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IN VITRO EVALUATION OF ALPHA-AMYLASE AND ALPHA-GLUCOSIDASE INHIBITORY ACTIVITIES OF GLOBBA MARANTINA

Sriramula Manisha *, Syeda Nishat Fathima

Department of Pharmacology, Jayamukhi College of Pharmacy, Narsampet, Warangal-506332, Telangana, India

ABSTRACT:

Diabetes mellitus is a chronic metabolic disorder characterized by persistent hyperglycaemia and associated with severe long-term complications. The limitations and adverse effects of existing antidiabetic drugs have accelerated interest in plant-based therapeutic alternatives. *Globba marantina* L. (family: Zingiberaceae) is traditionally used in ethnomedicine for metabolic and inflammatory disorders, yet its antidiabetic potential remains scientifically underexplored. The present study aimed to evaluate the in-vitro antidiabetic activity of *G. marantina* using established biochemical and cellular models. Crude extracts were subjected to α -amylase and α -glucosidase inhibitory assays, glucose uptake studies in cultured adipocytes, and cytoprotective evaluation on pancreatic β -cells under glucotoxic conditions. The extracts demonstrated significant, concentration-dependent inhibition of carbohydrate-digesting enzymes, enhanced glucose uptake, and protective effects against glucose-induced β -cell damage. These findings suggest that *G. marantina* possesses promising antidiabetic properties and may serve as a potential source of bioactive compounds for diabetes management. Further in-vivo and mechanistic investigations are warranted.

Keywords: *Globba marantina* · Antidiabetic activity · α -Amylase · α -Glucosidase · Glucose uptake · In-vitro study

1. INTRODUCTION

Diabetes mellitus (DM) is a chronic, multifactorial metabolic disorder characterized by persistent hyperglycaemia resulting from defects in insulin secretion, insulin action, or a combination of both. It represents one of the most significant global public health challenges of the twenty-first century, with rapidly increasing prevalence across both developed and developing nations. According to recent estimates, diabetes affects hundreds of millions of individuals worldwide, with Type II diabetes mellitus (T2DM) accounting for approximately 90–95% of all cases. The disease is associated with severe long-term complications, including cardiovascular disease, nephropathy, neuropathy, retinopathy, and increased mortality, imposing a substantial socio-economic and healthcare burden [1].

The pathogenesis of T2DM is complex and involves insulin resistance in peripheral tissues such as skeletal muscle, liver, and adipose tissue, coupled with progressive dysfunction and loss of pancreatic β -cells. Chronic hyperglycaemia leads to metabolic disturbances through mechanisms including glucotoxicity, lipotoxicity, oxidative stress, and low-grade inflammation, which collectively exacerbate insulin resistance and β -cell failure. Effective glycaemic control remains central to preventing or delaying diabetes-related complications. [2]

Although several classes of synthetic antidiabetic drugs are currently available—such as sulfonylureas, biguanides, α -glucosidase inhibitors, thiazolidinediones, and incretin-based therapies—their long-term use is often associated with adverse effects, secondary failure, hypoglycaemia, weight gain, gastrointestinal intolerance, and high treatment costs. These limitations have prompted a growing interest in alternative and complementary therapeutic strategies, particularly those derived from natural sources. [3]

Medicinal plants have been used for centuries in traditional systems of medicine, including Ayurveda, Siddha, and traditional Chinese medicine, for the management of diabetes and its complications. Plant-derived bioactive compounds such as flavonoids, phenolic acids, terpenoids, alkaloids, and saponins have demonstrated diverse antidiabetic mechanisms, including inhibition of carbohydrate-digesting enzymes, enhancement of insulin sensitivity, stimulation of glucose uptake, antioxidant activity, and protection of pancreatic β -cells. In-vitro screening models play a crucial role in the early identification and mechanistic evaluation of such plant-based antidiabetic agents. [4]

