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The Green Extraction of Mango (*Mangifera indica* L.) cv. 'Nam Dok Mai Si Thong' and the Evaluation of *In Vitro* Antioxidant and Anti-*α*-Glucosidase Activities: *In Silico* Molecular Docking Study

Chanjira Jaramornburapong [1], Muhammad Niyomdecha[2], Jirapast Sichaem[3*], Arthorn Loisruangsin[4], Nutthapol Funnimid[3] Suwimon Khwunsiriwong[5*]

[1]*Program of Chemistry, Faculty of Science and Technology, Nakhon Pathom Rajabhat University, Nakhon Pathom 73000, Thailand.*
[2]*Department of Chemistry, Faculty of Science, Silpakorn University, Sanam Chan, Muang, Nakhon Pathom 73000, Thailand.*
[3]*Department of Chemistry, Faculty of Science and Technology, Thammasat University Lampang Campus, Lampang 52190, Thailand*
[4]*Department of Physical and Material Sciences, Faculty of Liberal Arts and Science, Kasetsart University (Kamphaeng-Saen Campus), Nakhon Pathom 73140, Thailand*
[5]*Department of Chemistry, Faculty of Science and Technology, Rajabhat Rajanagarindra University, Chachoengsao 24000, Thailand.*

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ABSTRACT

Mango (*Mangifera indica* L.) cv. 'Nam Dok Mai Si Thong' is a premium Thai variety known for its golden skin, sweet fragrance, and rich, smooth flavor. This research focuses on extraction yields, as well as the antioxidant and anti-*α*-glucosidase activities of crude extracts obtained from various parts of the Nam Dok Mai Si Thong mango, using green extraction methods with ethanol and hot water as solvents. From this study, the highest extraction yield was observed in the hot water crude extract of the flowers (0.194%), followed by the shoots (0.118%) and leaves (0.112%). The ethanol extracts, particularly those from the flowers and leaves, exhibited stronger antioxidant activities, with the flower ethanol crude extract showing the highest DPPH radical scavenging activity (SC_{50} 0.030 ± 0.003 mg/mL). Additionally, the flower ethanol crude extract demonstrated the strongest inhibition against both maltase and sucrase, with IC_{50} values of 0.015 ± 0.002 and 0.0083 ± 0.001 mg/mL, respectively. Computational docking analysis revealed that mangiferin, a key bioactive compound, exhibited strong binding affinities with carbohydrate-digesting enzymes, suggesting its potential as a natural hypoglycemic agent. These findings highlight the potential pharmaceutical and nutraceutical applications of Nam Dok Mai Si Thong mango crude extracts, particularly in antioxidant therapy and diabetes management.

Keywords: Nam Dok Mai Si Thong mango, mangiferin, antioxidant activity, anti-*α*-glucosidase activity, molecular docking study.

Introduction

Mangoes belong to the Anacardiaceae family and have been traditionally used in medicine. For example, mango peels are brewed into tea to treat inflammation in Tonga and India.[1,2] Unripe fruit is used to alleviate fatigue and heatstroke, while ripe fruit is used to treat gastrointestinal, biliary, hematologic diseases, as well as scurvy. Fresh leaves are employed in the management of diabetes, and dried seeds ground into powder are traditional remedies for diarrhea and sore throats.[3] Nam Dok Mai Si Thong mango (*Mangifera indica* L.) is a premium Thai cultivar developed from the Nam Dok Mai mango variety through seed propagation in Phra Pradaeng District. Unlike the original, its fruit transitions from green to pale yellow within 1–2 months and eventually ripens to a rich golden hue, enhancing its ornamental appeal.

