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Saliva amylase inhibitory property of certain herbs and spices in Lampang, Thailand

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ABSTRACT

Salivary α-amylase inhibitors (SaAIs) derived from spices and herbs have shown promise for the effective management of type 2 diabetes. This study aimed to identify potential SaAIs from aqueous extracts of 15 commonly used plants. Among these, *Albizia lebbeck* (L.) Benth. exhibited the highest inhibitory activity, followed by *Mimosa pudica* L., *Ipomoea aquatica* Forssk., and *Gymnema inodorum* (Lour.) Decne. Phytochemical analysis indicated that *A. lebbeck* demonstrated the strongest amylase inhibition, which was attributed to its high content of phenolics, flavonoids, tannins, and saponins. *M. pudica* also showed a significant saponin concentration. *I. aquatica* displayed notable amylase inhibitory activity linked to its phenolic, flavonoid, and protein content, although it also contained a high amount of reducing sugars. *G. inodorum* exhibited inhibition through a combination of phenolics, flavonoids, saponins, and proteins. The findings provide compelling evidence that dietary intake of *A. lebbeck*, *I. aquatica*, and *G. inodorum* may positively influence glycemic control. These results suggest that all four plants are promising candidates for further *in vivo* studies and potential drug development in mammalian models and humans.

Keywords: Plant extract, amylase inhibitor, tannin content, phenolic content

INTRODUCTION

Type 2 diabetes mellitus (T2DM) represents a significant and escalating public health concern in Thailand, characterized by increasing prevalence, a substantial proportion of undiagnosed cases, and challenges in achieving optimal disease management. Over the past decade, the prevalence of T2DM among Thai adults has risen markedly, from 7.5% in 2009 to 11.6% in 2021. Notably, among individuals aged over 65 years, the prevalence increased from 10.1% in 2014 to 19.6% in 2020. By 2019, approximately 4.8 million Thai adults were living with T2DM, with projections estimating this number will reach 5.3 million by 2039 (Sodeno *et al*., 2022; Pitchalard *et al*., 2022).

Effective glycemic control is essential for individuals with T2DM to prevent complications and maintain metabolic health. Salivary amylase, also known as α-amylase or ptyalin, plays a pivotal role in the initial digestion of dietary starches by hydrolyzing the α-1,4-glycosidic bonds, converting starch into smaller sugars such as maltose. This enzymatic activity can influence the glycemic index of foods, thereby affecting postprandial blood glucose levels. Emerging research indicates that higher salivary amylase activity may contribute to more rapid and pronounced increases in blood glucose following the consumption of starch-rich foods.

Given the critical role of salivary α-amylase in carbohydrate metabolism, inhibitors of this enzyme have attracted attention for their potential therapeutic applications in managing T2DM. Salivary α-amylase inhibitors (SaAIs), particularly those derived from natural sources such as spices and herbs, have demonstrated promising antidiabetic properties. The present study aims to investigate and identify potent SaAIs from aqueous extracts of 15 commonly used medicinal plants, as detailed in Table 1.

Table 1. Fifteen commonly used medicinal plants selected for the salivary α-amylase inhibitory activity study.

Scientific name	Thai name	Family	Part	Used as	Reference
Acmella oleracea (L.)	Puk Pet	Asteraceae	leave	anti-inflammatory, antioxidative, antifungal, analgesic, and bacteriostatic effects	Kowalczyk *et al.* (2024)
Albizia lebbeck (L.) Benth.	Puk Tud	Leguminosae	leave	Antioxidative, anti-inflammatory, and neuroprotective effects	Phoraksa *et al*. (2023)
Azadirachta indica A. Juss	Sa Dao	Meliaceae	bark	Antioxidative, and antimicrobial activity	Baby *et al*. (2022)
Basella alba L.	Puk Pung	Basellaceae	leave	Anti-Melanogenic, Antioxidative, anti-inflammatory effects	Linsaenkart *et al.* (2024)
Casuarina junghuhniana Miq.	Son pradipat	Casuarinaceae	bark	not well-documented	-
Coccinia grandis (L.) Voigt	Tum Lueng	Cucurbitaceae	leave	α-glucosidase inhibitory	Bunyakitcharoen *et al*. (2024)
Dolichandrone serrulata (Wall. ex DC.) Seem.	Kae Na	Bignoniaceae	bark	Anti-pyretic, anti-inflammatory and anti-mutagenic effects	Chaimontri *et al.* (2021)
Gymnema inodorum (Lour.) Decne	Chiang Da	Asclepiadaceae	leave	hypoglycemic effect	Norkum *et al*. (2023)
Ipomoea aquatica Forssk.	Puk Bung	Convolvulaceae	leave	Antioxidative effect	Joshi *et al*. (2021)
Mimosa pudica L.	Mai Ya Rap	Fabaceae	leave	Antioxidant, Antimicrobial and Antacid effects	Gandhi *et al*. (2023)
Piper sarmentosum Roxb.	Cha Plue	Piperaceae	leave	Anthelmintic, antifungal, antibacterial and cytotoxic effects	Ware *et al*. (2023)
Samanea Saman (jacq.) Merr.	Chamchuri	Fabaceae	leave	Antidiabetic effect	Babin-Reejo *et al*. (2014)
Tamarindus indica L.	Ma Kham	Fabaceae	bark	Antibacterial and Hypoglycemic effect	Fagbemi,*et al*. (2022), Mohd Adnan *et al*. (2025)
Tectona grandis L. f.	Sak	Verbenaceae	bark	Antioxidant, antipyretic, analgesic, hypoglycemic, wound healing, cytotoxic effects	Asdaq *et al.* (2022)
Zanthoxylum limonella (Dennst.)	Ma Khwaen	Rutaceae	seed	blood glucose reduction	Pattanawongsa *et al*. (2021)

