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Investigating Boerhavia Diffusa: Phytochemical Analysis and Antioxidant Potential of Methanol, Ethyl Acetate, and Hexane Extracts

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ABSTRACT

Boerhavia diffusa, colloquially referred to as punarnava, is a perennial herb indigenous to tropical and subtropical regions, esteemed for its profound medicinal properties, particularly within the context of Ayurvedic practices. Renowned for its anti-inflammatory, diuretic, hepatoprotective, and antioxidant effects, this plant harbors bioactive constituents such as alkaloids, flavonoids, glycosides, and steroids, all of which contribute to its therapeutic efficacy in addressing ailments such as edema, liver dysfunction, renal disorders, and metabolic imbalances. Contemporary pharmacological investigations lend credence to its traditional uses, indicating its potential in the management of conditions like hypertension, diabetes, and rheumatoid arthritis. This study provides an in-depth examination of the plant's botanical characteristics, phytochemical constituents, and pharmacological activities, underscoring its therapeutic promise while advocating for further clinical exploration. The research delves into the phytochemical profile and antioxidant potential of *Boerhavia diffusa* through the analysis of methanol, ethyl acetate, and hexane extracts. Phytochemical screening revealed the presence of alkaloids, flavonoids, glycosides, saponins, and steroids across all extracts, with variable concentrations contingent upon the solvent employed. Antioxidant activity was assessed via in vitro assays, including the DPPH assay, which demonstrated considerable antioxidant potential in the methanol and ethyl acetate extracts. These extracts exhibited superior antioxidant activity, suggesting a correlation between polyphenolic compounds and enhanced antioxidant efficacy. Conversely, the hexane extract displayed moderate activity, primarily attributed to the presence of non-polar compounds. The results underscore *Boerhavia diffusa* as a promising natural source of antioxidants with potential applications in functional foods and therapeutic agents. Nevertheless, additional research is essential to elucidate the molecular mechanisms underlying its antioxidant properties and to evaluate its clinical viability, thereby confirming its safety and therapeutic efficacy in contemporary medical practice.

Keywords: *Boerhavia diffusa, Punarnava, Extraction, Phytochemical Composition, Antioxidant Activity, DPPH assay.*

1. INTRODUCTION

Boerhavia diffusa, a perennial herb indigenous to tropical and subtropical climates, is highly regarded for its therapeutic properties, particularly within the field of Ayurveda. The plant is rich in bioactive compounds, including alkaloids, flavonoids, glycosides, saponins, and steroids, which collectively contribute to its anti-inflammatory, diuretic, hepatoprotective, and antioxidant effects. Of particular significance are its antioxidant properties, which hold promise in addressing chronic diseases linked to oxidative stress, such as cancer, diabetes, and cardiovascular disorders. This study examines the antioxidant potential of *Boerhavia diffusa* extracts derived from methanol, ethyl acetate, and hexane solvents, analysing their phytochemical composition and antioxidant activity to assess their role in mitigating oxidative stress and related health concerns. Furthermore, the herb's efficacy in managing hypercholesterolemia and cardiovascular risks is explored, presenting a potential natural alternative to conventional therapeutic approaches.

2. MATERIALS AND METHODS

Collection of Sample:

The *Boerhavia diffusa* leaf samples were collected from the Sengadu Village, Tamil Nadu. They were washed with sterile distilled water, blotted dry, cut into small pieces, and shade-dried for five days. After drying, the leaves were ground into a fine powder and stored for analysis.

Hot Continuous Extraction (Soxhlet)

In this method, the powdered crude drug is placed in a thimble within the Soxhlet apparatus. The heated solvent vapor condenses in the condenser, drips onto the drug, and extracts active compounds. When the solvent reaches the siphon tube, it is returned to the flask. This process repeats until no residue remains. The hydro-alcoholic solution is then evaporated using a rotary evaporator, leaving the extracted plant material.

Phytochemical Qualitative Analysis

Test for Alkaloids:5 mg of the extract and a drop of Dragendorff's reagent produced an orange-red precipitate, confirming alkaloids.

Test for Carbohydrates:5 mg of extract mixed with Benedict's reagent and heated formed a reddish-brown precipitate, indicating carbohydrates.

Test for Glycosides:A brown ring at the interface of an extract, H_2SO_4, glacial acetic acid, and $FeCl_3$ confirmed cardiac glycosides.

