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In vitro evaluation of the antidiabetic potential of aqueous and ethanolic extracts of *Stemona tuberosa* tubers and leaves

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Abstract
Diabetes mellitus is a chronic metabolic disorder characterized by persistent hyperglycaemia and progressive complications. Inhibition of carbohydrate-digesting enzymes and prevention of protein glycation are important therapeutic strategies for controlling postprandial hyperglycaemia and delaying diabetic complications. The present study evaluated the in vitro antidiabetic potential of aqueous and ethanolic extracts of tubers and leaves of *Stemona tuberosa* by assessing α-amylase inhibitory activity and inhibition of non-enzymatic haemoglobin glycation. All extracts exhibited concentration-dependent α-amylase inhibition, with the ethanolic tuber extract showing the highest inhibitory activity, comparable to the standard drug acarbose. Similarly, the extracts significantly inhibited haemoglobin glycation, indicating their potential to prevent advanced glycation end product formation. Ethanolic extracts demonstrated superior activity compared to aqueous extracts, and tubers were more potent than leaves. These findings suggest that *Stemona tuberosa*, particularly its ethanolic tuber extract, possesses promising antidiabetic potential and may serve as a natural source of bioactive compounds for diabetes management.

Keywords: *Stemona tuberosa*, α-amylase inhibition, haemoglobin glycation, antidiabetic activity, medicinal plants

1. Introduction
Diabetes mellitus is one of the most prevalent metabolic disorders worldwide, characterized by chronic hyperglycaemia resulting from defects in insulin secretion, insulin action, or both. Persistent elevation of blood glucose levels leads to long-term complications, including nephropathy, neuropathy, retinopathy, and cardiovascular diseases. Despite the availability of several synthetic antidiabetic agents, their prolonged use is often associated with adverse effects and limited efficacy in preventing disease progression. Postprandial hyperglycaemia plays a crucial role in the development of type II diabetes and its complications. Inhibition of α-amylase, a key enzyme involved in carbohydrate digestion, is an established therapeutic approach to delay glucose absorption. In addition, non-enzymatic glycation of haemoglobin and other proteins leads to the formation of advanced glycation end products, which contribute significantly to diabetic complications. Therefore, agents capable of inhibiting both carbohydrate-digesting enzymes and protein glycation are of considerable therapeutic interest. [1]
Medicinal plants have long been used in traditional systems of medicine for the management of diabetes, owing to their diverse bioactive constituents and relatively low toxicity. *Stemona tuberosa* is a medicinal plant traditionally used for various therapeutic purposes; however, its antidiabetic potential has not been extensively investigated. The present study was designed to evaluate the in vitro antidiabetic activity of aqueous and ethanolic extracts of tubers and leaves of *S. tuberosa* by focusing specifically on α-amylase inhibition and inhibition of non-enzymatic haemoglobin glycation.

2. Materials and Methods
2.1 Plant Material Collection and Authentication
Fresh tubers and leaves of *Stemona tuberosa* were collected from their natural habitat during July 2025. The plant material was authenticated by Pharmacognosy faculty, and a voucher

Specimen was deposited in the institutional herbarium of Jayamukhi College of Pharmacy for future reference. The collected tubers and leaves were thoroughly washed with distilled water to remove adhering soil and impurities, shade-dried at room temperature for two weeks, and subsequently pulverized into coarse powder using a mechanical grinder. The powdered samples were stored in airtight containers until extraction.

2.2 Chemicals and Reagents
A-Amylase enzyme (porcine pancreatic), soluble starch, hemoglobin, glucose, acarbose, and all analytical-grade chemicals were procured from standard commercial suppliers. Distilled water was used throughout the experiments. All reagents were freshly prepared prior to use.

2.3 Preparation of Plant Extracts
The powdered tubers and leaves were subjected to extraction using aqueous and ethanolic solvents. For aqueous extraction, the plant material was macerated with distilled water in a ratio of 1:10 (w/v) for 24 h with intermittent shaking, followed by filtration through muslin cloth and Whatman No. 1 filter paper. The filtrate was concentrated by evaporation and dried to obtain the aqueous extract. For ethanolic extraction, the powdered material was extracted using ethanol (95%) by maceration under similar conditions. The extract was filtered and concentrated under reduced pressure using a rotary evaporator, followed by drying to obtain a solid residue. All extracts were weighed to calculate percentage yield and stored at 4 °C until further use.

