Comprehensive Analysis of the Anti-inflammatory and Anti-diabetic Properties of Aqueous-Alcoholic Extract of *Boerhavia diffusa*: An In Vitro Study

P. Harini[1], Priyadharshini R[2*], Rajeshkumar S[3]

[1]Department of Pathology, Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Sciences (SIMATS), Saveetha University, Chennai-600 077, Tamil Nadu, India. email: 151901095.sdc@saveetha.com

[2*]Department of Oral Pathology, Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Sciences (SIMATS), Saveetha University, Chennai-600 077, Tamil Nadu, India. email: priyadharshinir.sdc@saveetha.com

[3]Nanobiomedicine lab, Department of Pharmacology, Saveetha Dental College and hospitals, Saveetha Institute of Medical and Technical science (SIMATS), Saveetha University, Chennai-600077, Tamil Nadu, India. email: ssrajeshkumar@hotmail.com

Background

Chronic inflammation and diabetes mellitus are significant global health challenges. Inflammation exacerbates diabetic complications, necessitating therapeutic strategies targeting both conditions. *Boerhavia diffusa*, a traditional medicinal plant, is known for its anti-inflammatory and anti-diabetic properties. This study investigates the efficacy of its aqueous-alcoholic extract through in vitro assays.

Methods

Aqueous-alcoholic extracts of *Boerhavia diffusa* were prepared using standardized protocols. Anti-inflammatory activity was assessed using the albumin denaturation assay, with inhibition percentages calculated across various concentrations (10–50 µL). Anti-diabetic activity was determined through the alpha-amylase inhibition assay. Absorbance data were recorded at 666 nm and 540 nm for anti-inflammatory and anti-diabetic activities, respectively. Results were analyzed using Pearson correlation for concentration-dependent effects.

Results

The extract demonstrated a dose-dependent anti-inflammatory effect, with inhibition increasing from 33.0% (10 µL) to 76.1% (50 µL). A strong positive correlation between concentration and inhibition percentage was observed ($p < 0.05$). Similarly, consistent alpha-amylase inhibition was noted, ranging from 77.0% to 78.0%, with minor fluctuations in absorbance values. Both activities exhibited a high correlation with extract concentration, validating the extract's efficacy.

Conclusion

The findings reveal the significant anti-inflammatory and anti-diabetic properties of *Boerhavia diffusa* aqueous-alcoholic extract. The dose-dependent activity suggests its potential as a natural therapeutic agent for managing inflammation and diabetes. Future studies should focus on in vivo validation and clinical trials to establish its broader pharmacological applications.

Keywords

Boerhavia diffusa, anti-inflammatory, anti-diabetic, aqueous-alcoholic extract, in vitro.

Introduction

The increasing prevalence of chronic inflammatory and metabolic disorders, such as diabetes, poses significant challenges to global health [1]. Diabetes mellitus, characterized by persistent hyperglycemia, is closely associated with inflammation-driven complications, including neuropathy, nephropathy, and cardiovascular disorders [2]. Addressing these conditions necessitates the development of therapeutics that simultaneously target inflammation and glycemic control [3]. Traditional medicine offers promising avenues for identifying bioactive compounds with dual benefits. *Boerhavia diffusa* (commonly known as Punarnava) is a medicinal plant extensively used in Ayurvedic medicine for its multifaceted therapeutic properties [4].

Boerhavia diffusa, a member of the Nyctaginaceae family, has been recognized for its diverse pharmacological activities, including Antibacterial, Antioxidant and diuretic properties [5-7]. The plant roots and leaves are rich in bioactive compounds such as alkaloids, flavonoids, and glycosides, which have been documented to exhibit significant medicinal potential. While several studies have explored its traditional uses, there is limited in-depth analysis of its anti-inflammatory and antidiabetic effects, particularly using aqueous-alcoholic extracts [8].

Numerous studies have highlighted the pharmacological potential of *Boerhavia diffusa*. For instance, Gaur et al. (2022) reported its diuretic and adaptogenic properties, emphasizing its role in mitigating stress-induced metabolic imbalances [9]. From an anti-inflammatory perspective, the presence of flavonoids and phenolic acids in *Boerhavia diffusa* has been linked to its ability to inhibit key inflammatory mediators such as cyclooxygenase (COX) enzymes and pro-inflammatory cytokines [10]. Such mechanisms are vital in reducing chronic inflammation, a precursor to various metabolic disorders, including diabetes.

