RESEARCH ARTICLE

ETHNOMEDICINAL EXPLORATION, PHYTOCHEMICAL SCREENING AND ANTIMICROBIAL ACTIVITY OF *BOERHAVIA DIFFUSA* L.:

A MULTIPOTENTIAL MEDICINAL PLANT

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Abstract:

Boerhavia diffusa L. (family: Nyctanginaceae) is a medicinal herb commonly considered as a weed. The exploration of phytochemicals in different parts of B. diffusa with both the aqueous and ethanol extract will create awareness, along with the suitable solvent and method for extraction of pharmaceutical compounds. Hence, the present study focuses on phytochemical analysis of B. diffusa leaves, stems, and roots. In the antimicrobial test, the B. diffusa root ethanol extract inhibited the growth of Pseudomonas aeruginosa and Staphylococcus aureus with zones of inhibition of about 8.3 ± 0.6 mm and 20.7 ± 0.6 mm at 200 µg concentration, respectively. B. diffusa is highly nutritious, and the maceration and decoction extracts were similar except for the chloroform extract that was found to be weak. **Keywords:** Boerhavia diffusa L., Ethnomedicine, Phytochemicals, Antimicrobial Activity.

Introduction:

Boerhavia diffusa is a dicotyledonous herb or shrub, belongs to family Nyctaginaceae and commonly known as common hogweed. It is commonly known as Santh in Hindi, Thazhuthama in Malayalam, Punarnava in Bengali, Chattarani in Tamil, Khattan in Punjabi, Komma in Kannada, Purunee in Odia, Gadhapurni in Sambalpuri, etc. It is an herb that branches laterally at ground level with green leaves, pinkish or purplish stems, and purple flowers that are campanulate (Struwig et al., 2011). About 40 species of Boerhavia are widely distributed throughout the tropical and subtropical regions of the world, with many variations in South and North America (Douglas and Spellenberg, 2010). The plant grows in a creeping manner, and it has been found to grow more during the rainy season because the species Boerhavia tends to grow in wet soil and in sandy, stony, and clayey soils found near dried up water resources, as well as riverbeds, hill slopes, and mountains (Struwig and

Siebert, 2013). It is indigenous to India, where it is also referred to as Punarnava and used in traditional medicine (Selvaraj *et al.*, 2012). The plant is enriched with anti-inflammatory (Hiruma-Lima *et al.*, 2000), antibacterial (Olukoya *et al.*, 1993), antioxidant (Pari and Satheesh, 2004), and immunomodulatory (Mehrotra *et al.*, 2002) properties. The secondary metabolites present in the plants can act as effective pharmaceutical compounds (Manikandan *et al.*, 2016). The antioxidant properties of the plants help in reducing oxidative stress in humans (Bharathi *et al.*, 2018). Quercetin which is a flavonoid compound obtained from the plant can be used as a treatment for coronary heart disease and fatty liver (Vijayakumar *et al.*, 2018). The volatile compounds have been identified to have a variety of pharmaceutical roles when consumed in the form of aroma and fresh juice with anti-inflammatory, antimicrobial, antioxidant, anti-depressive, and analgesic properties (Antonelli *et al.*, 2020; Pinto *et al.*, 2021; Zhou *et al.*, 2020). *B. diffusa* has been identified among many other plants to confer protection against SARS CoV-2 (Anand *et al.*, 2021).

Both the *Staphylococcus aureus* and *Pseudomonas aeruginosa* are the bacterial types most commonly found to exhibit multidrug resistance (MacDougall *et al.*, 2005). Hence, in order to develop pharmaceutical products, the exploration of phytochemical profiling along with antimicrobial activity of various parts of plants were needed. The present study conducts ethnobotanical exploration, phytochemical screening and antimicrobial activity of both the aqueous and ethanol extracts of various parts like roots, stems and leaves of *B. diffusa*.

