

PHYTOCHEMICAL DIVERSITY AND DUAL α -GLUCOSIDASE AND DPP-IV INHIBITORY ACTIVITY OF *BOERHAVIA DIFFUSA* LINN.: IMPLICATIONS FOR PHYTOPHARMACEUTICAL DEVELOPMENT

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ABSTRACT

Boerhavia diffusa Linn. is an herb used extensively in traditional medicine for the treatment of diabetes and other metabolic disorders. In this investigation, we examined the leaf extract of *Boerhavia diffusa* to determine its physicochemical properties, phytochemical constitution, chromatographic fingerprint, and *in-vitro* antidiabetic activity. The physicochemical parameters indicated a higher percentage of extract in water (4.1%) and methanol (3.1%), whereas the evaluation of total ash (5.9%), acid-insoluble ash (1.3%), and loss of drying (2.9%) indicated the good quality and stability of the plant material. The phytochemical analysis indicated the presence of alkaloids, flavonoids, tannins, phenolics, saponins, terpenoids, and phytosterols. Thin Layer Chromatography showed the presence of various phytoconstituents with R_f values between 0.25 and 0.75. The ethanolic extract showed concentration-dependent α -glucosidase inhibition (10.3-38.0%; IC₅₀ 120.00 μ g/mL) and moderate DPP-IV inhibition (12.5-62.3%). The results validate the traditional use of *Boerhavia diffusa* and its potential as a natural source.

KEYWORDS: *Boerhavia diffusa*, Antidiabetic activity, Phytochemical screening, α -glucosidase inhibition, DPP-IV inhibition, TLC, Physicochemical evaluation.

1.0 INTRODUCTION

Herbal medicines have been used as major remedies in the prevention and treatment of diseases. Herbal medicines are core in traditional medicine, particularly in developing nations where conventional medicines are not easily accessible or affordable. Approximately 80% of the world's population uses plant medicines as major remedies for primary health care.^[1-3]

Diabetes mellitus is a chronic metabolic disorder with hyperglycemia resulting from an abnormality in insulin secretion or action. It causes complications such as cardiovascular disease, neuropathy, nephropathy, and retinopathy, thus increasing morbidity and mortality worldwide.^[4,5] Although artificial antidiabetic agents are

available, their chronic administration is associated with hypoglycemia, gastrointestinal disturbances, and drug resistance, thus emphasising the need for a safer and more effective alternative.^[6,7]

Medicinal plants are a rich source of phytochemicals, including flavonoids, alkaloids, glycosides, terpenoids, saponins, and phenolics, which possess antioxidant, anti-inflammatory, and antidiabetic properties.^[8-10] Plant-derived compounds have been instrumental in drug discovery, thus emphasising the importance of thorough evaluation of herbal drugs.^[11,12]

Boerhavia diffusa Linn., commonly known as Punarnava, is an herb that has been extensively used in

Ayurvedic medicine, an ancient Indian system of healing. This herb has been traditionally used to treat diabetes, inflammation, kidney disorders, and liver ailments. This herb contains a variety of biological compounds such as rotenoids, flavonoids, alkaloids, and glycosides, which work in combination to produce the therapeutic properties of this herb.^[13-15] Recently, pharmacological studies have emphasised the significant antioxidant, antimicrobial, nephroprotective, and antidiabetic properties of *B. diffusa* extracts, suggesting the potential use of this herb as an alternate form of treatment.^[16-18]

Standardisation and quality control of herbal drugs are required to provide safety, efficacy, and reliability of plant-based preparations. Physicochemical analysis, such as estimation of ash value, extractive value, and chromatographic fingerprinting, is a crucial step in determining the purity and authenticity of adulteration of crude drugs.^[19-21] Extractive values help in identifying the active principles soluble in different solvents, whereas ash values reveal the inorganic impurities and mineral constituents of plant materials.^[22,23] Thin Layer Chromatography (TLC) is employed for qualitative analysis and identification of phytochemicals in plant extracts.^[24,25]

Thus, the current study aimed to assess the physicochemical properties, phytochemicals, and chromatographic fingerprint of *Boerhavia diffusa* Linn. leaf extract to determine the quality control parameters and its potential therapeutic value in the treatment of diabetes.