Test for Saponins: Shaking 5 ml of distilled water with the extract produced foam, indicating saponins.

Test for Proteins: Adding Biuret reagent to 5 mg of extract and heating for 1-5 minutes showed a red/violet colour, indicating proteins.

Test for Amino Acids:A violet colour after heating 5 mg of extract with Ninhydrin solution indicated amino acids.

Test for Phenols: Adding ferric chloride to boiled leaf extract formed a brownish-green or blue colour, confirming phenols.

Test for Terpenoids: The appearance of a grey tint after heating the extract with chloroform and H_2SO_4 confirmed terpenoids.

Phytochemical Quantitative analysis

Determination of total phenolic content (Singleton and Rossi, 1965)

2g of plant powder was soaked in methanol for 24 hours, filtered, and the filtrate evaporated. The extract was centrifuged at 10,000 rpm for 15 minutes at 4°C, and the supernatant was used to prepare 20 mL of extract. This was mixed with 3 mL distilled water, and 0.5 mL of Folin-Ciocalteu reagent was added. After incubating for 3 minutes at 45°C, 2 mL of 20% Na_2CO_3 was added, and absorbance at 650 nm was measured to calculate total phenol content.

C (GAE) = c x V/M

where, c = concentration of sample from the curve obtained (mg/mL),

V = volume used during the assay (mL) and M = mass of the sample used during the assay (g)

Determination of total flavonoids

Flavonoid content was determined using a modified spectrophotometric method. 1g of dry powder was ground with 200 mL of 80% aqueous methanol, filtered, and 0.5 mL of the filtrate was mixed with 3 mL of distilled water, 0.3 mL of 5% sodium nitrite, and 0.6 mL of 10% aluminium chloride. After 6 minutes, 2 mL of 1 M sodium hydroxide was added, and the solution was diluted to 10 mL. Absorbance at 510 nm was measured, and total flavonoid content was calculated as quercetin equivalent (mg QE/g).

$X = (A\text{-}M_0/A_0\text{-}M)$

where, A= absorption of sample, A_0= absorption of standard (quercetin),

M= weight of sample (mg/mL) and M_0= weight of quercetin in solution (mg/mL)

Total Antioxidant assay

In each test tube, 1 mL each of distilled water, molybdate reagent, sodium phosphate, and sulfuric acid were added. The extract (10-100 µg/mL) was introduced and incubated at 95°C for 90 minutes. After cooling to room temperature, absorbance was measured at 695 nm.

DPPH assay

The antioxidant activity was determined using the DPPH assay. 0.5 mL of the sample, 1 mL of methanol, and 1 mL of 0.5 mM DPPH solution were mixed, and the colour change (from violet to yellow) was measured at 517 nm after 100 minutes. A control with methanol and DPPH, and a blank with extracts, were also prepared. The scavenging activity was calculated using a specific formula.

% of inhibition = Control O.D – Sample O.D/Control O.D X 100.

3. RESULTS

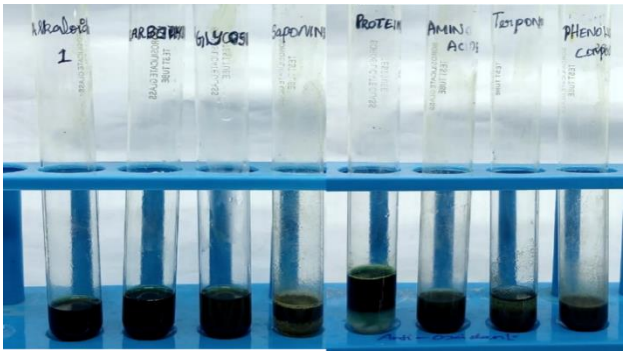
Phytochemical Qualitative Analysis Methanol Extract of *Boerhavia Diffusa*

This study investigated the phytocompounds in *Boerhavia diffusa* extracts. The methanol extract contained alkaloids, glycosides, saponins, proteins, amino acids, and phenolics, but lacked carbohydrates and terpenoids, as shown in Table 1 and Figure 1.

