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RESEARCH ARTICLE

Inhibitory effect of *Hibiscus rosa-sinensis* L. flower extract on α-glucosidase and α-amylase activities

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Abstract

Hibiscus rosa-sinensis flowers had traditionally been used in folk medicine to manage diabetes; however, the mechanisms underlying their glucose-lowering effects remained unclear. The aim of this study was to investigate phytochemical properties and *in vitro* inhibitory activity of *Hibiscus rosa-sinensis* flower extracts on starch-hydrolyzing enzymes. Ethanol (70 %) and aqueous extracts (EE and AE respectively) were prepared using an ultrasound-assisted extraction method. The phytochemical properties of the extracts were determined by colorimetric reactions. The antidiabetic activity of these extracts was examined via α-glucosidase and α-amylase inhibitory assays. The extracts were found to contain bioactive phytochemicals, including flavonoids, mucilage, tannins and reducing compounds. Moreover, the inhibitory effect of aqueous and ethanol 70 % extracts on α-glucosidase activity was observed at IC_{50} values of 1.40 ± 0.02 mg/mL and 0.92 ± 0.02 mg/mL, respectively. On the other hand, α-amylase activity was also inhibited by aqueous extract and ethanol 70 % extract at IC_{50} values of 1.39 ± 0.09 mg/mL and 0.95 ± 0.06 mg/mL, respectively. These findings suggested that the inhibitory activity of *H. rosa-sinensis* flower extracts on α-glucosidase and α-amylase might have contributed to the reduction of blood glucose levels, indicating potential for diabetes management.

Keywords: *Hibiscus rosa-sinensis*; α-glucosidase; α-amylase; diabetes

Introduction

Diabetes mellitus was a complex and chronic metabolic disorder that impairs the body's ability to regulate blood glucose levels, primarily due to defects in insulin secretion, insulin action, or both. It was broadly classified into type 1 diabetes (T1D), which was autoimmune-mediated and results in absolute insulin deficiency and type 2 diabetes (T2D), which accounts for over 90 % of all cases and is primarily associated with insulin resistance and β-cell dysfunction (1, 2). According to the International Diabetes Federation (IDF), approximately 537 million adults worldwide were living with diabetes in 2021 and this number was projected to rise to 643 million by 2030 and 783 million by 2045, highlighting a significant global public health challenge. The persistent hyperglycemia in diabetes could lead to severe complications, including cardiovascular diseases, neuropathy, nephropathy, retinopathy and impaired wound healing, which collectively contributed to increased morbidity and mortality (3). Management strategies typically included lifestyle modifications such as dietary regulation and physical activity, alongside pharmacological interventions including insulin therapy and various classes of oral hypoglycemic agents (OHA) like metformin, sulfonylureas, DPP-4 inhibitors and SGLT2 inhibitors (4). Despite their effectiveness, conventional antidiabetic drugs often presented limitations, such as adverse effects (e.g., gastrointestinal discomfort, hypoglycemia, weight gain, skin reactions), long-term toxicity, high treatment costs and reduced patient compliance (5). These concerns fueled a growing interest in exploring natural, plant-based therapies that might have offered safer, more accessible and cost-effective alternatives. Medicinal plants rich in bioactive phytochemicals-such as flavonoids, alkaloids, tannins and polyphenols-have demonstrated promising antidiabetic properties through mechanisms including inhibition of carbohydrate-hydrolyzing enzymes (α-amylase, α-glucosidase), stimulation of insulin secretion, enhancement of insulin sensitivity and protection against oxidative stress-induced β-cell damage (6, 7). Moreover, their antioxidant, anti-inflammatory and cytoprotective effects provided added therapeutic potential for mitigating diabetes-related complications (8). As a result, the investigation of herbal medicines as complementary or alternative strategies in diabetes treatment gained substantial attention in recent decades. Such studies not only contributed to the development

of novel therapeutic agents but also supported the integration of traditional knowledge with modern medical practices.