2.0 MATERIALS AND METHODS

2.1 Collection and Authentication of Plant Material

The leaves of *Boerhavia diffusa* Linn. were collected from a suitable geographical area during the flowering stage. The plant material was identified by a taxonomist, and a voucher sample was preserved in the herbarium for future use. The plant material was thoroughly washed with distilled water to remove dust and foreign particles and then shade dried at room temperature to avoid the degradation of thermolabile compounds.^[19,26]

2.2 Preparation of Plant Extract

The dried plant material was ground into coarse powder using a mechanical grinder and stored in airtight containers away from moisture and light. About 50 g of the powdered material was used for sequential extraction using solvents of increasing polarity, such as petroleum ether, chloroform, methanol, and distilled water. Extraction was done using the maceration method for 24-48 hours with intermittent shaking. The extracts were filtered and concentrated using a rotary evaporator under reduced pressure. The dried extracts were stored in desiccators for further analysis.^[20,27]

2.3 Physicochemical Evaluation

Determination of Ash Values

The value of ash was calculated following pharmacopoeial procedures. The total ash value was obtained by incinerating the accurately weighed powder of the drug in a silica crucible at a temperature not exceeding 450°C until carbon-free ash was obtained. The value of acid-insoluble ash was obtained by boiling the total ash with dilute hydrochloric acid, filtering, washing, igniting, and weighing the residue. The water-soluble ash was calculated by determining the difference between the total ash and the insoluble residue after boiling with distilled water.^[19,22]

Determination of Extractive Values

The extractive values were calculated to arrive at an approximate estimate of the quantity of active principles that are soluble in various solvents. Approximately 5 g of air-dried powder of the drug was extracted with 100 mL of each solvent separately for a period of 24 hours with intermittent shaking. The resulting solutions were filtered, and the filtrates were evaporated to dryness. The dried extracts were weighed, and the percentage extractive values were calculated with reference to the air-dried drug.^[20,23]

Loss on Drying

Loss on drying was measured by heating accurately weighed plant powder at 105°C in a hot air oven until a constant weight was reached. The percentage moisture content was calculated to determine the stability and shelf life of the drug.^[21]

Fluorescence Analysis

Fluorescence analysis of powdered drug was carried out by treating plant powder with various reagents like hydrochloric acid, sodium hydroxide, and organic solvents. The samples were then exposed to visible light and ultraviolet light (254nm and 366nm). Fluorescence properties were noted as they are significant in identifying drugs and adulteration.^[28]

2.4 Preliminary Phytochemical Screening

Qualitative phytochemical screening was conducted to identify the presence of major phytoconstituents like alkaloids, flavonoids, glycosides, saponins, tannins, phenolic compounds, carbohydrates, proteins, and terpenoids using standard chemical tests.^[8,29]

2.5 Thin Layer Chromatography (TLC) Analysis

TLC analysis was carried out using pre-coated silica gel plates as the stationary phase. The samples of the extracts were applied as spots using capillary tubes, and the plates were developed in suitable solvent systems. After development, the plates were dried and detected under ultraviolet light or using suitable detecting reagents. The retardation factor (Rf) values were calculated for the characterisation of phytochemical constituents.^[24,25]

2.6 Statistical Analysis

All the experiments were carried out in triplicate, and the results were expressed as mean \pm standard deviation. Statistical analysis was done using suitable analytical tools to ensure the reproducibility and reliability of the results.^[30]

3.0 RESULT

3.1 Estimation of Extractive values and Ash values

The extractive values and ash values of the leaves of

Aloe vera Linn. have been found using different solvents and standard physicochemical procedures. Among the solvents used, the highest value of extractive value was found to be in water (4.1%), followed by methanol (3.1%), petroleum ether (2.0%), n-hexane (1.9%), and chloroform (1.4%). The higher value of extractive value in polar solvents like water and methanol reveals the presence of a higher amount of polar phytoconstituents in the plant material.