This study was specifically conducted to investigate the anti-amylase inhibitory activity and to assess the total phenolic, flavonoid, tannin, saponin, terpenoid, alkaloid, and protein contents, along with the reducing sugar content, of the aqueous extracts.

MATERIALS AND METHODS

Plant materials

The spices and vegetables were collected from a local market close to Lampang Kanlayanee School in Thailand. An herbalist from Rajamangala University of Technology Lanna (RMUTL) verified the bark of the putative plants that were also harvested at this school, which is located at 18.28535942258579, 99.49865971349381, during October and November of 2024.

Sample preparation

A 100 g portion of the fresh sample was first rinsed with tap water, followed by distilled water upon arrival. It was then dried in a hot air oven at 55 °C until a constant weight was achieved. The dried material was ground using an electric blender. Fine powder was obtained by passing it through a 20-mesh sieves, and the resulting powder was stored at -20 °C

for subsequent analysis. To prepare the aqueous extract of each plant, 100 mg of dried plant powder was boiled with 5 mL of distilled water at 85 °C for 30 minutes, following the method described by Sassa-deepaeng *et al*. (2023). After cooling to room temperature, the mixture was filtered using a 0.22-micron nylon syringe filter (Merck KGaA, Darmstadt, Germany). The filtrate was then used for further experiments.

Anti-amylase Inhibitory Activity Assay

The carbon material was prepared without heating (Whitener, 2016). GBC GSBC FBC and FSBC were prepared by slowly adding 50 mL of concentrated sulfuric acid to each of the large beakers, which contained 50 g of glucose powder, saturated glucose solution (50 g of glucose), 50 g of fructose powder and saturated fructose solution (50 g of fructose), respectively. The individual carbon samples were separated, washed with deionized water, and dried in a hot-air oven at 95 °C for 24 h. Similarly, SJBC was prepared by adding concentrated sulfuric acid to sugarcane juice (12% of reducing sugar) in a 2 L beaker. The carbon from this process was separated by filter paper. It was washed and dried at 95 °C for 24 h in a hot air oven. All of the carbon samples were stored in a desiccator.

Anti-amylase Inhibitory Activity Assay

The inhibitory activity of salivary α-amylase was evaluated following the procedure described by Xiao *et al*. (2006). Written informed consent was obtained from Miss Apisara Moolphueng who participated as researcher in this experiment. Forty microliters of salivary α-amylase solution, diluted 40 times in 0.1 M phosphate buffer (pH 7), was mixed with 50 µL of the sample at various concentrations in microplate wells (Bibby Sterilin Ltd., Stone, UK) and incubated at 37 °C for 30 minutes. Then, 40 µL of 0.2% soluble starch was added to initiate the enzyme reaction, which continued at 37 °C for an additional 20 minutes. Afterward, 100 µL of iodine reagent (5 mM iodine (Univar, Ajax Finechem, Australia) and 5 mM Potassium iodide (RCI Labscan, Dublin, Republic of Ireland)) was added, and the absorbance was measured at 630 nm using microplate reader (BIOBASE-EL10, Biobase Biodustry (Shandong) Co., Ltd., China). The enzyme inhibitory activity was calculated using the formula:

$$\alpha\text{-amylase inhibitory activity (\%)} = (C / E) \times 100$$

where E represents the optical density of the reaction containing both the sample (inhibitor) and the enzyme, and C denotes the optical density of the reaction containing the enzyme with the same volume of distilled water instead of the sample.

Total phenolic content

The total phenolic content (TPC) was determined using the Folin-Ciocalteu (FC) colorimetric method as described by Sassa-deepaeng *et al*. (2023). A 20 µL aliquot of the extract at varying concentrations was mixed with 100 µL of FC reagent (Merck, Darmstadt, Germany) and 1,980 µL of deionized water, followed by incubation at ambient temperature for 5 minutes. Then, 300 µL of 7% sodium carbonate solution (QRëC, Auckland, New Zealand) were added, and the mixture was incubated for another 60 minutes in the dark at ambient temperature. Absorbance was measured at 765 nm using a Metash UV-5200 spectrophotometer with UV-Professional analysis software. The total phenolic content (TPC) analysis was performed in triplicate. A calibration curve was prepared using gallic acid standards (Bio Basic Inc., Ontario, Canada). at various concentrations. Results were expressed as milligrams of gallic acid equivalent (GAE) per gram of dry sample weight.