Previous studies have mainly relied on either aqueous or ethanolic extracts, potentially missing the synergistic effects of bioactive compounds extracted using both solvents. Additionally, inconsistent extraction techniques and in vitro methodologies have led to variable and non-reproducible results. The exact biochemical mechanisms underlying the dual anti-inflammatory and antidiabetic effects of *Boerhavia diffusa* remain inadequately defined. To overcome these drawbacks, this study utilizes an aqueous-alcoholic extraction method to obtain a more comprehensive phytochemical profile and employs standardized in vitro assays. By addressing these limitations, the study aims to establish a mechanistic understanding of *Boerhavia diffusa's* therapeutic potential for inflammation and diabetes management.

Materials and methods

Preparation of aqueous alcoholic plant extract of *Boerhaavia diffusa*

Boerhavia diffusa was freshly procured from Nature and Nurture HealthCare Pvt. Ltd., New Delhi. The random sampling method was employed to ensure unbiased sample selection, reducing the risk of manual errors during collection and processing, as suggested by Priya et al. 2021 [11]. The aqueous-alcoholic extract was prepared in the nanotechnology laboratory of Saveetha Dental College and Hospitals following a standardized protocol to maximize bioactive compound retention.

For extraction, 50 ml of ethanol was measured using a measuring cylinder, followed by the addition of 5 g of crushed and powdered *Boerhavia diffusa* leaves. The mixture was thoroughly homogenized and then transferred to a glass beaker, where an additional 50 ml of distilled water was incorporated. The beaker was covered with aluminum foil and placed in an orbital shaker at 79.20 rpm for 24 hours to ensure efficient extraction [12]. After 24 hours, the extract was transferred to a measuring cylinder and subjected to controlled heating at 50°C for 20 minutes, followed by cooling to room temperature to preserve the stability of active phytochemicals [13].

The extract preparation was validated by expert nano-researchers to ensure reproducibility and standardization. The study utilized the aqueous-alcoholic extract, excluding ethanol or methanol only extracts to maintain consistency in experimental outcomes. The samples were exclusively prepared using powdered leaves of *Boerhavia diffusa*, avoiding other aerial parts of the plant to maintain phytochemical specificity and relevance to previous studies (Figure 1) [14].

Reagents

Anti-inflammatory Activity

The anti-inflammatory activity was assessed using Bovine Serum Albumin (BSA) denaturation assay. A reaction mixture containing 0.45 mL of a 1% aqueous Bovine Serum Albumin solution and 0.05 mL of *Boerhavia diffusa* at various concentrations (10 µL, 20 µL, 30 µL, 40 µL, and 50 µL) was prepared. To induce denaturation, 1N hydrochloric acid was added to the mixture. Diclofenac sodium served as the positive control, while dimethyl sulfoxide (DMSO) was used as the negative control. The reaction was allowed to proceed under controlled conditions, and the extent of protein denaturation was measured to evaluate the anti-inflammatory potential of the extract.

Anti-diabetic Activity

The anti-diabetic activity was assessed using an alpha-amylase inhibition assay. A reaction mixture containing 100 µL of 1% w/v alpha-amylase solution and varying concentrations of the leaf extract (10 µL, 20 µL, 30 µL, 40 µL, and 50 µL) was prepared. To initiate the reaction, 100 µL of 96 mM 3,5-dinitrosalicylic acid (DNSA) reagent was added. Sodium phosphate was used as the positive control, while dimethyl sulfoxide (DMSO) served as the negative control. The reaction was incubated under appropriate conditions, and the inhibition of alpha-amylase activity was measured spectrophotometrically to determine the anti-diabetic efficacy of the extract.