*Corresponding authors; Email: jirapast @tu.ac.th, suwimonkhwunsiriwong@gmail.com
Tel: +66932978366, +66813058181

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The ripe fruit is highly valued for its sweet fragrance, smooth texture, thin seed, and low fiber content. Owing to these exceptional qualities, Nam Dok Mai Si Thong has gained widespread popularity and is now cultivated extensively across Thailand, particularly in the central, northern, northeastern, eastern, and western regions.[4] Regarding its antioxidant potential, Siri-amornpan et al. (2008) reported strong *in vitro* activity in the pulp of *M. indica* L. using both the ferric reducing antioxidant power (FRAP) assay and the DPPH radical scavenging assay, with scavenging activity reaching up to 82%.[5] In terms of *α*-glucosidase inhibition, Prashanth and coworkers evaluated the ethanolic extracts of *M. indica* L. bark for their *α*-glucosidase inhibitory activity and observed strong inhibition, with an IC_{50} value of 314 µg/mL.[6] Preliminary phytochemical studies of various parts of *M. indica* L. have identified phenolic compounds, flavonoids, and *C*-glycosyl xanthones, particularly mangiferin (2-*β*-D-glucopyranosyl-1,3,6,7-tetrahydroxy xanthen-9-one) (Figure 1).[7-10] Mangiferin exhibits notable biological activities, including anti-inflammatory and antidiabetic effects,[11,12] antioxidant properties,[13-16] and anticancer activity.[17-19] Therefore, this study aims to evaluate the effectiveness of green extraction methods on the leaves, shoots, and flowers of the Nam Dok Mai Si Thong mango from Chachoengsao Province. The obtained extracts were evaluated for their *α*-glucosidase inhibitory and antioxidant activities. Additionally, an *in silico* study was conducted to investigate the enzyme inhibitory activity of mangiferin, a key bioactive compound. The findings of this study will contribute to the development of sustainable extraction techniques and the potential application of Nam Dok Mai Si Thong mango-derived bioactive compounds in functional foods and pharmaceuticals.

Materials and Methods

Plant materials

The leaves, shoots, and flowers of Nam Dok Mai Si Thong mango (*Mangifera indica* L.) were collected in April 2015 from Suan Kaew Wong Nu Kun, located in Bang Khla District, Chachoengsao Province, Thailand (13°40'56.7"N 101°09'22.6"E), for use in this study.

Preparation of crude extracts

The different parts of the Nam Dok Mai Si Thong mango, including leaves, shoots, and flowers (Figure 2), were cleaned and air dried. The different parts were blended thoroughly with a blender. Then, 5 g of each part was placed in an Erlenmeyer flask, and 50 mL of 95% ethanol or hot water was added for each flask. The extracts were then subjected to sonication for 10 minutes. The extracts were filtered using Whatman No. 1 filter paper, and the solvents were evaporated using a rotary evaporator. The crude extract was then placed in a desiccant jar again to allow the solvent to completely evaporate, and the % yield was calculated. The crude extracts were separated using the thin-layer chromatography (TLC) technique, and mangiferin was identified based on its R_f value.

α-Glucosidase inhibitory activity assay

This assay, adapted from a previous method,[20] utilized α-glucosidase from rat intestinal acetone powders (Sigma-Aldrich, St. Louis, Missouri, USA), which contains maltase and sucrase with specific activities of 0.09 and 0.45 units/mg protein, respectively. The enzymatic reaction was assessed using a colorimetric method to measure the release of free glucose. Each crude extract and acarbose were prepared at concentrations of 0.04, 0.2, 1, 2.5, and 5 mg/mL (10 µL). The reaction was initiated by adding 20 µL of α-glucosidase enzyme, followed by incubation at 37°C for 10 minutes. Then, 20 µL of either maltose (0.58 mM) or sucrose (20 mM) (Merck, Darmstadt, Germany) was added as substrates. The reaction was incubated at 37°C for 10 minutes for maltose and 40 minutes for sucrose. To stop the reaction, the mixture was heated at 100°C for 15 minutes. A glucose concentration was measured through a glucose oxidase-based reaction using a Glu-kit (Sigma-Aldrich, St. Louis, Missouri, USA), with absorbance recorded at 540 nm using a microplate reader (Infinite® F50 Plus, Tecan, Switzerland). The percentage of inhibition was calculated using a previously established formula, and the IC_{50} value was obtained from a graph plotting inhibition percentage against the concentration of the sample. Acarbose (Bayer, Germany) served as the positive control.