Hibiscus rosa-sinensis, commonly known as the Chinese hibiscus or shoe flower, was a strikingly beautiful and versatile plant renowned for its vibrant and aesthetically appealing flowers (9). Beyond its ornamental value, this plant had a rich history of traditional use in various cultures for its potential health benefits (9). It had been used as a natural remedy for some diseases and painful symptoms, as well as in herbal cosmetics. A wide range of compounds from *H. rosa sinensis* had been reported, such as tannins, anthraquinones, quinines, phenols, flavanoides, alkaloids, terpenoids, saponins, cardiac glycosides, protein, free amino acids, carbohydrates, reducing sugars, mucilage, essential oils and steroids (10). Numerous scientific researches had highlighted the positive effects of *H. rosa-sinensis* on human health, making it a subject of increasing interest in the field of herbal medicine and natural remedies (9). I particular the flower extract of *H. rosa-sinensis* had been shown to lower blood glucose levels, enhance insulin sensitivity and alleviate some of the complications associated with diabetes has been determined in an in vivo model (9–13). However, an *in vitro* model for the determination of the mechanism of action of flower extract on antidiabetic activity was still needed for further investigation. Therefore, this study was conducted to investigate the inhibitory effect of *H. rosa-sinensis* flower extract on α-glucosidase and α-amylase activities.

Materials and Methods

Materials

Fresh flowers from *H. rosa-sinensis* were collected in Ho Chi Minh City, Vietnam. α-glucosidase, α-amylase, acarbose and p-NPG (4-Nitrophenyl β-D-galactopyranoside) were purchased from Sigma-Aldrich (USA). Ethanol and methanol were obtained from Xilong (China).

Preparation of the extract

Flowers were washed and dried at 50 °C until moisture content was reduced to below 12 %. The dried flower were then ground into fine powder. A total of 50 g of the powdered sample was extracted using 1000 mL of either 70 % EtOH or distilled water at 55 °C for 1 hr via ultrasound-assisted extraction (UAE), consist of 15 min of sonication followed by a 5 -min pause. The extraction process was repeated 3 times and all extract solutions were combined ad filtered. The solvent was subsequently evaporated under reduced pressure using a rotary vacuum evaporator. The final extracts were stored at 4 ° C for future use.

Phytochemical screening of the flower extracts from H. rosa-sinensis

The phytochemical constituents of the aqueous and 70 % ethanol flower extracts of *H. rosa-sinensis* were identified using the improved Ciulei's method (14).

Determination of α-glucosidase inhibitor assay

The α-glucosidase inhibition assay was assessed using a modified standard protocol (15). A mixture containing 100 µL α -glucosidase (0.2 U/mL), 50 µL of extracts at various concentrations (0.5, 0.75, 1.0, 1.25, 1.5, 1.75 mg/mL) and 50 µL of sodium phosphate buffer was incubated at 37 °C for 20 min. Subsequently, 50 µL of p-NPG 0.5 mM was added to this mixture and further incubated at 37 °C for 20 min. The reaction was stopped by adding 100 µL of 0.2 N Na_2CO_3. The absorbance of the resulting p-nitrophenol was measured at 405 nm. Acarbose was used as a positive control. Each assay was performed triplicate. The percentage inhibition of α-glucosidase was calculated using the following formula:

$$I\% = 100\% - \left(\frac{A_0 - A_1}{A_0} \right) X\ 100\%$$

A_0: Absorbance of the control (no extracts)

A_1: Absorbance of test sample

Determination of α-amylase inhibitor assay

The α-amylase inhibition assay was measured according to a modified protocols (16). A mixture of 30 µL of α-amylase (0.2 U/ mL), 50 µL of extracts at various concentrations (0.5, 0.75, 1.0, 1.25, 1.5, 1.75 mg/mL) and 80 µL of sodium phosphate buffer was incubated at 37 °C for 30 min. Then, 130 µL of 1 mg/mL starch solution was added and incubated for additional 10 min at 37 °C. The reaction was terminated by adding 50 µL of 0.1 N HCl, followed by 50 µL of iodine solution to determine the amount of remaining starch. Absorbance was measured at 660 nm. Acarbose was used as the positive control. Each experiment was conducted in triplicate. The percentage inhibition of α-amylase was calculated using the formula:

$$I\% = 100\% - \left(\frac{A_0 - A_1}{A_0} \right) X\ 100\%$$

A_0: Abs of the control (no extracts)

A_1: Abs of the sample

Statistical analysis

All data were expressed as mean ± standard deviation (SD). Statistical analysis was performed using one -way ANOVA in SPSS. Tukey's multiple range test was applied to assess difference among treatment groups. Differences were considered statistically significant at $p < 0.05$.