Materials and Methods:

Study Area

Bargarh is situated in the western region of Odisha with a latitude and longitude of 21.342585° North and 83.624199° East, respectively. It consists of two subdivisions, namely Bargarh and Padampur, 12 blocks, and four NACs. The land area of Bargarh district is about 5837 square kilometers; among them, about 1216.13 square kilometers (20.83%) are covered in forest area (Sahu *et al.*, 2010; Sahu *et al.*, 2013; Sahu *et al.*, 2016; Sahu and Sahu, 2020; Sahu *et al.*, 2021; Sahu and Ekka, 2021; Sahu and Mishra, 2022). The plants and ethnobotanical data were collected by frequent exploration throughout the area, but before that, a survey was done by taking observations and direct personal interviews of the locals and experts about the use of medicinal plants found in their locality. The information about the ethnomedicinal uses for selected plants was documented, and photographs were taken. The scientific names and family names were further checked by using local flora book (Saxena and Brahman, 1994-1996).

Plant Collection and Extraction

The plant parts of *B. diffusa* were collected from the various parts of Bargarh district, Odisha, India, during August and November of 2024 (Figure 1). After being separated, the roots, stems, and leaves were left to dry for roughly six weeks in the shade. After being shade-dried, the *B. diffusa* pieces were ground into a powder, sieved, and kept for later use in an airtight container. In an airtight container, 100 milligrams of *B. diffusa* powdered leaves, stems, and roots were dissolved in 100 milliliters of distilled water, or ethanol, for approximately 48 hours while being constantly stirred. Quantitative filter paper was used to filter the solvents after 48 hours, and they were then stored for further use.



Figure 1. Photographs showing leafy parts with flower of *Boerhavia diffusa* L. (a), roots of *B. diffusa* L (b).

Qualitative Tests

For phytochemical analysis, the following modified protocols were carried out (Nayak *et al.*, 2024; Sahu *et al.*, 2024; Sharma *et al.*, 2024; Bhoi et al., 2025; Rani *et al.*, 2025; Sahu *et al.*, 2025; Dash *et al.*, 2025; Mallik *et al.*, 2025; Padhan *et al.*, 2025; Sahu *et al.*, 2025).

- Carbohydrates (Molisch's test): 2 mL of Molisch's reagent was added to the extract, and the mixture was thoroughly agitated. After that, 2 mL of concentrated H₂SO₄ was carefully added to the test tube's side, and the interface was watched for the development of a violet ring.
- Proteins (Xanthoproteic test): In a test tube, 2-3 mL of test solution were mixed with 1 mL of strong nitric acid. The development of a white precipitate that, when heated, turns yellow and eventually dissolves, giving the solution a yellow hue, indicates a positive test. After cooling the solution, extra sodium hydroxide is carefully added, causing the yellow solution to turn deeper orange.
- Amino Acids (Ninhydrin Test): 1 mL of amino acid solution was mixed with five drops of a 0.2% ninhydrin solution in acetone. After two minutes of boiling over a water bath and letting it cool, the liquid was examined for the development of a blue hue.
- Alkaloids (Test for alkaloids): To 2 mL of extract, 2 mL of concentrated HCl was added. Then, a few drops of Mayer's reagent were added. The presence of green colour, white precipitate, or turbidity indicated the presence of alkaloids.
- Flavonoids (Alkaline reagent test): Aqueous plant crude extract was combined with 2 mL of 2.0% NaOH mixture; this resulted in an intense yellow tint that turned colourless when two drops of diluted acid were added. Flavonoids were found in this result.
- *Phenols (Lead Acetate Test)*: 0.2 mL of extract was mixed with 2 mL of aqueous sodium carbonate, and then 0.2 mL of Folin's reagent was added. Phenols were present when the colour turned blue.
- Terpenoids (Salkowski's test): About 5.0 mL of extract was mixed with 2.0 mL of chloroform and 3.0 mL of concentrated H₂SO₄. A reddish-brown colour at the interface indicated the presence of terpenoids.

