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Phytochemical profile, FTIR and antidiabetic properties of hydroethanolic leaf extract of *Boerhavia diffusa*

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Abstract
Here, we report on the phytochemical composition and the antioxidant, antimicrobial, and antidiabetic activities of a 70% hydroethanolic leaf extract of *Boerhavia diffusa*. Proximate analysis revealed the leaf powder to be rich in ash (15.70%) and crude protein (13.23%). Quantitative phytochemical analysis showed the extract contained high levels of total phenolics (130.01 mgGAE/g), flavonoids (70.04 mgRE/g), alkaloids (124.80 mg/g), tannins (94.22 mg/g) and terpenoids (86.02 mg/g), which were corroborated by FT-IR spectroscopy. The high bioactive compound content confirms *B. diffusa*'s potential as a natural antioxidant for health applications.

Keywords: α-Amylase inhibition, α-glucosidase inhibition, FT-IR, 70% ethanolic extract, phytocompounds, proximate composition

Introduction
Reactive oxygen species (ROS) and free radicals, though essential for normal cellular processes, can cause oxidative stress when produced in excess, leading to chronic conditions such as diabetes, cardiovascular diseases, neurodegenerative disorders and cancer (Christen *et al*, 2000; Droge, 2002) [8, 14]. Antioxidants mitigate these effects by neutralizing reactive molecules. However, concerns about the safety of synthetic antioxidants like butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA) have intensified the search for safe, plant-derived alternatives (Ito *et al*. 1985; Braca *et al*. 2002) [23, 6].

Boerhavia diffusa (Punarnava), a perennial herb of the Nyctaginaceae family, is widely distributed in tropical and subtropical regions and has long been used in Ayurveda, Unani and traditional medicine for managing inflammation, liver and kidney disorders and diabetes(Nutritionally, its leaves are rich in proteins, lipids, fibre and carbohydrates, while phytochemical analyses have revealed phenols, flavonoids, alkaloids, glycosides, saponins and terpenoids that contribute to antioxidant, antimicrobial, hepatoprotective and antidiabetic activities (Beegun *et al*. 2014; Pari and Satheesh, 2004; Cushnie *et al*. 2012; Oseni *et al*. 2024) [4, 33, 10, 32].

Comparative studies indicate that ethanolic extracts of *B. diffusa* exhibit stronger radical scavenging and reducing power than aqueous extracts, owing to their higher phenolic and flavonoid content (Singh *et al*. 2012) [38]. Given the global rise of diabetes and antimicrobial resistance, natural plant-based inhibitors of α-amylase, α-glucosidase and microbial pathogens hold promise as safer therapeutic alternatives (Krentz and Bailey, 2005; Cushnie *et al*. 2014) [25, 10].

This study was conducted to investigate the phytochemical composition, proximate profile and biological activities of the 70% ethanolic extract of *B. diffusa* (BDE). This approach was chosen to highlight the efficiency of hydroethanolic extraction in recovering both polar and semi-polar bioactive substances, thereby maximizing the biological activity of the plant extract.

2. Materials and methods
Material collection and preparation
Damage-free leaves of *B. diffusa* were collected from the College of Fishery Science, Muthukur

and identified by the Principal Scientist, Agricultural Research Station, ANGRAU and further deposited in the Herbarium Department of Botany, ANGRAU. Fresh leaves were washed thoroughly and dried in the shade at room temperature for about 10 days. Dried leaves were ground, sieved, and stored in moisture-free containers until analysis.

Proximate Composition

Proximate composition of the shade-dried leaf powder was determined following AOAC (2000) [2] methods, including the estimation of moisture, crude protein, crude fat and ash.

pH

The pH of the leaf powder was determined by following Zarandona *et al* (2021) [45] by using a pH meter (Eutech Instruments, Malaysia) in triplicate.