Results and discussion

Qualitative phytochemical screening of H. rosa-sinensis flower extracts

The phytochemical screening revealed that both 70 % ethanol extract (EE) and aqueous extract (AE) of *H. rosa-sinensis* flowers contained alkaloids, flavonoids, polyphenols, mucilage and reducing sugars (Table 1). Both extracts were found to be rich in flavonoids and polyphenols. AE contained higher amount of mucilage than EE; meanwhile, EE was found to contain anthraquinones. Similar phytochemical components, including reducing sugars, polyphenols, tannins, flavonoids, mucilage, anthraquinones and alkaloids had been reported (17–19). However, the components such as steroids, saponin and coumarin were also reported in those studies. In addition, flavonoid were not detected in EE (18). Variations in soil

Table 1. Phytochemical composition of *H. rosa-sinensis* flower extracts

No.	Reagents/Tests	Aqueous extract (AE)	Ethanol 70 % extract (EE)
1		**Alkaloid**	
	Mayer's reagent	+	+
	Dragendoff's reagent	+	+
	Bouchardart's reagent	+	+
	Bertrand's reagent	+	+
	Hager's reagent	-	+
2		**Flavonoid**	
	Con HCl + Mg ribbon	+++	+++
	Anthocyanidin's test	+++	+++
	Proanthocyanidin's test	+++	+++
3		**Polyphenols**	
	Ferric chloride solution	+++	+++
	Gelatine – NaCl 1 %	++	+
4		**Gums/Mucilage**	
	Alcoholic precipitation	+	+++
5		**Carbohydrate**	
	Fehling solution	++	++
6		**Saponin**	
	Foam test	-	-
7		**Anthraquinones**	
	KOH 10 %	+	-

(-) absence of phytochemical activities; (±) uncertain; numbers of (+) represent the positivity level of the test; EE; ethanol 70 % extract; AE: aqueous extract

conditions, geographic origin, harvest timing and extraction parameters might have contributed to differences in the phytochemical profiles. Additionally, the sensitivity of detection methods could have influenced qualitative outcomes.

Determination of α-glucosidase inhibitor activity of *H. rosa-sinensis* flower extracts

α-Glucosidase, an enzyme primarily located in the small intestine, played a crucial role in carbohydrate digestion by hydrolyzing disaccharides and oligosaccharides into absorbable monosaccharides such as glucose (20). Inhibiting α-glucosidase activity was considered important in managing conditions like diabetes, where controlling blood glucose levels was a key aspect of treatment. The inhibitory activity of extracts from *H. rosa-sinensis* and acarbose on α-glucosidase were presented in Table 2, Table 3 and Fig.1. The result showed that the extracts and acarbose inhibited α-glucosidase in a dose-dependent manner. The IC_{50} values for AE, EE and acarbose were determined to be 1.40, 0.92 and 0.23 mg/mL, respectively. These finding indicated that the inhibitory effect of EE on α-glucosidase activity was significantly stronger than that of AE. However, the inhibitory activities of both extracts were lower than that of a commercial drug acarbose.

