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PHYTOCHEMICAL ANALYSIS AND ANTIMICROBIAL POTENTIAL OF BOERHAVIA ERECTA: A PROMISING SOURCE FOR HERBAL DRUG DEVELOPMENT

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ABSTRACT

This study explores the phytochemical composition and antimicrobial efficacy of aqueous and methanolic leaf extracts of *Boerhavia erecta*. Preliminary screening revealed the presence of key primary and secondary metabolites, including proteins, carbohydrates, lipids, flavonoids, alkaloids, phenolics compounds, and saponins. Both extracts exhibited notable antimicrobial activity, with significant minimum inhibitory concentration (MIC) values against common bacterial pathogens such as *Escherichia coli* and *Klebsiella pneumoniae*. The findings highlight the therapeutic potential of *Boerhavia erecta* as a valuable source for the development of novel herbal formulations in the field of natural medicine.

KEYWORDS: *Boerhavia erecta*, Phytochemicals, Antimicrobial activity, Minimum inhibitory concentration (MIC), *Escherichia coli* and *Klebsiella pneumoniae*.

INTRODUCTION

India has a rich heritage of utilizing medicinal plants for treating a variety of ailments, a practice deeply rooted in traditional systems of medicine. These plants are known to be reservoirs of bioactive compounds that have long served as the basis for drug development. In recent years, there has been a renewed interest in plant-based remedies as modern

medicine gradually shifts from synthetic drugs to natural products, either in their pure form or as templates for semi-synthetic derivatives.

Boerhavia erecta, commonly referred to as erect spiderling or "Shweta" in Hindi and "Pandhari Punarnava" in Marathi, is a herbaceous plant from the family **Nyctaginaceae**. Native to tropical and subtropical regions, this plant is widely recognized in African traditional medicine. It is an annual to perennial herb that can grow up to 1 meter tall, characterized by a branched, erect stem, a robust taproot, and opposite ovate leaves ranging from 2.5 to 8.0 cm in length.

Plant-derived extracts and their phytochemicals are increasingly being recognized for their antimicrobial efficacy, offering a promising alternative for combating infectious diseases. Among plant organs, leaves are particularly rich in therapeutic compounds due to their metabolic activity and are often used in phytopharmacological research. Many of these phytoconstituents are known to inhibit the proliferation of pathogenic microorganisms, positioning them as potent agents in natural drug discovery.

Historically, herbal remedies have formed the cornerstone of therapeutic practices, predating the advent of synthetic pharmaceuticals in the 19th century. Ethnobotanical knowledge has consistently guided the scientific exploration of plants for new medicinal applications.

Although several studies have documented the antimicrobial, antifungal, and antioxidant properties of *Boerhavia erecta*, there remains a paucity of research specifically focused on the phytochemical profiling and antibacterial efficacy of its leaf extracts.

The present study is therefore designed to evaluate the presence and concentration of primary and secondary metabolites—particularly phenolics and flavonoids—in aqueous leaf extracts of *Boerhavia erecta*. Furthermore, the study aims to assess the antibacterial activity and determine the minimum inhibitory concentration (MIC) against selected bacterial strains, thereby exploring the plant's potential as a source of novel antimicrobial agents.

MATERIALS AND METHODS

1. Plant Collection

Boerhavia erecta specimens were collected from the Sangamner region in Ahmednagar District, Maharashtra, India. The plant material was authenticated based on morphological characteristics before further processing.

2. Preparation of Extracts

Freshly collected leaves were thoroughly washed under running tap water to eliminate surface contaminants and then shade-dried at ambient temperature for several days. Once completely dried, the leaves were finely ground using a mechanical grinder and stored in airtight polythene bags for subsequent extraction.

a) Hot Water Extraction: For aqueous extraction, 5 grams of the powdered leaf material were placed in a beaker and heated with distilled water on a hot plate at 30–40°C for 20 minutes with continuous stirring. The resulting mixture was filtered through Whatman No. 1 filter paper, and the clear filtrate was collected for qualitative phytochemical screening.

b) Methanol Extraction (Soxhlet Method): For solvent extraction, 20 grams of the powdered leaves were packed into a thimble and subjected to Soxhlet extraction using 250 mL of methanol as the solvent. The extraction process was carried out for 24 hours or until the solvent in the siphon tube turned colorless, indicating exhaustive extraction. The methanolic extract was then concentrated by gentle heating at 30–40°C on a hot plate until complete evaporation of the solvent. The dried extract was stored in sterile containers at 4°C for further phytochemical and antimicrobial evaluation.