2.4 Preliminary Phytochemical Screening
Qualitative phytochemical analysis of aqueous and ethanolic extracts of tubers and leaves was carried out using standard chemical tests to detect the presence of major secondary metabolites. The extracts were screened for alkaloids (Dragendorff's and Mayer's tests), flavonoids (Shinoda test), tannins (Ferric chloride test), saponins (foam test), glycosides (Keller-Killiani test), steroids and triterpenoids (Liebermann-Burchard test). The presence or absence of phytoconstituents was recorded based on characteristic color changes or precipitate formation. [2]

2.5 α-Amylase Inhibitory Assay
The α-amylase inhibitory activity of *Stemona tuberosa* extracts was evaluated using a spectrophotometric method. Briefly, different concentrations of the extracts (ranging from 100-1500 µg/mL) were pre-incubated with α-amylase solution in phosphate buffer (pH 6.9) at 37 °C for 10 min. Subsequently, soluble starch solution was added as the substrate, and the reaction mixture was incubated further. The reaction was terminated by the addition of a suitable color-developing reagent, and absorbance was measured at the appropriate wavelength using a UV-Visible spectrophotometer. [3]
Acarbose was used as the standard reference inhibitor. The percentage inhibition of α-amylase activity was calculated using the formula

$$\text{Percentage inhibition (\%)} = \frac{\text{Control absorbance} - \text{Sample absorbance}}{\text{Control absorbance}} \times 100$$

The concentration required to inhibit 50% of enzyme activity (IC_{50}) was determined from the dose-response curve.

2.6 Non-Enzymatic Haemoglobin Glycation Assay
The antiglycation activity of the extracts was assessed using an in vitro non-enzymatic haemoglobin glycation model. Haemoglobin solution was incubated with glucose in phosphate buffer in the presence and absence of various concentrations of the extracts. The reaction mixtures were incubated at 37 °C for a specified period under sterile conditions to allow glycation to occur. Following incubation, the extent of haemoglobin glycation was measured spectrophotometrically. The percentage inhibition of hemoglobin glycation was calculated relative to the control without extract. Lower absorbance values indicated greater inhibition of glycation. IC_{50} values were calculated to compare the antiglycation potency of different extracts. [4]

2.7 Statistical Analysis
All experiments were conducted in triplicate, and the results were expressed as mean±standard deviation (SD). IC_{50} values were calculated using concentration-response analysis.

3. Results
3.1 Extraction Yield
The percentage yield of aqueous and ethanolic extracts of *Stemona tuberosa* tubers and leaves varied significantly depending on the solvent and plant part used. Ethanolic extraction resulted in higher yields compared to aqueous extraction for both tubers and leaves. The ethanolic extract of tubers showed the highest yield (21.36%), whereas the aqueous tuber extract yielded 12.78%. Similarly, leaves extracted with ethanol produced a yield of 18.92%, while the aqueous leaf extract showed a comparatively lower yield of 11.45%. These findings indicate that ethanol is a more efficient solvent for extracting bioactive constituents from *S. tuberosa*, and that tubers possess a slightly higher extractable phytochemical content than leaves.

3.2 Preliminary Phytochemical Screening
Preliminary qualitative phytochemical screening of aqueous and ethanolic extracts of *Stemona tuberosa* tubers and leaves revealed the presence of a wide range of secondary metabolites associated with antidiabetic activity. Both aqueous and ethanolic extracts tested positive for alkaloids, carbohydrates, flavonoids, steroids, triterpenoids, amino acids, tannins, saponins, fixed oils and fats, and glycosides, including cardiac glycosides.
However, qualitative differences were observed between extraction solvents. Ethanolic extracts exhibited a broader and more consistent presence of phytoconstituents compared to aqueous extracts, particularly for proteins, tannins, fixed oils, saponins, and glycosides, which were weakly present or absent in aqueous extracts. These observations correlate with the higher extraction yields obtained with ethanol and suggest improved solubilization of bioactive compounds in ethanolic media. Overall, the phytochemical profile supports the pharmacological potential of *S. tuberosa* in antidiabetic applications.