Determination of α-amylase inhibitor activity of *H. rosa-sinensis* flower extracts

Besides α-glucosidase, α-amylase - produced by the salivary glands and pancreas-played a critical role in the initial digestion of complex carbohydrates such as starch and glycogen into smaller oligosaccharides and maltose (21). Likewise, the inhibition of this enzyme can led to a slower and more controlled release of glucose into the bloodstream after meals, which was particularly beneficial for individuals with diabetes. In this study, the inhibitory activity of *H. rosa-sinensis* flower extracts and acarbose against α-amylase enzyme was investigated in an *in vitro* model. Similarly, both extracts and acarbose exhibited inhibitory activity on α-amylase in a dose-dependent manner (Table 4 and 5 and Fig. 2). The IC_{50} values for AE, EE and acarbose

Table 2. Percentage inhibition of α-glucosidase activity by *H. rosa sinensis* extracts

Concentration of extract (mg/mL)	% Inhibition	
	Aqueous extract (AE)	EtOH 70 % extract (EE)
0.5	$20.23^{f} \pm 1.21$	$27.45^{f*} \pm 2.44$
0.75	$29.90^{e} \pm 1.96$	$43.55^{e*} \pm 1.31$
1	$37.87^{d} \pm 1.19$	$51.32^{d*} \pm 0.41$
1.25	$43.80^{c} \pm 0.26$	$65.88^{c*} \pm 1.94$
1.5	$52.17^{b} \pm 0.73$	$77.96^{b*} \pm 2.74$
1.75	$62.15^{a} \pm 0.61$	$92.76^{a*} \pm 0.49$
Regression equation	$y = 32.27x + 4.72$, $R^2 = 0.99$	$y = 50.78x + 2.70$, $R^2 = 0.99$
IC_{50} value (mg/mL)	1.40 ± 0.01	0.92 ± 0.02

Means in columns and rows with different letters (a-f) are significantly different at the $p \leq 0.05$.

Table 3. Percentage inhibition of α-glucosidase activity by acarbose

Concentration (mg/mL)	0.15	0.2	0.25	0.3	0.35	0.4
Percentage inhibition (%)	$36.17^{f} \pm 1.24$	$46.04^{e} \pm 0.80$	$53.00^{d} \pm 1.41$	$61.33^{c} \pm 0.22$	$70.27^{b} \pm 1.24$	$77.57^{a} \pm 0.24$
Regression equation	$y = 164.59x + 12.13$, $R^2 = 0.99$					
IC_{50} value (mg/mL)	0.23 ± 0.00					

Means in columns with different letters (a-f) are significantly different at the $p \leq 0.05$.

