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RESEARCH ARTICLE

Exploring the therapeutic potential of leaf extract of *Nephrolepis brownii* (Desv.) Hovenkamp & Miyam.: An integrative study on antioxidant and anti-diabetic activities

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Abstract

Diabetes mellitus is a major worldwide health problem that is frequently associated to elevated oxidative stress. Medicinal herbs, recognized for their high antioxidant content, provide a potential approach to diabetes therapy. Despite their long history of usage in traditional medicine, ferns such as *Nephrolepis brownii* have received little attention in terms of therapeutic potential. Hence, the current study investigates the antioxidant activity of *N. brownii* leaf extract in water using the DPPH radical scavenging assay. The anti-diabetic activities of the leaf extract are evaluated using α-amylase and α-glucosidase inhibition tests, with effectiveness compared to the standard medication acarbose. The results reveal substantial antioxidant potential in *N. brownii* leaf extract in water (70.0 % inhibition at 1000 µg/mL), comparable to the antioxidant activity of ascorbic acid. The extract showed a concentration-dependent inhibition of α-amylase (IC_{50}: 67.70 µg/mL) and α-glucosidase (IC_{50}: 76.37 µg/mL), albeit slightly less potent than acarbose. These findings highlight the potential *N. brownii* as a natural antioxidant and anti-diabetic agent and this is the first report on this plant. This work contributes valuable insights into the diverse therapeutic properties of *N. brownii*, indicating its potential as a natural source of antioxidants and a subject for further investigation in anti-diabetic research.

Keywords: acarbose; α-amylase; α-glucosidase; antioxidants; anti-diabetic agent; DPPH; diabetes mellitus; *Nephrolepis brownii*; RSA

Introduction

Diabetes mellitus (DM) is a common metabolic condition defined by high blood glucose levels caused by insulin deficiency or insulin resistance in target tissues (1). The global incidence of this chronic and severe condition has been steadily increasing, particularly in metropolitan areas of developing countries. The expectation that, by 2030, the prevalence of Type II diabetes will rise by 10.1 %, with nearly 90 % of those affected living in developing nations, is a major source of concern (2).

The harmful consequences of persistent hyperglycemia in diabetes are compounded by an increase in the generation of free radicals, notably reactive oxygen species (ROS) and reactive nitrogen species (RNS) (3). This increased oxidative stress has a major impact on diabetes complications, insulin resistance and pancreatic beta cell malfunction. Recognizing the critical need for effective therapeutic treatments, the World Health Organization (WHO) emphasizes medicinal plants as a rich source of different bioactive chemicals for drug development (4).

Many underdeveloped nations rely heavily on traditional medicine, with over 80 % of the population using plant-based substances for healthcare needs (5). Medicinal plants include a variety of bioactive compounds, including tannins, alkaloids, polysaccharides, terpenoids, steroids and flavonoids, as discovered by phytochemical screening (6). These chemicals show promise in countering free radicals' harmful effects on cellular components, indicating a possible path for diabetes treatment and the avoidance of related problems (7).

Numerous studies have found a relationship between diabetes and increased free radical production, decreased antioxidant capacity and oxidative damage to critical biological components (8). Hyperglycemia-induced oxidative stress has been shown to play a critical role in the development of diabetes complications, insulin resistance and endothelial cell death (9). As a result, therapies that reduce intracellular free radical generation using antioxidants may have therapeutic effects in avoiding oxidative stress-related diseases (10).

Although pharmacological therapies like acarbose, which inhibits α-amylase and α-glucosidase are available, they can cause liver damage and gastrointestinal issues. Exploring alternate sources with low side effects is important for inhibiting α-amylase and α-glucosidase, which are critical in managing blood glucose levels (11).

Ferns have a long history of use in folklore medicine, dating back to antiquity. Despite their widespread use in folk treatments, the medicinal qualities and practical benefits of most ferns are not well understood (12). Although recent studies are limited, accumulating data shows that ferns have a wide range of bioactivities and therapeutic qualities including antibacterial, antiviral, antioxidant, anti-inflammatory, antitussive, anticancer and anti-HIV capabilities. This study aims to assess the therapeutic potential of aqueous leaf extract from *N. brownii,* which is an underexploited fern species by evaluating its antioxidant activity (DPPH radical scavenging assay) and anti-diabetic properties (α-amylase and α-glucosidase inhibition assays).