Water-Holding Capacity

The water-holding capacity (WHC) of the leaf powder was evaluated according to Beuchat (1977) [5] with slight modifications. 1:10 ratio of powder and distilled water is centrifuged (3000 rpm) for 30 min and the obtained supernatant was carefully decanted. The volume of water retained with the sample in the sediment was measured. WHC was expressed as

$$\text{WHC (\%)} = \frac{W2 - W1}{W1} \times 100$$

Where, W1 = centrifuge tube + dry sample
W2 = centrifuge tube + wet sample

Preparation of Ethanolic Extract

Plant Extract was prepared according to Kusumaningsih *et al* (2021) [26] with slight modifications. To prepare extract, leaf powder was immersed in 70% ethanol in 1:10 ratio for 48 h with intermittent stirring. The mixture was centrifuged and the extract was separated from the residue. The filtrate was concentrated in rotary evaporator at 60 °C and freeze-dried to obtain a powdered extract (yield: 7% w/w), which was stored in amber bottles at 4 °C until analysis.

Fourier Transform Infrared (FTIR) Spectroscopy analysis

BDE extract was tested using FTIR scanning (4000-400 cm^{-1}) and the peaks obtained were compared with standard charts to determine the functional groups present.

Quantitative tests

The total phenolic content (TPC) was determined using the Folin-Ciocalteu reagent, as described by Singleton and Rossi (1965) [39]. Total Flavonoid content (TFC) was quantified according to Chang *et al*. (2002) [7] and tannin content was measured according to Makkar *et al* (1993) [28]. Alkaloid and steroid contents were estimated following the method described by Harborne (1998) [21]. The terpenoid content was analysed according to the method described by Ghorai *et al* (2012) [17].

Anti-diabetic activity

α-amylase inhibition and α-glucosidase inhibition

The α-amylase inhibitory activity and the α-glucosidase inhibitory activity of the extract were determined according to the standard procedure described by Wongsa *et al* (2012) [44].

Statistical Analysis

All the experiments were done in triplicate and the results are shown as mean ± SD. Data were analysed using one-way ANOVA and significant differences ($p < 0.05$) were determined by Duncan's Multiple Range Test (DMRT) using SPSS 20.

Results and Discussion

Proximate Composition and Physicochemical Properties

Nutritional and physicochemical profiles of *B. diffusa* leaf powder are represented in Table 1. The moisture content was within a favourable range for the stability and shelf life of the powdered material, as it inhibits microbial growth and enzymatic degradation (Fellows, 2022) [16]. This value is consistent with the findings of Ezeabara and Nwiyi (2017) [15], who reported a moisture content of 11.53% in shade-dried leaves for 21 days. In contrast, Ujowundu *et al*. (2008) [42] applied oven drying at 60 °C, which resulted in markedly different proximate values, including very high moisture (82.22 ± 4.16%) and low protein (2.26 ± 0.02%), fat (1.61 ± 0.06%) and ash (0.96±0.01) content, supporting the view that processing methods, particularly drying, significantly influence the proximate composition of the plant material.

The ash content, an indicator of total mineral matter, was considerably high, suggesting that *B. diffusa* leaves are a rich reservoir of inorganic nutrients and electrolytes. This value is lower than the 20.42% reported by Ezeabara and Nwiyi (2017) [15] but significantly higher than the 0.96% reported by Ujowundu *et al*. (2008) [42], further emphasizing the dramatic influence of drying techniques on mineral content preservation. The crude protein content reinforces the nutraceutical potential of this plant, supporting its traditional use as a dietary supplement. The crude fat content is comparable to that reported in previous studies. The water-holding capacity (WHC) indicates a moderate ability of the leaf powder to bind water, a functional property that could be valuable in food formulation. The slightly acidic nature can be attributed to the presence of natural organic acids, including phenolic acids, flavonoids and other secondary metabolites.

Table 1: Proximate composition and physicochemical properties of *B. diffusa* leaf powder.