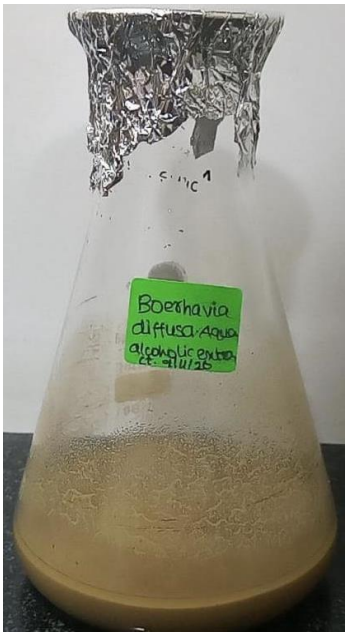

Figure 1: Preparation of the aqueous-alcoholic extract of *Boerhavia diffusa*

Anti inflammatory activity

Albumin denaturation assay

The anti-inflammatory activity of *Boerhavia diffusa* was evaluated using a modified protocol based on the method proposed by Muzushima and Kabayashi [15]. Various concentrations of *Boerhavia diffusa* extract (10 µL, 20 µL, 30 µL, 40 µL, and 50 µL) were added to 0.45 mL of 1% aqueous bovine serum albumin (BSA). The pH of the mixture was adjusted to 6.3 using a small amount of 1N hydrochloric acid. The samples were incubated at room temperature for 20 minutes, followed by heating at 55°C in a water bath for 30 minutes. After cooling to room temperature, the absorbance of the samples was measured

spectrophotometrically at 660 nm. Diclofenac sodium was used as the standard, and dimethyl sulfoxide (DMSO) served as the control (Figure 2).

Percentage of protein denaturation was determined utilizing the following equation,

$$\% \text{ inhibition} = \frac{\text{Absorbance of control - Absorbance of sample}}{\text{Absorbance of control}} \times 100$$

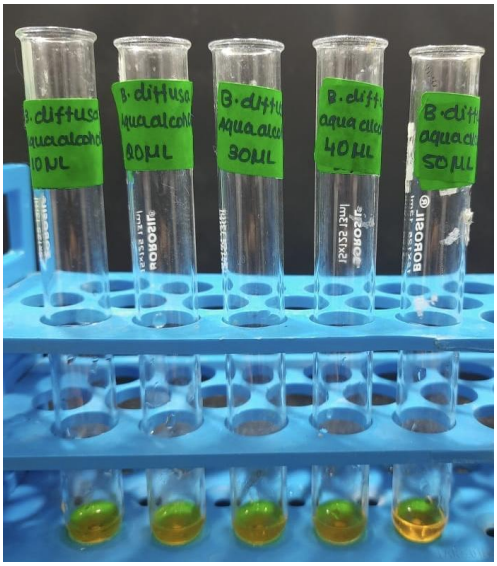

Figure 2: Test tubes with varying concentrations (10 µL, 20 µL, 30 µL, 40 µL, and 50 µL) of aqueous-alcoholic extract of *Boerhavia diffusa* prepared for in vitro experimentation. The color gradient reflects the different concentrations of the extract utilized in the setup.

Antidiabetic activity

Alpha amylase inhibition assay

Alpha-amylase inhibition was determined by quantifying the amount of maltose liberated during the experiment. The method reported by Bhutkar and Bhise has been followed [16]. Different concentrations of nanoparticles (20µL, 40µL, 60µL, 80µL, 100µL) were pre-incubated with 100 µL of α- amylase solution (1U/mL) at room temperature for 30 minutes. 100 µL of starch solution (1% w/v) was further added to it and the mixture was incubated at room temperature for 10 minutes. 100 µL of 96 mM (3,5-dinitrosalicylic acid solution) DNSA reagent was added to it to stop the reaction and the solution was heated in a water bath for 5 minutes. Control was maintained where the equal quantity of enzyme extract was replaced by sodium phosphate buffer maintained at a pH value of 6.9. Reading was measured at 540 nm. The experiment was performed in triplicate. Acarbose was used as a positive control (Figure 3).

The % inhibition was calculated using the formula

$$\% \text{ inhibition} = \frac{C - T}{C} \times 100$$

Where, C= control, T= test sample

Figure 3 Test tubes containing varying concentrations (10 µL, 20 µL, 30 µL, 40 µL, and 50 µL) of aqueous-alcoholic extract of *Boerhavia diffusa* prepared for analysis. The changes in turbidity among the samples indicate differences in concentration levels used for the experimental procedure.