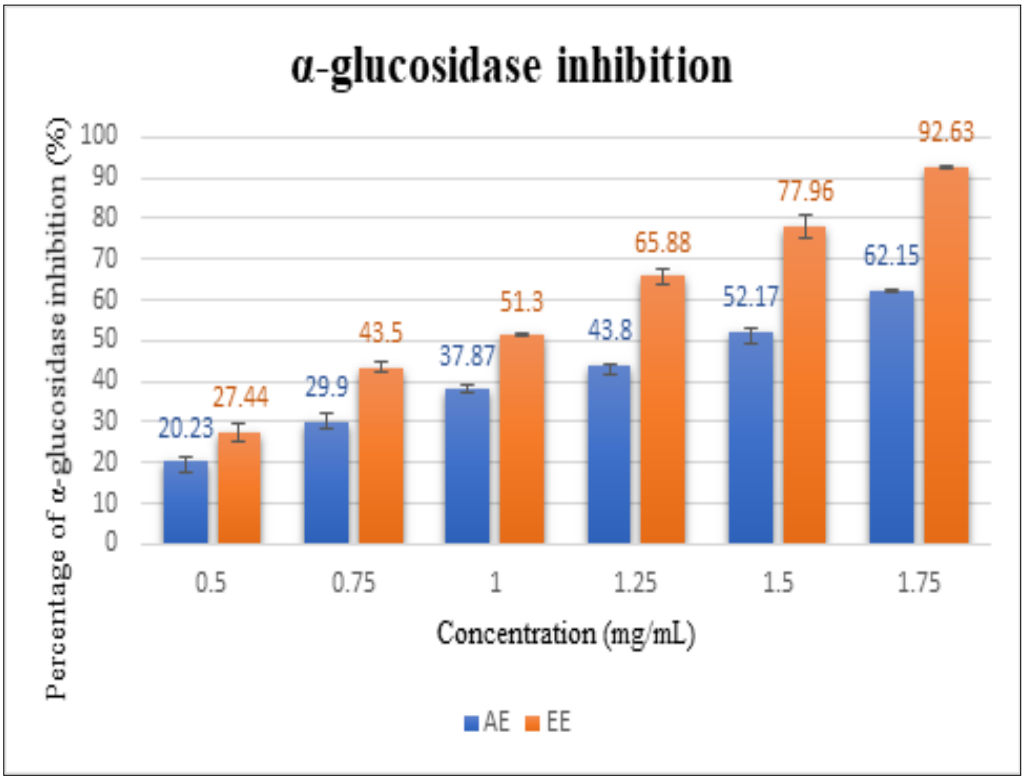

Fig. 1. Inhibitory effect of Aqueous extract (AE) and Ethanol extract (EE) on α-glucosidase activity.

Table 4. Percentage inhibition of α-amylase activity by *H. rosa-sinensis*

Concentration (mg/mL)	Percentage of α-amylase inhibition (%)	
	Aqueous extract (AE)	**Ethanol 70 % extract (EE)**
0.5	$33.32^{f} \pm 0.37$	$39.60^{f*} \pm 1.48$
0.75	$37.26^{e} \pm 2.01$	$43.18^{e*} \pm 0.70$
1	$40.24^{d} \pm 0.01$	$52.57^{d*} \pm 2.43$
1.25	$45.21^{c} \pm 2.15$	$58.71^{c*} \pm 1.91$
1.5	$53.07^{b} \pm 2.10$	$62.20^{b*} \pm 2.00$
1.75	$57.84^{a} \pm 1.56$	$68.30^{a*} \pm 2.35$
Regression equation	$y = 20.00x + 21.99$, $R^2 = 0.98$	$y = 23.62x + 27.52$, $R^2 = 0.98$
IC_{50} value (mg/mL)	1.39 ± 0.09	0.95 ± 0.06

Means in columns with different letters *(a-d) and mean in row with the asterisk* are significant different at the $p \leq 0.05$.

Table 5. Percentage inhibition of α-amylase activity by Acarbose

Concentration (mg/mL)	0.15	0.2	0.25	0.3	0.35	0.4
Percentage of α - amylase inhibition (%)	$46.67^{e} \pm 2.12$	$56.69^{d} \pm 1.94$	$61.26^{c} \pm 1.27$	$67.30^{b} \pm 1.01$	$71.32^{a} \pm 1.47$	$74.69^{a} \pm 2.09$
Regression equation	$y = 108.61x + 33.12$, $R^2 = 0.97$					
IC_{50} value (mg/mL)	0.16 ± 0.02					

Means in columns with different letters (a-d) are significant different at the $p \leq 0.05$.

were 1.39, 0.95 and 0.16 mg/mL, respectively. EE demonstrated stronger inhibitory activity than AE, although both were again less potent compared to acarbose.

The results clearly demonstrated that the ethanol extract (EE) exhibited stronger inhibitory activity than the aqueous extract (AE) against both α-glucosidase and α-amylase. Similar findings had been reported in which it was observed that ethanol extracts of *Hibiscus sabdariffa* showed higher inhibitory effects than aqueous extracts on these enzymes (22). This result suggested that a mixture of highly polar compounds was less effective in inhibiting α-glucosidase and α-amylase compared to compounds with lower polarity, such as those extracted by 70 % ethanol. High total polyphenol and anthocyanin content in *H. rosa-sinensis* flowers were previously reported at levels of 735 ± 46 mg/ 100 g extract and 284 ± 17 mg/ 100 g extract, respectively (23). Key bioactive constituents such as cyanidin 3-sophoroside, rutin, quercetin, kaempferol and myricetin had also been identified (24, 25). The presence of various polyphenols, flavonoids and anthocyanins in anthocyanin-rich extract *H. rosa-sinensis* flowers was confirmed (26). These compounds had been demonstrated as potential inhibitors of α-glucosidase and α-amylase (27–29). As a result, the inhibitory activity of *H. rosa-sinensis* on α-glucosidase and α-amylase might have been attributed to the presence of these bioactive compounds in the flower extract. According to Sachdewa and Khemani, an ethanol flower extract of *H. rosa sinensis* was shown reduce blood glucose after 21 days of oral administration. The hypoglycemic activity of this extract was found to be comparable to that of glibenclamide, but its mechanism did not involve the release of insulin (30). Therefore, the antidiabetic activity of the ethanol