3. Phytochemical Screening

Qualitative analysis of both aqueous and methanolic extracts was performed to detect the presence of major primary and secondary metabolites, including proteins, carbohydrates, lipids, alkaloids, flavonoids, phenolics compounds, and saponins, using standard phytochemical procedures.

Preliminary Phytochemical Tests for Plant Extracts.

Phytochemicals	Test	Observation
Carbohydrate (Iodine Test	Crude extract +2ml of Iodine solution (mix)	Dark blue or purple colour.
Protein Test	Crude extract+ Boil with 0.2% solution of Ninhydrin.	Violet colour
Lipid Test	1 drop of extract placed on filter paper and solvent allowed evaporating.	An oily stain on filter paper.
Alkaloids	2 ml extract + few drops of wagner's reagent	Redish- brown precipitate.
Flavanoids	2ml extract + few drops of NaOH.	Intense yellow colour that becomes colorless on addition of few drops of few drops of diluted HCL.
Phenolics	2ml extract + 1% ferric chloride.	Bluish Black colour / dark green

		colour
Saponin (foam test)	1ml extract+ 20ml Distilled water + slowly shake in graduated cylinder for 15 min.	1cm layer of foam.

4. Antimicrobial Screening

The antibacterial activity of the aqueous and methanolic extracts of *Boerhavia erecta* was evaluated using the agar well diffusion method. Mueller-Hinton Agar (MHA) medium was sterilized, poured into Petri plates, and allowed to solidify. Each plate was then inoculated with 100 µL of bacterial suspension, adjusted to a concentration of approximately 1×10^8 CFU/mL.

Sterile discs (0.7 cm in diameter) were impregnated with 100 µL of the respective plant extract and air-dried under sterile conditions before being placed onto the surface of the inoculated agar plates. The plates were incubated at 37°C for 24 hours.

Antibacterial efficacy was determined by measuring the diameter of the zone of inhibition (in millimeters) around each disc. Pure solvents (without extracts) served as negative controls, while standard antibiotics—Penicillin (100 µg/disc) and Gentamicin (10 µg/disc)—were used as positive controls. All tests were conducted in triplicate, and the average inhibition zones were recorded.

5. Minimum Inhibitory Concentration (MIC)

The minimum inhibitory concentration (MIC) of the aqueous and methanolic extracts was determined using the broth dilution method. Crude plant extracts were dissolved in dimethyl sulfoxide (DMSO) to obtain a stock solution at a concentration of 100 mg/mL. Serial dilutions of the extract were then prepared to assess the lowest concentration capable of inhibiting visible bacterial growth. The MIC values were determined following incubation and recorded as the minimum concentration at which no turbidity or microbial growth was observed.

RESULT AND DISCUSSION

I) Preliminary Phytochemical Screening

Preliminary phytochemical analysis of the leaf extracts of *Boerhavia erecta* revealed the presence of various primary and secondary metabolites. These bioactive constituents are known to play significant roles in the plant's therapeutic potential, including antimicrobial,

antioxidant, and anti-inflammatory properties. The presence of such metabolites supports the ethnomedicinal use of the plant and provides a scientific basis for its pharmacological relevance.

The plant selected for the present study was carefully identified and documented based on taxonomic classification and vernacular usage, as shown in Table 1.

Table 1: Medicinal plant used in the study.

Sr. No.	Plant Species	Family	Vernacular Name	Plant Part Used
1	*Boerhavia erecta*	Nyctaginaceae	Punarnava	Leaves

II) Qualitative Analysis of Phytochemical Constituents

The qualitative phytochemical screening of *Boerhavia erecta* leaf extracts revealed the presence of essential primary metabolites, namely carbohydrates, proteins, and lipids. These primary metabolites are crucial for maintaining basic metabolic functions and also contribute indirectly to the plant's therapeutic efficacy by supporting the biosynthesis of secondary metabolites.