Total flavonoid content

The total flavonoid content (TFC) was assessed following the method of Sassa-deepaeng *et al*. (2023). A mixture of 20 µL of extract and 380 µL of deionized water was prepared, followed by the addition of 100 µL of 5% sodium nitrite (Univar, Ajax Finechem, Australia) solution. After 5 minutes of incubation, the 100 µL of 10% aluminum chloride (Univar, Ajax Finechem, Australia) solution was added, and the mixture was left to stand for 6 minutes at room temperature. Finally, the 400 µL of 1M sodium hydroxide (Labscan, Bangkok, Thailand) solution was added. After a 15-minute incubation at ambient temperature in the dark, absorbance was recorded at 415 nm. The TFC was calculated using a calibration curve prepared with quercetin (Sigma–Aldrich, Germany) at different concentrations and expressed as milligrams of quercetin equivalent (QE) per gram of dry weight.

Total tannin content

The total tannin content (TTC) was also determine as described by Sassa-deepaeng *et al*. (2023), the 250 µL of extract was vigorously mixed with 450 µL of 1% vanillin (Merck KGaA, Darmstadt, Germany) reagent. After a 5-minute incubation, 300 µL of concentrated hydrochloric acid (Labscan, Bangkok, Thailand) was added, and the mixture was incubated for an additional 30

minutes at ambient temperature. The solution developed a red color. Absorbance was then measured at 500 nm. The TTC was calculated using a calibration curve prepared with epigallocatechin gallate (Myskinrecipes, Bangkok, Thailand) at different concentrations and expressed as milligrams of epigallocatechin gallate equivalent (EGCGE) per gram of dry sample weight.

Saponin content

The saponin content was determined following the method of Singh *et al*. (2019). A solution of 0.5% para-anisaldehyde (Myskinrecipes, Bangkok, Thailand) in ethyl acetate (Labscan, Bangkok, Thailand) and a solution of 50% sulfuric acid (Labscan, Bangkok, Thailand) in ethyl acetate (Labscan, Bangkok, Thailand) were the two reagents mixed in equal amounts. The standard and the tentative plant sample's aqueous extracts were dissolved in two milliliters of ethyl acetate followed by the addition of 1 mL each of reagent A and B. The solution was mixed and then incubated for 10 minutes at 60°C in a dry bath incubator (Major Science Co., Ltd., Taoyuan, Taiwan). After cooling at room temperature, the absorbance was measured at 430 nm. The saponin content was calculated using a calibration curve prepared with saponin from quillaja bark (Sigma Aldrich, Saint Louis, MO, USA) at different concentrations and expressed as milligrams of saponin equivalent (SE) per gram of dry sample weight.

Terpenoid content

The terpenoid content was determined following the method of Das *et al*. (2022). One milliliter of aqueous extract was taken in test tube and vortexed thoroughly with 3 mL chloroform (Labscan, Bangkok, Thailand) and 0.4 mL Concentrated Sulfuric acid (Labscan, Bangkok, Thailand) in controlled cooling box in the dark for 4 hours. The solution developed a reddish-brown color appeared at the bottom of the tubes. After decant supernatant, the 4 mL methanol (Labscan, Bangkok, Thailand) was added and absorbance was measured at 538 nm. the terpenoid content was calculated using a calibration curve prepared with myrcene (Myskinrecipes, Bangkok, Thailand) solution and expressed as milligrams of myrcene equivalent (ME) per gram of dry sample weight.

Alkaloid content

The alkaloid content was determined following the method of Shamsa *et al*. (2008). After 69.8 mg of bromocresol green (Myskinrecipes, Bangkok, Thailand) was completely dissolved in 3 mL of 2N sodium hydroxide (Labscan, Bangkok, Thailand) and 5 mL of distilled water, the mixture was diluted to 1000 mL with distilled water to create a bromocresol green solution. In order to prepare the phosphate buffer (pH 4.7), 0.2 M citric acid (42.02 g citric acid (Univar, Ajax Finechem, Australia) in 1 L of distilled water) was used to bring the pH of 2 M sodium phosphate solution (71.6 g di-Sodium hydrogen phosphate dihydrate (Kemaus, Cherrybrook, Australia) in 1 L of distilled water) down to 4.7. The 1 mL bromocresol green, 1 mL Phosphate buffer, and 0.1 mL aqueous extract were mixed and place in ambient temperature for 10 minutes. After adding 1.5 mL chloroform (Labscan, Bangkok, Thailand), the mixture was more incubated at the ambient temperature for 10 minutes prior to the solution developed a yellow color. The absorbance was measured at 470 nm. The alkaloid content was calculated using a calibration curve prepared with pure atropine sulphate (A.N.B. Laboratories Co., Ltd., Thailand) and expressed as milligrams of atropine equivalent (AE) per gram of dry sample weight.

Protein content

Protein assay was performed according to the Lowry's method described by Mæhre *et al*. (2018). The 0.1 mL aqueous extract was mixed with 0.9 mL of solution A (composed of 2 g/L potassium sodium tartrate (Univar, Ajax Finechem, Australia) and 100 g/L sodium carbonate (Univar, Ajax Finechem, Australia) in 0.5 M sodium hydroxide (Labscan, Bangkok, Thailand)), followed by incubation at 50 °C for 10 minutes. After cooling the samples to room temperature, 1 mL of solution B (containing 0.2 g/L potassium sodium tartrate tetrahydrate (Univar, Ajax Finechem, Australia) and 0.1 g/L copper sulfate pentahydrate (QRëC, Auckland, New Zealand) in 0.1 M sodium hydroxide) was added and place in ambient temperature to react for another 10 minutes. Subsequently, 3 mL of solution C (Folin–Ciocalteu phenol (Merck KGaA, Darmstadt, Germany) reagent diluted 1:16 v/v with water) was mixed in, and the samples were incubated again at 50 °C for 10 minutes. Absorbance was then measured at 650 nm. A protein content was calculated using a calibration curve prepared using bovine serum albumin fraction V (Sigma-Aldrich, St Louis, MO, USA) at concentrations of 0, 0.0625, 0.125, 0.25, 0.5, and 1 g/L and results were expressed in micrograms per gram of dry sample weight.