- Saponins (Foam test): A fraction of the extract was vigorously shaken with 20 mL of water in a graduated cylinder for 15 min, which was observed for the presence of persistent foam.
- Quinone (HCl method): To 1.0 mL of extract, a few drops of concentrated HCl were added. A yellowish-brown colour indicated the presence of quinone.
- *Phytosterols (Salkowski's test)*: The chloroform extract was treated with concentrated H₂SO₄ and observed for the formation of a red colour.

Antimicrobial Assay

Agar Disc Diffusion Method

Both the aqueous and ethanol extracts of *B. diffusa* leaves, stems, and roots at doses of 100, 150, and 200 µg were used in the antimicrobial experiment (Nayak et al. 2024) against the Gramnegative bacterium *P. aeruginosa* (ATCC 27853) and the Gram-positive bacterium *S. aureus* (ATCC 25923). The 0.5 McFarland standard was followed in maintaining the bacterial strain's colony suspensions. Mueller-Hinton agar was the medium. The medium-containing plate was covered with roughly 100 mL of microbial culture. The discs immersed in the extracts of various concentrations were immediately placed on the plates after spreading the microbial culture. The plates were then incubated in an inverted position for about 16–18 and 24 h at 37°C. After incubation, the plates were observed for the zone of inhibition, and its diameter was measured in mm. All replica were done thrice to get better results.

Statistical Analysis

The values in the tables are expressed as the mean \pm the standard deviation (n = 3) and were calculated by using MS-Office 2019.

Results:

Ethnomedicinal uses of B. diffusa

The leaves of the *B. diffusa* are taken as leafy vegetables by the native of Bargarh district. Traditionally this plant is used as medicinal plants to cure a number of diseases, the commonly used few methods and dosages are described as follows:

- ➤ Root powders are mixed with a required amount of sugar and orally taken to get relief from dry cough.
- ➤ 3 grams of Root powder of *B. diffusa* mixed with 500 mg of turmeric taken orally twice a day to cure asthma.
- ➤ Equal amount of root powder of both *B. diffusa* and Ashwagandha with mishri is taken to relieve leucorrhea.
- ➤ 10 gram each of root of both *B. diffusa* and Mutha mixed with 500 ml of lukewarm cow milk and taken orally to get relief from inflammation.
- ➤ 2 gm of root powder taken with a glass of lukewarm cow milk and taken orally to get relief from cold fever.
- > The leaf juice along with black pepper powder are recommended to be taken orally to cure frequent urination.
- A paste made by taking whole plants and 20 numbers of black pepper and recommended to take orally to get relief from poisonous insect bite and sting.

Qualitative Tests

In the qualitative tests, the aqueous and ethanol extracts of *B. diffusa* leaves, stems, and roots showed the presence of most phytochemicals, such as proteins, flavonoids, terpenoids, carbohydrates, phenols, and phytosterols (Table 1). Both the aqueous and ethanol extracts of *B. diffuse* leaves, stems, and roots showed the presence of carbohydrate. Protein is present in only the aqueous extract of all three parts, while absent in the ethanol extracts of all three parts. Amino acids are present in both the aqueous and ethanol extracts of leaf and stem, while amino acids are absent in the aqueous extract of root. Alkaloids are present in ethanol extracts of all three parts, while absent in aqueous extracts. Flavonoids are present in the aqueous extract of all three parts and also present in the ethanol extract of the root, while absent in the ethanol extract of the leaves and stems. Phenols are present in all the aqueous extracts of all three parts, i.e., leaves, stem, and roots; they are also present in the ethanol extract of leaves while absent in the rest. Terpenoids are present in all the aqueous extracts of three parts and also present in the ethanol extract of both stem and root, while absent in the ethanol extract of leaves. Saponins are present in both aqueous extracts of stems and roots, while absent in the other extracts. Quinone is present in both the aqueous extracts of stems and roots, while absent in other extracts. Sterols are absent in aqueous extracts of all three parts and absent in all the ethanol extracts.