This study is significant because it sheds information on the development of natural medicines for the treatment of DM and related problems. By filling a knowledge gap on the therapeutic characteristics of *N. brownii*, the study hopes to pave the way for future research into plant-derived medicines with low side effects. Phytochemical studies on this plant have not yet been reported; hence such investigations could pave the way for the development of effective pharmaceuticals. Phytochemical studies are the backbone of drug discovery. Therein lies the significance of the present study. Phytohemical work on related species of this genus is available and is discussed later in the paper. The objectives of the present study include the evaluation of the antioxidant and anti-diabetic properties of the pteridophyte *N.brownii*.

Materials and Methods

Plant material

The plant material, *Nephrolepis brownii* was collected from the Chemmunji Forest Division located at the Bonacaud hill station between August and September. Following identification, the species was systematically archived in the herbarium of the Botanical Survey of India with accession number 144830.

Preparation of *N. brownii* leaflet extract in water

To prepare the water extract (WE) from *N. brownii* leaflet, the powdered fern was mixed with deionized water in a ratio of 1:20 (dry weight to volume). Subsequently, the mixture underwent a 1 hr incubation in a water bath with periodic agitation every 15 min. Following this, the mixture was subjected to vacuum filtration and the resulting filtrate underwent centrifugation at 7830 rpm for 5 min. The obtained supernatant was freeze-dried. The freeze-dried WE was subsequently reconstituted in water, divided into aliquots and stored at 4 °C for future analysis, following the procedure outlined by Lam (13). The extract method chosen was Soxhlet method. Soxhlet extraction uses the solvent reflux and siphon principle simultaneously. Soxhlet extraction method was used as it saves the solvent extraction efficiency and promotes accuracy.

Methods

Antioxidant activity

DPPH radical scavenging assay: In this study, the antioxidant activity of *N. brownii* leaflet WE was evaluated using the DPPH radical scavenging assay, with ascorbic acid serving as the reference standard. The ascorbic acid stock solution was prepared at a concentration of 1 mg/mL in distilled water. A 60 µM solution of DPPH in methanol was freshly prepared and various concentrations (1.56, 3.12, 6.25, 12.5, 25, 50, 100, 200, 400, 800 µg/mL) of the *N. brownii* leaflet WE were tested. The reaction mixtures were incubated in the dark for 15 min at room temperature, followed by the measurement of the decrease in absorbance at 517 nm using a spectrophotometer. A control was established with DPPH solution alone and 95 % methanol served as the blank. The antioxidant activity was assessed by calculating the percentage inhibition of DPPH radicals, with statistical analysis conducted as necessary. All experiments were conducted in triplicate to ensure reliability and safety precautions were observed throughout the experimental procedures. The methodology followed a systematic approach to assess the antioxidant potential of the *N. brownii* leaflet WE through the DPPH radical scavenging assay, providing a robust framework for data analysis and interpretation (14).

Anti-diabetic activity

α-Amylase inhibition assay: The anti-diabetic activity of the *N. brownii* leaflet WE was assessed through the α-amylase inhibition assay, following a modified protocol from the Worthington Enzyme Manual (15). In this assay, 500 µL of 0.02 M sodium phosphate buffer (pH 6.9 with 0.006 M NaCl) containing 0.5 mg/mL of α-amylase enzyme and various concentrations (in µg) of the test sample as an enzyme inhibitor were pre-incubated at 37 °C for 10 min. Following pre-incubation, 500 µL of a 1 % starch solution in 0.02 M sodium phosphate buffer (pH 6.9) was added to each tube and incubated at room temperature for 5 min. The enzymatic reaction was halted using 1.0 mL of dinitrosalicylic acid (DNSA) reagent. Subsequently, the test tubes were subjected to a 5 min incubation in a boiling water bath and then cooled to room temperature. The reaction mixture was diluted to a final volume of 10 mL with distilled water and the absorbance was measured at 540 nm using a UV-Visible spectrophotometer. The absorbance values were compared with controls, including a control with starch but without **α**-amylase (C) and a control with starch and **α**-amylase (B).

The percentage inhibition was calculated using the formula:

$$\text{Percentage Inhibition} = (B - A) \times 100 / (B - C)$$

Where,

A is the absorbance of the test; B is the absorbance of the control with starch and **α**-amylase; C is the absorbance of the control with starch and without **α**-amylase.

This methodology was employed as a systematic approach to evaluate the potential α-amylase inhibitory activity of the test samples, offering insights into their anti-diabetic properties.