Reducing sugar content

The reducing sugar content was measured using the dinitrosalicylic acid (DNS method) described by Tharawatchruk *et al*. (2023). The DNS (Fluka Chemie GmbH, Buchs, Switzerland) reagent was prepared by combining a solution of DNS (2.5 g in 100 mL of 1 M sodium hydroxide) with a hot sodium potassium tartrate solution (75 g in 125 mL of distilled water). The mixture was then diluted to a total volume of 500 mL with distilled water. For the assay, the 0.9 mL of DNS reagent was added to 0.1 mL of an aqueous tentative plant extract in 1.5-mL microcentrifuge tube. The tubes were heated in boiling water for 5 minutes. The solution developed a red-brown color. Absorbance was measured at 540 nm. Reducing sugar content was calculated using a calibration curve prepared with 2 mM glucose (Univar, Ajax Finechem, Australia) solution and expressed as milligrams per gram of dry sample weight.

Statistical analysis

The data were processed using the Analysis ToolPak add-in of Microsoft Excel 2016 (Microsoft Corporation, CA, USA). One-way analysis of variance (ANOVA) and the least significant difference (LSD) test were employed to identify statistically significant differences, with a threshold of $p < 0.05$ considered significant.

RESULTS AND DISCUSSION

The saliva alpha-amylase inhibitory activity of 15-tentative plants was investigated. It was found that *A. lebbeck* exhibited the highest inhibitory activity at concentration of 20 mg/mL (81.59±5.84%), followed by *M. pudica* (32.26±2.17%), *I. aquatica* (26.27±0.94%), and *G. inodorum* (22.63±6.03%), respectively. The α-amylase inhibitory activity of samples was indicated in Figure 1.

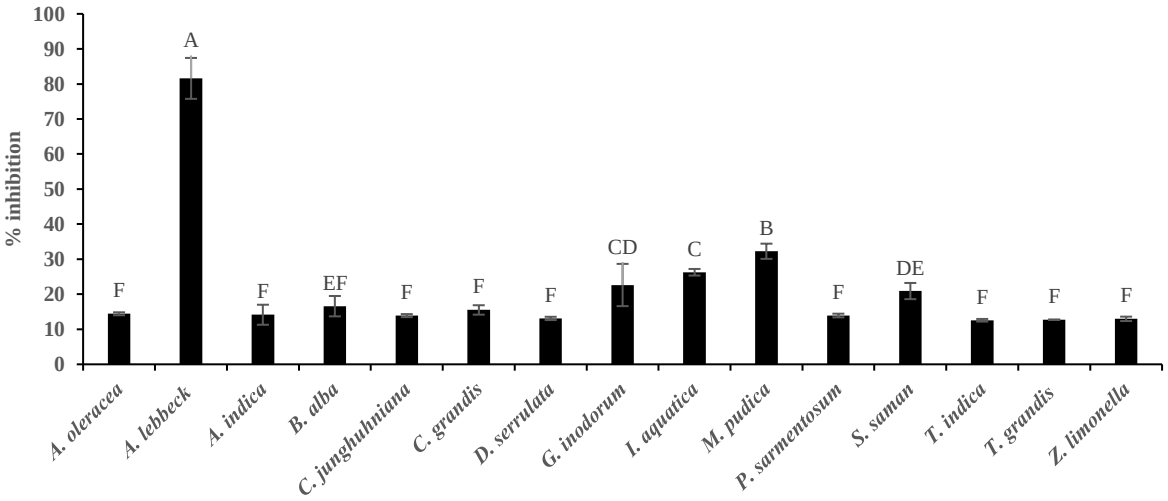

Figure 1. The α-amylase inhibitory activity of 15-tentative plants. Different letters indicate significant differences ($p < 0.05$).

Effect of pH on the adsorption of methylene blue

A. lebbeck exhibited the highest enzyme inhibitory activity, as previously reported by Jaiswal and Kumar (2017), followed by *M. pudica* (Shrestha *et al*., 2022), *I. aquatica* (Saikia *et al*., 2023), and *G. inodorum* (Phanjaroen *et al*., 2024). To investigate the correlation with specific phytochemicals, quantitative phytochemical tests were subsequently conducted, as reported below.

The secondary metabolites including total phenolic compounds, total flavonoids, tannins, saponins, terpenoids, alkaloids, reducing sugars, and proteins were extracted using water from the leaves, seeds, and stem bark of selected plant species. Comparative analyses revealed that the concentrations of total phenolic content were significantly higher in all samples exhibiting positive amylase inhibitory activity. Notably, *I. aquatica* leaves contained the highest phenolic content (2.75 ± 0.16 mg GE/g DW, followed by *G. inodorum* (1.95 ± 0.06 mg GE/g DW), *A. lebbeck* (1.12 ± 0.06 mg GE/g DW), and *M. pudica* (1.01 ± 0.24 mg GE/g DW), as illustrated in Figure 2.

