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Research Article

HYPOGLYCEMIC POTENTIAL OF *HIBISCUS ROSA-SINENSIS* AND *MANGIFERA INDICA* LEAVES BY *IN VITRO* METHODS

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ABSTRACT

Objective: The objective of the present study was to provide *in vitro* evidence for the potential inhibitory activity of extracts of *Mangifera indica* (MI) and *Hibiscus rosa-sinensis* on α-amylase and α-glucosidase enzymes.

Methods: The plant extracts were prepared with methanol by maceration. Different concentrations (10, 20, 40, 80, and 100 µg/mL) of both the extracts were made using distilled water, and the extracts were subjected to α-amylase and α-glucosidase inhibitory assay. The absorbance was measured at 540 nm and 405 nm using spectrophotometer. Using this method, the percentage of α-amylase and α-glucosidase inhibitory activity and IC_{50} values of each extract were calculated.

Results: The methanolic extracts of MI and *H. rosa-sinensis* exhibited significant α-amylase and α-glucosidase inhibitory activity. The inhibitory effect of α-amylase inhibitory assay was observed up to 89% and 90% at a concentration of 100 µg/mL in MI and *H. rosa-sinensis,* respectively. The inhibitory effect of α-glucosidase inhibitory assay was observed up to 89% and 91% at a concentration of 100 µg/mL in MI and *H. rosa-sinensis,* respectively, and is comparable to the standard acarbose (94% at 100 µg/mL). The IC_{50} values of α-amylase inhibitory assay of MI and *H. rosa-sinensis* are 35.33 and 30.97, respectively. The IC_{50} values of α-glucosidase inhibitory assay of MI and *H. rosa-sinensis* are 31.67 and 25.86, respectively. The IC_{50} value of acarbose is 6.94.

Conclusion: The results obtained support that MI and *H. rosa-sinensis* extracts exhibit considerable α-amylase and α-glucosidase inhibitory activity which can be attributed to the phytochemical constituents present in them. Further, this study supports its usage in ethno medicines for the management of diabetes.

Keywords: *Mangifera indica*, *Hibiscus rosa-sinensis*, α-amylase, α-glycosidase.

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INTRODUCTION

Diabetes mellitus (DM) is a complex and diverse group of disorders that disturb the metabolism of the biomolecules such as carbohydrates, fats, and proteins [1]. Insulin dysfunction results from a lack of pancreatic cells to release insulin (Type 1 DM) or an inadequate insulin response (Type 2 DM) [2]. Regulating plasma glucose levels is vital for delaying or preventing T2D. α-amylase and α-glucosidase are one of the therapeutic approaches for decreasing postprandial hyperglycemia. The ability of a drug or diet to delay the production or absorption of glucose by inhibiting carbohydrate hydrolyzing enzymes is of greater importance today [3]. There are many synthetic drugs available as oral hypoglycemic agents to treat diabetes but continuous use of synthetic drugs causes severe side effects and highly expensive [4].

α-amylase and α-glucosidase are the digestive enzymes that hydrolyze starch and are implicated in postprandial hyperglycemia [5]. An effective means of lowering the levels of postprandial hyperglycemia have been offered by α-amylase and α-glucosidase inhibitors [6].

Retarding the absorption of glucose by inhibiting the carbohydrate hydrolyzing enzymes such as pancreatic amylase and α-glucosidase in the intestine is effectively done by medicinal plants [7].

Mangifera indica (MI), also known as mango, is an important plant in the Ayurvedic and indigenous medical systems for over 4000 years [8]. Different parts of the MI tree have been demonstrated to exert anticancer, anti-inflammatory, antioxidant, antibacterial, antifungal, anthelmintic, gastroprotective, hepatoprotective, immunomodulatory, antiplasmodial, and antihyperlipidemic effects [9].

