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SCIENTIFIC VALIDATION OF THE ANTIOXIDANT AND ANTIBACTERIAL PROPERTIES OF BOERHAAVIA DIFFUSA LINN ROOT EXTRACT USED IN TRADITIONAL MEDICINE

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ABSTRACT

Background of the study: *Boerhaavia diffusa Linn.* Is widely used in traditional medicine and is reputed for its therapeutic properties, particularly antimicrobial and antioxidant effects. Despite its traditional use, comprehensive scientific studies evaluating the antioxidant and antimicrobial potential of hydroalcoholic extract of the entire plant remain limited. This study aimed to evaluate the in vitro antioxidant and antibacterial activities of a hydroalcoholic extract derived from the root of the *Boerhaavia diffusa Linn.* **Materials and Methods:** The antioxidant activity of the hydroalcoholic extract was assessed using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical-scavenging assay, Nitric oxide scavenging activity of root of *Boerhaavia diffusa Linn* with ascorbic acid serving as the reference standard. Antimicrobial activity was evaluated using the cup-plate agar diffusion method against two model bacterial species for Gram Negative and Gram Positive. **Results:** The hydroalcoholic Root extract of *Boerhaavia diffusa Linn* contained a broad spectrum of phytochemicals, including alkaloids, flavonoids, phenolic compounds, tannins, saponins, glycosides, steroids, terpenoids, carbohydrates, proteins, amino acids, and reducing sugars, while anthraquinones and fixed oils/fats were absent. The extract exhibited moderate antibacterial activity against Gram-positive bacteria (Staphylococcus, Streptococcus, Bacillus, Enterococcus) and selective activity against Gram-negative bacteria (Escherichia coli and Salmonella), showing no inhibition of Pseudomonas and Proteus spp. **Conclusion:** The presence of diverse bioactive phytochemicals in *B. diffusa* leaves supports its traditional medicinal use, and the observed antibacterial activity indicates potential for further development as a natural antimicrobial agent, particularly against Gram-positive and selective Gram-negative pathogens.

KEYWORDS: *Boerhaavia diffusa linn*, Antioxidant activity, Antimicrobial activity, hydroalcoholic extract, Phytochemicals, DPPH radical scavenging, Agar diffusion method, Gram-positive bacteria, Gram-negative bacteria, Traditional medicine, *In vitro studies.*

INTRODUCTION

Infectious diseases pose significant threats to health and are major causes of morbidity and mortality worldwide.[1] *Boerhaavia diffusa linn*, commonly known as Punarnava or "the rejuvenator," is revered in Ayurveda for its wide-ranging therapeutic potential. Traditionally, it has been used for centuries to restore and revitalize the body and treat various ailments.[2&3] The rise of multi-drug resistant pathogens underscores the urgent need for novel antimicrobial compounds, with natural products offering diverse chemical structures and promising drug leads.[4] Due to rising antibiotic

resistance and chemotherapeutic failures, scientists are increasingly exploring medicinal plants as sources of novel antimicrobial agents.[5] Nowadays, secondary plant metabolites (phytochemicals) with previously unknown biological activities are being extensively explored as potential medicinal agents with antibacterial efficacy. Historically, humans have used numerous plants about 1500 documented in ancient texts and 800 in traditional medicine with *Boerhaavia diffusa Linn* being one of the most widely utilized.[6&7] The roots of *Boerhaavia diffusa Linn* are a rich source of bioactive chemical constituents, including alkaloids such as Punarnavine, rotenoids like Boeravinones A–F, flavonoids, phenolic glycosides (e.g., Punarnavoside), steroids, triterpenoids, and various other phenolic compounds. These diverse phytochemicals contribute to the plant's medicinal properties.[8] Quantitative studies have shown that both aqueous and hydroalcoholic root extracts contain significant amounts of proteins, carbohydrates, ascorbic acid, carotenoids, and total phenolics, highlighting their nutritional and antioxidant potential. In various antioxidant assays, including DPPH, ABTS, and FRAP, the hydroalcoholic root extract demonstrated strong radical-scavenging activity, often exceeding that of other plant parts.[9] This underscores the root's potential as a natural source of antioxidants and bioactive compounds for therapeutic applications.[10] In the present study was under taken to investigate anti-bacterial and oxidant activities of hydroalcoholic extract of the *Boerhaavia diffusa Linn* root of the plant. To evaluate the preliminary phytochemical screening and antibacterial potential of different solvent extracts of *Boerhaavia diffusa Linn* leaves on several Gram-positive and Gram-negative microorganisms of medical importance.