α-Glucosidase inhibition assay: The impact of the *N. brownii* leaf extract in water on α-glucosidase inhibition activity was evaluated (16). In this assay, 400 µL of α-glucosidase (0.067 U/

mL) was preincubated with various concentrations of the sample for 30 min. Subsequently, 200 µL of 3.0 mM para-nitrophenyl-α-D-glucopyranoside (pNPG), used as a substrate and dissolved in 0.1 M sodium phosphate buffer (pH 6.9), was added to initiate the reaction. The reaction mixture was incubated at 37 °C for 30 min and then halted by adding 2 mL of 0.1 M Na_2CO_3. The α-glucosidase activity was determined by measuring the yellow-coloured para-nitrophenol released from pNPG at 400 nm.

The inhibitory activity percentage was calculated using the formula:

Inhibitory activity (%) = [(B - T) / (B - C)] ×100

Where,

B is the absorbance of blank; T is the absorbance in the presence of test substance; C is the absorbance of control.

The same procedure was followed using acarbose (1 mg/mL stock) as the standard (16). This methodology was employed as a systematic approach to assess the inhibitory effect of the *N. brownii* leaf extract in water on α-glucosidase activity, offering valuable insights into its potential anti-diabetic properties.

Results

Antioxidant activity

DPPH radical scavenging assay

The antioxidant activities of *N. brownii* leaflet extract in water and ascorbic acid, as assessed through the DPPH radical scavenging assay, provide valuable insights into their potential applications in combating oxidative stress-related conditions.

Table 1 presents the antioxidant activity of ascorbic acid (standard) at various concentrations in the DPPH radical scavenging assay. The control group exhibited an average optical density (OD) of 0.8654 at 517 nm. Ascorbic acid demonstrated a concentration-dependent decrease in OD, indicating its ability to scavenge DPPH radicals. Percentage inhibition increased with higher concentrations, reaching 94.95 % at 800 µg/mL. The IC_{50} value, representing the concentration at which 50 % of DPPH radicals were scavenged, was calculated to be 28.19 µg/mL. These results illustrate the potent antioxidant properties of ascorbic acid, serving as a reference standard for comparison with the *N. brownii* leaflet extract in water, presented in Table 2.

Table 2 presents the antioxidant activity of *N. brownii* leaflet extract in water at various concentrations. The control group exhibited an average OD of 0.8654 at 515 nm. The leaf extract demonstrated a concentration-dependent decrease in OD, indicating its ability to scavenge DPPH radicals. Percentage inhibition increased with higher concentrations, reaching 70.0 % at 1000 µg/mL. The IC_{50} value, representing the concentration at which 50 % of DPPH radicals were scavenged, was calculated to be 219.64 µg/mL. These findings highlight the considerable antioxidant potential of the *N. brownii* leaflet extract in water, suggesting its efficacy in neutralizing free radicals and supporting its potential therapeutic applications. Fig. 1 depicts the DPPH radical scavenging activity of the standard and the leaf extract in water.

Anti-diabetic activity

α-Amylase inhibition assay

The anti-diabetic activity of *N. brownii* leaf extract in water was investigated and compared with acarbose, a known α-amylase inhibitor, through the α-amylase inhibition assay.

Table 1. Antioxidant activity of ascorbic acid

	Concentration (µg/mL)	OD at 515 nm			Average OD at 515 nm	% of inhibition	Standard error
		OD1	OD2	OD3			
Control	-	0.8655	0.8651	0.8655	0.8654		
	1.56	0.7987	0.7989	0.7881	0.7952	3.36	0.005774
	3.12	0.7272	0.7274	0.7275	0.7274	11.61	0.450333
	6.25	0.6813	0.6810	0.6813	0.6812	17.22	0.415692
	12.5	0.5518	0.5514	0.5514	0.5515	32.98	0.565803
	25	0.4585	0.4588	0.4588	0.4587	44.26	0.637321
	50	0.1465	0.1466	0.1466	0.1466	82.19	0.005774
	100	0.0926	0.0929	0.0922	0.0926	88.75	0.721688
Ascorbic acid	200	0.0841	0.0848	0.0842	0.0844	89.75	0.005774
	400	0.0654	0.0648	0.0647	0.0650	92.11	0.005774
	800	0.0418	0.0411	0.0417	0.0415	94.95	0.548483
IC_{50}				**28.19**			