Hibiscus rosa-sinensis (shoe flower) is a perennial shrub belonging to the family Malvaceae and genus *Hibiscus* [10]. The pharmacological activities of *H. rosa-sinensis* include anti-bacterial activity, anti-fungal activity, anti-oxidant activity, anti-cancer activity, anti-fertility activity, wound healing activity, hair growth promoting activity, neuroprotective action, anti-inflammatory activity, cardioprotective activity, gastroprotective activity, anti-pyretic activity, anti-hyperlipidemic activity, hepatoprotective activity, and immune responses [11].

This study was carried out to evaluate *in vitro* inhibitory effect of extracts of MI and *H. rosa-sinensis* on α-amylase and α-glucosidase enzymes.

METHODS

Materials

α-amylase, α-glucosidase, soluble starch, sodium potassium tartrate tetrahydrate, sodium hydroxide, potassium dihydrogen phosphate, disodium hydrogen phosphate, sodium dihydrogen phosphate, sodium chloride, sodium carbonate, p-nitrophenyl glucopyranoside, and 3,5-dinitrosalicylic acid were purchased from SD Fine.

Plant materials

MI and *H. rosa-sinensis* leaves were collected from Tirupati in November, 2022. The plants were botanically identified and authenticated by Madhav Shetti, botanist, SV University, Tirupati. Specimens were deposited at herbarium, SVCP. The plant materials were washed and dried under shade, coarsely powdered, and stored in airtight containers.

Preparation of plant extracts

Dried powdered (500 g) plant leaves were subjected to maceration with methanol for 7 days. The obtained extracts were filtered and the filtrates

were dried completely. The extracts were evaporated using rotary evaporator (Buchi E-210) under reduced pressure. The percentage yield of MI and *H. rosa-sinensis* was 4.2% and 5.6%, respectively. Plant extracts were prepared at different concentrations and subjected to α-amylase and α-glucosidase inhibitory assay.

Phytochemical screening

Phytochemical composition of the leaves was determined using the standard methods. Phenolic compounds and flavonoids were tested to be present in the extracts.

***In vitro* α-amylase inhibitory assay**

α-amylase inhibitory assay was carried out according to the standard method (DNSA method) with minor modification [12]. 0.2 mL of different concentrations of test extracts were allowed to react with 0.4 mL of α-amylase enzyme and 0.2 mL of 0.2 M phosphate buffer (pH 6.9) and were incubated for 30 min at 250°C. 0.4 mL of 1% starch solution was added and incubated for 10 min. The reaction was stopped by addition of 1 mL 3,5-dinitrosalicylic acid and was heated in boiling water for 10 min. The mixture was cooled and the volume was made up to 10 mL with distilled water. DNS is a coloring reagent and the reducing groups released from starch by α-amylase action were measured. The boiling water was used to stop the α-amylase activity and catalyzing the reaction between DNS and reducing groups of starch. Blank was prepared without enzyme. Acarbose was used as the standard. The absorbance was recorded at 540 nm using spectrophotometer and the percentage inhibition of α-amylase enzyme was calculated using the formula.

$$\text{Inhibition } \% = (Abs_{control} - Abs_{extract} / Abs_{control}) \times 100$$

Fig. 1: *Mangifera indica* leaves

Fig. 2: *Hibiscus rosa-sinensis* leaves

***In vitro* α-glucosidase inhibitory assay**

α-glucosidase inhibitory assay was carried out according to the standard method (pNPG method) with minor modification [13]. 100 µL of α-glucosidase (1.0 U/mL) was pre-incubated with 50 µL of the different concentrations of the extracts for 10 min. Then, 50 µL of 3.0 mM (pNPG) as a substrate dissolved in 20 mM phosphate buffer (pH 6.9) was added to start the reaction. The reaction mixture was incubated at 37°C for 20 min and stopped by adding 2 mL of 0.1 M Na_2CO_3. Blank was prepared without enzyme. Acarbose was used as the standard. The α-glucosidase activity was determined by measuring the yellow-colored paranitrophenol released from pNPG at 405 nm.

Percentage inhibition was calculated as

$$\% \text{ Inhibition} = (Abs_{control} - Abs_{extract} / Abs_{control}) \times 100$$

Statistical analysis

All values were expressed mean±SD. Statistical difference and linear regression analysis were performed using GraphPad prism 5 statistical software.