Compositions	(%)
Moisture	12.93 ± 0.97
Protein	13.23 ± 0.53
Fat	4.84 ± 0.95
Ash	15.70 ± 0.79
Dry matter	87.07 ± 0.13
Water holding capacity	27.96 ± 0.13
pH	6.43 ± 0.01

Phytochemical Composition of the 70% Ethanolic Extract

For quantitative estimation, plant secondary metabolite contents were analysed in mg/g dry weight of the BDE. The extract was a rich source of diverse secondary metabolites, as quantified in Figure 1. The choice of hydroethanolic solvent was validated by the high yield of bioactive compounds, as it efficiently extracted both polar and medium-polarity molecules.

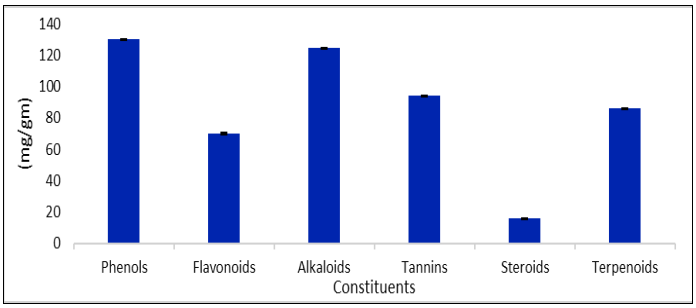

Fig. 1: Phytochemical composition of the BDE.

The TPC was remarkably high at 130.01 mg of GAE/g of extract. Phenolic compounds are well-known for their redox characteristics, which give them strong antioxidant properties. This value is consistent with the findings of Gophane and Khobragade (2019) [20], who reported a high TPC (155.35 mg GAE/g) in ethanolic root extracts of *B. diffusa*, indicating the systemic distribution of these valuable compounds throughout the plant. Similarly, the flavonoid content was also substantial, at 70.04 mg RE/g. Flavonoids contribute significantly to antioxidant activity through mechanisms such as hydrogen donation and metal chelation (Heim *et al*. 2002) [22].

Notably, the extract was exceptionally rich in alkaloids (124.80 mg/g) and tannins (94.22 mg/g) content. Alkaloids are associated with a wide range of pharmacological activities, including antimicrobial and antidiabetic effects (Litwinienko *et al*. 2014; Tiong *et al*. 2013) [27, 41], whereas tannins are known for their astringent, antimicrobial and radical-scavenging properties (Chung *et al*. 1998) [9]. The significant terpenoid content (86.02 mg/g) and the presence of steroids (15.63 mg/g) further broaden the therapeutic potential of the extract, as these compound classes exhibit anti-inflammatory, anticancer and antimicrobial activities (Thoppil and Bishayee, 2011; Cássia *et al*. 2013; Vundru *et al*. 2013) [40, 12, 43].

The observed divergence in phytochemical concentrations compared to other studies, such as the lower values reported by Adeku *et al*. (2022) [1], can be attributed to factors such as genetic variability, environmental conditions, plant age and crucially, the extraction protocol (Gobbo-Neto and Lopes, 2007) [19]. The 70% ethanol concentration used in this study appears to be optimal for solubilizing this specific profile of bioactive compounds, striking a balance between polarity and extraction efficiency, as supported by Olvera-Aguirre *et al*. (2022) [31], who found hydroethanolic solvents to be superior for phenolic recovery from Moringa leaves.

FT-IR Analysis

FTIR analysis of the extract (Fig. 3) revealed a complex spectral profile with several distinct absorption peaks, indicating a multifaceted chemical composition. Key vibrational bands were observed at 3897-3595 cm^{-1}, which may be attributed to O-H or N-H stretching vibrations, potentially confirming the presence of alcohols, phenols, or amines. A notable peak at 2356 cm^{-1} is characteristic of O=C=O asymmetric stretching, often associated with atmospheric CO_2 adsorption. Strong absorptions at 1745 and 1699 cm^{-1} are indicative of C=O stretching vibrations that are typical of carbonyl groups in esters, ketones, or carboxylic acids. The band at 1648 cm^{-1} likely corresponds to amide I (C=O stretch) or C=C stretching in alkenes. Peaks at 1540 and 1517 cm^{-1} may represent amide II (N-H bending) or aromatic C=C vibrations. Additional bands at 1462, 1397 and 984 cm^{-1} are consistent with C-H bending, O-H deformation, or C-O stretching modes, commonly found in carbohydrates, polyphenols and organic acids. These spectral features collectively suggest the presence of bioactive compounds, such as polyphenols, flavonoids, organic acids, or polysaccharides, aligning with the typical phytochemical profiles of plant-derived (Silverstein *et al*. 1962; Kizil *et al*. 2002) [37, 24]. These functional groups, particularly hydroxyl and carbonyl groups, are recognized for their hydrogen-donating and radical-scavenging properties, which explains the potent antioxidant activity observed in the DPPH and ABTS assays.

