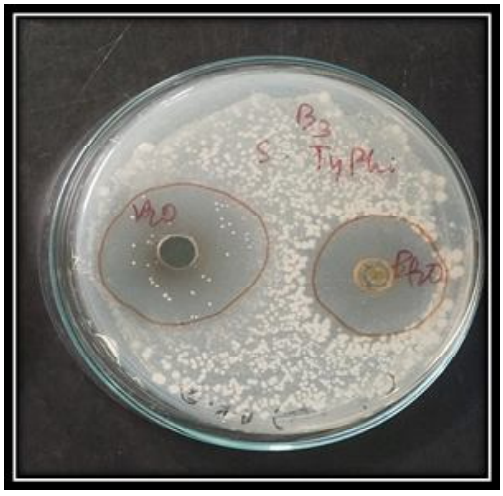
		+ve Control	44.33±1.15					
		-ve Control	0					
		100mg/ml	H_2O	30±2	0	32±2	15.67±1.15	15.33±0.58	42.33±3.21
			EtOH	0	15.67±0.58	12±2	26.33±2.31	17.67±0.58	0
		+ve Control	46±1						
		-ve Control	0						
	4.	*E.coli*	50mg/ml	H_2O	0	0	28±1	22±1.73	21±1	0
				EtOH	11.1±1	0	24.67±0.58	21.33±0.58	19±1	27.33±2.08
		+ve Control	32±2.65						
		-ve Control	0						
		100mg/ml	H_2O	0	0	0	0	19±1	0	15±1
			EtOH	13.67±1.53	15.33±1.53	25±3.61	27±1.73	22.33±0.58	0
		+ve/ Control	37.33±1.53						
		-ve/ Control	0						

Note : Values represented here are the mean of Triplicate±Standard deviation

highest Zone of Inhibition (ZOI) at 50mg/ml was obtained for *Boerhavia diffusa* L. root (water extract) 37±1.73 against *Salmonella typhi* and highest Zone of Inhibition (ZOI) at 100mg/ml was obtained *Boerhavia erecta* L. root (water extract) 42.33±3.21 against *Salmonella typhi*.



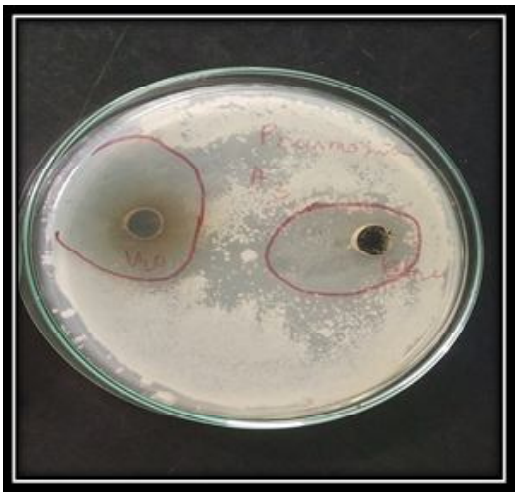
Boerhavia diffusa L. root in H_2O against *S. typhi*



Boerhavia diffusa L. root in EtOH against *S. typhi*

Figure 1. Highest anti-microbial activity at 50 mg/ml in H_2O and EtOH extract

Boerhavia erecta L. root in H_2O against *S. typhi*



Boerhavia erecta L. root in EtOH against *S. pneumoniae*

Figure 2. Highest anti-microbial activity at 100mg/ml in H_2O and EtOH extract

The antibacterial activity of *Boerhavia diffusa* L. and *Boerhavia erecta* L. suggests that both species possess bioactive constituents capable of inhibiting the growth of pathogenic bacteria. The variation in antimicrobial efficacy between the two species at different extract concentrations indicates that antibacterial activity is influenced by both the concentration of the extract and the distribution of phyto-chemicals within the plant.

The comparatively higher activity of *B. diffusa* L. root extract at 50 mg/mL against *Salmonella typhi* suggests the presence of potent antimicrobial compounds that remain effective even at lower concentrations. In contrast, the enhanced activity of *B. erecta* L. root extract at 100 mg/mL indicates a concentration-dependent response, where a higher extract concentration may provide sufficient levels of active metabolites to exert greater antibacterial effects. Such differences may arise from variations in the qualitative and quantitative composition of secondary metabolites, including phenolic compounds, flavonoids, alkaloids, tannins, and terpenoids, which are known to disrupt bacterial cell membranes, interfere with enzyme activity, and inhibit essential metabolic pathways.

The strong inhibitory effect against *S. Typhi* is noteworthy, as this pathogen is responsible for typhoid fever and the increasing emergence of antimicrobial resistance has created a demand for alternative therapeutic agents. The present findings support the traditional medicinal use of *Boerhavia* species and suggest that their root extracts may serve as promising sources of natural antimicrobial compounds.

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