Table 1: Phytochemical analysis of different extracts of B. diffusa leaves, stems, and root

Phytochemical	Leaf		Stem		Root	
Analysis	LAE	LEE	SAE	SEE	RAE	REE
Carbohydrates	++	++	++	++	++	++
Protein	++	-	++	-	+	-
Amino acids	++	++	++	++	-	++
Alkaloids	-	+	-	+	-	+
Flavonoids	++	-	+	-	++	+
Phenols	++	+	++	-	+	-
Terpenoids	+	-	+	++	++	+
Saponins	+	-	-	-	+	-
Quinone	-	-	++	-	+	-
Sterols	+	-	++	-	+	-

LAE: Leaf aqueous extract, LEE: Leaf ethanol extract, SAE: Stem aqueous extract,

SEE: Stem ethanol extract, RAE: Root aqueous extract, REE: Root ethanol extract,

+: lower presence of particular compound, ++: higher presence, -: absence.

Antimicrobial Activity

Antimicrobial activity was detected in the *B. diffusa* decoctions of leaves, stems, and roots, as well as the ethanolic extracts of leaves and roots (Table 2). The aqueous extracts did not show any antimicrobial resistance towards *P. aeruginosa* (ATCC 27853). The maximum inhibition was found in the ethanolic extract of roots. The maximum inhibition of bacterial growth was found at 150 μ g and 200 μ g concentrations of the extracts, with zones of about 7.3 \pm 0.6 mm and 8.3 \pm 0.6 mm, respectively. In terms of the antimicrobial activity against *S. aureus* (ATCC 25923), all extracts showed antibacterial activity. The aqueous extracts of leaves having 100 μ g, 150 μ g, and 200 μ g concentrations show zones

of inhibition of 5.7 ± 0.6 , 7.7 ± 0.6 , and 8.7 ± 0.6 , respectively. The aqueous extracts of stems having 100 µg concentration did not show any zone of inhabitation, while 150 µg and 200 µg concentrations show zone of inhibition of 4.3 ± 0.6 and 6.3 ± 0.6 , respectively. The aqueous extracts of roots having 200 µg concentrations show zone of inhibition of 9.3 ± 0.6 , while the aqueous extracts of root having 100 µg, and 150 µg concentrations did not show zone of inhibition. The ethanolic extract of leaves having 100 µg, and 150 µg concentrations did not show zone of inhibition, while 200 µg concentrations show zone of inhibition of 17.3 ± 0.6 . The ethanolic extract of stems having 100 µg concentrations did not show zone of inhibition, while 150 µg and 200 µg concentrations show zone of inhibition of 5.3 ± 0.6 and 8.7 ± 1.2 respectively. The ethanolic extract of roots having 100 µg concentrations did not show zone of inhibition, while 150 µg and 200 µg concentrations show zone of inhibition of 6.3 ± 0.6 and 20.7 ± 0.6 respectively. In this finding, the root ethanol extract exhibited the greatest antimicrobial activity, with an inhibition zone of about 20.7 ± 0.6 mm.

Table 2: Zone of inhibition of both the aqueous and ethanol extracts of various plant parts of *B. diffusa* against *P. aeruginosa* and *S. aureus*

Plant Parts	Concentration of extracts	Diameter of Zone of inhibition against P. aeruginosa in mm (Mean ± SE)		Diameter of Zone of inhibition against S. aureus. in mm (Mean ± SE)	
		Aqueous	Ethanol	Aqueous	Ethanol
		Extract	Extract	Extract	Extract
Leaf	100μg	-	-	5.7 ± 0.6	-
	150μg	-	-	7.7 ± 0.6	-
	200μg	-	-	8.7 ± 0.6	17.3 ± 0.6
Stem	100μg	-	-	-	-
	150μg	-	-	4.3 ± 0.6	5.3 ± 0.6
	200μg	-	-	6.3 ± 0.6	8.7 ± 1.2
Root	100μg	-	-	-	-
	150μg	-	7.3 ± 0.6	-	6.3 ± 0.6
	200μg	-	8.3 ± 0.6	9.3 ± 0.6	20.7 ± 0.6