Antioxidant activity assay

This assay was modified from a previously established method.[20] Sample solutions were prepared in DMSO at concentrations of 0.04, 0.1, 0.2, 0.5, and 1 mg/mL. In a 96-well plate, 20 µL of DMSO was added to both the blank and sample wells, followed by 100 µL of a 0.05 mM DPPH solution in methanol. The reaction mixtures were incubated in the dark at room temperature for 15 minutes. Absorbance was measured at 520 nm using a microplate reader, and the percentage of DPPH radical scavenging was calculated using the following equation

$$\%\ \text{DPPH radical scavenging} = \frac{A_{blank} - A_{sample}}{A_{blank}} \times 100 \qquad (1)$$

Where A_{blank} is the absorbance of the blank solution and A_{sample} is the absorbance of the sample solution.

The half maximal scavenging concentration (SC_{50}) value was determined from the calibration curve of % DPPH radical scavenging at various sample concentrations. SC_{50} represents the sample concentration required to scavenge 50% of DPPH radicals.

Statistical data analysis

Data analysis was conducted in triplicate and expressed as mean ± standard deviation (SD), analysis of variance (ANOVA), Least Significant Difference (LSD), and Duncan Multiple Range Test (DMRT). Data correlation was searched using the SPSS program.

Enzyme inhibitory activity of mangiferin using the in silico docking analysis method

The *in silico* enzyme inhibitory activity of mangiferin, acarbose, and enzyme substrates with key carbohydrate-digesting enzymes relevant to antidiabetic activity, including salivary α-amylase, α-glucosidase, lactase, maltase-glucoamylase (maltase site), sucrase-isomaltase (isomaltase site), and sucrase-isomaltase (sucrase site), was analyzed using AutoDock4.[21] These enzymes were chosen because they play crucial roles in the digestion and absorption of carbohydrates in the human digestive tract.[22,23] Inhibiting their activity is a well-established strategy for managing postprandial hyperglycemia, a key aspect of type 2 diabetes management, by slowing down the breakdown of complex carbohydrates into absorbable monosaccharides.[24,25] The binding affinities of these small molecules to the enzymes were compared in terms of free energy of binding (ΔG_{bind}). The enzyme structures were obtained from the U.S. data center for the Global Protein Data Bank (PDB) (https://www.rcsb.org). Ligands and other small molecules associated with the enzyme structures were removed. The structures of the small molecules were retrieved from PubChem, an open chemistry database maintained by the National Institutes of Health (NIH), and subsequently converted to PDB format using Open Babel.[26]

Results and Discussion

Extraction yield

The extraction yield and characteristics of different parts of Nam Dok Mai Si Thong mango using ethanol and hot water as solvents are summarized in Table 1. The highest extraction yield was obtained from the hot water crude extract of flowers (0.194%), followed by the hot water crude extract of shoots (0.118%) and leaves (0.112%). The lowest yield was observed in the ethanol crude extract of shoots (0.036%). The physical characteristics of the extracts varied according to the solvent used. Ethanol extracts exhibited different colors and consistencies, with leaf crude extract being a green solid, shoot crude extract being a yellow solid, and flower crude extract being a dark brown viscous liquid. In contrast, all hot water extracts were dark brown solids. The color difference may be attributed to the types of phytochemicals present (Figure 3), such as chlorophyll in leaves and flavonoids or tannins in shoots and flowers. Overall, the results suggest that hot water is more effective in extracting bioactive compounds from the flowers, shoots, and leaves of Nam Dok Mai Si Thong mango, as indicated by the higher yields. Ethanol, on the other hand, may be more selective for non-polar to moderately polar constituents, which is reflected in the variations in crude extract color and consistency. The presence of mangiferin in the crude extracts was identified using the TLC technique (Figure 4). The ethanol and hot water extracts of shoots and leaves showed spots corresponding to standard mangiferin (R_f 0.68; EtOAc:MeOH:H_2O, 4:1:1 v/v/v), indicating that mangiferin is a major constituent in these parts of Nam Dok Mai Si Thong mango. In contrast, only faint spots were detected in the flower extracts, suggesting that mangiferin may be present as a minor constituent.