3.3 α-Amylase Inhibitory Activity
All aqueous and ethanolic extracts of *Stemona tuberosa* tubers and leaves exhibited concentration-dependent inhibition of α-amylase activity over the tested range (100-1500 µg/mL). Among the extracts, the ethanolic tuber extract showed the highest inhibitory activity, achieving 77.34±2.19% inhibition at 1500 µg/mL, followed by the ethanolic leaf extract (75.09±2.24%). Aqueous extracts demonstrated comparatively lower inhibition, with maximum values of 70.56±2.04% for tubers and

68.87±2.01% for leaves. The standard drug acarbose produced 81.56±1.79% inhibition at 500 µg/mL.

IC_{50} analysis indicated that the ethanolic tuber extract was the most potent inhibitor (465.52±8.47 µg/mL), followed by ethanolic leaves (498.32±9.87 µg/mL), while aqueous tuber and leaf extracts showed higher IC_{50} values, reflecting lower inhibitory potency.

Table 1: α-Amylase Inhibitory Activity and IC_{50} Values of Aqueous and Ethanolic Extracts of *Stemona tuberosa* Tubers and Leaves

Extract	Concentration (µg/mL)	% Inhibition (Mean ± SD)	IC_{50} (µg/mL)
Ethanolic Tubers	100	28.41±1.28	465.52±8.47
	250	40.74±1.54	
	500	53.26±1.86	
	1000	66.59±2.07	
	1500	77.34±2.19	
Aqueous Tubers	100	22.13±1.07	624.68±10.23
	250	34.67±1.34	
	500	47.08±1.79	
	1000	59.23±1.96	
	1500	70.56±2.04	
Ethanolic Leaves	100	25.87±1.12	498.32±9.87
	250	38.98±1.41	
	500	51.75±1.67	
	1000	63.42±2.08	
	1500	75.09±2.24	
Aqueous Leaves	100	20.79±1.03	647.67±12.33
	250	33.09±1.26	
	500	45.50±1.67	
	1000	57.64±1.84	
	1500	68.87±2.01	
Standard (Acarbose)	500	81.56±1.79	480.24±7.84

Note: Values are expressed as mean ± SD (n = 3). IC_{50} values represent the concentration required to inhibit 50% of α-amylase activity.

3.4. Inhibition of Haemoglobin Glycosylation

The aqueous and ethanolic extracts of *Stemona tuberosa* tubers and leaves showed significant, concentration-dependent inhibition of non-enzymatic haemoglobin glycation. The ethanolic tuber extract exhibited the highest antiglycation activity, with 61.79±1.68% inhibition at 800 µg/mL, followed closely by the ethanolic leaf extract (60.22±1.89%). Aqueous extracts showed moderate inhibition, reaching 55.03±1.75% for tubers and 54.18±1.65% for leaves at the same concentration. Trolox, used as the standard, showed 68.75±1.79% inhibition at 100 µg/mL.

IC_{50} values further confirmed the superior antiglycation potency of ethanolic extracts, with ethanolic tubers showing the lowest IC_{50} (395.32±7.85 µg/mL), followed by ethanolic leaves (410.91±8.74 µg/mL), while aqueous extracts exhibited higher IC_{50} values.

Table 2: Inhibition of Non-Enzymatic Haemoglobin Glycosylation and IC_{50} Values of Aqueous and Ethanolic Extracts of *Stemona tuberosa* Tubers and Leaves

Extract	Concentration (µg/mL)	% Inhibition (Mean ± SD)	IC_{50} (µg/mL)
Ethanolic Tubers	50	18.24±0.95	395.32±7.85
	100	27.56±1.19	
	200	38.67±1.44	
	400	50.32±1.68	
	800	61.79±1.68	
Ethanolic Leaves	50	15.87±0.96	410.91±8.74
	100	25.96±1.27	
	200	36.47±1.44	
	400	48.59±1.62	
	800	60.22±1.89	
Aqueous Tubers	50	12.96±0.87	465.24±9.84
	100	21.74±0.92	
	200	32.47±1.31	
	400	43.55±1.58	
	800	55.03±1.75	
Aqueous Leaves	50	11.37±0.83	487.27±10.84
	100	20.42±1.05	
	200	31.28±1.34	
	400	42.64±1.56	
	800	54.18±1.65	
Standard (Trolox)	100	68.75±1.79	—

Note: Values are expressed as mean ± SD (n = 3). IC_{50} values represent the concentration required to inhibit 50% of non-enzymatic haemoglobin glycosylation.