The data from the anti-inflammatory and antidiabetic activity assays were recorded in Excel sheets and analyzed statistically using Spearman correlation in IBM SPSS software (version 23).

Results

Anti inflammatory activity

The anti-inflammatory activity of *Boerhavia diffusa* aqueous-alcoholic extract was analyzed across different concentrations (10 µL, 20 µL, 30 µL, 40 µL, and 50 µL). The percentage inhibition of anti-inflammatory activity increased significantly with higher extract concentrations. At 10 µL, the percentage

inhibition was 33.0%. It decreased slightly to 30.3% at 20 µL but showed a sharp increase at higher concentrations. At 30 µL, inhibition was 71.1%, further rising to 74.3% at 40 µL. The maximum inhibition of 76.1% was observed at 50 µL. These results suggest that the anti-inflammatory potential of the extract improves significantly with increasing concentration, indicating a dose-dependent effect Spearman correlation analysis revealed a strong negative correlation (r = -1) with a statistically significant p-value of less than 0.05 (Figure 4,5).

Absorbance values (Wt%) recorded at 666 nm showed an inverse relationship with the percentage inhibition. At lower concentrations (10 µL and 20 µL), absorbance was higher (0.481 and 0.518, respectively), indicating lower inhibition. At 30 µL, a steep decrease in absorbance to 0.148 was observed, aligning with a sharp rise in inhibition. Further reductions in absorbance were noted at 40 µL (0.129) and 50 µL (0.119), corresponding to the highest inhibition values. This inverse correlation between absorbance and inhibition confirms the effectiveness of *Boerhavia diffusa* in reducing protein denaturation at higher concentrations (Table 1).

The experimental results validate the anti-inflammatory properties of *Boerhavia diffusa*. Higher concentrations demonstrated greater efficacy, with a strong dose-dependent inhibition of protein denaturation. This supports the potential of *Boerhavia diffusa* as a natural anti-inflammatory agent, with its effectiveness being most pronounced at concentrations of 30 µL and above.

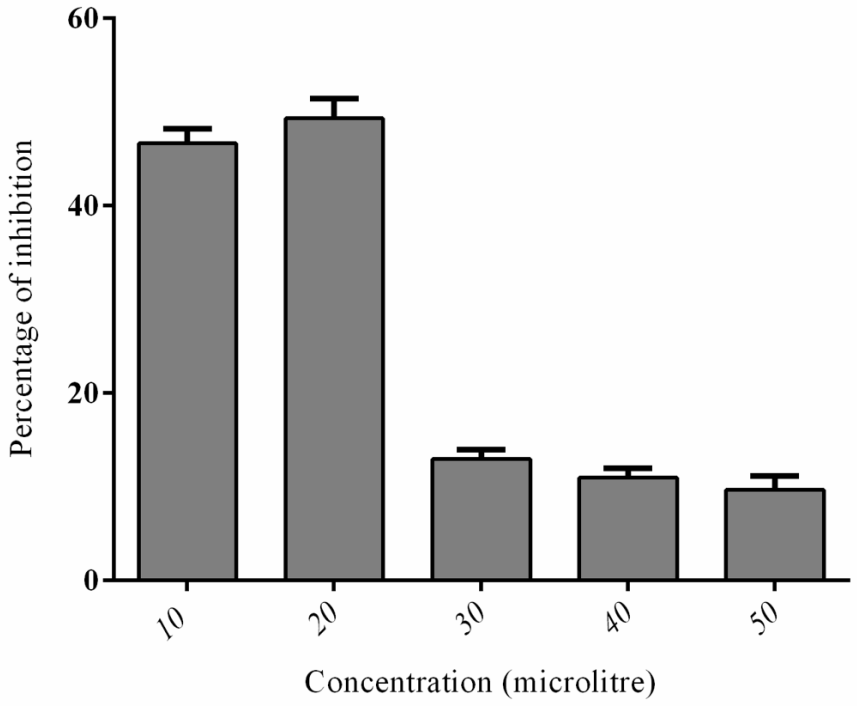

Figure 4: The bar graph represents the percentage inhibition of protein denaturation at varying concentrations (10 µL, 20 µL, 30 µL, 40 µL, and 50 µL) of *Boerhavia diffusa* aqueous-alcoholic extract. Pearson correlation analysis indicates a strong positive correlation between concentration and inhibition percentage, demonstrating the extract's dose-dependent anti-inflammatory effectiveness.