Antioxidant activity of crude extracts

All crude extracts exhibited DPPH radical scavenging activity with varying SC_{50} values (Table 2). Among the samples, the flower ethanol extract showed the strongest antioxidant activity (SC_{50} 0.030 ± 0.003 mg/mL), followed closely by the flower hot water extract (SC_{50} 0.031 ± 0.004 mg/mL). Other extracts with notable activity included leaf ethanol (SC_{50} 0.062 ± 0.008 mg/mL) and shoot ethanol (SC_{50} 0.072 ± 0.006 mg/mL), while the leaf hot water and shoot hot water extracts demonstrated comparatively weaker activity. Butylated hydroxytoluene (BHT), a synthetic antioxidant, was used as a standard to evaluate the antioxidant potential of the extracts. BHT exhibited an SC_{50} value of 0.360 ± 0.010 mg/mL, which was significantly higher than that of all crude extracts, confirming the superior natural antioxidant potential of the mango extracts. The strong antioxidant activity of the ethanol extracts, particularly from flowers and leaves, suggests the presence of phenolic compounds, flavonoids, and other bioactive antioxidants.[6,9,13]

These findings indicate that the flower and leaf extracts, especially the ethanol extracts, possess strong natural antioxidant properties that could be valuable in the pharmaceutical, nutraceutical, and food industries.[27]

α-Glucosidase inhibitory activity of crude extracts

All crude extracts exhibited α-glucosidase inhibitory effects against both maltase and sucrase enzymes, as shown in Table 2. The flower ethanol extract demonstrated the most potent inhibition of both enzymes, with IC_{50} values of 0.015 ± 0.002 mg/mL for maltase and 0.0083 ± 0.001 mg/mL for sucrase. The flower hot water extract also showed strong activity (IC_{50} 0.025 ± 0.003 mg/mL for maltase and 0.018 ± 0.002 mg/mL for sucrase). Moderate inhibition was observed in the ethanol and hot water extracts of leaves and shoots, with IC_{50} values ranging from 0.036 to 0.061 mg/mL for maltase and 0.023 to 0.032 mg/mL for sucrase. In comparison, the standard drug acarbose exhibited significantly higher potency, with IC_{50} values of 0.00065 ± 0.0001 mg/mL for maltase and 0.0019 ± 0.0002 mg/mL for sucrase. These results suggest that the flower extracts, particularly the ethanol extract, exhibit promising α-glucosidase inhibitory potential. The strong inhibitory activity observed in both flower ethanol and hot water extracts may be attributed to the presence of flavonoids, tannins, or

phenolic compounds.[9,13,27] The higher activity found in ethanol extracts, especially from flowers, implies the presence of more non-polar bioactive compounds with enzyme-inhibitory effects. In contrast, the relatively lower activity of the leaf and shoot extracts indicates that their phytochemical compositions may be less effective against α-glucosidase enzymes. These findings highlight the flower extracts, especially the ethanol extract, as promising natural α-glucosidase inhibitors, potentially useful for managing postprandial hyperglycemia in diabetic patients. To the best of our knowledge, the α-glucosidase inhibitory activity of crude extracts from Nam Dok Mai Si Thong mango parts has not been previously reported. This study thus provides new insights into the potential therapeutic application of Nam Dok Mai Si Thong mango flower extracts in diabetes management.

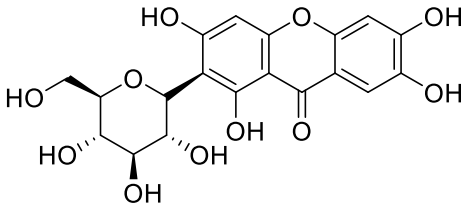

Figure 1: Structure of mangiferin.

Figure 2: Characteristics of the leaves, shoots, and flowers of the Nam Dok Mai Si Thong mango from Chachoengsao Province.