Table 1 Phytochemical Qualitative Analysis Methanol Extract of *Boerhavia Diffusa*

SI.NO	Test	Result
1	Alkaloids Test	+
2	Carbohydrates Test	–
3	Glycosides Test	+
4	Saponins Test	+
5	Proteins Test	+
6	Amino Acid Test	+
7	Phenolic Compounds Test	+
8	Terpenoids Test	–

Figure 1Phytochemical Qualitative Analysis Methanol Extract of *Boerhavia Diffusa*



Phytochemical Qualitative Analysis Ethyl acetate extract of *Boerhavia Diffusa*

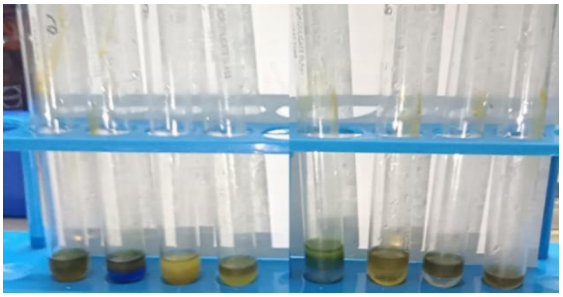
This study examined the ethyl acetate extract of *Boerhavia diffusa* for medicinally important phytocompounds. The extract contained alkaloids, proteins, and phenolics, but lacked carbohydrates, glycosides, saponins, amino acids, and terpenoids, as shown in Table 2 and Figure 2.

Table 2 Phytochemical Qualitative Analysis Ethyl acetate Extract of *Boerhavia Diffusa*

SI.NO	Test	Result
1	Alkaloids Test	+
2	Carbohydrates Test	–

3	Glycosides Test	–
4	Saponins Test	–
5	Proteins Test	+
6	Amino Acid Test	–
7	Phenolic Compounds Test	+
8	Terpenoids Test	–

Figure 2 Phytochemical Qualitative Analysis Ethyl acetate Extract of *Boerhavia Diffusa*



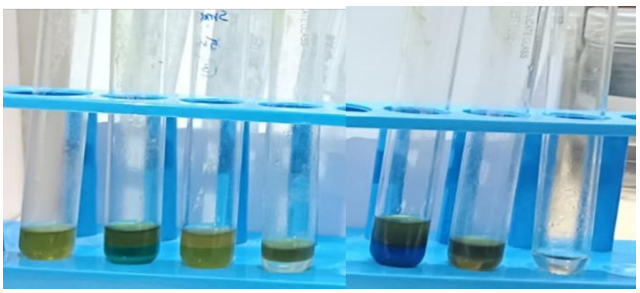
Phytochemical Qualitative Analysis Hexane extract of *Boerhavia Diffusa*

This study examined the hexane extract of *Boerhavia diffusa* for medicinally significant phytocompounds. The extract contained alkaloids but lacked carbohydrates, glycosides, saponins, proteins, amino acids, phenolics, and terpenoids, as shown in Table 3 and Figure 3.

Table 3 Phytochemical Qualitative Analysis Hexane Extract of *Boerhavia Diffusa*

SI.NO	Test	Result
1	Alkaloids Test	+
2	Carbohydrates Test	–
3	Glycosides Test	–
4	Saponins Test	–
5	Proteins Test	–
6	Amino Acid Test	–
7	Phenolic Compounds Test	–
8	Terpenoids Test	–

Figure 3 Phytochemical Qualitative Analysis Hexane Extract of *Boerhavia Diffusa*



Phytochemical Quantitative analysis

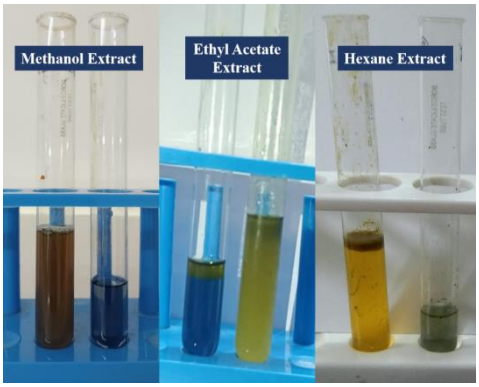
The total phenol and flavonoid contents in *Boerhavia diffusa* extracts are as follows:

- Methanol extract: 0.52 mg/g phenols (gallic acid equivalent), 1.39 mg/g flavonoids (quercetin equivalent)
- Ethyl acetate extract: 0.80 mg/g phenols (gallic acid equivalent), 1.68 mg/g flavonoids (quercetin equivalent)
- Hexane extract: 0.20 mg/g phenols (gallic acid equivalent), 0.61 mg/g flavonoids (quercetin equivalent), as shown in Table 4 and Figure 4.