Table 2. Antioxidant activity of aqueous extract of *N. brownii* leaflet

Sample code	Concentration (µg/mL)	OD1	OD2	OD3	Average OD at 515 nm	% of inhibition	Standard error
Control	-	0.8655	0.8651	0.8655	0.8654		
	1.56	0.8147	0.8147	0.8143	0.8146	5.87	0.005774
	3.12	0.7655	0.7654	0.7650	0.7653	11.57	0.617765
	6.25	0.7406	0.7400	0.7406	0.7404	14.44	0.005774
	12.5	0.7077	0.7078	0.7070	0.7075	18.25	0.721688
	25	0.6586	0.6585	0.6586	0.6586	23.90	0.005774
	50	0.5343	0.5347	0.5342	0.5344	38.25	0.721688
	100	0.4750	0.4752	0.4757	0.4753	45.08	0.623538
N. brownii leaflet extract in water	200	0.4361	0.4363	0.4367	0.4364	49.58	0.005774
	400	0.3721	0.3723	0.3729	0.3724	56.96	0.554256
	800	0.3210	0.3215	0.3211	0.3212	62.88	0.023094
	1000	0.2599	0.2591	0.2595	0.2595	70.0	0.173205
	IC_{50}				**219.64**		

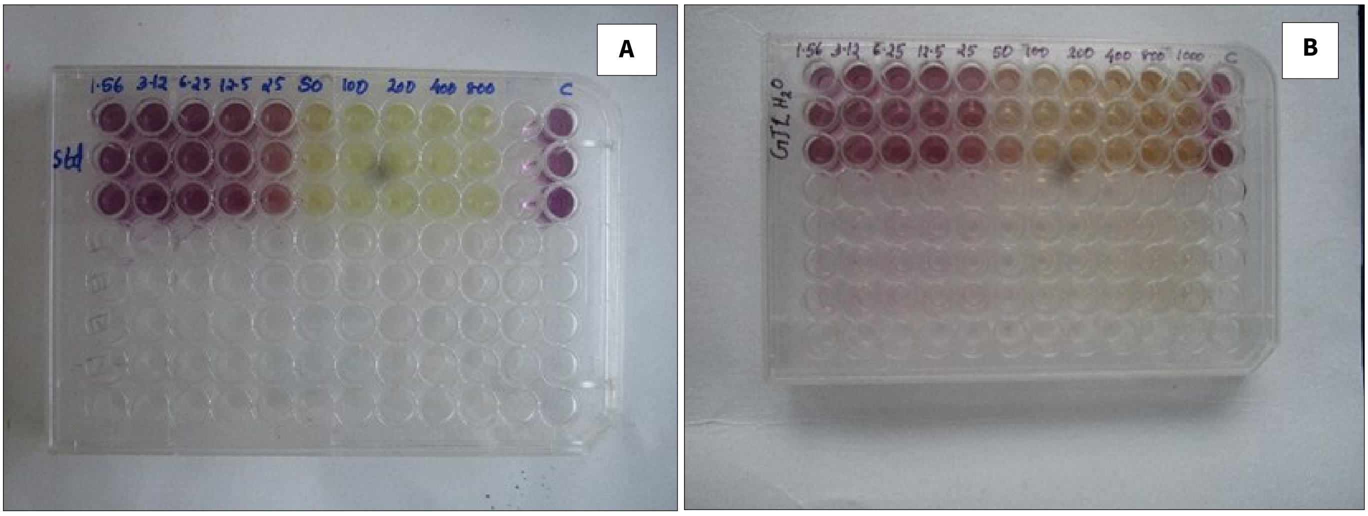

Fig. 1. DPPH radical scavenging activity: A - standard; B - leaf extract in water.

Fig. 2 indicates α-amylase inhibition activity of the standard and the leaf extract in water.The results presented in Table 1, 2 demonstrate the concentration-dependent inhibition of α-amylase by both acarbose and the *N. brownii* leaf extract in water.

In Table 3, acarbose exhibited significant α-amylase inhibition with an IC_{50} value of 49.52 µg/mL. The percentage of inhibition increased progressively with higher concentrations, reaching 89.57 % at 100 µg/mL. These findings are consistent with the expected inhibitory effect of acarbose on α-amylase activity.

Table 4 presents the α-amylase inhibition activity of *N. brownii* leaf extract in water. The extract demonstrates concentration-dependent inhibition, with an IC_{50} value of 67.70 µg/mL. Percentage inhibition ranged from 7.99 % at 6.25 µg/mL to 60.43 % at 100 µg/mL. While the extract showed notable α-amylase inhibitory activity, the IC_{50} value was higher than that of acarbose, suggesting that acarbose is more potent in inhibiting α-amylase under these assay conditions.

α-Glucosidase inhibition assay

The α-glucosidase inhibition assay was conducted to evaluate the anti-diabetic potential of *N. brownii* leaf water extract, comparing its activity with the standard drug acarbose.