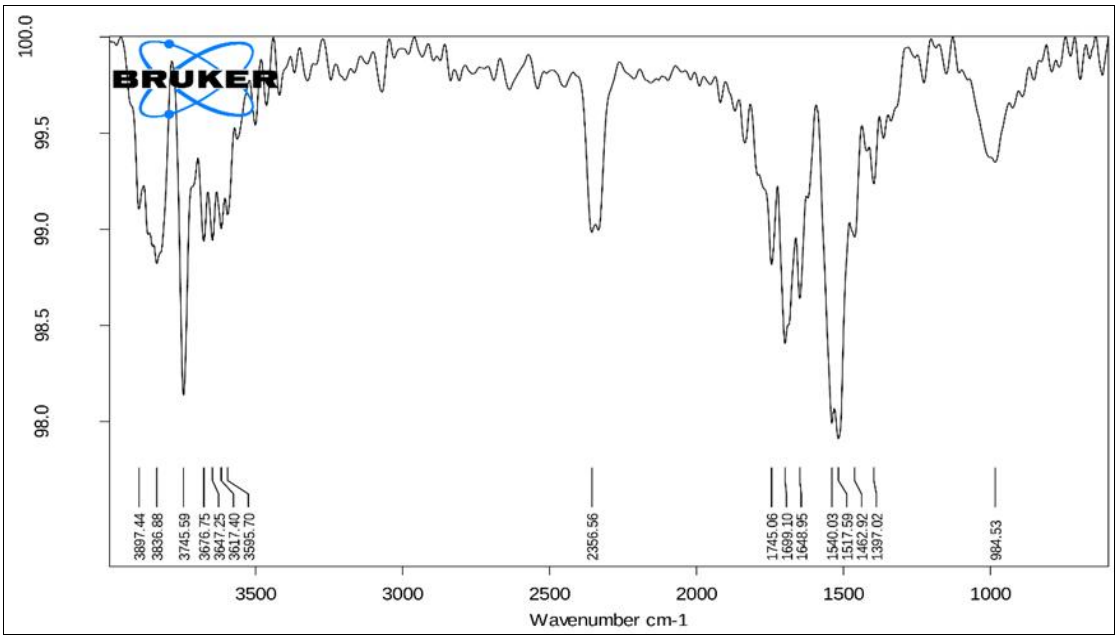

Fig 2: FT-IR spectrum of the BDE.

Anti-diabetic activity

The antidiabetic potential of BDE was evaluated by measuring its inhibitory effects on the carbohydrate-digesting enzymes α-amylase and α-glucosidase. The extract demonstrated strong, dose-dependent inhibition of both enzymes, as detailed in Figure 5. α-Amylase inhibition increased from 11.95% at 20 µg/mL to 69.60% at 100 µg/mL, with an IC_{50} value of 58.25 µg/mL, while α-glucosidase inhibition rose from 12.45% to 71.30% over the same concentration range, yielding an IC_{50} of 71.15 µg/mL.

Tannins are effective enzyme inhibitors due to their strong protein-precipitating properties (Chung *et al*. 1998) [9]. The high alkaloid content also contributes significantly, as many alkaloids have been reported to possess strong antidiabetic properties through various mechanisms, including enzyme inhibition (Tiong *et al*. 2013) [41]. Pari and Satheesh (2004) [33] reported the antidiabetic activity of *B. diffusa* in diabetic rats, attributing it to enhanced hepatic glycolysis and glycogen synthesis.