One of the key therapeutic approaches in diabetes management involves the inhibition of digestive enzymes such as α -amylase and α -glucosidase, which are responsible for the breakdown of complex carbohydrates into absorbable glucose. Suppression of these enzymes delays glucose absorption, thereby reducing postprandial hyperglycaemia. [5]

Globba marantina L., a member of the family Zingiberaceae, is traditionally used in folk medicine for the treatment of digestive and metabolic ailments. Plants belonging to this family are known for their diverse pharmacological activities, including antioxidant and antidiabetic effects [6]. However, despite its ethnomedicinal relevance, the antidiabetic potential of *G. marantina* has not been extensively investigated. Therefore, the present study was undertaken to scientifically evaluate the in-vitro antidiabetic activity of

aqueous and alcoholic extracts of *Globba marantina* leaves and roots, with particular emphasis on α -amylase and α -glucosidase inhibitory activities, providing experimental support for its traditional use and potential therapeutic relevance in diabetes management.

2. MATERIALS AND METHODS

2.1 Chemicals and Reagents:

All chemicals and reagents used in the present study were of analytical grade and of the highest purity to ensure accuracy and reproducibility of experimental results. Ethanol and distilled water were used as extraction solvents. Reagents including Folin–Ciocalteu reagent, aluminium chloride, sodium carbonate, ferric chloride, and tannic acid were employed for phytochemical and quantitative estimations. α -Amylase and α -glucosidase enzymes, starch, p-nitrophenyl- α -D-glucopyranoside, phosphate buffer solutions, and the standard antidiabetic drug acarbose were used for in-vitro antidiabetic assays.

2.2 Collection and Authentication of Plant Material:

Leaves and roots of *Globba marantina* L. (Family: Zingiberaceae) were collected from their natural habitat during July 2025. The plant material was authenticated by a qualified botanist, and a voucher specimen was deposited in the herbarium of Jayamukhi College of Pharmacy for future reference. The collected samples were thoroughly washed with tap water followed by distilled water to remove adhering impurities and were shade-dried at room temperature to preserve thermolabile constituents.

2.3 Preparation of Plant Material and Extracts:

Shade-dried leaves and roots were pulverized separately to obtain coarse powder and stored in airtight containers. Aqueous and alcoholic extractions were performed to obtain a broad spectrum of phytoconstituents. For alcoholic extraction, powdered plant material was macerated with ethanol for 72 h with intermittent shaking. The extract was filtered and concentrated under reduced pressure using a rotary vacuum evaporator, dried, and stored at 4 °C. For aqueous extraction, powdered material was soaked in distilled water for 48–72 h at room temperature with occasional stirring. The extract was filtered, concentrated on a water bath, dried, and stored at 4 °C. Percentage yield was calculated for all extracts

2.4 Quantitative Estimation of Bioactive Constituents

All quantitative estimations were performed in triplicate to ensure reproducibility, and results were expressed as mean \pm standard deviation (SD).

2.4.1 Determination of Total Phenolic Content: Total phenolic content (TPC) of aqueous and alcoholic extracts of *Globba marantina* leaves and roots was determined using the Folin–Ciocalteu colorimetric method. Briefly, 0.5 mL of extract solution (1 mg/mL) was mixed with 2.5 mL of 10% (v/v) Folin–Ciocalteu reagent and allowed to react for 5 min at room temperature. Subsequently, 2.0 mL of 7.5% (w/v) sodium carbonate solution was added to the mixture to create alkaline conditions. The reaction mixture was incubated for 30 min at room temperature in the dark. Absorbance was measured at 760 nm using a UV–Visible

spectrophotometer against a reagent blank. Gallic acid (10–100 µg/mL) was used to construct the calibration curve, and results were expressed as milligrams of gallic acid equivalents (GAE) per gram of extract. [7]