Fig. 3 indicates the α-glucosidase inhibition activity of the standard and the leaf extract in water. In Table 5, acarbose exhibited concentration-dependent inhibition of α-glucosidase, with an IC_{50} value of 16.72 µg/mL. This aligns with expectations, as acarbose is a known α-glucosidase inhibitor. The significant inhibitory effect observed at lower concentrations underscores its potency in suppressing the activity of the enzyme.

In Table 6, *N. brownii* leaf extract in water displayed a similar concentration-dependent inhibition of α-glucosidase. However, the IC_{50} value for the leaf extract was found to be 76.37 µg/mL, indicating a relatively lower inhibitory potential compared to acarbose. Nevertheless, the extract demonstrated notable inhibitory activity at higher concentrations, suggesting its potential as a natural α-glucosidase inhibitor.

Discussion

Research on fern species' antioxidant activity is crucial for identifying natural sources of antioxidants and understanding their health benefits against oxidative stress-related diseases. Additionally, ferns exhibit various pharmacological activities, including antibacterial, antirheumatic, anti-diabetic, antitumor, antifungal, anti-inflammatory and anti-tussive properties, mainly attributed to phenolics, flavonoids, alkaloids and terpenoids. Presence of hydroxyl functional group in flavonoids empower them to act as antioxidant. Phenols are one of the most commonly occurring groups of phytochemicals, with significant

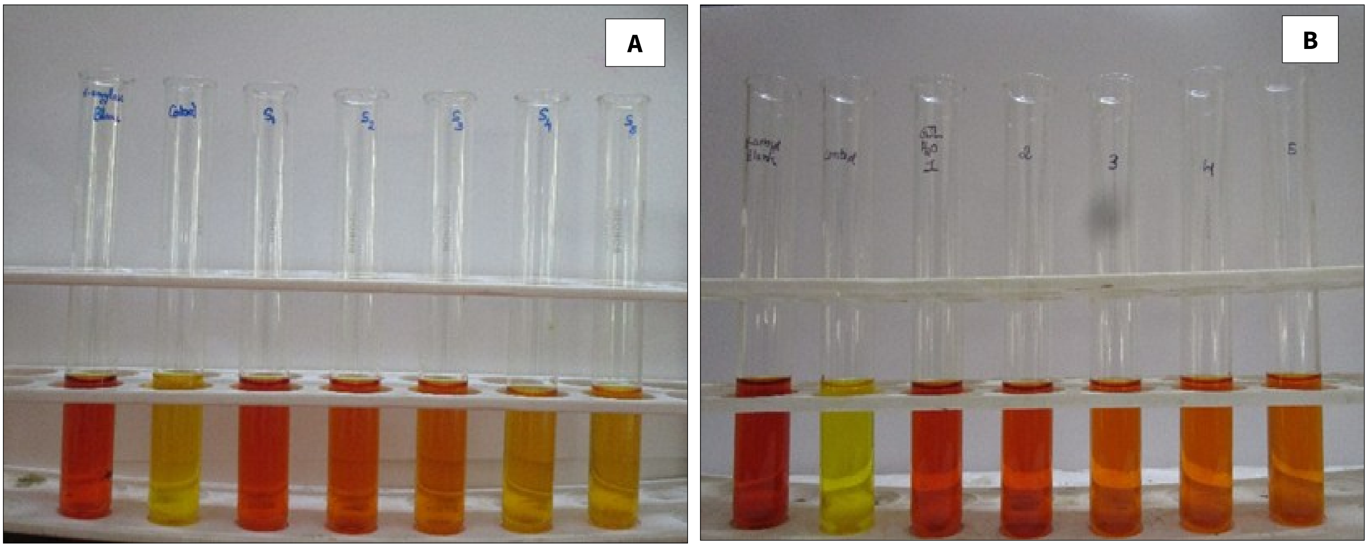

Fig. 2. α-Amylase inhibitory activity: A - standard; B - leaf extract in water.