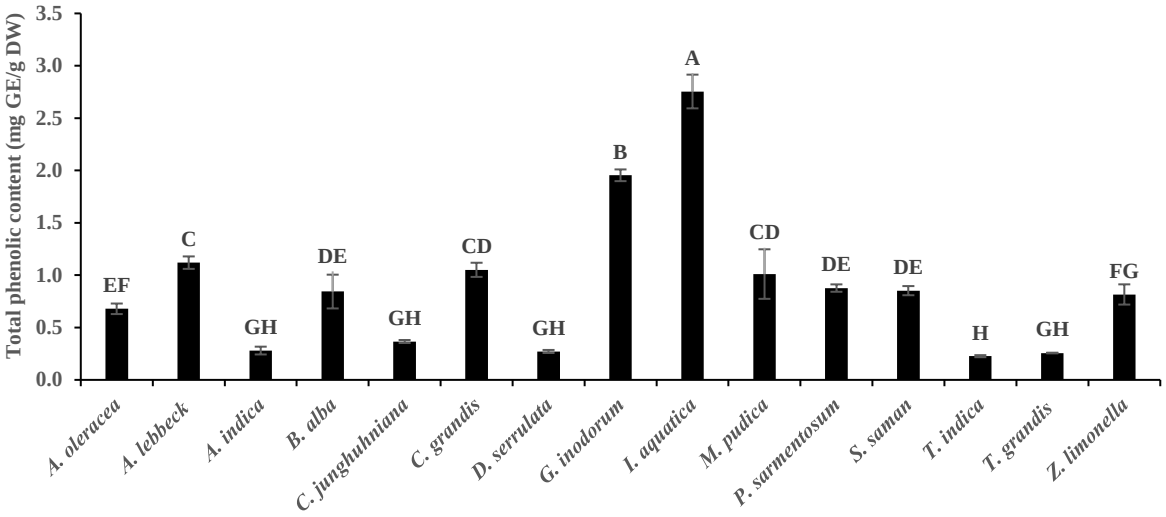

Figure 2. Total phenolic content of 15 plant extracts. Bars represent the standard deviation from triplicate determinations. Different letters indicate significant differences ($p < 0.05$).

These findings are consistent with those of Saikia *et al*. (2023), who reported that polyphenolic compounds, including polyphenol glycosides and phenolics in *I. aquatica*, are closely associated with amylase inhibitory activity. Similarly, Haideri *et al*. (2024) demonstrated that the phenolic composition of *G. inodorum* represents a major active constituent responsible for amylase inhibition. Kajaria *et al*. (2013) also documented that the polyphenolic compounds present in *A. lebbeck* play a potentially important role in managing diabetes through the inhibition of α-amylase. Interestingly, although *M. pudica* is not traditionally recognized as a food source in Thailand, data from Bohara *et al*. (2022) indicated that its aqueous extract, rich in phenolic compounds, also exhibits strong amylase inhibitory activity.

To examine the potential involvement of flavonoids in amylase inhibitory activity, the total flavonoid content (TFC) was quantified using the aluminum chloride ($AlCl_3$) colorimetric method. The results indicated that the concentrations of total flavonoid content were significantly higher in the three samples that demonstrated positive amylase inhibitory activity. Notably, *I.aquatica* leaves exhibited the highest flavonoid content (2.93 ± 0.29 mg QE/g DW), followed by *A. lebbeck* (1.99 ± 0.29 mg QE/g DW) and *G. inodorum* (2.03 ± 0.08 mg QE/g DW). In contrast, *M. pudica* did not show a comparable increase in flavonoid content, as illustrated in Figure 3.