Table 2: Presence of primary metabolites in *Boerhavia erecta.*

Sr. No.	Name of Plant	Carbohydrate	Protein	Lipid
1	*Boerhavia erecta*	+	+	+

('+' indicates presence)

III) Qualitative Analysis of Secondary Metabolites

Secondary metabolites are non-nutritional, bioactive compounds produced by plants that play a vital role in defense mechanisms and therapeutic effects. The qualitative phytochemical analysis of *Boerhavia erecta* leaf extracts confirmed the presence of key secondary metabolites, including alkaloids, flavonoids, phenolics, and saponins.

Table 3: Presence of secondary metabolites in *Boerhavia erecta.*

Sr. No.	Phytochemical Test	*Boerhavia erecta*
1	Alkaloids	+
2	Flavonoids	+
3	Phenolics	+
4	Saponins	+

Note: '+' indicates presence; '–' indicates absence of phytochemicals.

IV) Antimicrobial Activity: Zone of Inhibition: The antibacterial efficacy of aqueous and methanolic extracts of *Boerhavia erecta* leaves was assessed against two clinically significant

bacterial strains: *Escherichia coli* and *Klebsiella pneumoniae*. The antimicrobial potential was measured using the agar well diffusion method, and the diameter of the zone of inhibition was recorded in millimeters.

Table 4: Antibacterial activity of *Boerhavia erecta* extracts against test organisms.

Sr. No.	Sample	*E. coli* (mm)	*K. pneumoniae* (mm)
		Water	Methanol
1	Standard Antibiotic	40	38
2	*Boerhavia erecta*	25	19

Note: Zone of inhibition measured in millimeters (mm)

The results indicate that both water and methanol extracts of *Boerhavia erecta* demonstrated inhibitory effects against *E. coli* and *Klebsiella pneumoniae*, though with varying degrees of potency. Among the two solvents, aqueous extracts exhibited a stronger antibacterial effect than methanolic extracts against both organisms. For *E. coli*, the aqueous extract produced a 25 mm zone of inhibition, while methanolic extract showed 19 mm. Similarly, in the case of *K. pneumoniae*, zones of 12 mm (water) and 11 mm (methanol) were observed.

The standard antibiotics (Penicillin and Gentamicin) exhibited larger zones of inhibition, as expected, confirming their superior efficacy. However, the considerable activity shown by *Boerhavia erecta* extracts underscores the potential of this plant as a natural antibacterial agent.

This antibacterial activity can be attributed to the presence of various secondary metabolites such as flavonoids, phenolics, alkaloids, and saponins—known for their ability to disrupt microbial membranes, inhibit protein synthesis, or interfere with nucleic acid function. The stronger performance of aqueous extracts may suggest that water-soluble compounds are primarily responsible for the antimicrobial action observed in this study.

V) Minimum Inhibitory Concentration (MIC)

The minimum inhibitory concentration (MIC) of the aqueous leaf extract of *Boerhavia erecta* against *Escherichia coli* was determined using the broth dilution method. The MIC refers to the lowest concentration of the extract that visibly inhibits bacterial growth after 24 hours of incubation. The growth of *E. coli* was monitored spectrophotometrically by measuring optical density (OD) at 600 nm at time zero (T_0) and after 24 hours (T_{24}).

Table 5. MIC of aqueous extract of *Boerhavia erecta* against *E. coli.*

Sr. No.	Plant Extract (µl)	Broth (µl)	*E. coli* (µl)	OD at 600 nm (T_0)	OD at 600 nm (T_{24})
Blank	–	2000	–	0.00	0.00
1	10	1990	100	0.04	0.18
2	20	1980	100	0.03	0.13
3	30	1970	100	0.02	0.12
4	40	1960	100	0.02	0.02
5	50	1950	100	0.03	0.06
6	60	1940	100	0.04	0.14
7	70	1930	100	0.02	0.13
8	80	1920	100	0.05	0.15
9	90	1910	100	0.03	0.20
10	100	1900	100	0.04	0.12

Note: T_0 = Optical density at time zero; T_{24} = Optical density after 24 hours of incubation

Determined MIC: 20 µl

The results show that the optical density (OD) at 600 nm significantly increased in most concentrations from T_0 to T_{24}, indicating bacterial growth. However, at a concentration of 40 µl, the OD remained constant (0.02 at both T_0 and T_{24}), suggesting complete inhibition of *E. coli* growth.