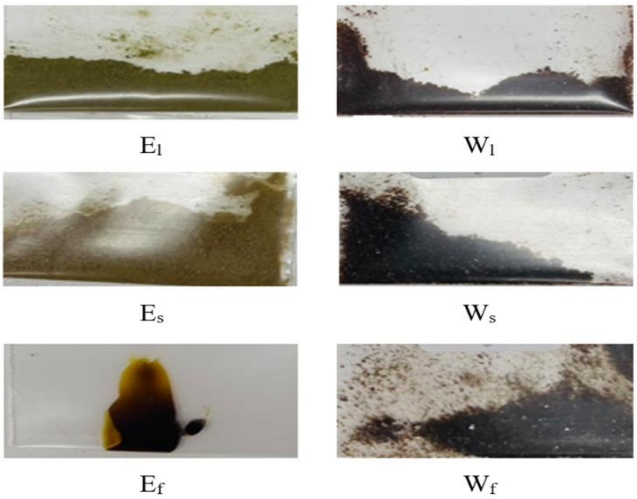

Figure 3: The characteristics of crude extracts from the leaves, shoots, and flowers of Nam Dok Mai Si Thong mango: E_l (ethanol crude extract of leaves), W_l (hot water crude extract of leaves), E_s (ethanol crude extract of shoots), W_s (hot water crude extract of shoots), E_f (ethanol crude extract of flowers), and W_f (hot water crude extract of flowers).

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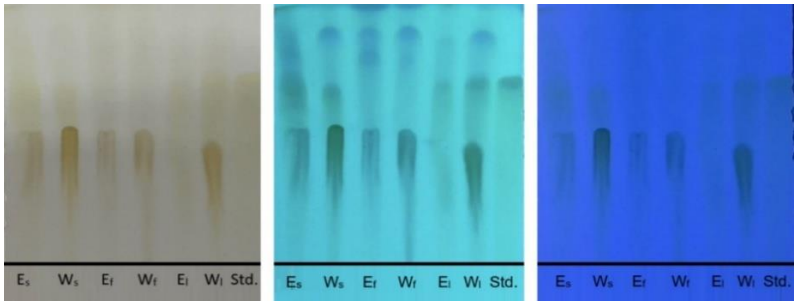

Figure 4: Identification of mangiferin in crude extracts by TLC technique using EtOAc:MeOH:H_2O (4:1:1, v/v/v).

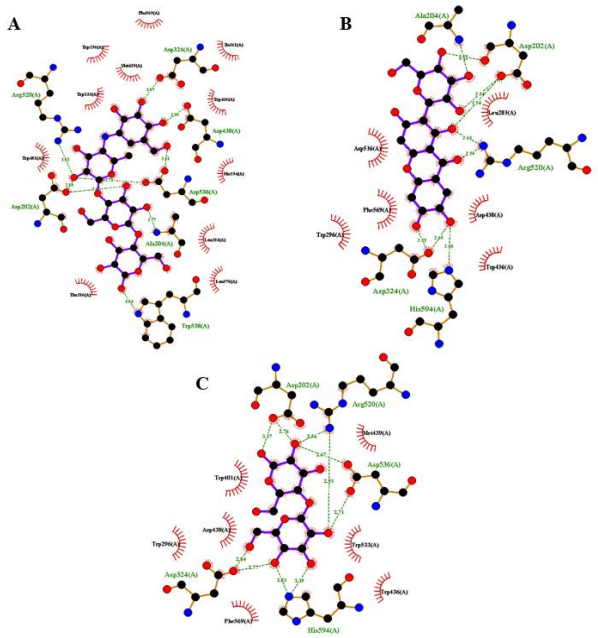

Figure 5: A 2D ligand-protein interaction diagram generated by LigPlot$^+$. α-Glucosidase binds to A) acarbose, B) mangiferin, and C) maltose (substrate).