MATERIALS AND METHODS

Collection of Plant: The roots of *Boerhaavia diffusa Linn* were collected from the Yercaud foothills, Salem district, Tamil Nadu, India. The plant material was taxonomically authenticated, and a voucher specimen was deposited in the Department for future reference. The freshly collected roots were thoroughly washed with running tap water to remove adhering soil and debris, followed by rinsing with distilled water. The cleaned roots were then shade-dried at room temperature for one week to preserve thermo labile phytoconstituents. After complete drying, the roots were cut into small pieces and coarsely powdered using a mechanical blender. The powdered root material was passed through a suitable mesh to ensure uniform particle size and subsequently stored in a sterile, airtight container under dry conditions until further experimental use.

Soxhlet extraction: The air-dried plant material was finely powdered, and a total quantity of 730 g of the powdered sample was subjected to hot continuous extraction using a Soxhlet apparatus with hydroalcoholic as the extraction solvent. The extraction process was carried out until the solvent in the siphon tube became colorless, indicating exhaustive extraction of the phytoconstituents. Following completion of extraction, the hydroalcoholic extract was filtered, and the solvent was removed under reduced pressure using a rotary vacuum evaporator at a controlled temperature to prevent thermal degradation of bioactive compounds. This process yielded a concentrated crude extract, which was further dried to constant weight to ensure complete removal of residual solvent. The dried extract was then carefully weighed to determine the percentage yield and subsequently transferred into a sterile, airtight container. The extract was stored in a desiccator to protect it from moisture and light until further experimental use.[11]

Extraction of *Boerhaavia diffusa Linn* plant component- root: The plant root extract was prepared by soaking 50 g of shade-dried root powder in 50% hydroalcoholic at 40 °C for 24 h with intermittent stirring, filtered through muslin cloth, concentrated under reduced pressure using a rotary vacuum evaporator (~5% yield), and all shade-dried powdered extracts of Root, stem, and root were used undiluted or diluted as required in normal saline solution.[20]

Test Organisms: The bacterial strains selected for the present study included both Gram-positive and Gram-negative organisms commonly implicated in human infections and widely used in antimicrobial screening studies. The Gram-positive bacteria comprised species of Staphylococcus, Streptococcus, Bacillus, and Enterococcus. The Gram-negative bacteria included Escherichia coli, Pseudomonas spp., Salmonella spp., and Proteus spp. These bacterial strains were chosen based on their clinical relevance and differential cell wall characteristics, allowing for the evaluation of the broad-spectrum antimicrobial potential of the test extract. All strains were maintained under appropriate laboratory conditions and sub-cultured prior to use in antimicrobial assays.[12]

Agar Disc Diffusion Method: The agar disc diffusion method was employed to evaluate the antibacterial activity of the Root extract against the selected test organisms. Mueller Hinton agar (MHA) was prepared by dissolving 3.1 g of the dehydrated medium in 100 mL of distilled water in a sterile conical flask. The medium was sterilized by autoclaving at 121 °C and 15 lb pressure for 15 minutes, allowed to cool to room temperature, and aseptically poured into sterile Petri plates. The plates were left undisturbed to allow the medium to solidify.

Fresh bacterial cultures were prepared and uniformly swabbed onto the surface of the solidified MHA plates using sterile cotton swabs to obtain a confluent lawn of microbial growth. Sterile filter-paper discs (6 mm diameter) were impregnated with 50 µL of the plant extract and allowed to dry under sterile conditions to ensure complete absorption of the extract. The impregnated discs were then carefully placed onto the inoculated agar plates using sterile forceps.

The plates were incubated at 37 °C for 18–24 hours. After incubation, antibacterial activity was assessed by measuring the diameter of the zones of inhibition (in millimeters) surrounding the discs. For each bacterial strain, negative control discs impregnated with the corresponding pure solvent were included to confirm that any observed inhibition was due solely to the plant extract. All experiments were performed in triplicate, and the results were recorded as mean zone-of-inhibition values.[13]

Determination of Antioxidant activity: DPPH Assay: The antioxidant activity of different extracts was evaluated using the DPPH radical scavenging assay, where various concentrations of the extracts (10 to 320 µg/ml) were reacted with 0.1 mM hydroalcoholic DPPH solution and the decrease in absorbance at 517 nm after 20 min incubation in the dark was measured as an indicator of free radical scavenging activity. Ascorbic acid served as the standard, all experiments were performed in triplicate, and percentage scavenging was calculated using the standard formula based on absorbance values.