Table 4 Phytochemical Quantitative analysis of *Boerhavia diffusa*

SI NO	Test Sample (*Boerhavia diffusa*)	Result
1	Total Phenol Methanol Extract	0.52 mg/g
2	Total Flavonoids Methanol Extract	1.39 mg/g
3	Total Phenol Ethyl Acetate Extract	0.80 mg/g
4	Total Flavonoids Ethyl Acetate Extract	1.68 mg/g
5	Total Phenol Hexane Extract	0.20 mg/g
6	Total Flavonoids Hexane Extract	0.61 mg/g

Figure 4 Phytochemical Quantitative analysis of *Boerhavia diffusa*



Antioxidant Activity

DPPH Assay Methanol Extract of *Boerhavia diffusa*

The methanol extract of *Boerhavia diffusa* showed dose-dependent activity, with a maximum inhibition of 83.1% at 200 µg/ml, compared to the standard quercetin, as shown in Table 5, Figure 5, and Graph 1.

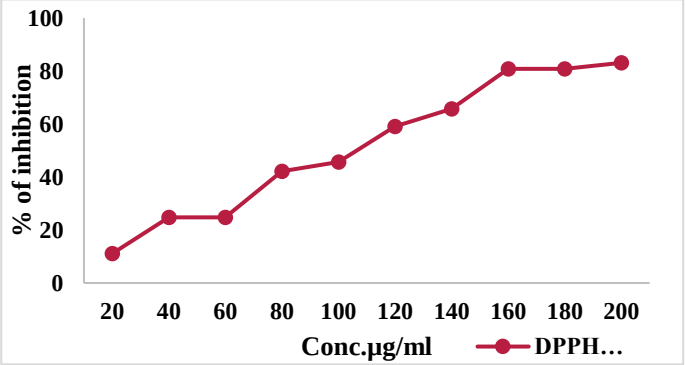
Table 5 DPPH Assay methanol extract of *Boerhavia diffusa*

SI NO	Concentration (µg/ml)	DPPH Activity %
1	20	11.1
2	40	24.8
3	60	24.8
4	80	42.2
5	100	45.7
6	120	59.1
7	140	65.7

8	160	80.8
9	180	80.8
10	200	83.1

Figure 5 DPPH Assay Methanol Extract of *Boerhavia diffusa*

Graph 1 DPPH Assay Methanol Extract of *Boerhavia diffusa*



DPPH Assay Ethyl Acetate Extract of *Boerhavia diffusa*

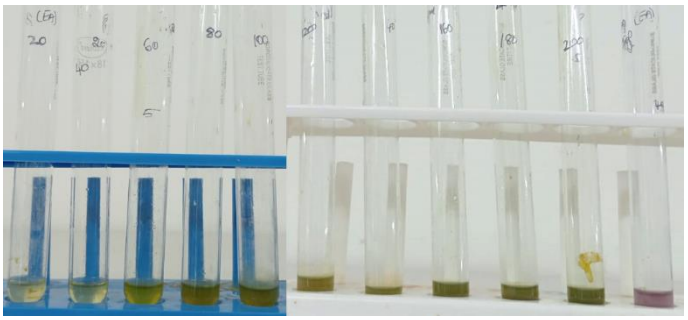
The ethyl acetate extract of *Boerhavia diffusa* showed dose-dependent activity, with a maximum inhibition of 64.4% at 200 µg/ml, compared to quercetin, as shown in Table 6, Figure 6, and Graph 2.

Table 6 DPPH Assay Ethyl Acetate Extract of *Boerhavia diffusa*

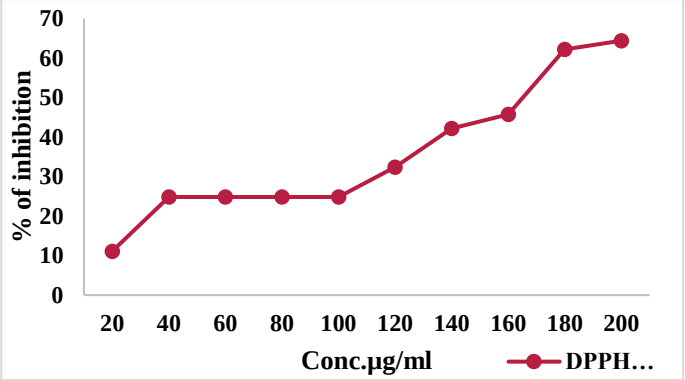
SI NO	Concentration (µg/ml)	DPPH Activity %
1	20	11.1
2	40	24.8
3	60	24.8
4	80	24.8
5	100	24.8
6	120	32.4
7	140	42.2
8	160	45.7