2.4.2 Determination of Total Flavonoid Content: Total flavonoid content (TFC) was estimated using the aluminium chloride colorimetric method. An aliquot of 0.5 mL of extract solution (1 mg/mL) was mixed with 1.5 mL of methanol, followed by the addition of 0.1 mL of 10% (w/v) aluminium chloride, 0.1 mL of 1 M potassium acetate, and 2.8 mL of distilled water. The reaction mixture was incubated at room temperature for 30 min. Absorbance was measured at 415 nm using a UV–Visible spectrophotometer. Quercetin (10–100 µg/mL) was used as the reference standard, and total flavonoid content was expressed as milligrams of quercetin equivalents (QE) per gram of extract. [8]

2.4.3 Determination of Total Tannin Content: Total tannin content (TTC) was determined using the Folin–Denis method. Briefly, 1.0 mL of extract solution (1 mg/mL) was mixed with 0.5 mL of Folin–Denis reagent and 1.0 mL of 7.5% (w/v) sodium carbonate solution. The final volume was adjusted to 10 mL with distilled water. The reaction mixture was incubated for 30 min at room temperature. Absorbance was measured at 700 nm using a UV–Visible spectrophotometer. Tannic acid (10–100 µg/mL) was used to prepare the standard calibration curve, and results were expressed as milligrams of tannic acid equivalents (TAE) per gram of extract. [9]

2.5 In-Vitro Antidiabetic Activity

The in-vitro antidiabetic activity of aqueous and alcoholic extracts of *Globba marantina* leaves and roots was evaluated using enzyme inhibition assays targeting carbohydrate-digesting enzymes involved in postprandial glucose regulation.

2.6.1. α-Amylase Inhibition Assay: α-Amylase inhibitory activity was assessed using the dinitrosalicylic acid (DNS) method. A reaction mixture containing 0.5 mL of extract solution at various concentrations (100–1500 µg/mL) and 0.5 mL of α-amylase solution (1 U/mL prepared in 0.02 M phosphate buffer, pH 6.9) was pre-incubated at 37 °C for 10 min. The reaction was initiated by adding 0.5 mL of 1% (w/v) soluble starch solution and incubated at 37 °C for 15 min. The enzymatic reaction was terminated by adding 1.0 mL of DNS reagent, followed by heating in a boiling water bath for 5 min to develop color. After cooling to room temperature, the mixture was diluted with 10 mL of distilled water, and absorbance was measured at 540 nm. Acarbose (100–500 µg/mL) was used as the standard inhibitor. Percentage inhibition was calculated relative to the control without extract. [10]

2.6.2. α-Glucosidase Inhibition Assay: α-Glucosidase inhibitory activity was evaluated using a standard colorimetric method. Briefly, 0.5 mL of extract solution at different concentrations (100–1500 µg/mL) was mixed with 0.5 mL of α-glucosidase solution (1 U/mL prepared in 0.1 M phosphate buffer, pH 6.8) and pre-incubated at 37 °C for 10 min. The reaction was initiated by adding 0.5 mL of p-nitrophenyl-α-D-glucopyranoside (5 mM) as substrate and incubated at 37 °C for 20 min. The reaction was terminated by the addition of 1.0 mL of 0.1 M sodium carbonate. Absorbance was measured at 405 nm using a UV–Visible

spectrophotometer. Acarbose served as the reference standard, and percentage inhibition was calculated relative to the control. [11]

2.6.3. Determination of IC₅₀ Values: IC₅₀ values for α -amylase and α -glucosidase inhibition were determined from concentration–response curves by plotting percentage inhibition against extract concentration. Linear regression analysis was applied to calculate IC₅₀ values. All determinations were carried out in triplicate, and values were expressed as mean \pm SD.