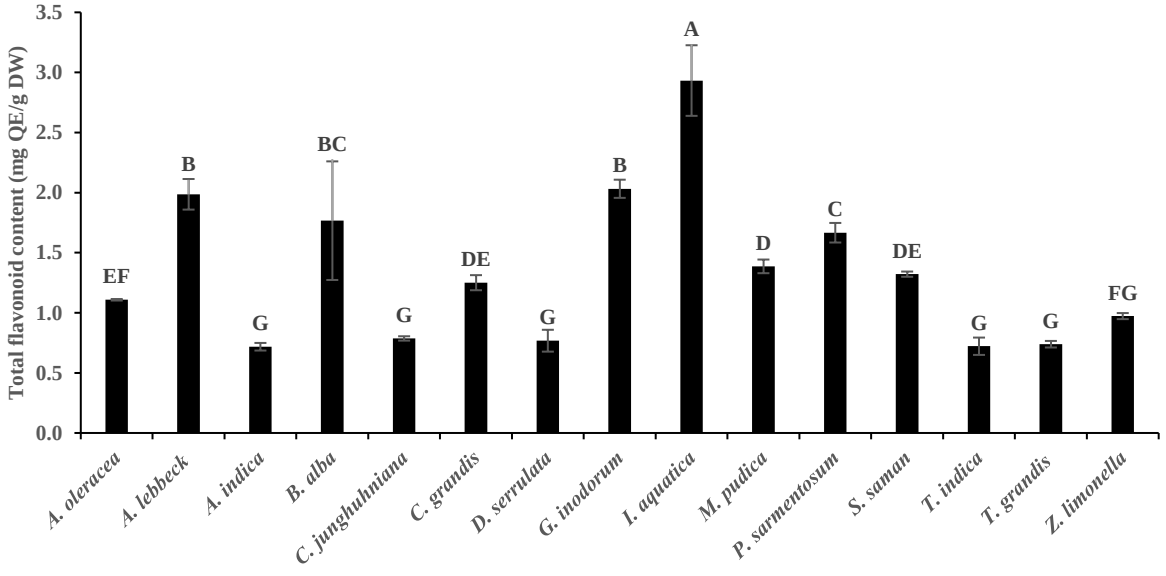

Figure 3. Total flavonoid content of 15 plant extracts. Bars represent the standard deviation from triplicate determinations. Different letters indicate significant differences ($p < 0.05$).

It was observed that *I. aquatica* exhibited the strongest amylase inhibitory activity, which was attributed to its high flavonoid content, including the presence of flavins and flavin-like compounds, as reported by Saikia *et al*. (2023). The extract of *A. lebbeck* was found to contain flavonols such as kaempferol and quercetin 3-*O*-α-rhamnopyranosyl (1→6)-β-glucopyranosyl(1→6)-β-galactopyranosides. Furthermore, amylase inhibition has also been documented in protein isolates derived from the seeds (Ekun *et al*., 2024) and bark (Jaiswal & Kumar, 2017) of this species. *G. inodorum* demonstrated strong α-amylase inhibitory activity, which has been attributed to the presence of myricetin (Haideri *et al*., 2024).

To evaluate the effect of tannins on amylase inhibitory activity, the total tannin content was quantified. The analysis revealed that *A. lebbeck* extract contained a significantly high concentration of tannins (0.46±0.01 mg EGCGE/g DW), which was positively correlated with its amylase inhibitory activity as showed in Figure 4.

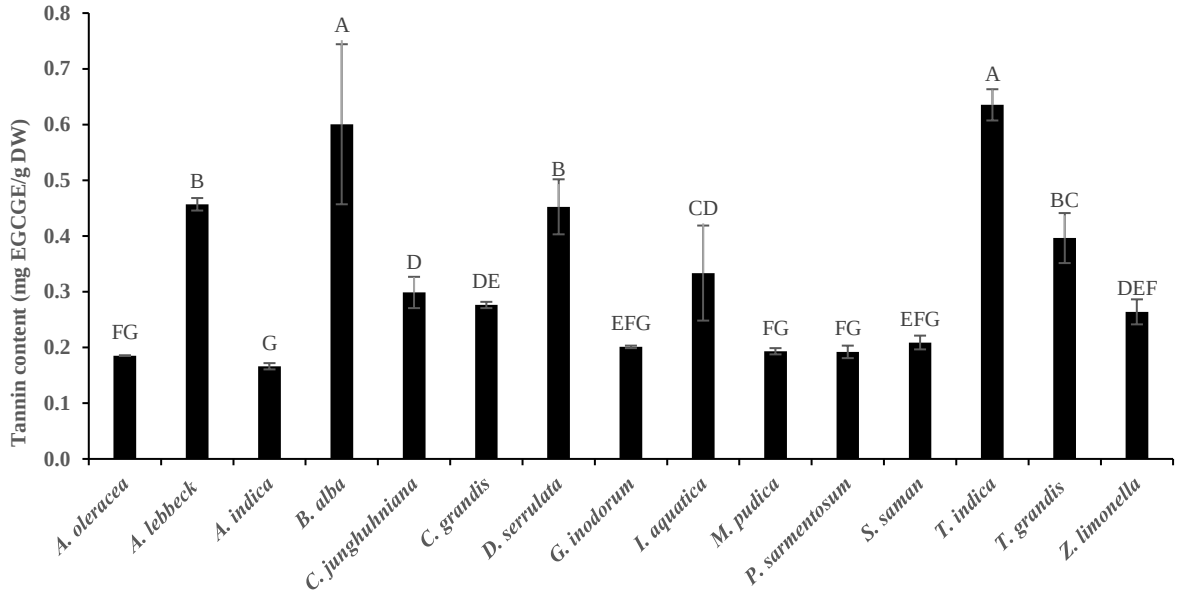

Figure 4. Tannin content of 15 plant extracts. Bars represent the standard deviation from triplicate determinations. Different letters indicate significant differences ($p < 0.05$).

This phenomenon can be explained by Zhang *et al*. (2023), who reported that tannins act as co-inhibitors of salivary α-amylase. Additionally, tannins have been shown to exert an inhibitory effect on α-glucosidase, an enzyme that plays a crucial role in the regulation of type 2 diabetes.

The saponin content was also analyzed, and the results indicated that *A. lebbeck* extract contained a significantly high concentration of saponins (79.62 ± 1.90 mg SE/g DW), which was positively correlated with its amylase inhibitory activity. This was followed by *M. pudica* (58.16 ± 1.51 mg SE/g DW) and *G. inodorum* (56.64 ± 1.53 mg SE/g DW), as presented in Figure 5.