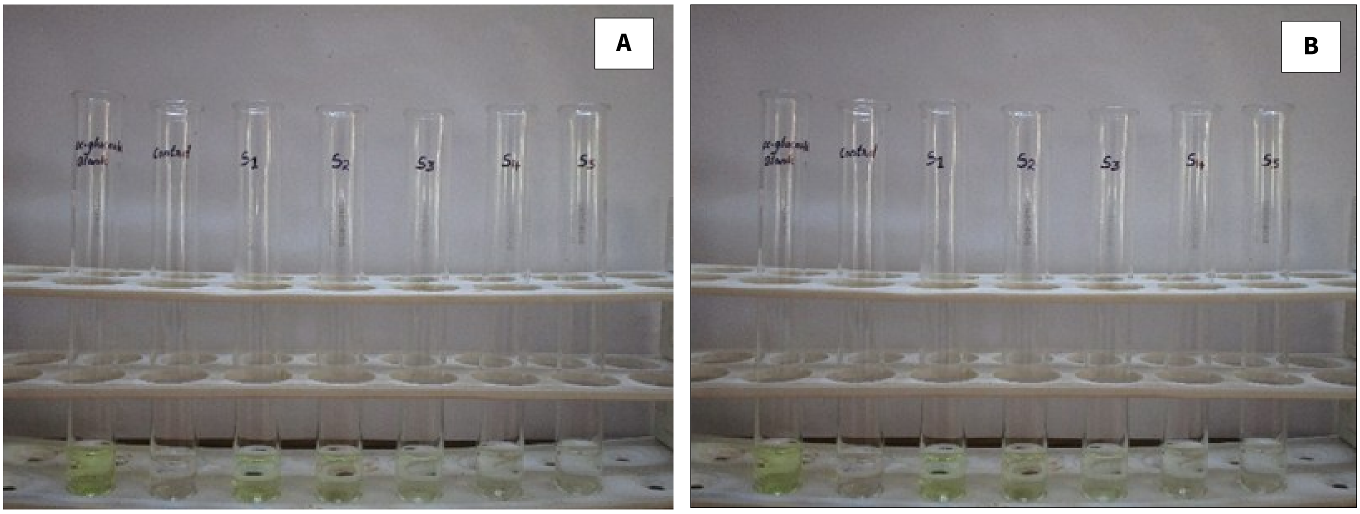

Fig. 3. α-Glucosidase inhibitory activity: A - standard; B - leaf extract in water.

Table 3. α-Amylase inhibitory activity of acarbose

Standard	Concentration (µg/mL)	OD at 540 nm	% of inhibition	Standard error
Blank		0.980		
Control		0.031		
	6.25	0.900	8.43	0.536936
	12.5	0.850	13.70	0.057735
Acarbose (standard)	25	0.640	35.83	0.057735
	50	0.340	67.44	0.173205
	100	0.130	89.57	0.173333
IC_{50}		**49.52**		

Table 4. α-Amylase inhibitory activity of *N. brownii* leaf extract in water

Standard	Concentration (µg/mL)	OD at 540 nm	% of inhibition	Standard error
Blank		1.37		
Control		0.018		
	6.25	1.262	7.99	0.568712
	12.5	0.994	27.81	0.112596
N. brownii leaf water extract	25	0.889	35.58	0.57735
	50	0.765	44.75	0.57735
	100	0.553	60.43	0.57735
IC_{50}		**67.70**		

Table 5. α-Glucosidase inhibitory activity of acarbose

Sample	Concentration (µg/mL)	OD at 400 nm	% of inhibition	Standard error
Blank		0.704		
Control		0.026		
	6.25	0.567	20.20	0.57735
	12.5	0.442	38.64	0.57735
Acarbose (standard)	25	0.208	73.15	0.57735
	50	0.050	96.46	0.57735
	100	0.042	97.64	0.57735
IC_{50}		**16.72**		

Table 6. α-Glucosidase inhibitory activity of *N. brownii* leaf extract in water

Sample	Concentration (µg/mL)	OD at 400 nm	% of inhibition	Standard error
Blank		1.486		
Control		0.028		
	1.5	1.415	4.87	0.57735
	3.125	1.273	14.61	0.57735
	6.25	1.107	25.99	0.57735
	12.5	0.992	33.88	0.587736
N. brownii leaf water extract	25	0.879	41.63	0.57735
	50	0.811	46.30	0.57735
	100	0.705	53.57	0.57735
IC_{50}		**76.37**		

morphological and physiological importance in plants. Phenolic compounds have been reported as major group of compounds that contribute to the antioxidant activity of plant extracts and has been correlated with DPPH scavenging assay (17).

N. brownii contains a notable amount of secondary metabolites such as flavonoids and phenols, which demonstrate significant potential in counteracting oxidative stress by effectively quenching ROS (18). These compounds play a vital role in protecting the plant against environmental stressors and may offer therapeutic benefits for human health by mitigating oxidative stress-related diseases. Therefore, an attempt was made to analyse the antioxidant activity of *N. brownii* using DPPH radical scavenging assay to uncover its free radical scavenging potential.