RESULTS

In the present study, the methanolic (alcoholic) extracts of MI and *H. rosa-sinensis* were evaluated for their inhibitory effect on α-amylase and α-glucosidase enzymes by *in vitro* methods.

The methanolic extract of MI has exhibited increase in % inhibition of α-amylase with increase in concentration. At 10, 20, 40, 80, and 100 µg/mL, the % inhibition was found to be 30, 43, 58, 74, and 89%, respectively, as shown in Table 1. Among all the concentrations,

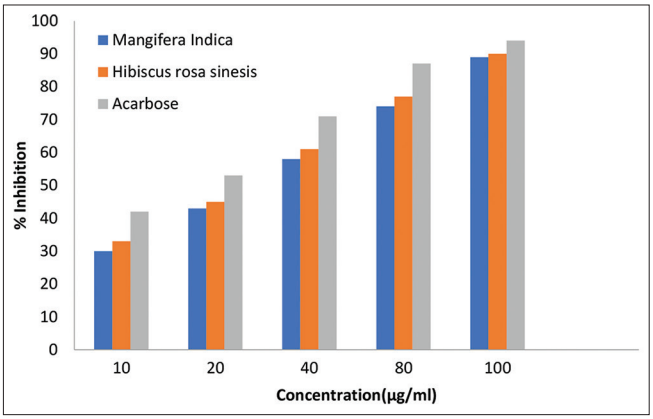

Fig. 3: % inhibition of the extracts at various concentrations in alpha-amylase inhibitory assay

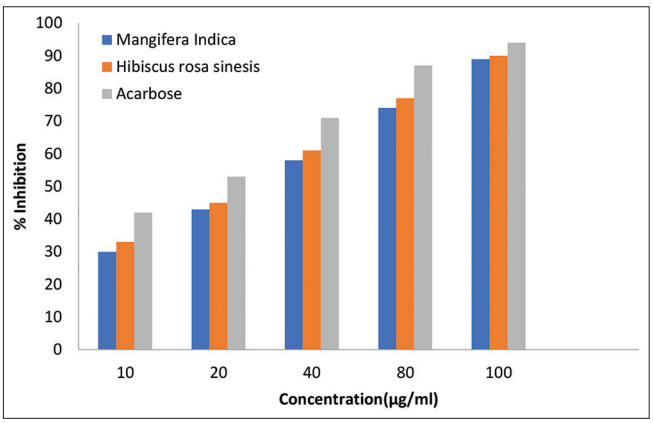

Fig. 4: % inhibition of the extracts at various concentrations in alpha-glucosidase inhibitory assay

maximum inhibition was seen at 100 µg/mL with 89% which was comparable with that of standard acarbose (94%). The methanolic extract of *H. rosa-sinensis* has exhibited increase in % inhibition of α-amylase with increase in concentration. At 10, 20, 40, 80, and 100 µg/mL, the % inhibition was found to be 33, 45, 61, 77, and 90%, respectively, as shown in Table 1. Among all the concentrations, maximum inhibition was seen at 100 µg/mL with 91% which was comparable with that of standard acarbose (95%) shown in Fig. 3.

The methanolic extract of MI has exhibited increase in % inhibition of α-glucosidase with increase in concentration. At 10, 20, 40, 80, and 100 µg/mL, the % inhibition was found to be 32, 46, 60, 76, and 89%, respectively, as shown in Table 2. Among all the concentrations, maximum inhibition was seen at 100 µg/mL with 89% which was comparable with that of standard acarbose (94%). The methanolic extract of *H. rosa-sinensis* has exhibited increase in % inhibition of α-glucosidase with increase in concentration. At 10, 20, 40, 80, and 100 µg/mL, the % inhibition was found to be 34, 49, 65, 82, and 91%, respectively, as shown in Table 2. Among all the concentrations, maximum inhibition was seen at 100 µg/mL with 91% which was comparable with that of standard acarbose (94%) shown in Fig. 4.