Calculate % Scavenging (Radical Inhibition):
% RSA= ($\mathbf{A}_{control}$ - A_{sample}) divided by $\mathbf{A}_{control}$ x 100, where $\mathbf{A}_{control}$ is absorbance of DPPH + hydroalcoholic and A_{sample} is absorbance of DPPH + sample (Blank Corrected)

Determination of Antioxidant activity: Nitric oxide radical scavenging (NO) assay: Nitric oxide scavenging activity was assessed by incubating various concentrations (10–320 µg/mL) of hydroalcoholic root extracts with 10 mM sodium nitroprusside at room temperature for 180 min, followed by reaction with Griess reagent and measurement of absorbance at 546 nm, using ascorbic acid as the positive control. The experiment was performed in triplicate, and percentage nitric oxide scavenging activity was calculated by comparing the absorbance of test samples with that of the control.

Calculate % Scavenging (Radical Inhibition):
% RSA= ($\mathbf{A}_{control}$ - A_{sample}) divided by $\mathbf{A}_{control}$ x 100, where $\mathbf{A}_{control}$ is absorbance of DPPH + hydroalcoholic and A_{sample} is absorbance of DPPH + sample (Blank Corrected)

RESULTS AND DISCUSSION

Qualitative phytochemical screening of the hydroalcoholic Root extract of *Boerhaavia diffusa Linn* revealed the presence of a wide range of bioactive secondary metabolites (Table 1). The extract tested positive for alkaloids, flavonoids, phenolic compounds, tannins, saponins, glycosides, steroids, terpenoids, carbohydrates, proteins and amino acids, and reducing sugars, while anthraquinones and fixed oils and fats were absent.

The presence of alkaloids is of pharmacological significance, as alkaloids such as punarnavine reported in *Boerhaavia diffusa Linn* are known to exhibit antimicrobial, anti-inflammatory, and diuretic activities. The detection of flavonoids and phenolic compounds suggests strong antioxidant potential, since these compounds are well recognized for their ability to scavenge free radicals and protect against oxidative stress-induced cellular damage.[14]

Tannins identified in the extract contribute to antimicrobial and astringent properties by forming complexes with microbial proteins and enzymes, thereby inhibiting microbial growth. The presence of saponins supports the traditional use of *B. diffusa*, as saponins are reported to possess antimicrobial, anti-inflammatory, and immunomodulatory activities. The detection of glycosides, steroids, and terpenoids further indicates the therapeutic potential of the plant. Steroids and terpenoids are known for their anti-inflammatory, cardioprotective, and hepatoprotective properties, while glycosides contribute to various biological activities including antioxidant and antimicrobial effects.[15]

Table 1: Qualitative analysis of phytochemicals in Boerhaavia diffusa Linn leaves extracts.

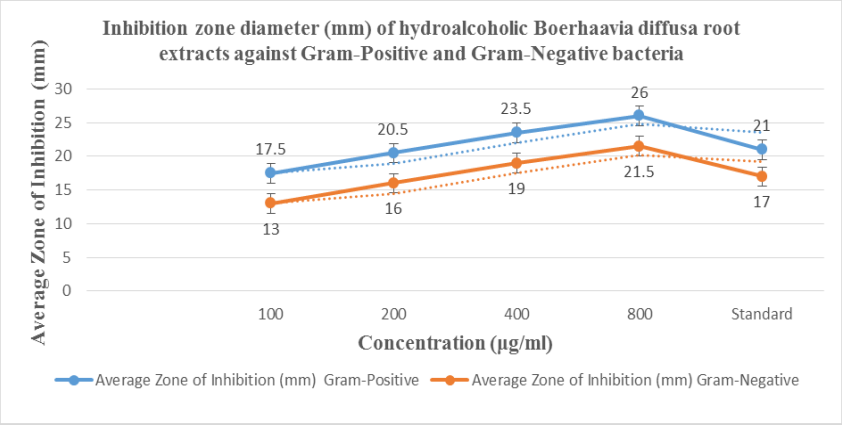
S. No.	*Extract*	*Hydroalcoholic extract*
1	Alkaloids	Present (+)
2	Flavonoids	Present (+)
3	Phenolic compounds	Present (+)
4	Tannins	Present (+)
5	Saponins	Present (+)
6	Glycosides	Present (+)
7	Steroids	Present (+)
8	Terpenoids	Present (+)
9	Carbohydrates	Present (+)
10	Proteins & Amino acids	Present (+)
11	Reducing sugars	Present (+)
12	Anthraquinones	Absent (-)
13	Fixed oils & fats	Absent (-)
Absent (-), Present (+)		