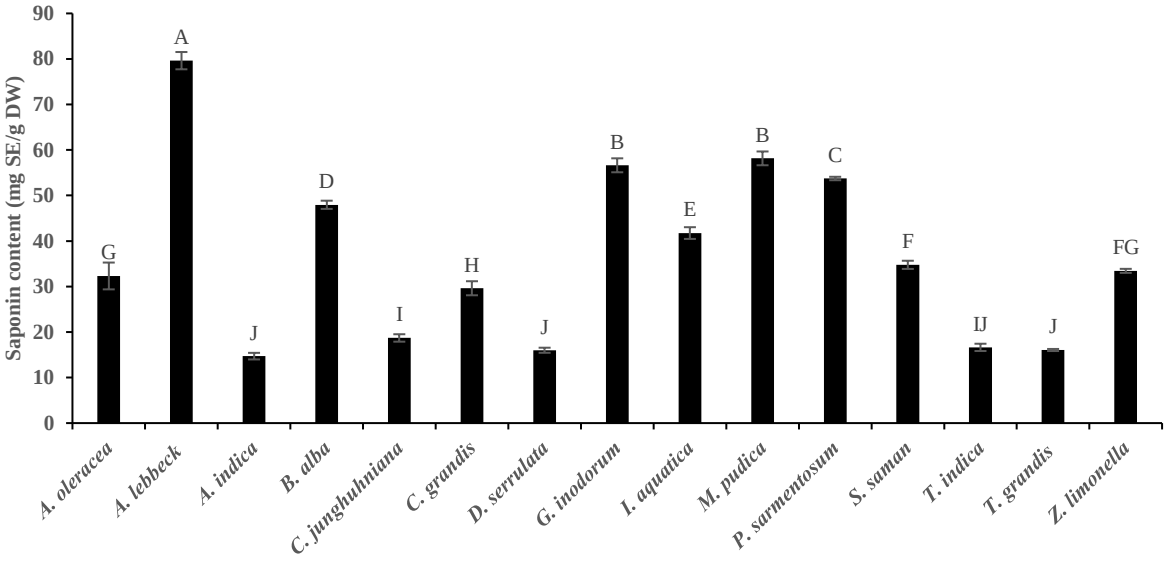

Figure 5. Saponin content of 15 plant extracts. Bars represent the standard deviation from triplicate determinations. Different letters indicate significant differences ($p < 0.05$).

The high saponin content in *A. lebbeck* was first documented by Desai & Joshi (2019). Saponins have also been reported in *M. pudica* (Rizwan *et al*., 2022) and *G. inodorum* (Jeytawan *et al*., 2022), although not specifically as amylase inhibitors. Nonetheless, saponins have been shown to moderately inhibit α-amylase activity (Ngoc *et al*., 2023; Hanh *et al*., 2016). In contrast, alkaloids and terpenoids were not detected in any of the samples, likely due to their presence in concentrations below the detection limit of the colorimetric method used. Thus, it can be inferred that these compounds do not contribute to the observed amylase inhibitory activity.

Protein was also detected in all samples. Based on protein estimation using Lowry's method, the inhibitory plant extract of *I. aquatica* extract exhibited the highest protein concentration (31.32 ± 0.31 µg/g DW), followed by *G. inodorum* (23.86 ± 0.83 µg/g DW), as presented in Figure 6.

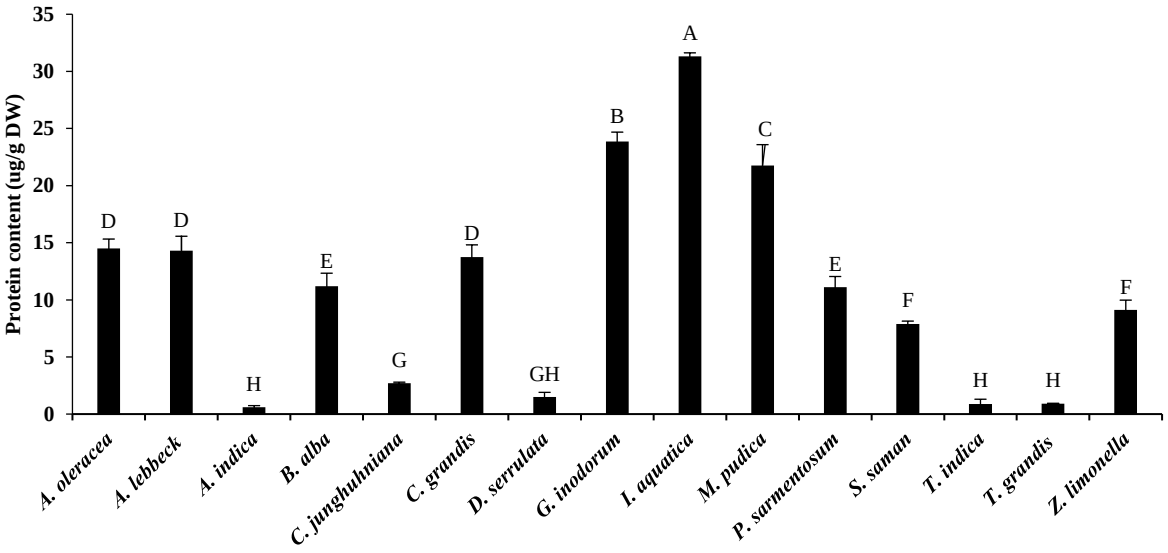

Figure 6. Protein content of 15 plant extracts. Bars represent the standard deviation from triplicate determinations. Different letters indicate significant differences ($p < 0.05$).

