



α -AMYLASE AND α -GLUCOSIDASE INHIBITORY ACTIVITY OF MAGNOLIA CHAMPACA SEED EXTRACT: AN IN-VITRO APPROACH TO ANTIDIABETIC POTENTIAL

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ABSTRACT

Diabetes mellitus is a metabolic disorder characterized by chronic hyperglycaemia resulting from defects in insulin secretion, insulin action, or both. α -Amylase and α -glucosidase inhibitors are commonly used to achieve better control of postprandial hyperglycaemia in type 2 diabetes mellitus. The present study aimed to screen novel α -amylase and α -glucosidase inhibitors from natural sources, such as *Magnolia champaca* seeds, to reduce the toxicity and side effects associated with currently available synthetic inhibitors. The in vitro antidiabetic activity of the ethanolic extract of *Magnolia champaca* seeds was evaluated at various concentrations. The extract exhibited a significant α -glucosidase inhibitory effect in a concentration-dependent manner ($IC_{50}=102.66\pm4.72$ mg/mL) and α -amylase inhibitory activity ($IC_{50} = 223.52\pm5.36$ mg/mL). These findings suggest that the *Magnolia champaca* seeds extract possesses promising potential for the management of hyperglycaemia in type 2 diabetes mellitus.

KEYWORDS: Antidiabetic activity, Alpha amylase inhibitor, Alpha glucosidase inhibitor, *Magnolia champaca*, *Michelia champaca*, Medicinal plants.

INTRODUCTION

Diabetes mellitus is a metabolic disorder with multiple causes, characterized by chronic high blood sugar (hyperglycaemia) and disturbances in the metabolism of carbohydrates, fats, and proteins. It results from defects in insulin secretion, insulin action, or both.^[1] Diabetes is one of several lifestyle-related disorders and serves as a breeding ground for many life-threatening conditions. It is a major contributor to premature illness, particularly due to cardiovascular disease, blindness, and kidney failure.^[2] Researchers are increasingly focusing on medicinal plants due to their proven effectiveness, wide availability, and generally lower incidence of side effects.^[3]

Michelia Champaca Linn., a member of the *Magnoliaceae* family, is widely recognized as Champa, an evergreen plant prized for its fragrant blooms and attractive foliage, commonly cultivated in Indian gardens and temple grounds.^[4] *Michelia champaca* is native to tropical Asia, specifically found in countries such as Bangladesh, India (Arunachal Pradesh, Assam, Bihar), Myanmar, Thailand, Vietnam, Sumatra, and Malaysia.^[5] *Michelia champaca* has been a traditional remedy for various ailments, including fever, colic, leprosy, coughs, rheumatism, and other disorders. This plant has demonstrated a broad spectrum of pharmacological activities, including anti-microbial, anti-pyretic, anti-inflammatory, anti-oxidant, insecticidal, anti-uretic,

carminative, and anti-diabetic properties, showcasing its potential as a versatile medicinal agent.^[6] *Michelia champaca* has been found to possess a diverse array of phytoconstituents, including alkaloids, tannins, sterols, flavonoids, phenols, and saponins. The presence of these bioactive compounds suggests that the whole plant of *Michelia champaca* has the potential to serve as a valuable source of medicinal compounds with various therapeutic applications.^[7]

Inhibition of α -glucosidase and α -amylase, enzymes involved in carbohydrate digestion, can significantly reduce the postprandial rise in blood glucose following a mixed carbohydrate meal. This makes their inhibition an important strategy for managing postprandial blood glucose levels in type 2 diabetic and borderline patients. In recent years, there has been a growing interest in functional foods and plant-based medicines capable of modulating physiological processes for the prevention and management of diabetes and obesity. Consequently, in vitro inhibition of α -glucosidase and α -amylase has become an attractive area of research.^[8] Therefore, natural alpha amylase and glucosidase inhibitors from the dietary plants can be used as an effective therapy for treating post prandial hyperglycaemia with minimal side effects.^[9]

The traditional use of *Magnolia champaca* seeds for managing diabetes has not yet been scientifically validated. Therefore, the present study aims to evaluate the antidiabetic potential of *Magnolia champaca* seeds through in vitro to provide scientific evidence for their antihyperglycemic activity.