Discussion:

In this present manuscript we had reported the ethnomedicinal uses, phytochemical screening and antimicrobial activity from various parts of *B. diffusa*. Many authors also reported ethnomedicinal works, phytochemical screening and antimicrobial activities of other medicinal plants from different region of Odisha, India. Sahu and Ekka (2021) reported that tender leaves of *B. diffusa* are cooked with mustard oil, chilly, salt and then eaten by the native of Bargarh district, western Odisha, India. Behera *et al.* (2021) reported that the roots of *B. diffusa* are used for the treatment of cough by the native of Chandli reserve forest, Balangir district, Odisha. Sahu *et al.* (2021) reported that the roots of *B. diffusa* are used to treat cough by the Sahara tribal group of Kangaon village of Bargarh district, Odisha. Sahu and Sahu (2022) reported that the leaves of *B. diffusa* is used as leafy vegetables by the tribal peoples of Jharigaon block of Nabarangpur district, Odisha Dash and Sahu (2023) reported that the leaves of *B.*

diffusa are used as food by cooking and also used to treat accumulation of fluids in the body by the native of Balangir district, western Odisha. Patra et al. (2025) reported that tender leaves of B. diffusa are cooked with mustard oil, chilly, salt and then eaten by the native of Barpali NAC, Bargarh district, Odisha, India.

Nayak et al. (2024) reported the presence of alkaloids, tannins, terpenoids and flavonoids from the Tridax procumbens leaf aqueous. Sahu et al. (2024) reported the distribution and morphological description of the Hibiscus plant, along with its phytochemical as well as various bioactive chemical composition and their structures, the ethno-medicinal and botanical benefits on human health, and toxicity research. Sharma et al. (2024) investigated the phytochemical constituents of the leaves of Marsilea minuta, which are responsible for various reported pharmacological properties. The phytochemical screening detected various bioactive compounds present in three different extracts (aqueous, methanol, and ethanol), such as tannin, saponin, flavonoids, phenolic compounds, and reducing sugars. These secondary metabolites exhibited antioxidant, anti-inflammatory, antidiabetic, and antimicrobial properties. Bhoi et al. (2025) reported that the native of Bargarh district used the leaves of Justicia adhatoda L. for the treatment of asthma, arthritis, colds, coughs, eczema, malaria, tuberculosis and rheumatism, and as a uterine tonic. The phytochemical screening revealed presence of phytochemicals like alkaloids, steroids, terpenoids, saponins, phenols and tannins in the ethanol extract, while flavonoids, cardiac glycosides, amino acids, proteins, carbohydrates and reducing sugar were found to be absent. Meanwhile, In the aqueous extracts, alkaloids, flavonoids, terpenoids, saponins, tannins, phenols, amino acids and proteins showed positive result, but steroids, tannins, cardiac glycosides, carbohydrates and reducing sugars showed negative result.

Rani et al. (2025) reported the qualitative phytochemical analysis of the hydroethanolic extract of *Drimia indica* bulbs revealed the presence of tannins, saponins, flavonoids, phenolic compounds, reducing sugars, and steroids. Sahu et al. (2025) reported phytochemicals screening were carried out by using various extracts of Achyranthes aspera prepared by shaking and boiling method and documented. Steroid was found to be absent in all extracts prepared by using solvents (methanol, ethanol and water) by both methods. Tannin is present in the shoot and root of several extracts isolated by different solvent using shaking as well as boiling method. Terpenoids are absent in all parts of the plant. Alkaloid is present in methanolic, ethanolic and water extracts of both methods. Phenol was absent in all extract made by shaking method and present in extract using methanol, ethanol and water as solvent and using boiling method. Leaf and inflorescence contain flavonoid. Saponin was present in root and stem parts by shaking method whereas it was found in leaf and inflorescence extract by boiling method. Coumarin was found in all the three extracts. Dash et al. (2025) reported the ethnobotanical exploration and Phytochemical screening of Tinospora cordifolia (Willd.) Miers. belongs to the family Menispermaceae, a climbing shrub from Bargarh district, Odisha India. Both the water and ethanol solvents were used to obtain extracts of leaves and stems i.e. used for qualitative phytochemical screening by using standard methods. In this finding alcohol shows a higher solubility rate as compared to water. Present finding shows the presence of carbohydrates, proteins, alkaloids, flavanoids, saponins, glycosides, steroids, terpenoids, phenolics and tannins.