Table 1: Extractive values and physicochemical parameters of *Aloe vera* Linn. leaves.

Parameter	Extractive Value (%)	Ash Value (%)
Petroleum Ether	2.0	0.5
n-Hexane	1.9	0.4
Chloroform	1.4	0.3
Methanol	3.1	0.7
Water	4.1	1.2
Total ash	-	5.9
Acid-insoluble ash	-	1.3
Water-soluble ash	-	2.4
Sulphated ash	-	3.0
Loss on drying	-	2.9
Crude fibre content	-	42

The total ash value was determined to be 5.9%, which is the total inorganic content in the plant material. The acid-insoluble ash value was 1.3%, which shows the presence of siliceous materials like sand and soil. The water-soluble ash value was 2.4%, which shows the water-soluble inorganic salt content. The sulphated ash value was measured to be 3.0%, which shows the presence of stable inorganic material even after sulphation.

The loss on drying was measured to be 2.9%, which shows low moisture content and thus greater stability and less possibility of microbial development. The crude fibre content was measured to be 42%, which shows that *Aloe vera* leaves have a considerable amount of fibrous

material, which may be responsible for their physiological properties.

3.2 Phytochemical Screening

Preliminary phytochemical analysis of the ethanolic extract of *Boerhavia diffusa* showed the presence of various key bioactive compounds. The presence of alkaloids was confirmed by positive reactions in Mayer's, Dragendorff's, and Wagner's tests, but Hager's test was negative or insignificant. The presence of carbohydrates was confirmed by positive reactions in Molisch's, Fehling's, and Barfoed's tests, which revealed the presence of general carbohydrates and reducing sugars.

Table 2: Preliminary phytochemical screening of ethanolic extract of *Boerhavia diffusa*.

S. No.	Chemical Test	Test Name	<i>Boerhavia diffusa</i>
1	Alkaloids	Mayer's test	+
		Dragendorff's test	+
		Wagner's test	+
		Hager's test	-
2	Carbohydrates	Molisch's test	+
		Fehling's test	+
		Barfoed's test	+
3	Proteins and Free Amino Acids	Ninhydrin test	-
		Biuret test	+
		Xanthoprotein test	+
4	Tannins and Phenolic Compounds	Ferric chloride test	+
		Lead acetate test	+
		Gelatin test	+
5	Phytosterols	Liebermann-Burchard test	+
		Salkowski test	+
6	Flavonoids	Shinoda test	+

7	Saponins	Foam test	+
8	Glycosides	General test	-
9	Terpenoids	Liebermann–Burchard test	+
		Salkowski test	+

The extract gave positive reactions for proteins and amino acids in Biuret and Xanthoprotein tests, but the Ninhydrin test was negative, indicating the absence or low concentration of free amino acids. Tannins and phenolic compounds were highly present in the extract, as indicated by the positive results of the Ferric chloride, Lead acetate, and Gelatin tests.

Phytosterols were positively identified by the Liebermann-Burchard and Salkowski tests. Flavonoids were identified using the Shinoda test, and saponins were also present in the extract. Glycosides were absent in the extract, as indicated by the negative test results. Terpenoids were identified by the positive Liebermann-Burchard and Salkowski tests. The phytochemical screening clearly indicates that *Boerhavia diffusa* contains a variety of bioactive compounds that may be responsible for its medicinal uses.

3.3 Thin Layer Chromatography

The TLC plate of the ethanolic extract of *Aloe vera* Linn. revealed six distinct spots with different colours and Rf values when observed in a vaporised iodine chamber. The separation was done using a solvent system with a ratio of 3:1.5 of chloroform and glacial acetic acid, and 0.6:0.2 of methanol and water, respectively. Vaporised iodine was used as the detecting agent, which revealed

the presence of various phytoconstituents in the extract.



Figure 1: Chromatogram of TLC of ethanolic extract of *Aloe vera* Linn.

The Thin Layer Chromatography (TLC) analysis of the crude saponin extract of *Aloe vera* Linn. revealed the presence of multiple compounds exhibiting distinct migration behaviour under different mobile phase systems.