Primary metabolites such as carbohydrates, reducing sugars, proteins, and amino acids were also present, reflecting the nutritional and metabolic importance of the plant material and supporting cellular growth and repair mechanisms. The absence of anthraquinones indicates a low likelihood of laxative or irritant effects, while the absence of fixed oils and fats suggests that non-polar lipid constituents are minimal in the hydroalcoholic Root extract. This profile indicates that hydroalcoholic solvent systems are particularly effective in extracting polar and moderately polar phytoconstituents from *Boerhaavia diffusa* leaves.[16]

Overall, the qualitative phytochemical profile observed in this study is consistent with earlier reports on *B. diffusa* and provides a strong scientific basis for its reported antioxidant, antimicrobial, anti-inflammatory, and therapeutic activities, thereby justifying its use in traditional medicine and further pharmacological investigations.

Table 2: Inhibition zone diameter (mm) of hydroalcoholic *Boerhaavia diffusa* root extracts against Gram-Positive and Gram-Negative bacteria

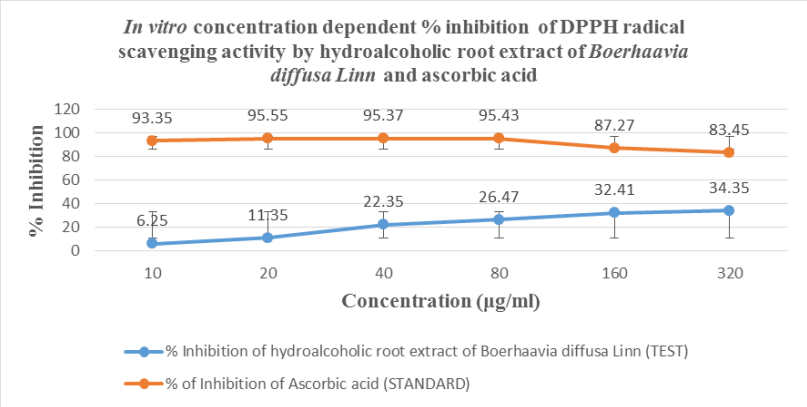
S.No:	Average Zone of Inhibition (mm) Gram-Positive	Average Zone of Inhibition (mm) Gram-Negative	*Concentration* (µg/ml)
1	17.5	13	100
2	20.5	16	200
3	23.5	19	400
4	26.0	21.5	800
5	21	17	Standard
6	*No Zone*	*No Zone*	Control



The hydroalcoholic root extract of *Boerhaavia diffusa Linn* exhibited dose-dependent antibacterial activity against both Gram-positive and Gram-negative bacteria (Table 2). As the concentration increased from 100 µg/mL to 800 µg/mL, the average zone of inhibition (ZOI) increased from 17.5 mm to 26.0 mm against Gram-positive strains and from 13 mm to 21.5 mm against Gram-negative strains, indicating that higher concentrations of bioactive compounds enhance antibacterial potency. At the standard control, the ZOI was 21 mm (Gram-Positive) and 17 mm (Gram-Negative), while the negative control showed no inhibition, confirming that the observed antibacterial effects were attributable to the plant extract. The slightly smaller zones observed against Gram-negative bacteria at lower concentrations may reflect the inherent resistance due to their outer membrane structure, which limits the diffusion of bioactive phytochemicals. These results suggest that the Root extract possesses broad-spectrum antibacterial activity, with efficacy comparable to standard antibiotics at higher concentrations. The findings are consistent with previous reports emphasizing that solvent extracts, extract concentration, and solubility of active compounds significantly influence observed antibacterial activity.[16,17,18,19&20]

Table 3: Anti oxidative activity of *Boerhaavia diffusa* using DPPH scavenging activity

S.No:	*Concentration* (µg/ml)	% Inhibition of hydroalcoholic root extract of *Boerhaavia diffusa Linn* (TEST)	% of Inhibition of Ascorbic acid (STANDARD)
1	10	6.25	93.35
2	20	11.35	95.55
3	40	22.35	95.37
4	80	26.47	95.43
5	160	32.41	87.27
6	320	34.35	83.45