In the present study, the WE of the plant was used to encompass a wide array of bioactive compounds, maintain biological relevance, ensure safety in handling and offer cost-effectiveness, thereby offering valuable insights into the potential therapeutic attributes of plants. The DPPH assay is crucial for quantifying antioxidants because it provides a simple, rapid and reliable method to measure the free radical scavenging capacity of the extracts (19). This assay helps in determining the potential health benefits of plant-derived compounds by evaluating their ability to neutralize harmful free radicals. In the DPPH radical scavenging assay, the extract exhibited significant antioxidant activity (70.0 % at 1000 µg/mL), comparable to the standard ascorbic acid (94.95 % at 800 µg/mL). This finding indicates that *N. brownii* has the potential to serve as a rich source of natural antioxidants and this study represents the first report in this regard. The concentration-dependent trend observed in both ascorbic acid and the leaf extract implies the presence of a diverse range of compounds with varied antioxidant potentials in the natural extract.

Previous studies have highlighted the significant potential of ferns as sources of bioactive compounds with antioxidant and anti-diabetic properties. A comparative analysis of *Diplazium esculentum* and *Marsilea minuta* both commonly used as vegetables, revealed that *D. esculentum* possesses superior nutritional and antioxidative properties (20). This study underscores the importance of considering ferns not only for their medicinal value but also as potential dietary supplements. An evaluation of several fern species was conducted and *Aleuritopteris flava* and *Lindsaea odorata* exhibited the highest antioxidant activity among those studied (21). This suggests that a variety of ferns possess significant potential as sources of natural antioxidants, which could be utilized in preventing oxidative stress-related disorders. The antioxidative potential of leaf extracts from various medicinal ferns was studied and *Blechnum orientale* demonstrated the highest total polyphenol content and strongest antioxidative potential (22). Among the indigenous fern species in East Kalimantan, *Acrostichum aureum* has the highest total phenolic and flavonoid content, along with potent antioxidant activity (23). These findings highlight the diversity of bioactive compounds present in ferns.

The enzymes α-amylase and α-glucosidase play pivotal roles in the breakdown of carbohydrates into glucose (24). Inhibition of these enzymes constitutes a key strategy for managing hyperglycemia and treating type 2 diabetes (DM2). Acarbose is a commonly prescribed drug for DM2 treatment, functioning by inhibiting both α-amylase and α-glucosidase (25). However, being a synthetic drug, acarbose can lead to undesirable effects such as diarrhea, stomach pain and digestion difficulties (26). The present study also revealed the potential anti-diabetic activity of *N. brownii* leaf extract in water by analyzing its inhibitory effects on α-amylase and α-glucosidase enzymes. The α-amylase inhibition assay revealed that *N. brownii* leaf extract in water exhibited notable inhibition of α-amylase (from 7.99 % at 6.25 µg/mL to 60.43 % at 100 µg/mL). While acarbose demonstrated a slightly lower IC_{50} value (49.52 µg/mL), the natural origin of the leaf extract (67.70 µg/mL) and its observed inhibitory activity make it a promising candidate for further investigation as a natural anti-diabetic agent. The IC_{50} value is crucial in α-amylase inhibition assays for evaluating the efficacy of extracts. It quantifies the concentration needed to inhibit 50 % of enzyme activity, thus indicating the potential of the extract as a natural anti-diabetic agent by moderating carbohydrate digestion and glucose absorption (27). The α-glucosidase inhibition assay further supported this potential, with the extract displaying significant inhibitory activity at elevated concentrations (53.57 % of inhibition at concentration of 100 µg/mL), albeit with a higher IC_{50} compared to acarbose (IC_{50} value, 16.72 µg/mL).

The comparison of both assays highlights the consistent potency of acarbose in inhibiting α-amylase and α-glucosidase compared to the WE of *N. brownii* leaves. However, the natural origin of the extract and its significant inhibitory effects indicate a promising avenue for further exploration in anti-diabetic research. The slightly higher IC_{50} values for the leaf extract suggest that higher concentrations may be needed to achieve comparable inhibitory effects, emphasizing the importance of dosage considerations in potential therapeutic applications.

When compared with previous research involving various ferns, promising findings have been revealed regarding their antioxidant and anti-diabetic properties. Studies utilizing ferns as sources of bioactive compounds targeting antioxidant properties and α-amylase and α-glucosidase inhibitory activities have also reported encouraging results. For instance, both leaf and rhizome extracts of *Phymatopteris triloba* and *Gleichenia truncata* were identified for their remarkable α-glucosidase inhibitory activity (28). The antihyperglycemic potential of the methanolic extract of *Christella dentata* is commendable (29). Through evaluation experiments on glucose-challenged mice, the study demonstrated a dose-dependent reduction in blood sugar levels upon oral administration of the extract. Notably, doses of 100, 200 and 400 mg/ kg body weight resulted in substantial decreases in blood glucose levels by 48.02 %, 49.44 % and 54.52 % respectively, compared to control mice. These findings underscore the promising therapeutic prospects of *C. dentata* extract in managing hyperglycemia.