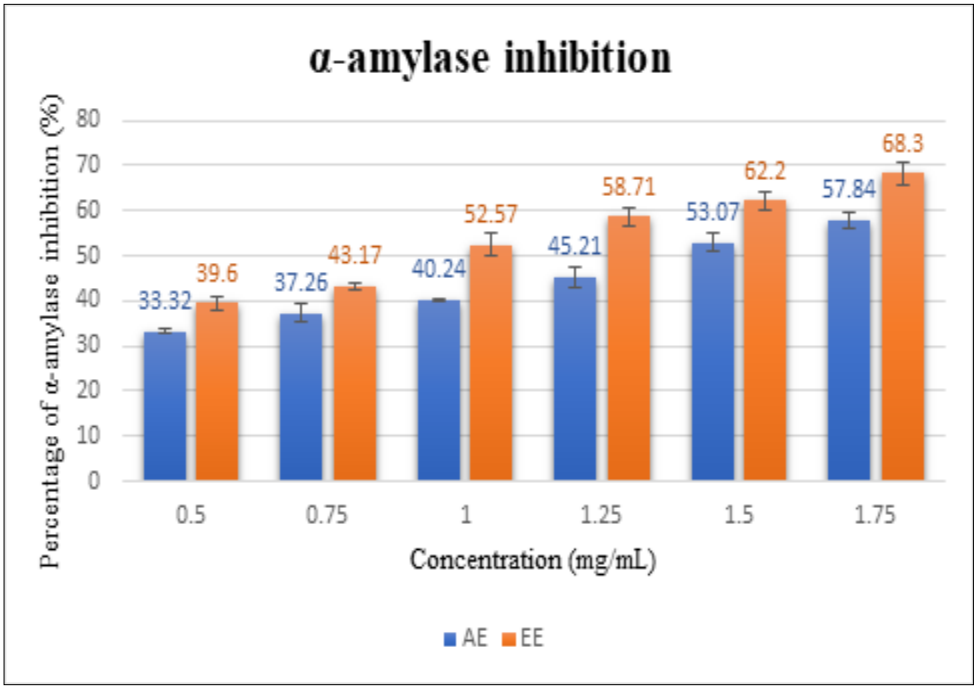

Fig. 2. Inhibitory effect of Aqueous extract (AE) and Ethanol extract (EE) on α-amylase activity.

flower extract might have primarily resulted from the inhibition of carbohydrate-hydrolyzing enzymes.

Conclusion

This study demonstrated that *Hibiscus rosa-sinensis* flower extracts possessed significant *in vitro* inhibitory activity against the carbohydrate-hydrolyzing enzymes α-glucosidase and α-amylase, which were critical in postprandial glucose regulation. Among the tested extracts, the 70 % ethanol extract exhibited stronger inhibitory effects compared to the aqueous extract, with lower IC_{50} values for both enzymes. These inhibitory activities were likely attributed to the presence of polyphenols, flavonoids and anthocyanins identified in the extracts. Based on these findings, *H. rosa-sinensis* flower extract, particularly the ethanol-based extract, appeared to be a promising natural therapeutic agent for managing type 2 diabetes by modulating carbohydrate metabolism. Further in vivo studies and compound isolation were warranted to validate these effects and clarify the precise mechanisms of action.

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Authors' contributions

Surgical and medical practices were performed by VTNM. The study concept was developed by VTNM and NTYL, while the study design was completed by NTN. Data collection and processing were carried out by NTYL and LTT. Data analysis and interpretation were performed by VTS. LTT conducted the literature review. The manuscript was written by VTNM.

Compliance with ethical standards

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

Ethical issues: None

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