Potential therapeutic properties of the plant reported by present scientific examine comprise antioxidant, anti-stress, anti-inflammatory, anti-allergic, anti-rheumatic, adaptogenic, antipyretic, antimalarial, anti-leprotic, hypoglycemic, hepatoprotective, immunomodulatory, anticancer activities, etc. Mallik et al. (2025) reported Various phytochemical tests of Murraya koenigii (Linn.) Spreng., including the Wagner's test for alkaloids, Lead acetate test for flavonoids, Ferric chloride test for phenols and tannins, Foam test for saponins, and the Keller-Killiani test for glycosides, confirm the presence of these bioactive compounds in M. koenigii. Additionally, tests for carbohydrates, proteins, and steroids also reveal the plant's diverse chemical makeup. These findings support the plant's medicinal use in local and traditional healing practices, particularly in regions like Odisha, where it plays an integral role in managing health conditions. Padhan et al. (2025) reported that for both aqueous and ethanolic extracts revealed the presence of the following phytochemical constituents saponins, flavonoids, terpenoids, cardiac glycosides and alkaloids (aqueous extract) and tannins, saponins, flavonoid, steroids, cardiac glycosides, anthroquinones and alkaloids (ethanolic extract) in Moringa oleifera leaves. This is an indication that the M. oleifera leaves contained tannins, saponins, flavonoid, steroids, terpenoids, cardiac glycosides, anthroquinones and alkaloids as secondary metabolites. Sahu et al. (2025) reported that for phytochemical analysis ten selected fresh parts of species were collected.

The results revealed that alkaloids showed positive results in the ethanol extracts of all the selected spice/plant species, while quinones and anthraquinones showed negative results in all. Phenolic compounds, flavonoids, and saponins were also quite abundant in most species. The ethanol extract of Allium sativum (bulb) showed positive results for alkaloids, flavonoids, phenolic compounds, tannins, glycosides, saponins, steroids and carbohydrates. The ethanol extract of Brassica juncea (seeds) showed positive results for alkaloids, flavonoids, phenolic compounds, tannins, terpenoids and triterpenoids, glycosides and proteins. The ethanol extract of Cinnamomum verum (bark) showed positive results for alkaloids, phenolic compounds, tannins, saponins and protein. The ethanol extract of Cuminum cyminum (seeds) showed positive results for alkaloids, flavonoids, phenolic compounds, tannins, terpenoids and triterpenoids, glycosides, saponins and steroids. The ethanol extract of Curcuma longa (rhizome) showed positive results for alkaloids, flavonoids, phenolic compounds, tannins, terpenoids and triterpenoids, glycosides, saponins and carbohydrates. The ethanol extract of Coriander sativum (seeds and leaves) showed positive results for alkaloids, flavonoids, phenolic compounds, terpenoids and triterpenoids, saponins, steroids and carbohydrates. The ethanol extract of Elettaria cardamomum (fruits and seeds) showed positive results for alkaloids, flavonoids, phenolic compounds, tannins, terpenoids and triterpenoids, saponins, carbohydrates and proteins.

The ethanol extract of *Syzygium aromaticum* (flower buds) showed positive results for alkaloids, flavonoids, phenolic compounds, tannins, terpenoids % triterpenoids, steroids, carbohydrates, and proteins. The ethanol extract of *Trigonella foenum-graceum* (leaves) showed alkaloids, phenolic compounds, tannins, saponins, and proteins. The ethanol extract of *Zingiber officinale* (rhizome) showed positive results for alkaloids, flavonoids, tannins, glycosides, saponins, and carbohydrates.

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