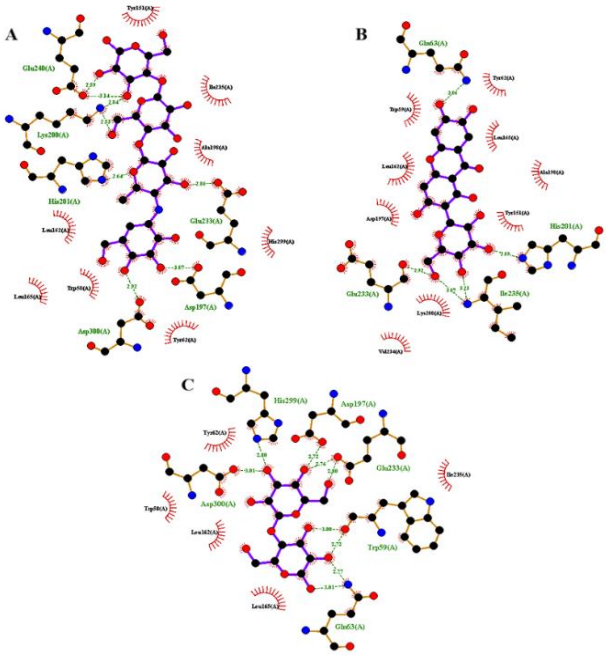

Figure 6: A2D ligand-protein interaction diagram generated by LigPlot$^+$. Salivary α-amylase binds to A) acarbose, B) mangiferin, and C) maltose (substrate).

Table 1: Physical characteristics and percentage yield of crude extracts.

Crude extract	Characteristics	% Extraction Yield (w/w)
Leaf ethanol	Green solid	0.068
Leaf hot water	Dark Brown solid	0.112
Shoot ethanol	Yellow solid	0.036
Shoot hot water	Dark Brown solid	0.118
Flower ethanol	Dark Brown viscous liquid	0.099
Flower hot water	Dark Brown solid	0.194

Table 2*:* Antioxidant and *α*-glucosidase inhibitory activities of crude extracts.

Crude extract	DPPH (SC_{50}, mg/mL)	*α*-Glucosidase	
		Maltase (IC_{50}, mg/mL)	Sucrase (IC_{50}, mg/mL)
Leaf ethanol	0.062 ± 0.008	0.037 ± 0.004	0.023 ± 0.002
Leaf hot water	0.120 ± 0.012	0.061 ± 0.006	0.024 ± 0.002
Shoot ethanol	0.072 ± 0.006	0.036 ± 0.003	0.023 ± 0.006
Shoot hot water	0.106 ± 0.005	0.043 ± 0.002	0.032 ± 0.003
Flower ethanol	0.030 ± 0.003	0.015 ± 0.002	0.0083 ± 0.001
Flower hot water	0.031 ± 0.004	0.025 ± 0.003	0.018 ± 0.002
BHT[a]	0.360 ± 0.010		
Acarbose[a]		0.00065 ± 0.0001	0.0019 ± 0.0002

[a] Positive control

Table 3: Free energy of binding (ΔG_{bind}) for docking acarbose, mangiferin, and substrates with carbohydrate-digesting enzymes.

Enzyme	ΔG_{bind} (kcal/mol)		
	Acarbose	Mangiferin	Substrate
Salivary *α*-amylase	-5.50	-6.84	-4.33
Pancreatic *α*-amylase	-5.48	-7.22	-4.81
α-Glucosidase	-7.79	-6.86	-5.57
Lactase	-6.95	-6.79	-5.88
Maltase-glucoamylase (maltase site)	-6.90	-6.58	-5.33
Sucrase-isomaltase (isomaltase site)	-5.48	-7.22	-6.60
Sucrase-isomaltase (sucrase-site)	-6.20	-7.13	-3.85