Moreover, solvent extracts from *Cyathea latebrosa*, *Cibotium barometz*, *Drynaria quercifolia*, *Blechnum orientale* and *Dicranopteris linearis* known for their high total phenol contents, have been recognized as potential antioxidants (30). *Pyrrosia lingua* and *Osmunda cinnamomea* have demonstrated inhibition toward α-glucosidase (31). *Pteris vittata* possess α-amylase inhibitory potential and it was found that the ethanolic extract of *P. vittata* exhibited superior α-amylase inhibitory activity compared to the aqueous extract (32). This enhanced inhibitory potential was attributed to the phenolic content present in the plant extract. These results suggest the potential of *P. vittata* as a natural source of compounds that could aid in the regulation of blood glucose levels, possibly through the inhibition of α-amylase activity.

The reported antioxidant and anti-diabetic activities in various fern species belonging to the genus *Nephrolepis* provide a foundation for further exploration and understanding of the therapeutic potential of *Nephrolepis* species extracts in managing oxidative stress and diabetes. *N. undulata* leaf extract was found to possess both anti-diabetic and antioxidant properties, with its likely mechanism of action attributed to its inhibitory effect on glucose hydrolyzing enzymes and its ability to facilitate cellular detoxification (33). The α-glucosidase and α-amylase inhibitory assays conducted on the methanolic extract of *Nephrolepis auriculata* (L.) Trimen revealed potent anti-diabetic properties, with IC_{50} values of 55.79 ± 1.01 µg/mL and 57.54 ± 1.52 µg/mL respectively. It was uncovered that both aqueous and methanolic extracts of *Nephrolepis auriculata* are rich in phenolics and flavonoids, which may be responsible for their antioxidant and anti-diabetic activities (34). In the antioxidant assay, significant results were demonstrated with 67.02 % inhibition at the concentration of 30 µg/mL of the ethanolic extract of leaves of *N. cordifolia* (35).

In summary, the results collectively suggest that the WE of *N. brownii* leaves possesses significant antioxidant and anti-diabetic potential. While acarbose remains a potent standard, the natural origin of the extract and its observed inhibitory activity present a promising foundation for further investigations. Future research should focus on isolating specific bioactive compounds, understanding their mechanisms of action and conducting *in vivo* studies to assess the overall efficacy and safety of the WE of *N. brownii* leaves as a potential natural antioxidant and anti-diabetic agent.

Antioxidant activity is exhibited by the plant extract by neutralising free radicals, by either donating a hydrogen atom or by a single electron transfer mechanism. Anti-diabetic agent present in the extract controls the activity of some metabolic enzymes such as amylase that breaks starch into glucose and pancreatic α-amylase inhibition that give an effective strategy to lower hyperglycemia via starch breakdown.

Conclusion

This comprehensive study delved into the therapeutic potential of *N. brownii* leaf extract in water, emphasizing its dual efficacy as an antioxidant and a prospective anti-diabetic agent. The DPPH radical scavenging assay revealed the extract's significant antioxidant capabilities, demonstrating a concentration-dependent decrease in optical density. Although the IC_{50} value was higher compared to the standard ascorbic acid, the extract exhibited substantial antioxidant potential. In the context of anti-diabetic activities, both the α-amylase and α-glucosidase inhibition assays indicated promising results for the WE of *N. brownii* leaves. While acarbose exhibited stronger inhibitory effects, the natural origin of the leaf extract makes it an intriguing candidate for further investigation as a potential anti-diabetic agent. This study contributes valuable insights into the multifaceted therapeutic properties of *N. brownii*, suggesting its potential application as a natural source of antioxidants and a candidate for further anti-diabetic research. Future work will focus on the isolation of active compounds responsible for antioxidant and anti-diabetic activities. Limitation of the work includes the absence of clinical trials, which are of prime importance in confirming the therapeutic property. In future investigations, clinical trials will be executed.

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Authors' contributions

GMD carried out the experimentation and received support in manuscript writing from GGP, FSS, HJB, VV, SM, JSK, MKA and SKS. RA contributed by assisting with the identification of the plant species. All authors read and approved the manuscript.

Compliance with ethical standards

Conflict of interest: The authors have no conflicts of interest to declare.

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