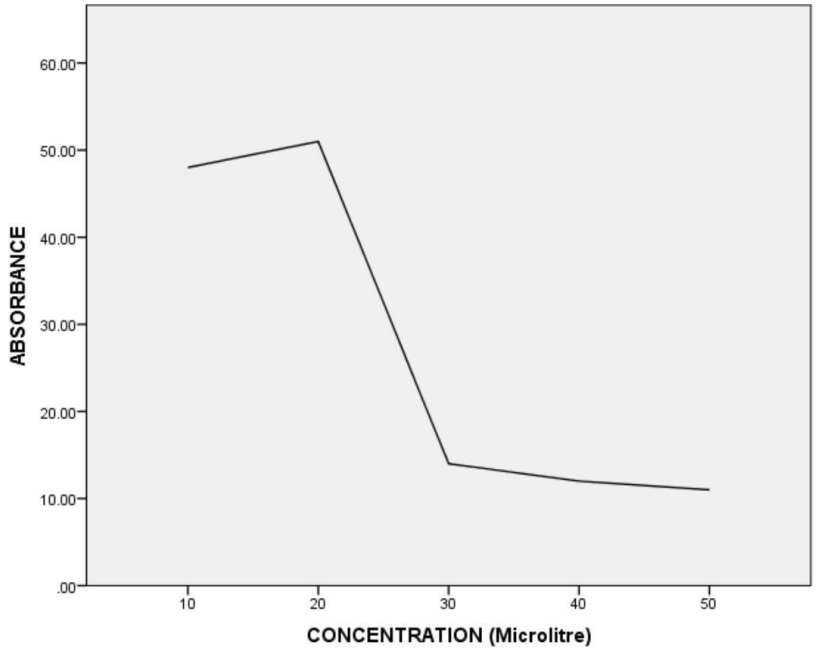

Figure 5: The line graph illustrates the absorbance values (Wt%) at 666 nm for varying concentrations of *Boerhavia diffusa* extract, showing a decrease in absorbance as the concentration increases.

Table 1 The table shows the Anti-inflammatory activity along with the Percentage of inhibition

S.No	**Concentration (µl)**	**Wavelength (nm)**	**Absorbents (Wt%)**	**Percentage of inhibition**
1	10 µl	666.0	0.481	33.0
2	20 µl	666.0	0.518	30.3
3	30 µl	666.0	0.148	71.1
4	40 µl	666.0	0.129	74.3
5	50 µl	666.0	0.119	76.1

Antidiabetic activity

The anti-diabetic activity of the aqueous-alcoholic extract of *Boerhavia diffusa* was analyzed across different concentrations (10 µL, 20 µL, 30 µL, 40 µL, and 50 µL). At 10 µL, the percentage inhibition was 77.9%. Slight variations were observed at 20 µL (77.0%), 30 µL (77.8%), and 40 µL (77.7%). The highest inhibition was recorded at 50 µL, reaching 78.0%. These results suggest that the extract exhibits strong and stable anti-diabetic activity across all tested concentrations, with minimal variability. Spearman correlation analysis revealed a strong negative correlation (r = 1) with a statistically significant p-value of less than 0.05 (Figure 6,7).

The absorbance values (Wt%) recorded at 540 nm showed minor fluctuations across the concentrations. The absorbance was 0.108 at 10 µL and 50 µL, corresponding to the highest percentage inhibition. At 20 µL, the absorbance increased slightly to 0.114, reflecting a small dip in inhibition (77.0%). At 30 µL and 40 µL, the absorbance stabilized at 0.109, indicating consistent anti-diabetic activity. This consistency in absorbance values aligns well with the observed inhibition percentages, highlighting the extract's anti-diabetic effect (Table 2).