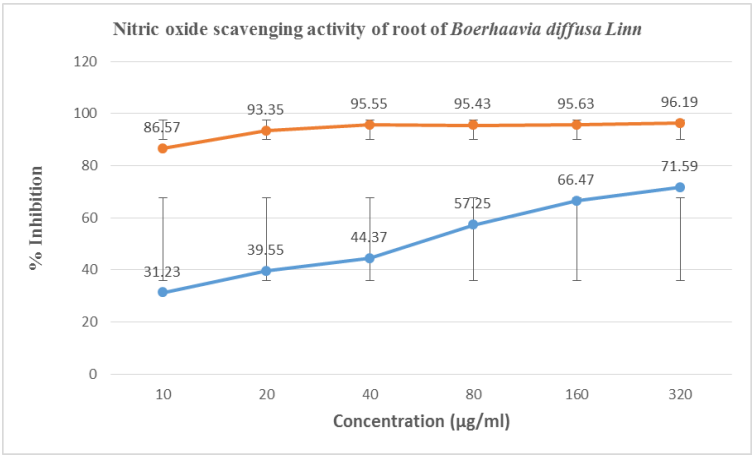


The hydroalcoholic root extract of *Boerhaavia diffusa* showed a concentration-dependent increase in DPPH radical-scavenging activity, with % inhibition rising from 6.25% at 10 µg/mL to 34.35% at 320 µg/mL.**[21]** In contrast, the standard antioxidant ascorbic acid exhibited consistently high scavenging activity (>83%) across all tested concentrations, indicating its strong free-radical neutralizing potential. The moderate antioxidant activity of the extract may be attributed to the presence of phenolics, flavonoids, and rotenoids reported in *B. diffusa* roots. Lower activity compared to ascorbic acid is expected for crude plant extracts, as they contain complex mixtures with varying antioxidant efficiencies. These findings support previous reports that *B. diffusa* possesses notable antioxidant potential, validating its traditional therapeutic use.[23]

Nitric oxide scavenging activity of root of *Boerhaavia diffusa Linn*

Table 4: Nitric oxide scavenging activity of root of *Boerhaavia diffusa Linn.*

S.No:	*Concentration* (µg/ml)	% Inhibition of hydroalcoholic root extract of *Boerhaavia diffusa Linn* (TEST)	% of Inhibition of Ascorbic acid (STANDARD)
1	10	31.23	86.57
2	20	39.55	93.35
3	40	44.37	95.55
4	80	57.25	95.43
5	160	66.47	95.63
6	320	71.59	96.19



The hydroalcoholic root extract of *Boerhaavia diffusa* exhibited a marked, concentration-dependent nitric oxide (NO) scavenging activity, with inhibition increasing from 31.23% at 10 µg/mL to 71.59% at 320 µg/mL.[22] Ascorbic acid, used as the standard, showed consistently high NO scavenging activity (>86%) across all concentrations, confirming assay reliability. The significant NO inhibitory potential of the extract suggests effective interaction with nitric oxide or reactive nitrogen species generated from sodium nitroprusside. This activity may be attributed to bioactive phytoconstituents such as flavonoids, phenolics, and alkaloids known for their nitrosative stress–modulating properties. The results support earlier findings that plant-derived antioxidants can effectively scavenge nitric oxide and related species, contributing to anti-inflammatory and cytoprotective effects.[24]

CONCLUSION

The present investigation demonstrates that the hydroalcoholic root extract of *Boerhaavia diffusa* Linn is rich in diverse bioactive phytochemicals, including alkaloids, flavonoids, phenolics, tannins, saponins, glycosides, steroids, and terpenoids, which collectively contribute to its biological activities. The extract exhibited dose-dependent antioxidant potential, as evidenced by its DPPH and nitric oxide radical scavenging activities, indicating its ability to neutralize free radicals and reactive nitrogen species. Additionally, significant antibacterial activity was observed against Gram-positive bacteria and selective Gram-negative pathogens, supporting its broad-spectrum antimicrobial potential.

Although the antioxidant activity was moderate compared with ascorbic acid, the results are notable given the crude nature of the extract and the synergistic effects of multiple phytoconstituents. Overall, these findings scientifically validate the traditional use of *B. diffusa* and suggest that its hydroalcoholic root extract may serve as a promising natural source for developing antioxidant and antimicrobial agents, warranting further isolation, characterization, and in vivo studies

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