MATERIALS AND METHODOLOGY

Collection of plant material and Authentication

The fresh seeds of *Magnolia champaca* were collected during the month of September 2024 from the local area of Mandya District and Karnataka state and authenticated by Dr. Thejesh Kumar, M P. M.Sc., Ph.D. Co-Ordinator, Department of botany (PG), Bharathi College (PG & RC), Bharathinagara. The fresh seeds were dried under shade and crushed into coarse powder using an electrical grinder. The powdered plant material stored in an air tight container for future use.

Extraction

After being dried in the shade, the fresh *Magnolia champaca* seeds were ground into a coarse powder and extracted. Hot Soxhlet continuous process: Using a Soxhlet apparatus, the dried coarse powder of *Magnolia champaca* seeds was extracted with 70% ethanol at a temperature of 50–60°C. Until the solvent in the thimble turned clear, extraction was continued. Following full extraction, a rotary evaporator was used to concentrate the extract, which was then kept in a refrigerator until it was needed.

IN-VITRO ANTI-DIABETIC ACTIVITY

In vitro α -amylase inhibitory studies^[10]

The α -amylase inhibition assay was performed using the 3,5-dinitrosalicylic acid (DNSA) method. The ethanolic seeds extract of *Magnolia champaca* was dissolved in of 10% DMSO to give concentrations ranging from 20 to 100 μ g/ml. A volume of 50 μ l of α -amylase solution (2 units/ml) was mixed with 50, 100, 150, 200, 250, 300 μ g of the extract and was incubated for 10 min at 30°C. Thereafter 50 μ l of the starch solution 1% in buffer (w/v) ((Na₂HPO₄/NaH₂PO₄ (0.02 M), NaCl (0.006 M) at pH (6.9) was added to each tube and incubated for 3 min. The reaction was terminated by the addition of 50 μ l DNSA reagent (12 g of sodium potassium tartrate tetrahydrate in 8.0 mL of 2 M NaOH and 20 mL of 96 mM of 3,5-dinitrosalicylic acid solution) and was boiled for 10 min in a water bath at 85–90 °C. The mixture was cooled to ambient temperature and was diluted with 5 ml of distilled water, and the absorbance was measured at 540 nm using a UV-Visible spectrophotometer. The blank was prepared by replacing the plant extract with 50 μ l of buffer. A blank reaction was similarly prepared using the plant extract at each concentration in the absence of the enzyme solution. A positive control sample was prepared using Acarbose (50 μ g/ml–300 μ g/ml) and the reaction was performed similarly to the reaction with plant extract as mentioned above and each experiment was performed in triplicate. The α -amylase inhibitory activity was expressed as percent inhibition and was calculated using the equation given below: The % α -amylase inhibition was plotted against the extract concentration and the IC₅₀ values were obtained from the graph. The results are recorded in **Table 1**.

$$\% \alpha \text{ amylase inhibition} = \frac{\text{Abs (Control)} - \text{Abs (Extract)}}{\text{Abs (Control)}} \times 100$$

Alpha Glucosidase Inhibitory Activity^[9,11]

The ethanolic seeds extract of *magnolia champaca* was dissolved in of 10% DMSO to give concentrations ranging from 20 to 100 μ g/ml., The alpha glucosidase was dissolved in 100 mM phosphate buffer pH 6.8. A volume of 10 μ L alpha glucosidase (1 U/mL) was mixed with concentration range from 20, 40, 60, 80, 100, 120 μ L of the extract and was pre incubated at 37°C for 15 min. Then, 20 μ L P-NPG (5 mM) was added as a substrate and further incubated at 37°C for 20 min. Add 3 ml of 50 mM sodium hydroxide was added to the mixture and the absorbance was measured at 410 nm using a UV-Visible spectrophotometer. The blank was prepared by replacing the plant extract with 50 μ l of buffer. A blank reaction was similarly prepared using the plant extract at each concentration in the absence of the enzyme solution. A positive control sample was prepared using Acarbose (20 μ g/ml–120 μ g/ml) and the reaction was performed similarly to the reaction with plant extract as mentioned above and each experiment was performed in triplicate. The α -glucosidase inhibitory activity was expressed as percent inhibition and was calculated using the equation given below: The % α - glucosidase

inhibition was plotted against the extract concentration and the IC₅₀ values were obtained from the graph. The results are recorded in **Table 2**.

$$\% \alpha \text{ glucosidase inhibition} = \frac{\text{Abs (Control)} - \text{Abs (Extract)}}{\text{Abs (Control)}} \times 100$$