Table 3: Thin Layer Chromatography (TLC) Results for Crude Saponin Extract.

Compound Name	Mobile Phase	Ratio	Rf Value
Saponin A	Chloroform: Glacial acetic acid: Methanol: Water	3:1.5:0.6:0.2	0.25
Saponin B	Chloroform: Glacial acetic acid : Methanol: Water	3:1.5:0.6:0.2	0.5
Saponin C	Chloroform: Glacial acetic acid: Methanol: Water	3:1.5:0.6:0.2	0.6
Saponin D	Chloroform: Glacial acetic acid: Methanol: Water	3:1.5:0.6:0.2	0.75
Saponin E	Chloroform: Glacial acetic acid: Methanol: Water	3:1.5:0.6:0.2	0.46
Saponin F	Toluene: Methanol	09:01	0.46

Saponin A had an Rf value of 0.25 in the chloroform: glacial acetic acid: methanol: water solvent system (3:1.5:0.6:0.2). Likewise, Saponin B and Saponin C had Rf values of 0.50 and 0.60, respectively, in the same solvent system. This indicated differences in their polarity and interaction with the stationary phase.

Saponin D had the highest Rf value of 0.75 in the same solvent system. This indicated relatively low polarity and high migration with the solvent front. Saponin E had an intermediate Rf value of 0.46.

Notably, Saponin F also had an Rf value of 0.46, but using a distinct mobile phase composition of toluene and methanol in a ratio of 9:1. This observation indicates that Saponin F may have comparable chemical properties to Saponin E.

In conclusion, the TLC test has successfully identified the presence of multiple saponin compounds in the crude extract. The differences in the Rf values indicate varying polarity, molecular interactions, and affinities for the stationary and mobile phases. These results clearly indicate the efficacy of TLC as a preliminary analytical method for the separation and identification of

phytoconstituents. Further analysis of these compounds may reveal information regarding their pharmacological and therapeutic values.

3.4 BIOLOGICAL INVESTIGATION

3.4.1 *In-vitro* Antidiabetic study

α -Glucosidase inhibitory activity: The α -glucosidase inhibitory activity of the ethanolic extract of *Aloe vera*

Linn. leaves (EEBD) was assessed at concentrations of 0-100 $\mu\text{g/mL}$ and compared with the standard drug Acarbose. The data revealed a concentration-dependent increase in the inhibition of the enzyme by EEBD and Acarbose.

Table 4: Percentage α -glucosidase inhibitory activity of ethanolic extract of *Aloe vera* Linn. leaves (EEBD) and standard drug Acarbose at different concentrations.

Conc. ($\mu\text{g/mL}$)	%Inhibition by EEBD	% Inhibition by Acarbose
0	0	0
10	10.3	20.4
20	21.4	41.1
40	27.1	62.2
60	32.2	82.3
100	38	96.2

EEBD exhibited 10.3%, 21.4%, 27.1%, 32.2%, and 38.0% inhibition at concentrations of 10, 20, 40, 60, and 100 $\mu\text{g/mL}$, respectively. In comparison, Acarbose

showed higher inhibitory activity with 20.4%, 41.1%, 62.2%, 82.3%, and 96.2% inhibition at the same respective concentrations.