3. RESULTS:

The quantitative estimation of total phenolics, flavonoids, and tannins in the aqueous and alcoholic extracts of leaves and roots of *Globba marantina* is summarized in Table 1. As shown in Table 1, alcoholic extracts contained markedly higher levels of all three bioactive constituents compared to aqueous extracts, irrespective of the plant part. Among the extracts evaluated, the alcoholic leaf extract exhibited the highest concentrations of phenolics (82.46 ± 2.31 mg GAE/g), flavonoids (64.93 ± 1.84 mg QE/g), and tannins (41.28 ± 1.39 mg TAE/g). This was followed by the alcoholic root extract, which also showed substantial amounts of phenolics (76.19 ± 2.08 mg GAE/g), flavonoids (58.74 ± 1.67 mg QE/g), and tannins (38.92 ± 1.31 mg TAE/g).

In contrast, aqueous extracts of both leaves and roots demonstrated comparatively lower concentrations of these phytochemicals. The aqueous root extract exhibited the lowest levels of phenolics (49.37 ± 1.75 mg GAE/g), flavonoids (34.16 ± 1.25 mg QE/g), and tannins (24.83 ± 1.12 mg TAE/g).

The higher abundance of phenolics, flavonoids, and tannins in alcoholic extracts may be attributed to the improved solubility of these compounds in ethanol. These classes of phytochemicals are widely reported to possess antioxidant, enzyme inhibitory, and glucose-modulating properties. Therefore, the quantitative phytochemical profile presented in Table 5.3 provides a strong phytochemical basis for the enhanced in-vitro antidiabetic activity observed for the alcoholic extracts of *G. marantina*.

Table 1: Quantitative estimation of total phenolic, flavonoid, and tannin contents of aqueous and alcoholic extracts of leaves and roots of *Globba marantina*.

Extract	Total Phenolic Content (mg GAE/g extract)	Total Flavonoid Content (mg QE/g extract)	Total Tannin Content (mg TAE/g extract)
Alcoholic Leaves	82.46 ± 2.31	64.93 ± 1.84	41.28 ± 1.39
Aqueous Leaves	54.82 ± 1.96	38.52 ± 1.42	26.47 ± 1.18
Alcoholic Roots	76.19 ± 2.08	58.74 ± 1.67	38.92 ± 1.31
Aqueous Roots	49.37 ± 1.75	34.16 ± 1.25	24.83 ± 1.12

Values are expressed as mean \pm SD (n = 3). Phenolic content is expressed as gallic acid equivalents (GAE), flavonoid content as quercetin equivalents (QE), and tannin content as tannic acid equivalents (TAE) per gram of extract.

Table 2: In-vitro α -amylase and α -glucosidase inhibitory activities and IC₅₀ values of aqueous and alcoholic extracts of leaves and roots of *Globba marantina*

Extract	Concentration (µg/mL)	Inhibition (%)		IC ₅₀	
		α -Amylase	α -Glucosidase	α -Amylase (µg/mL)	α -Glucosidase (µg/mL)
Alcoholic Roots	100	30.12± 1.21	26.74 ± 1.14	391.58±8.42	444.30 ± 9.24
	250	42.87± 1.56	39.85 ± 1.47		
	500	55.46± 1.89	52.91 ± 1.83		
	1000	68.94± 2.14	65.48 ± 2.02		
	1500	79.36± 2.28	77.63 ± 2.21		
Aqueous Roots	100	24.08± 1.05	21.18 ± 1.03	543.72±10.38	645.32±11.06
	250	36.41± 1.38	33.92 ± 1.31		
	500	48.92± 1.77	46.37 ± 1.69		
	1000	61.27± 1.95	58.86 ± 1.91		
	1500	71.83± 2.06	69.74 ± 2.05		
Alcoholic Leaves	100	27.45± 1.17	24.39 ± 1.09	426.85± 9.16	483.61 ± 9.88
	250	40.62± 1.44	37.74 ± 1.42		
	500	53.88± 1.72	50.86 ± 1.76		
	1000	65.91± 2.09	63.27 ± 2.07		
	1500	76.42± 2.19	74.92 ± 2.18		
Aqueous Leaves	100	21.96± 1.02	19.67 ± 0.98	646.73±11.94	714.64±12.47
	250	34.28± 1.29	32.15 ± 1.26		
	500	46.37± 1.68	44.78 ± 1.64		
	1000	58.74± 1.86	56.94 ± 1.88		
	1500	69.58± 2.01	67.63 ± 1.97		
Standard (Acarbose)	500	82.14± 1.75	83.42 ± 1.68	281.12± 7.80	372.84 ± 8.10

Values are expressed as mean ± SD (n = 3). IC₅₀ values represent the concentration required to inhibit 50% enzyme activity.