The high protein content in *I. aquatica* extract has been documented by Ali & Kaviraj (2018), and similarly in *G. inodorum* by Norkum *et al*. (2023). This may be related to their observed

amylase inhibitory activity. Although no studies have specifically reported that proteins in these species act as α-amylase inhibitors, seed protein extracts from *A. lebbeck* have demonstrated inhibitory effects against both α-amylase and α-glucosidase (Ekun *et al*., 2024). Therefore, it can be hypothesized that proteins in these extracts may contribute as co-inhibitors of amylase. Further analysis is required to clarify the inhibitory role of proteins in these extracts.

The reducing sugar content was also analyzed to assess whether its presence could contribute to elevated blood sugar levels if the extracts are considered for regular dietary use. Notably, *I. aquatica* extract exhibited the highest reducing sugar content (1690.57 ± 43.17 mg/g DW), as shown in Figure 7.

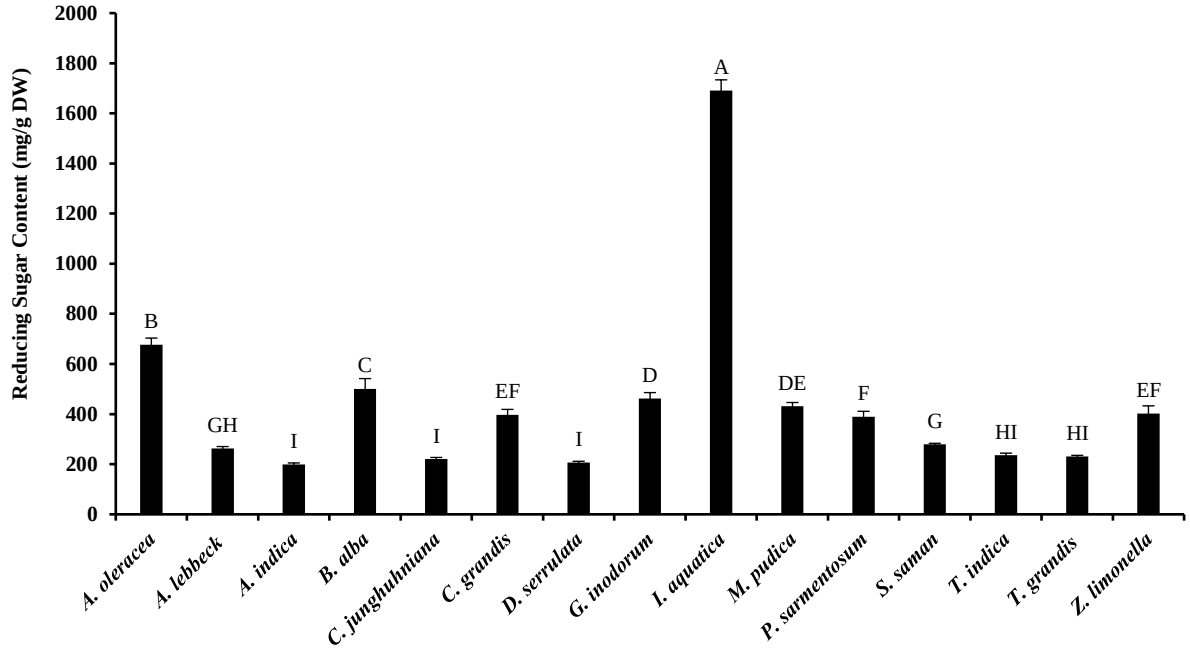

Figure 7. Reducing sugar content of 15 plant extracts. Bars represent the standard deviation from triplicate determinations. Different letters indicate significant differences ($p < 0.05$).

The presence of reducing sugars in *I. aquatica* leaves has been reported by Bokolo & Adikwu (2018). However, in contrast, consumption of the edible portions of *I. aquatica* over a one-week period was shown to effectively reduce fasting blood sugar levels (Malalavidhane *et al*., 2003). Therefore, it can be hypothesized that *I. aquatica* leaves may possess additional mechanisms for lowering blood sugar, which have yet to be fully elucidated.

CONCLUSIONS

This study demonstrated that *A. lebbeck* exhibited the highest salivary amylase inhibitory activity, followed by *M. pudica*, *I. aquatica*, and *G. inodorum*, respectively. The inhibitory effect of *A. lebbeck* was attributed to the presence of phenolics, flavonoids, tannins, and saponins, while the activity observed in *M. pudica* was primarily linked to its saponin content. *I. aquatica* also displayed amylase inhibitory activity, associated with its phenolic, flavonoid, and protein content, whereas *G. inodorum* demonstrated inhibition through a combination of phenolics, flavonoids, saponins, and proteins. Notably, a high reducing sugar content was identified in *I. aquatica*, highlighting the need for further investigation into its potential implications for blood sugar regulation.

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