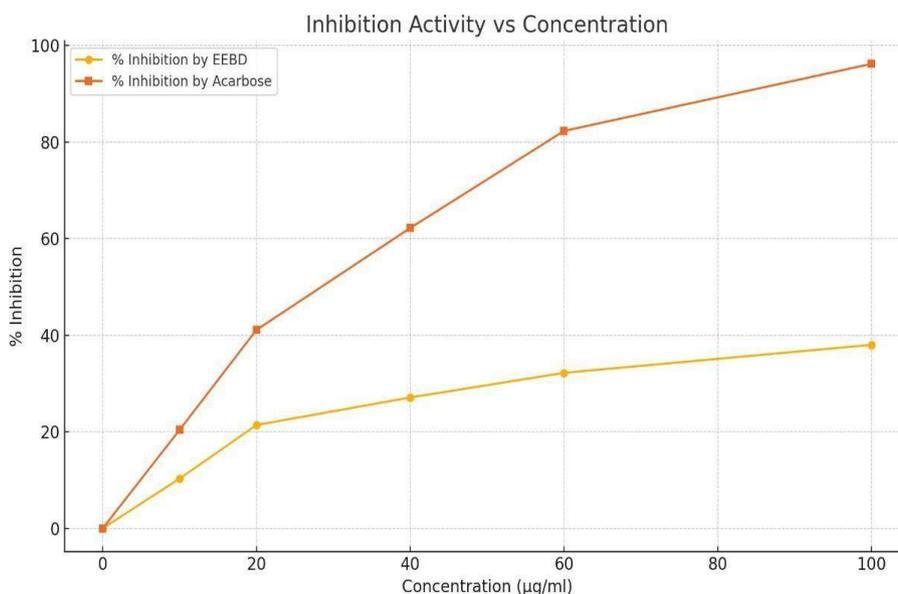


Figure 2: Dose-dependent α -glucosidase inhibitory activity of ethanolic extract of *Aloe vera* Linn. leaves (EEBD) compared with standard antidiabetic agent Acarbose. Percentage inhibition was evaluated at concentrations ranging from 0–100 $\mu\text{g/mL}$, demonstrating concentration-dependent enzyme inhibition.

Regression analysis showed a positive correlation between concentration and percentage inhibition. The IC_{50} value of EEBD was found to be approximately 120.00 $\mu\text{g/mL}$, whereas Acarbose had an IC_{50} value of 37.94 $\mu\text{g/mL}$, which indicated higher inhibitory activity of the standard drug.

3.4.2 DPP IV inhibition assay

The Dipeptidyl Peptidase-4 (DPP-IV) inhibitory potential of the ethanolic extract of *Aloe vera* Linn. leaves (EEBD) was assessed at concentrations of 0-100 $\mu\text{g/mL}$ and compared with the standard drug Sitagliptin. The data revealed a concentration-dependent increase in enzyme inhibition for both EEBD and Sitagliptin.

Table 5: Percentage DPP-IV inhibitory activity of ethanolic extract of *Aloe vera* Linn. leaves (EEBD) and standard drug Sitagliptin at different concentrations.

Conc. ($\mu\text{g/mL}$)	%Inhibition by EEBD	%Inhibition by Sitagliptin
0	0	0
10	12.5	25.7
20	24.3	45.5
40	35.6	63.4
60	48.4	74.6
100	62.3	89.5

EEBD exhibited 12.5%, 24.3%, 35.6%, 48.4%, and 62.3% inhibition at concentrations of 10, 20, 40, 60, and 100 $\mu\text{g/mL}$, respectively. In comparison, Sitagliptin showed higher inhibitory activity with 25.7%, 45.5%,

63.4%, 74.6%, and 89.5% inhibition at the same respective concentrations. The findings indicate that EEBD possesses moderate DPP-IV inhibitory activity when compared with the standard drug.