The anti-diabetic activity of *Boerhavia diffusa* extract, as measured by alpha-amylase inhibition, demonstrated consistently high efficacy across all concentrations, with inhibition values ranging from 77.0% to 78.0%. The close correlation between percentage inhibition and absorbance confirms the extract's potential as a natural alpha-amylase inhibitor.

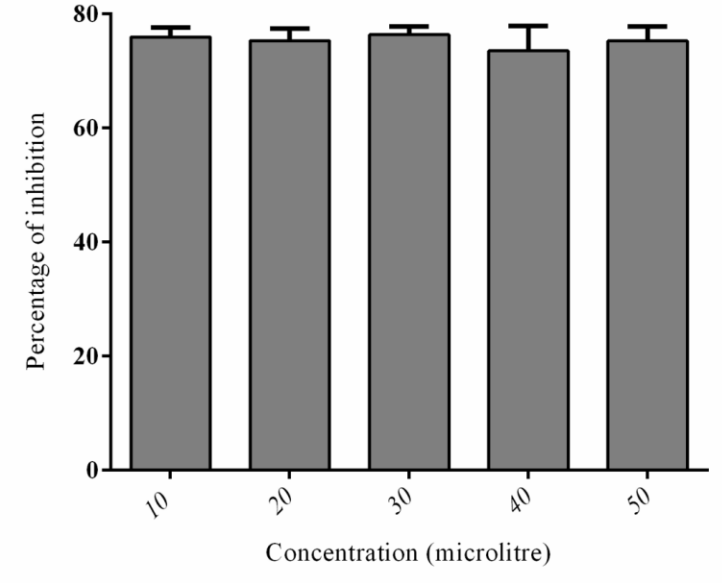

Figure 6: The bar graph illustrates the percentage inhibition of alpha-amylase activity at varying concentrations (10 µL, 20 µL, 30 µL, 40 µL, and 50 µL) of *Boerhavia diffusa* aqueous-alcoholic extract. Based on Pearson correlation analysis, a strong and positive correlation was observed between extract concentration and alpha-amylase inhibition, confirming the extract's effectiveness as a natural anti-diabetic agent.

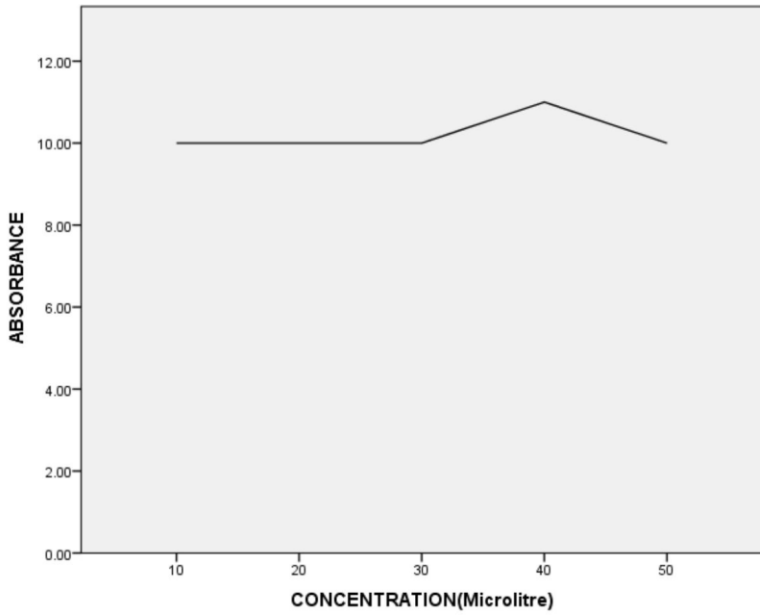

Figure 7: The bar graph illustrates the percentage inhibition of alpha-amylase activity at varying concentrations (10 µL, 20 µL, 30 µL, 40 µL, and 50 µL) of *Boerhavia diffusa* aqueous-alcoholic extract. Based on Pearson correlation analysis, a strong and positive correlation was observed between extract concentration and alpha-amylase inhibition, confirming the extract's effectiveness as a natural anti-diabetic agent.