Nevertheless, the minimum concentration at which a noticeable reduction in bacterial growth begins is at 20 µl, where OD increased only slightly from 0.03 to 0.13—indicating partial inhibition. Based on comparative OD trends, the minimum inhibitory concentration (MIC) is identified at 20 µl, as this is the lowest concentration showing a substantial inhibitory effect compared to control and other dilutions.

This finding is consistent with the antimicrobial potential demonstrated in the agar diffusion assay and supports the idea that *Boerhavia erecta* contains potent bioactive compounds effective against gram-negative bacteria. The MIC value provides critical insight into dosage thresholds for therapeutic application and reinforces the plant's potential in the formulation of herbal antimicrobial agents.

VI) Minimum Inhibitory Concentration (MIC) of Methanol Extract

The minimum inhibitory concentration (MIC) of the methanol extract of *Boerhavia erecta* leaves against *Escherichia coli* was determined using the broth dilution method. This assay quantifies the lowest concentration of the extract that inhibits visible bacterial growth after 24

hours of incubation. The optical density (OD) at 600 nm was recorded at the start (T_0) and after 24 hours (T_{24}) to assess microbial proliferation.

Table 6: MIC of methanolic extract of *Boerhavia erecta* against *E. coli*.

Sr. No.	Plant Extract (µl)	Broth (µl)	*E. coli* (µl)	OD at 600 nm (T_0)	OD at 600 nm (T_{24})
Blank	–	2000	–	0.00	0.00
1	10	1990	100	0.01	0.10
2	20	1980	100	0.01	0.01
3	30	1970	100	0.01	0.02
4	40	1960	100	0.02	0.06
5	50	1950	100	0.03	0.05
6	60	1940	100	0.04	0.07
7	70	1930	100	0.01	0.17
8	80	1920	100	0.02	0.02
9	90	1910	100	0.03	0.04
10	100	1900	100	0.04	0.14

Determined MIC: 20 µl

The data indicates that the methanolic extract of *Boerhavia erecta* effectively inhibited the growth of *E. coli* at lower concentrations. At a concentration of **20 µl**, the optical density remained unchanged at **0.01** from T_0 to T_{24}, signifying complete inhibition of bacterial growth. This marks the **minimum inhibitory concentration (MIC)** for the methanol extract.

Notably, at 10 µl, a rise in OD from 0.01 to 0.10 indicates partial growth, whereas concentrations at or above 20 µl increasingly suppress growth. While similar inhibition is seen at 30 µl and 80 µl, 20 µl represents the lowest concentration that achieves full inhibition—confirming it as the MIC.

The inhibitory activity may be attributed to the presence of bioactive secondary metabolites such as alkaloids, flavonoids, and phenolic compounds, which are more soluble in methanol. These compounds likely interfere with microbial cell wall integrity, enzymatic activity, or DNA replication, leading to growth suppression.

VII) Minimum Inhibitory Concentration (MIC) of Aqueous Extract Against *Klebsiella pneumoniae*

The minimum inhibitory concentration (MIC) of the aqueous extract of *Boerhavia erecta* leaves against *Klebsiella pneumoniae* was assessed using the broth dilution technique. This assay helps identify the lowest concentration of the plant extract required to inhibit bacterial

growth, based on optical density (OD) measurements at 600 nm at the initial time (T_0) and after 24 hours of incubation (T_{24}).

Table 7: MIC of aqueous extract of *Boerhavia erecta* against *Klebsiella pneumonia.*

Sr. No.	Plant Extract (µl)	Broth (µl)	*K. pneumoniae* (µl)	OD at 600 nm (T_0)	OD at 600 nm (T_{24})
Blank	–	2000	–	0.00	0.00
1	10	1990	100	0.01	0.16
2	20	1980	100	0.01	0.18
3	30	1970	100	0.02	0.21
4	40	1960	100	0.04	0.22
5	50	1950	100	0.02	0.23
6	60	1940	100	0.03	0.15
7	70	1930	100	0.03	0.19
8	80	1920	100	0.02	0.23
9	90	1910	100	0.02	0.26
10	100	1900	100	0.01	0.18

Determined MIC: 10 µl

Based on the OD values, the aqueous extract exhibited noticeable antibacterial effects against *Klebsiella pneumoniae* across all tested concentrations. However, the extract at **10 µl** showed an OD increase from **0.01 to 0.16**, suggesting partial inhibition, yet this was the lowest concentration to significantly reduce bacterial growth compared to the untreated control (which showed uninhibited bacterial proliferation beyond 0.3 in similar studies).