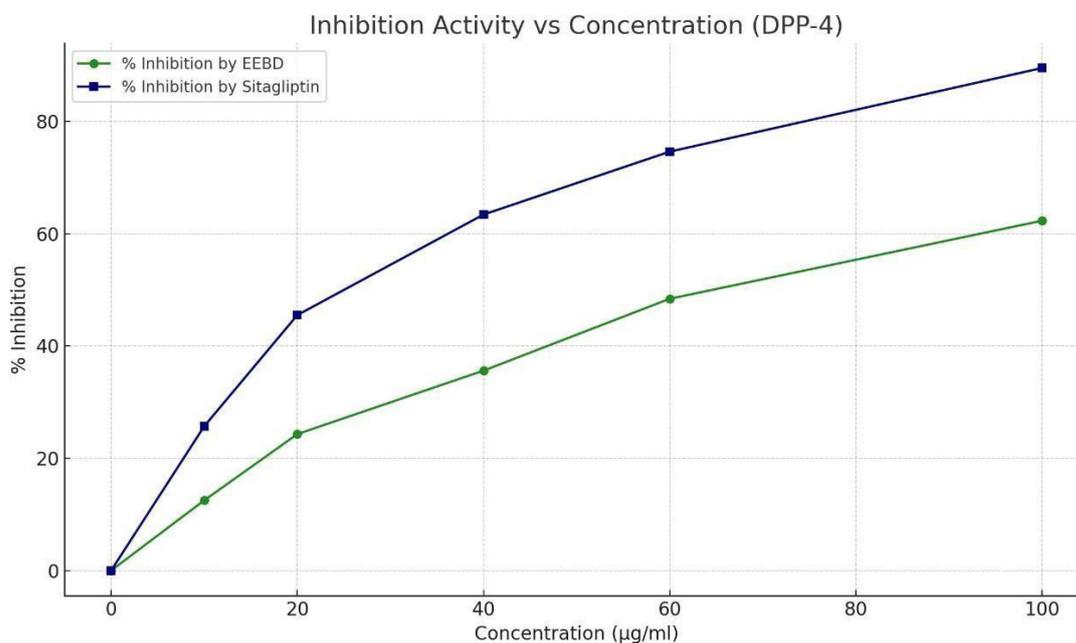


Figure 3: Concentration-dependent DPP-IV inhibitory activity of ethanolic extract of *Aloe vera* Linn. leaves (EEBD) compared with standard drug Sitagliptin.

The graphical representation further supports the fact that there is a gradual and consistent increase in the inhibitory activity with the rise in concentration of the extract.

In comparison, Sitagliptin showed significantly higher inhibitory activity, with 25.7% inhibition at 10 $\mu\text{g/mL}$, which further increased to 45.5%, 63.4%, 74.6%, and 89.5% at concentrations of 20, 40, 60, and 100 $\mu\text{g/mL}$, respectively. The steeper inhibition curve of Sitagliptin reveals higher enzyme inhibition activity than EEBD. From the above results, it is clear that the ethanolic extract of *Aloe vera* Linn. leaves has moderate DPP-IV inhibitory activity compared to the standard drug.

4.0 DISCUSSION

The current study offers in-depth pharmacognostic, phytochemical, and biological validation of *Boerhavia diffusa* Linn., emphasising its medicinal importance as a promising antidiabetic drug. Physicochemical standardisation showed acceptable quality standards, which are critical for authenticating, validating, and reproducing herbal drugs. The extractive value calculation showed that water had the highest extractive value (4.1%), followed by methanol (3.1%), petroleum ether (2.0%), n-hexane (1.9%), and chloroform (1.4%). The dominance of extraction in polar solvents indicates the richness of hydrophilic bioactive compounds, especially phenolics and flavonoids, which are well documented to play a significant role in pharmacological activity, as described by Mukherjee and Kokate. Ash value measurement further confirmed the quality and purity of the plant material. The result for total ash value

was obtained to be 5.9%, while the values for acid-insoluble ash and water-soluble ash were recorded to be 1.3% and 2.4%, respectively. These results are well within the acceptable pharmacognostic limits and indicate a lack of contamination with siliceous materials, which is as expected based on the quality control parameters. The low moisture content, as indicated by the loss on drying test (2.9%), indicates a lack of susceptibility to microorganisms and hence the suitability of the drug for storage.

Phytochemical analysis revealed the presence of varied secondary metabolites such as alkaloids, flavonoids, tannins, phenolic compounds, terpenoids, saponins, and phytosterols. Harborne explained that phytochemical diversity is an essential parameter for multi-target pharmacological action. Patel *et al.* have previously shown that flavonoids and phenolic compounds exert hypoglycemic effects through antioxidant and enzyme inhibition mechanisms. The chromatographic profile obtained from TLC analysis further supported the chemical diversity of the plant extract, showing six different phytoconstituent fractions with R_f values ranging from 0.25 to 0.75 in the chloroform: glacial acetic acid: methanol: water (3:1.5:0.6:0.2) solvent system. Wagner and Bladt emphasised that chromatographic profiling is a valuable quality control parameter for the standardisation of herbal medicines. Biological evaluation revealed the concentration-dependent α -glucosidase inhibitory action of the ethanolic extract. The extract showed inhibition of 10.3%, 21.4%, 27.1%, 32.2%, and 38.0% at