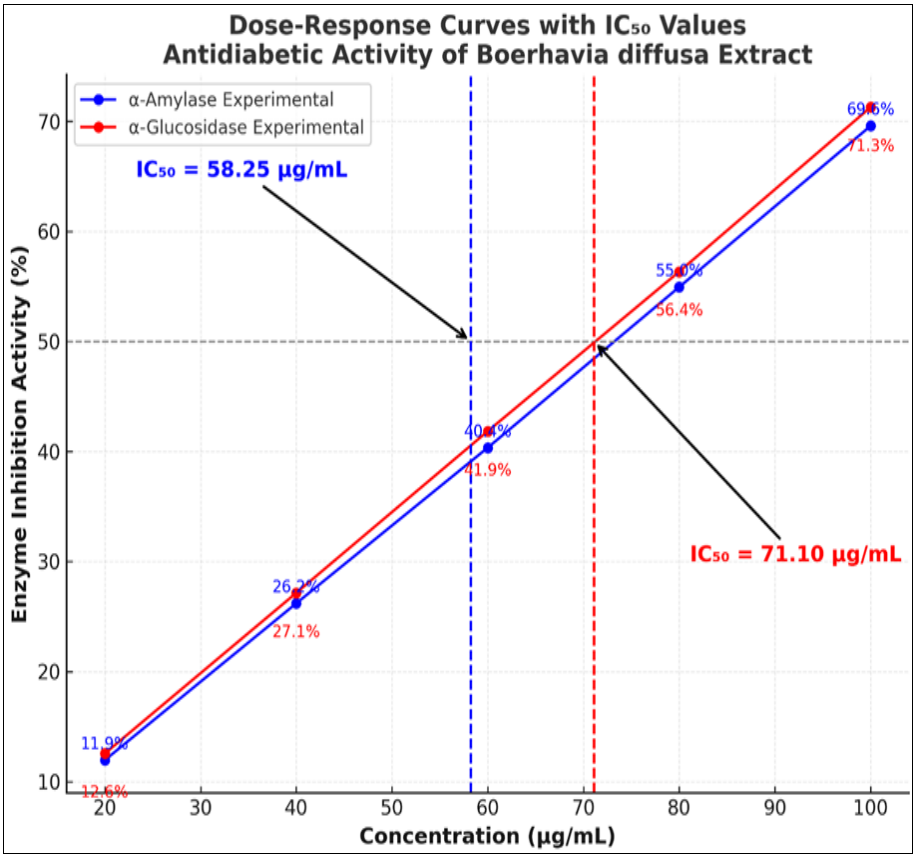

Fig 3: Dose-response relationships and IC_{50} determination for α-Amylase Inhibition and α-Glucosidase Inhibition Assays

Conclusion

This study presents a detailed evaluation of the nutritional and bioactive composition of *B. diffusa* leaves. Proximate analysis confirmed its value as a nutrient-dense material rich in minerals and proteins. The 70% ethanolic extract was found as a rich source of pharmacologically active phytochemicals, including exceptionally high amounts of phenolics, flavonoids, alkaloids and tannins, as confirmed by quantitative assays and FT-IR spectroscopy. The extract showed strong, dose-dependent antidiabetic properties through the effective inhibition of α-amylase and α-glucosidase enzymes. This activity can be directly linked to its abundant phytochemical composition. These results scientifically validate the traditional ethnomedicinal uses of *B. diffusa* and position its hydroethanolic leaf extract as a promising natural anti-diabetic agent. The rich phytochemical profile suggest that *B. diffusa* leaf extract could be effectively used as a core ingredient in nutraceutical formulations aimed at combating oxidative stress.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Author Contributions

All authors critically reviewed the manuscript for intellectual content, approved the final version to be published, and agreed to be accountable for all aspects of the work.

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