4. Discussion

The present study provides comprehensive in vitro evidence supporting the antidiabetic potential of aqueous and ethanolic extracts of *Stemona tuberosa* tubers and leaves. The evaluation was focused on two key therapeutic targets relevant to diabetes management: inhibition of α-amylase, which regulates postprandial glucose levels, and suppression of non-enzymatic haemoglobin glycation, a major contributor to long-term diabetic complications. The findings collectively demonstrate that *S. tuberosa*, particularly its ethanolic tuber extract, exhibits significant antidiabetic activity through these mechanisms.

Extraction yield analysis revealed that ethanolic extracts of both tubers and leaves yielded substantially higher percentages than aqueous extracts, with tubers showing the maximum yield. This observation suggests that ethanol is a more efficient solvent for extracting a broad range of bioactive secondary metabolites from *S. tuberosa*. Ethanol is known to solubilize both moderately polar and non-polar compounds, including flavonoids, alkaloids, phenolics, and glycosides, which are often implicated in antidiabetic activity. The comparatively higher yield obtained from tubers further indicates that this plant part may serve as a richer reservoir of pharmacologically active constituents than leaves.

Preliminary phytochemical screening supported these findings by revealing the presence of multiple bioactive compounds in both aqueous and ethanolic extracts. Alkaloids, flavonoids, tannins, saponins, steroids, triterpenoids, amino acids, fixed oils, and glycosides were detected, with ethanolic extracts exhibiting a broader and more consistent phytochemical profile. Many of these phytoconstituents have been previously reported to exert antidiabetic effects through enzyme inhibition, antioxidant activity, and modulation of glucose metabolism. The richer phytochemical composition of ethanolic extracts likely underlies their superior biological activity observed in subsequent assays. [5]

α-Amylase inhibition is a well-established strategy for controlling postprandial hyperglycaemia by delaying the enzymatic breakdown of dietary starch into absorbable glucose. In the present study, all extracts of *S. tuberosa* demonstrated concentration-dependent inhibition of α-amylase activity, indicating their potential to interfere with carbohydrate digestion. Among the tested samples, the ethanolic tuber extract showed the highest inhibitory activity, approaching that of the standard drug acarbose. The lower IC_{50} value of the ethanolic tuber extract further confirms its higher potency compared to other extracts. [6]

The superior α-amylase inhibitory activity of ethanolic extracts may be attributed to the presence of flavonoids, tannins, and alkaloids, which are known to bind to enzyme active sites or alter enzyme conformation, thereby reducing catalytic activity. Tannins and polyphenols, in particular, can form complexes with digestive enzymes, leading to reduced starch hydrolysis. The comparatively lower activity of aqueous extracts suggests that water alone may be less effective in extracting these enzyme-inhibitory compounds in sufficient concentrations. [7]

Non-enzymatic hemoglobin glycation represents another critical pathological process in diabetes, contributing to the formation of advanced glycation end products (AGEs) that are associated with microvascular and macrovascular complications. The ability of *S. tuberosa* extracts to inhibit hemoglobin glycation indicates their potential role in mitigating protein damage caused by prolonged hyperglycaemia. In this study, all extracts exhibited dose-dependent antiglycation activity, with ethanolic tuber extract again demonstrating the strongest effect, as evidenced by the highest percentage inhibition and lowest IC_{50} value.[8]

The antiglycation activity observed may be linked to the antioxidant and carbonyl-trapping properties of phytoconstituents such as flavonoids, phenolic compounds, and alkaloids. These compounds can inhibit early glycation reactions by scavenging reactive oxygen species, chelating metal ions, or directly interacting with glucose and reactive carbonyl intermediates. The moderate but significant antiglycation activity of aqueous extracts further supports the contribution of water-soluble constituents, although their lower efficacy compared to ethanolic extracts highlights the importance of solvent selection in phytopharmacological studies. [9]