Table 2: The table shows the anti-diabetic activity along with the Percentage of inhibition

S.No	**Concentration (µl)**	**Wavelength (nm)**	**Absorbents (Wt%)**	**Percentage of inhibition**
1	10 µl	540.0	0.108	77.9
2	20 µl	540.0	0.114	77.0
3	30 µl	540.0	0.109	77.8
4	40 µl	540.0	0.109	77.7

5	50 µl	540.0	0.108	78.0

Discussion

The current study demonstrates the significant anti-inflammatory and anti-diabetic potential of the aqueous-alcoholic extract of *Boerhavia diffusa*, evaluated through in vitro assays. The findings align with and expand upon recent literature, providing new insights into the therapeutic applications of the plant. The results from the albumin denaturation assay revealed a strong dose-dependent inhibition of protein denaturation, with the highest inhibition of 76.1% observed at a concentration of 50 µL. These findings are consistent with recent studies that highlight the anti-inflammatory properties of *Boerhavia diffusa*. For instance, Dinesh et al. (2021) reported a significant reduction in cyclooxygenase (COX) activity and pro-inflammatory cytokine levels when *Boerhavia diffusa* extracts were tested on in vitro models [17]. Similarly, Jalan et al. (2021) found that flavonoids and phenolic acids in *Boerhavia diffusa* contribute to its ability to mitigate inflammatory responses by stabilizing cellular membranes and inhibiting inflammatory mediators [18].

Study by Sinan et al. (2021), who observed a similar correlation in their investigation of anti-inflammatory bioactivity using combined extraction methods [19]. The alpha-amylase inhibition assay demonstrated consistently high efficacy across all concentrations, with inhibition percentages ranging between 77.0% and 78.0%. These results are comparable to those of Oyebode et al. (2021), who found that *Boerhavia diffusa* extracts significantly inhibited alpha-amylase activity, reducing starch hydrolysis and postprandial glucose levels [20]. Additionally, Nisha et al. (2018) reported similar findings, linking the plant's anti-diabetic properties to its rich content of alkaloids and flavonoids, which improve insulin sensitivity and glycemic control [21].

The minor fluctuations in absorbance values at 540 nm observed in this study indicate the stability and robustness of the extract's anti-diabetic activity. The spearman correlation analysis revealed a strong positive relationship between extract concentration and inhibition percentage, supporting the hypothesis that *Boerhavia diffusa* acts as a natural alpha-amylase inhibitor. This is consistent with Suvarna et al. (2021), who identified similar inhibitory trends in polyherbal formulations containing *Boerhavia diffusa* [22]. The combined anti-inflammatory and anti-diabetic properties observed in this study explained the therapeutic potential of *Boerhavia diffusa* for managing chronic metabolic disorders. Compared to previous studies that utilized either aqueous or ethanolic extracts, the aqueous-alcoholic extraction method employed here appears to enhance the synergistic bioactivity of the plant's compounds. This study use of standardized

protocols further strengthens the reliability of the findings, addressing inconsistencies reported in earlier research [23,24].

The observed dose-dependent trends and high efficacy suggest that *Boerhavia diffusa* could serve as a valuable component in integrative therapies targeting inflammation and diabetes. Future research should focus on in vivo validation and clinical trials to further explore its pharmacological mechanisms and therapeutic applications.

Conclusion

The findings of this study revealed significant anti-inflammatory and anti-diabetic properties of the aqueous-alcoholic extract of *Boerhavia diffusa*, demonstrating its potential as a natural therapeutic agent. The dose-dependent inhibition of protein denaturation and alpha-amylase activity indicates the plant's effectiveness in reducing inflammation and managing glycemic control. These results align with recent literature, further validating the synergistic bioactivity of the compounds extracted using a combined aqueous-alcoholic method. The study also addresses inconsistencies in previous research by employing standardized protocols, enhancing the reliability of the findings. Future in vivo studies and clinical trials are recommended to explore the full pharmacological potential and therapeutic applications of *Boerhavia diffusa*.

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Conflict of Interest

The authors declare that there are no conflicts of interest in the present study**.**

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