RESULTS

Invitro antidiabetic activity

In vitro α -amylase inhibitory activity

The *Magnolia champaca* ethanol extract revealed a significant inhibitory action on α -amylase enzyme. The percentage inhibition at 50-300 $\mu\text{g/ml}$ concentrations of

Magnolia champaca seeds extract showed a concentration dependent increase in percentage inhibition. The percentage inhibition varied from 66.08 ± 0.70 to 8.29 ± 0.70 for highest concentration (300 $\mu\text{g/ml}$) to the lowest concentration (50 $\mu\text{g/ml}$). The concentration required for 50% inhibition (IC₅₀) was found to be 223.52 ± 5.36 $\mu\text{g/ml}$ whereas the α -amylase inhibitory activity of positive control Acarbose produced percentage of 16.43 ± 3.51 for 50 $\mu\text{g/ml}$ and 91.68 ± 3.63 for 300 $\mu\text{g/ml}$. The IC₅₀ value of standard drug Acarbose against α -amylase was found to be 161.01 ± 11.60 $\mu\text{g/ml}$.

Table 1: α -amylase inhibition by *Magnolia champaca* ethanol extract.

Sample	Concentration ($\mu\text{g/ml}$)	% inhibition	IC ₅₀ $\mu\text{g/ml}$
<i>Magnolia champaca</i>	50	8.29 ± 0.70	223.52 ± 5.36
	100	16.71 ± 0.82	
	150	34.09 ± 0.34	
	200	46.21 ± 0.73	
	250	57.93 ± 0.65	
	300	66.08 ± 0.70	
Acarbose (standard)	50	16.43 ± 3.51	161.01 ± 11.60
	100	30.11 ± 4.39	
	150	47.27 ± 4.90	
	200	62.56 ± 5.22	
	250	78.24 ± 3.13	
	300	91.68 ± 3.63	

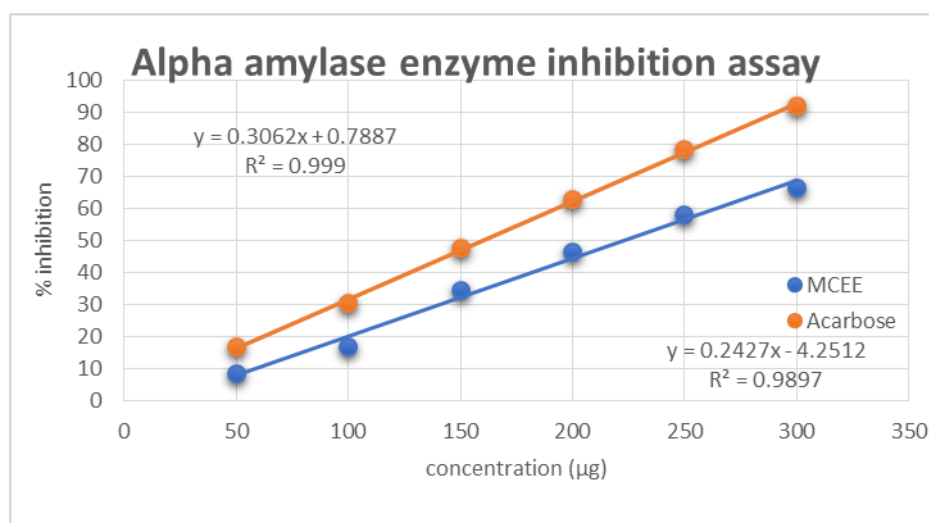


Figure 1: Percentage inhibition by Alpha amylase inhibition assay.

Alpha Glucosidase Inhibitory Activity

The *Magnolia champaca* ethanol extract revealed a significant inhibitory action on α -glucosidase enzyme. The percentage inhibition at 20-120 $\mu\text{g/ml}$ concentrations of *magnolia champaca* extract showed a concentration dependent increase in percentage inhibition. The percentage inhibition varied from 56.95 ± 1.10 to 8.21 ± 1.40 for highest concentration (120 $\mu\text{g/ml}$) to the lowest concentration of (20 $\mu\text{g/ml}$). The concentration required for 50% inhibition (IC₅₀) was found to be 102.66 ± 4.72 $\mu\text{g/ml}$ whereas the α -glucosidase inhibitory activity of positive control

Acarbose produced percentage of 14.37 ± 2.00 for 20 $\mu\text{g/ml}$ and 91.21 ± 3.40 for 120 $\mu\text{g/ml}$. The IC₅₀ value of standard drug Acarbose against α -glucosidase was found to be 69.05 ± 3.48 $\mu\text{g/ml}$.