9	180	62.2
10	200	64.4

Figure 6 DPPH Assay Ethyl Acetate Extract of *Boerhavia diffusa*



Graph 2 DPPH Assay Ethyl Acetate Extract of *Boerhavia diffusa*



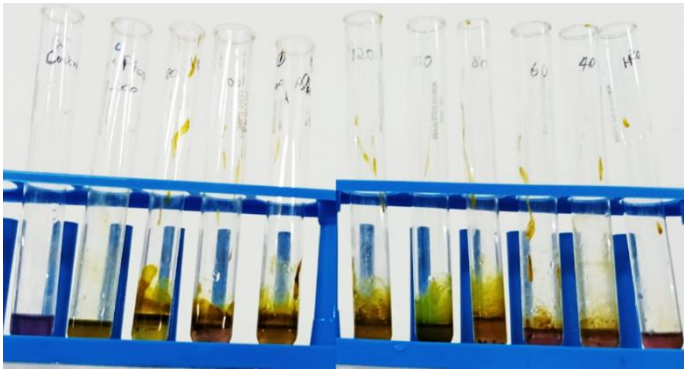
DPPH Assay Hexane Extract of *Boerhavia diffusa*

The hexane extract of *Boerhavia diffusa* showed dose-dependent activity, with a maximum inhibition of 68.8% at 200 µg/ml, compared to quercetin, as shown in Table 7, Figure 7, and Graph 3.

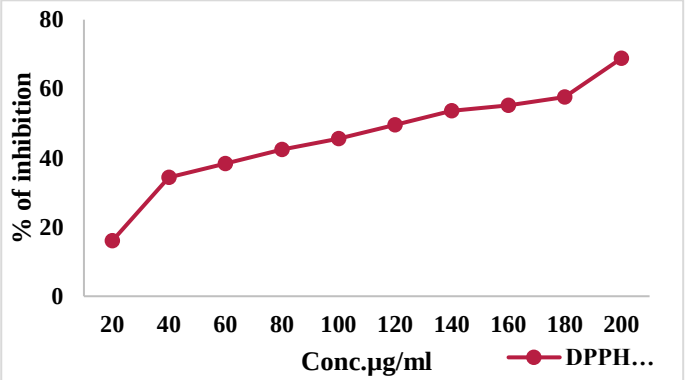
Table 7 DPPH Assay Hexane Extract of *Boerhavia diffusa*

SI NO	Concentration (µg/ml)	DPPH Activity %
1	20	16.0
2	40	34.4
3	60	38.4
4	80	42.4
5	100	45.6
6	120	49.6
7	140	53.6
8	160	55.2
9	180	57.6
10	200	68.8

Figure 7 DPPH Assay Hexane Extract of *Boerhavia diffusa*



Graph 3 DPPH Assay Hexane Extract of *Boerhavia diffusa*



4. DISCUSSION

This study investigated the phytochemical composition and antioxidant potential of *Boerhavia diffusa*, focusing on methanol, ethyl acetate, and hexane extracts. Qualitative analysis identified key bioactive compounds, including phenols, flavonoids, and alkaloids, in all extracts. The ethyl acetate extract had the highest total phenol (0.80 mg/g) and flavonoid (1.68 mg/g) content, suggesting its significant contribution to the plant's bioactivity. Antioxidant assays showed dose-dependent activity, with the methanol extract achieving 83.1% inhibition at 200 µg/ml, the ethyl acetate extract 64.4%, and the hexane extract 68.8%. These results highlight *Boerhavia diffusa* as a potential natural antioxidant source, with solvent choice influencing extraction efficiency. Ethyl acetate was most effective in extracting phenolic compounds, while methanol and hexane extracts showed lower yields.

5. CONCLUSION

This study highlights the strong antioxidant potential of *Boerhavia diffusa*, driven by high concentrations of phenols and flavonoids, with the methanol extract showing the highest levels. In vitro assays confirmed dose-dependent antioxidant effects, with the methanol extract achieving 83.1% inhibition. These findings support its traditional use for oxidative stress and suggest potential applications in managing health issues like hypercholesterolemia. Future research should explore its effects on lipid metabolism and cholesterol regulation.

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