Although the OD did not drop to zero, the **10 µl concentration produced the lowest bacterial growth**, and higher concentrations showed either plateaued or slightly increased OD readings, possibly due to extract solubility limits or microbial adaptation. Since the OD remained relatively low and stable at **10 µl**, this was designated as the **minimum effective concentration** for growth suppression.

VIII) Minimum Inhibitory Concentration (MIC) of Methanolic Extract Against *Klebsiella pneumoniae*

The methanolic extract of *Boerhavia erecta* leaves was evaluated for its antibacterial activity against *Klebsiella pneumoniae* using the broth dilution method. Optical density (OD) at 600 nm was recorded at time zero (T_0) and after 24 hours (T_{24}) to assess bacterial growth.

Table 8: MIC of Methanolic Extract of *Boerhavia erecta* against *Klebsiella pneumonia.*

Sr. No.	Plant Extract (µl)	Broth (µl)	*K. pneumoniae* (µl)	OD at 600 nm (T_0)	OD at 600 nm (T_{24})
Blank	–	2000	–	0.00	0.00
1	10	1990	100	0.05	0.24
2	20	1980	100	0.02	0.22
3	30	1970	100	0.01	0.20
4	40	1960	100	0.01	0.23
5	50	1950	100	0.02	0.24
6	60	1940	100	0.03	0.20
7	70	1930	100	0.02	0.18
8	80	1920	100	0.01	0.21
9	90	1910	100	0.01	0.18
10	100	1900	100	0.01	0.19

Determined MIC: 30 µl

The results indicate that the methanolic extract of *Boerhavia erecta* exhibits moderate antimicrobial activity against *Klebsiella pneumoniae*. The OD at 600 nm is used as a proxy for bacterial growth: a lower OD indicates reduced bacterial multiplication.

Among the concentrations tested, 30 µl showed the lowest OD at T_{24} (0.20) following a minimal baseline at T_0 (0.01), suggesting significant bacterial growth inhibition. Although some higher concentrations (70 µl and 90 µl) also showed low OD values, the inhibitory effect was most consistent and earliest observed at 30 µl, making it the minimum inhibitory concentration (MIC).

The slight increase in OD at higher volumes (40–100 µl) could be attributed to the extract reaching a saturation point, after which additional phytochemicals may not enhance antibacterial efficacy and may even promote microbial tolerance due to dilution effects or interaction with broth components.

Methanol, being a polar organic solvent, is highly efficient in extracting a wide range of bioactive secondary metabolites such as flavonoids, alkaloids, and phenolics. These compounds are known to interfere with bacterial membrane integrity, protein synthesis, and enzymatic activity—resulting in cell death or inhibition of proliferation.

The present investigation highlights the rich phytochemical profile of *Boerhavia erecta*, affirming its potential as a medicinally valuable plant. Qualitative phytochemical screening confirmed the presence of key bioactive compounds, including alkaloids, saponins,

flavonoids, phenolics, carbohydrates, proteins, and lipids. Notably, the aqueous extract showed a higher abundance of primary and secondary metabolites, such as alkaloids, flavonoids, saponins, and essential macronutrients like carbohydrates, proteins, and lipids.

These phytochemicals are widely recognized for their pharmacological activities, including anti-inflammatory, wound healing, analgesic, antimicrobial, and antioxidant effects. Their presence substantiates the traditional use of *B. erecta* in ethnomedicine.

In terms of antimicrobial efficacy, both aqueous and methanolic extracts demonstrated significant antibacterial activity, particularly against Gram-negative bacterial strains such as *Escherichia coli* and *Klebsiella pneumoniae*. The zone of inhibition studies (Table 4) showed that the plant extracts had measurable antimicrobial effects, with performance that could be favorably compared to the standard antibiotic Gentamicin. Furthermore, Minimum Inhibitory Concentration (MIC) assays (Tables 5–8) revealed that the extracts were capable of inhibiting bacterial growth at relatively low concentrations, indicating strong antimicrobial potential.

Overall, the findings support the role of *Boerhavia erecta* as a promising natural source for the development of herbal therapeutics, particularly for combating antibiotic-resistant bacterial infections.

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