Table 2: α -glucosidase inhibition by *Magnolia champaca* seeds ethanol extract.

Sample	Concentration ($\mu\text{g/ml}$)	% inhibition	IC ₅₀ $\mu\text{g/ml}$
<i>Magnolia champaca</i>	20	8.21 $\pm$ 1.40	102.66 $\pm$ 4.72
	40	26.53 $\pm$ 1.23	
	60	33.21 $\pm$ 1.08	
	80	41.65 $\pm$ 1.27	
	100	46.15 $\pm$ 0.87	
	120	56.95 $\pm$ 1.10	
Acarbose (standard)	20	14.37 $\pm$ 2.00	69.05 $\pm$ 3.48
	40	26.91 $\pm$ 4.01	
	60	41.47 $\pm$ 4.01	
	80	55.78 $\pm$ 4.83	
	100	72.78 $\pm$ 4.51	
	120	91.21 $\pm$ 3.40	

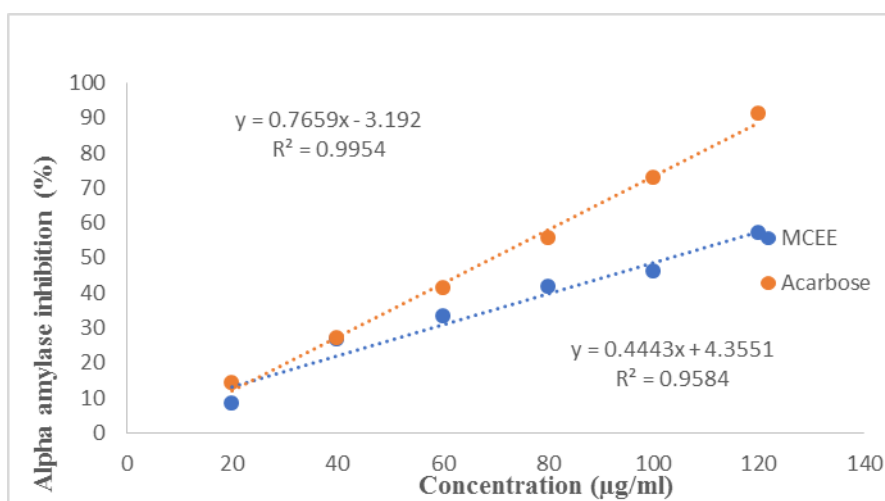


Figure 2: Percentage inhibition by alpha glucosidase assay.

DISCUSSION

The ethanol extract of *Magnolia champaca* seeds displayed significant inhibitory effects on both α -amylase and α -glucosidase enzymes. In the case of α -amylase, Acarbose had an IC₅₀ value of 161.01 $\pm$ 11.60 $\mu\text{g/ml}$, indicating strong inhibitory potency. Conversely, the ethanolic extract of *Magnolia champaca* showed an IC₅₀ value of 223.52 $\pm$ 5.36 $\mu\text{g/ml}$, reflecting its considerable inhibitory potential. For α -glucosidase inhibition, Acarbose recorded an IC₅₀ value of 69.05 $\pm$ 3.48 $\mu\text{g/ml}$, while the extract of *Magnolia champaca* seeds demonstrated an IC₅₀ value of 102.66 $\pm$ 4.72 $\mu\text{g/ml}$. These findings collectively suggest that both possess appreciable inhibitory activity relative to the standard, highlighting their potential therapeutic applications in managing postprandial hyperglycemia.

CONCLUSION

The extract exhibited potent inhibitory effects on carbohydrate-hydrolysing enzymes, with IC₅₀ values of 223.52 $\pm$ 5.36 $\mu\text{g/ml}$, for α -amylase and 102.66 $\pm$ 4.72 $\mu\text{g/ml}$ for α -glucosidase. The lower IC₅₀ value for α -glucosidase indicates stronger inhibition compared to α -amylase, confirming a dose-dependent increase in inhibitory activity and suggesting the extract's potential as a natural antidiabetic agent.

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