Enzyme inhibitory activity of mangiferin using in silico docking analysis

Although the *in vitro* inhibitory assays involved testing the enzyme inhibition of complex crude extracts, the computational docking analysis is based specifically on mangiferin due to several compelling reasons. Firstly, preliminary phytochemical studies have consistently identified mangiferin as a major *C*-glycosyl xanthone present in *M. indica* L. This study further confirmed the presence of mangiferin in our crude extracts using the TLC technique, where it appeared as a major constituent in the ethanol and hot water extracts of shoots and leaves. Although it was detected only as a minor constituent in the flower extracts, mangiferin was nonetheless present. Secondly, mangiferin is a well-documented bioactive compound with extensive research highlighting its potent antidiabetic effects.[28] It has been shown to enhance insulin sensitivity, modulate lipid profiles, regulate blood glucose levels, and delay intestinal glucose absorption.[29,30] This strong literature support makes mangiferin a logical primary candidate for initial *in silico* mechanistic investigations into the antidiabetic potential of mango extracts. While we acknowledge that crude extracts contain a diverse array of phytochemicals beyond mangiferin that may collectively contribute to the observed *α*-glucosidase activity, focusing on a prominent and well-characterized compound like mangiferin provides valuable preliminary insights into the potential molecular mechanisms. It serves as a representative marker for the extract's antidiabetic properties, allowing for a focused computational exploration of its direct interaction with carbohydrate-digesting enzymes (Figures 5 and 6). The binding affinities of acarbose, mangiferin, and various enzyme substrates with carbohydrate-digesting enzymes are shown in Table 3. While *α*-glucosidase and *α*-amylase are pivotal enzymes in the metabolism of dietary carbohydrates, other carbohydrate-digesting enzymes also play a significant role in breaking down specific disaccharides and oligosaccharides, ultimately releasing glucose units. Moreover, other monosaccharides, such as fructose and galactose, can present challenges for diabetic patients, as the body converts them into glucose before they enter the bloodstream.The more negative ΔG_{bind} values for mangiferin, compared to those of the enzyme substrates, indicate a stronger binding affinity of mangiferin for the enzyme's active site. This suggests that mangiferin may slow glucose absorption and metabolism, potentially acting as a hypoglycemic agent similar to acarbose. Furthermore, the ΔG_{bind} values for sucrase-isomaltase (isomaltase site) are noteworthy. The ΔG_{bind} value for acarbose (-5.48 kcal/mol) is less negative than that for isomaltose (-6.60 kcal/mol), indicating that acarbose is not an effective inhibitor of sucrase-isomaltase at the isomaltase site. In contrast, mangiferin exhibits stronger binding to sucrase-isomaltase at the isomaltase site (ΔG_{bind} -7.22 kcal/mol) compared to isomaltose. This finding is significant because Thai glutinous rice, a staple in northeastern and northern Thailand, consists almost entirely of amylopectin.[31] The structure of amylopectin includes *α*-1,6-glycosidic bonds at branch points, the same type of bond found in isomaltose. Therefore, mangiferin could potentially slow the digestion of amylopectin by targeting these *α*-1,6-glycosidic bonds. Slower digestion may lead to a more gradual release of glucose, which could be particularly beneficial for managing blood sugar levels.

Conclusion

This study highlights the potential of Nam Dok Mai Si Thong mango as a natural source of bioactive compounds with antioxidant and *α*-glucosidase inhibitory activities. Hot water extraction yielded a higher quantity of extracts than ethanol extraction. Among all extracts, the flower ethanol crude extract exhibited the highest antioxidant and *α*-glucosidase inhibitory activities, suggesting the presence of potent bioactive constituents. In addition, *in silico* molecular docking analysis revealed that mangiferin binds strongly to carbohydrate-digesting enzymes, particularly to the isomaltase subunit of sucrase-isomaltase. This interaction suggests that mangiferin may interfere with carbohydrate breakdown, thereby potentially slowing glucose absorption in the small intestine. These findings support the use of Nam Dok Mai Si Thong mango crude extracts, particularly the flower ethanol crude extract and mangiferin, as promising candidates for functional food and pharmaceutical applications aimed at diabetes management and oxidative stress reduction. Further research is warranted to isolate active compounds, clarify their mechanisms of action, and assess their therapeutic potential in clinical settings. In addition, *in silico* analysis of other bioactive constituents may provide insights into their individual functions and synergistic effects on overall bioactivity.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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