Table 1: Alpha-amylase inhibitory effects of *Mangifera indica* and *Hibiscus rosa-sinensis* extracts

Plant extracts	Concentration (µg/mL)	% Inhibition	IC_{50}
Mangifera indica	10	30	35.33
	20	43	
	40	58	
	80	74	
	100	89	
Hibiscus rosa-sinensis	10	33	30.97
	20	45	
	40	61	
	80	77	
	100	90	

Table 2: Alpha-glucosidase inhibitory effects of *Mangifera indica* and *Hibiscus rosa-sinensis* extracts

Plant extracts	Concentration (µg/mL)	% Inhibition	IC_{50}
Mangifera indica	10	32	31.67
	20	46	
	40	60	
	80	76	
	100	89	
Hibiscus rosa-sinensis	10	34	25.86
	20	49	
	40	65	
	80	82	
	100	91	

Table 3: Alpha-amylase inhibitory effects and alpha-glucosidase inhibitory effects acarbose

Assay	Concentration (µg/mL)	% Inhibition	IC_{50}
Alpha-amylase	10	42	15.04
	20	53	
	40	71	
	80	87	
	100	94	
Alpha-glucosidase	10	46	5.91
	20	59	
	40	74	
	80	91	
	100	95	

The IC_{50} values of α-amylase inhibitory assay of MI and *H. rosa-sinensis* are 35.33 and 30.97, respectively. The IC_{50} values of α-glucosidase inhibitory assay of MI and *H. rosa-sinensis* are 31.67 and 25.86, respectively. The IC_{50} value of acarbose is 15.04 and 5.91, respectively, for α-amylase inhibitory assay and α-glucosidase inhibitory assay shown in Table 3.

The results were depicted in the bar graphs as shown in Figs. 3 and 4.

DISCUSSION

Alpha amylase is responsible for hydrolyzing the starch, which breaks down into glucose before absorption. Alpha-amylase is the most important digestive enzyme that catalyzes the hydrolysis of alpha-1, 4 glycosidic linkages of carbohydrates. In a healthy person, excess levels of sugar will be converted to energy sources. However, in some cases, high levels of blood glucose due to excess activity of alpha-amylase result in hyperglycemia.

At present, the mainstay of treatment involves insulin secretagogues and sensitizers, however, the use of carbohydrate-digesting enzyme inhibitors plays a vital role in controlling hyperglycemia by reducing the intestinal absorption of glucose. Acarbose is one of the leading inhibitors of carbohydrate metabolic enzymes in the gastrointestinal tract, but it is linked with side effects such as diarrhea and other intestinal disturbances such as bloating, flatulence, cramping, and abdominal pain. Postprandial hyperglycemia is primarily attributed to two carbohydrate hydrolyzing enzymes, namely α-amylase and α-glucosidase. α-amylase begins the process of carbohydrate digestion by hydrolysis of 1, 4-glycosidic linkages of polysaccharides (starch, glycogen) to disaccharides and α-glucosidase catalyzes the disaccharides to monosaccharides, which leads to postprandial hyperglycemia. Therefore, α-amylase and α-glucosidase inhibitors are useful in the control of hyperglycemia as they delay carbohydrate digestion, which consequently reduces the postprandial plasma glucose level. Herbal drugs have been widely used globally for diabetic treatment over thousands of years due to their traditional acceptability and less side effects.

Due to the presence of phytochemical constituents such as phenolic compounds and flavonoids, MI and *H. rosa-sinensis* leaf extracts have inhibited both the enzymes and shown anti diabetic activity *in vitro*.

CONCLUSION

The selected plants – MI and *H. rosa-sinensis* leaf extracts – have exhibited *in vitro* antidiabetic activity. *H. rosa-sinensis* extracts are more potent when compared to MI extracts. The *in vitro* antidiabetic activity may be due to the presence of chemical constituents like polyphenols. However, the principle compounds responsible for the inhibitory action of α-amylase and α-glucosidase need to be further identified and characterized.

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CONFLICTS OF INTEREST

There are no conflicts of interest by the authors.

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