A consistent pattern emerged across both α-amylase inhibition and haemoglobin glycation assays, wherein ethanolic extracts were more potent than aqueous extracts, and tubers were more active than leaves. This concordance strengthens the reliability of the findings and suggests that the antidiabetic effects of *S. tuberosa* are not assay-specific but rather reflect a genuine pharmacological potential mediated by multiple bioactive compounds. The dual activity against postprandial glucose elevation and protein glycation is particularly relevant, as it addresses both immediate glycaemic control and long-term complication prevention. [10]

Overall, the results of this study align well with existing literature on medicinal plants rich in polyphenols, alkaloids, and glycosides, which have been shown to exert antidiabetic effects through enzyme inhibition and antiglycation mechanisms. However, scientific reports on the antidiabetic activity of *Stemona tuberosa* remain limited, and the present investigation contributes novel evidence supporting its therapeutic relevance.

5. Conclusion

Stemona tuberosa extracts exhibited notable in vitro antidiabetic activity through α-amylase inhibition and suppression of haemoglobin glycation, with the ethanolic tuber extract showing the highest potency. These findings support its potential as a natural antidiabetic agent.

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Conflict of Interest

The authors declare no conflict of interest.

References

1. Alam S, Hasan MK, Neaz S, Hussain N, Hossain MF, Rahman T. Diabetes mellitus: Insights from epidemiology, biochemistry, risk factors, diagnosis,

complications and comprehensive management. Diabetology. 2021;2(2):36-50. DOI:10.3390/diabetology2020004.
2. Trease GE, Evans WC. Pharmacognosy. 15th ed. London: Saunders Publishers; 2002. p. 42-44, 221-229, 246-249, 304-306, 331-332, 391-393.
3. Tamil IG, Dineshkumar B, Nandhakumar M, Senthilkumar M, Mitra A. *In vitro* study on α-amylase inhibitory activity of an Indian medicinal plant, *Phyllanthus amarus*. Indian J Pharmacol. 2010;42(5):280-282. DOI:10.4103/0253-7613.70107.
4. Tupe RS, Kemse NG, Khaire AA, Shaikh SA. Attenuation of glycation-induced multiple protein modifications by Indian antidiabetic plant extracts. Pharm Biol. 2017;55(1):68-75. DOI:10.1080/13880209.2016.1228683.
5. Rao A, Kumari S, Laura JS, Dhania G. Qualitative phytochemical screening of medicinal plants using different solvent extracts. Orient J Chem. 2023;39(3):621-626. DOI:10.13005/ojc/390312.
6. Hasaninezhad F, Tavaf Z, Panahi F, Nourisefat M, Khalafi-Nezhad A, Yousefi R. Assessment of antidiabetic properties of novel synthetic curcumin analogues: α-amylase and α-glucosidase as target enzymes. J Diabetes Metab Disord. 2020;19(2):1505-1515. DOI:10.1007/s40200-020-00685-z.
7. Ali H, Houghton PJ, Soumyanath A. α-Amylase inhibitory activity of some Malaysian plants used to treat diabetes; with particular reference to *Phyllanthus amarus*. J Ethnopharmacol. 2006;107(3):449-455. DOI:10.1016/j.jep.2006.04.004.
8. Perera HK, Handuwalage CS. Detection of protein glycation inhibitory potential of nine antidiabetic plants using a novel method. Asian J Med Sci. 2014;6(2):1-6. DOI:10.3126/ajms.v6i2.10584.
9. Tijjani H, Imam SA. Inhibition of haemoglobin glycosylation, glucose uptake and *in vitro* antidiabetic activities of solvent fractions from *Daucus carota* seed. Ann Sci Technol. 2021;6(1):26-33. DOI:10.2478/ast-2021-0004.
10. Dalli M, Daoudi NE, Abrigach F, Azizi SE, Bnouham M, Kim B, Gseyra N. *In vitro* α-amylase and hemoglobin glycation inhibitory potential of *Nigella sativa* essential oil and molecular docking studies of its principal components. Front Pharmacol. 2022;13:1036129. DOI:10.3389/fphar.2022.1036129.