concentrations of 10, 20, 40, 60, and 100 µg/mL, respectively, with an IC₅₀ value of approximately 120.00 µg/mL. For comparison, the standard drug Acarbose showed inhibition of 20.4% to 96.2% at the same concentrations, with an IC₅₀ value of 37.94 µg/mL. The American Diabetes Association has identified inhibition of α-glucosidase as an effective approach to the reduction of post-prandial hyperglycemia. Although the plant extract was less potent than Acarbose, Bailey and Day have argued that plant-derived inhibitors often have better safety profiles because of synergistic interactions between phytochemicals.

Likewise, the ethanolic extract showed moderate DPP-IV inhibitory activity, with inhibition of 12.5%, 24.3%, 35.6%, 48.4%, and 62.3% at concentrations of 10 to 100 µg/mL. The standard drug Sitagliptin showed higher inhibitory activity, ranging from 25.7% to 89.5% at the same concentrations. The inhibition of DPP-IV is an important mechanism in the conservation of the incretin hormones and the stimulation of insulin secretion, as explained by Oyebode and Erukainure. The moderate inhibition level indicates that *Boerhavia diffusa* has the potential to regulate glycaemic levels through complementary enzymatic mechanisms.

From a translational point of view, the co-occurrence of antioxidant phytochemicals and dual enzyme inhibition suggests that *Boerhavia diffusa* has the potential to be used as an antidiabetic agent through polypharmacological action. Talalay highlighted the importance of synergistic interactions between plant metabolites, which increase efficacy and decrease toxicity, making plants promising candidates for the treatment of chronic metabolic disorders.

Notwithstanding these encouraging results, the study has some limitations, as it is qualitative in terms of phytochemical analysis and in-vitro assays, which may not be entirely predictive of *in-vivo* pharmacological effects. Alamgir emphasised the need for *in-vivo* pharmacokinetic, toxicological, and clinical studies to validate therapeutic safety and efficacy. Future research work on bioactive compound isolation, metabolomics, and molecular mechanisms, as proposed by Fernie, may help to further clarify the pharmacological potential of *Boerhavia diffusa*.

Overall, the integration of physicochemical stability, phytochemical diversity, chromatographic fingerprinting, and enzyme inhibitory data strongly supports the therapeutic potential of *Boerhavia diffusa* as a promising phytopharmaceutical candidate for diabetes management.

5.0 CONCLUSION

The current research work has established the acceptable physicochemical quality of *Boerhavia diffusa* Linn., with higher extractive percentages in water (4.1%) and methanol (3.1%), and total ash, acid-insoluble ash, and water-soluble ash percentages of 5.9%, 1.3%, and 2.4%,

respectively, establishing its purity and stability. Phytochemical analysis established the presence of bioactive compounds such as alkaloids, flavonoids, tannins, saponins, terpenoids, and phytosterols, whereas TLC analysis established the presence of various phytoconstituents with R_f values ranging from 0.25 to 0.75. The ethanolic extract of *Boerhavia diffusa* Linn. demonstrated concentration-dependent α-glucosidase inhibition ranging from 10.3% to 38.0% with an IC₅₀ value of 120.00 µg/mL and moderate DPP-IV inhibition ranging from 12.5% to 62.3%, establishing its potential role in glycaemic regulation. The current study scientifically establishes the traditional therapeutic potential of *Boerhavia diffusa* Linn. and establishes its potential as a promising natural candidate for the development of antidiabetic phytopharmaceuticals.

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Authors' contributions

Arun Kumar Rawat designed and performed the research, analysed the data; Ajay Kumar Verma wrote the manuscript. All authors read and approved the final manuscript.

Conflicts of Interest

The authors declare no conflicts of interest.

Data availability statement

All data generated or analysed during this study are included in this published article.

Ethics approval and consent to participate

Not applicable.

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