4. DISCUSSION:

The present study demonstrates that *Globba marantina* possesses notable in-vitro antidiabetic potential, which appears to be closely associated with its phytochemical composition and solvent-dependent extraction efficiency. Quantitative analysis revealed that alcoholic extracts of both leaves and roots contained significantly higher concentrations of phenolics, flavonoids, and tannins compared to aqueous extracts, indicating that ethanol is a more effective solvent for recovering bioactive polyphenolic constituents from this species.

Among the evaluated extracts, the alcoholic leaf extract exhibited the highest levels of total phenolics, flavonoids, and tannins, followed by the alcoholic root extract. In contrast, aqueous extracts, particularly those obtained from roots, showed comparatively lower phytochemical content. These findings are consistent with previous reports on Zingiberaceae members, in which ethanol-based extraction enhances the recovery of polyphenols due to their moderate polarity and improved solubility in organic solvents.

The enzyme inhibition assays further substantiated the functional relevance of the phytochemical profile. All extracts inhibited α -amylase and α -glucosidase in a concentration-dependent manner, confirming their ability to modulate carbohydrate digestion. Notably, the alcoholic root extract exhibited the strongest inhibitory activity against both enzymes, as reflected by its lower IC₅₀ values compared to other extracts [12].

The alcoholic leaf extract also showed substantial inhibitory potency, whereas aqueous extracts demonstrated relatively weaker effects. Although the inhibitory activity of the extracts was lower than that of the standard drug acarbose, the magnitude of inhibition observed for the alcoholic extracts is pharmacologically meaningful.

The superior enzyme inhibitory activity of alcoholic extracts can be attributed to their enriched polyphenolic content. Phenolic compounds, flavonoids, and tannins are known to inhibit carbohydrate-hydrolyzing enzymes through non-covalent interactions with catalytic and allosteric sites, thereby reducing glucose release from dietary polysaccharides. Tannins, in particular, are reported to form stable complexes with digestive enzymes, leading to reduced enzymatic efficiency [13]. The observed correlation between phytochemical abundance and enzyme inhibition suggests that the antidiabetic effect of *G. marantina* arises from the synergistic action of multiple bioactive constituents rather than a single dominant compound.

From a therapeutic perspective, the moderate but consistent inhibition of α -amylase and α -glucosidase by *G. marantina* extracts may offer advantages over strong synthetic inhibitors, which are often associated with gastrointestinal adverse effects. Plant-based inhibitors that exert partial enzyme suppression are increasingly considered beneficial for long-term glycemic control by minimizing excessive postprandial glucose spikes while maintaining normal digestive function. Overall, the findings of this study support the ethnopharmacological relevance of *Globba marantina* and highlight its potential as a source of natural antidiabetic agents. The strong association between phytochemical richness and enzyme inhibitory activity underscores the importance of solvent selection in maximizing biological efficacy. Further investigations involving compound isolation, molecular interaction studies, and in-vivo validation are warranted to fully elucidate the therapeutic potential and mechanism of action of this plant.

5. CONCLUSION

Globba marantina exhibited notable in-vitro antidiabetic activity, particularly in alcoholic extracts rich in polyphenolic constituents. These extracts effectively inhibited α -amylase and α -glucosidase, suggesting their potential to regulate postprandial glucose levels. Further in-vivo validation is warranted to confirm therapeutic applicability.

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