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Research Article

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In Silico Study of *Caesalpinia sappan* bark Extract as an α-Glucosidase Enzyme Inhibitor in Preventing Hyperglycemia and Antioxidant Capacity

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ABSTRACT

Background: *Caesalpinia sappan* L. bark is traditionally consumed as a herbal beverage and is known for its antidiabetic and antioxidant properties. Inhibition of α-glucosidase represents an important therapeutic strategy to control postprandial hyperglycemia in diabetes management.

Aims: This study aimed to evaluate the α-glucosidase inhibitory potential of selected *C. sappan* bark compounds using an in silico molecular docking approach and to assess their antioxidant activity through an in vitro assay.

Methods: Molecular docking analysis was conducted on the α-glucosidase enzyme (PDB ID: 5NN8) using brazilin, protosappanin A, protosappanin B, and sappanchalcone as test ligands, with acarbose serving as the reference inhibitor. Binding affinities and interactions with key catalytic residues were analyzed. Antioxidant activity was evaluated using the DPPH radical scavenging assay, and IC_{50} values were determined.

Results: Among the tested compounds, brazilin exhibited the strongest binding affinity toward α-glucosidase (ΔG = −7.1 kcal/mol) and formed interactions with important catalytic residues, comparable to acarbose. The antioxidant assay demonstrated strong radical scavenging activity, with an IC_{50} value of 16.62 ppm, indicating high antioxidant capacity.

Conclusion: The findings suggest that *C. sappan* bark, particularly brazilin, holds promising potential as a natural α-glucosidase inhibitor with significant antioxidant properties, supporting its traditional use as an antidiabetic herbal beverage.

INTRODUCTION

Diabetes mellitus is a major public health priority in Indonesia and is listed among the leading non-communicable diseases because of its high morbidity, mortality, and economic burden. It is a metabolic disorder characterized by impaired carbohydrate metabolism and persistent hyperglycaemia due to insufficient insulin secretion or reduced tissue sensitivity to insulin, which eventually leads to serious, often life-threatening complications such as cardiovascular disease, neuropathy, nephropathy, and blindness (IDF, 2021).

The progressive rise in diabetes cases each year is accompanied by an increased incidence of chronic complications and diabetes related deaths, highlighting the need for effective prevention, early control strategies, and continuous research on novel therapeutic approaches (Mazumdar et al., 2020).

One promising approach is the exploration of medicinal plants as sources of antidiabetic agents, including compounds that inhibit α-glucosidase, a key

enzyme in carbohydrate digestion. α-Glucosidase catalyses the conversion of dietary carbohydrates into glucose, and excessive activity contributes to postprandial hyperglycaemia and chronic oxidative stress, which, in turn, triggers the generation of reactive oxygen species, the release of inflammatory mediators such as interleukin-6 and TNF-α, and apoptosis in various organs. Inhibiting intestinal α-glucosidase can slow glucose absorption and help attenuate postprandial blood glucose spikes, as demonstrated by current α-glucosidase-inhibiting drugs such as acarbose, voglibose, and miglitol. However, these agents are often associated with gastrointestinal side effects, including flatulence and diarrhea (Simamora et al., 2019; Kumar et al., 2018).

Oxidative stress driven by chronic hyperglycaemia can be counteracted by antioxidant agents, including vitamins and flavonoids, that scavenge free radicals and modulate redox-sensitive pathways (Dirir et al., 2021). In this context, the bark of *Caesalpinia sappan* L., a traditional Southeast Asian medicinal herb, has garnered considerable interest (Tulin et al., 2017). Its bark contains bioactive constituents, including brazilin, flavonoids, and polyphenols, with reported antioxidant, antidiabetic, and anti-inflammatory activities. Previous in silico work indicated that brazilin has inhibitory potential against α-amylase, suggesting a broader role in the modulation of carbohydrate metabolism. However, the inhibitory effect of *C. sappan* bark extract on α-glucosidase and its mechanistic implications for preventing hyperglycaemia remain insufficiently characterized (Artanti et al., 2023).

Therefore, this study aims to evaluate the α-glucosidase inhibitory activity and antioxidant capacity of *C. sappan* bark extract, and to identify the most promising bioactive constituents using an in silico approach complemented by in vitro antioxidant assays. The findings are expected to contribute to the development of safer and more effective herbal-based candidates for the management of type 2 diabetes mellitus.

MATERIALS AND METHODS

Materials

Human lysosomal acid α-glucosidase (PDB ID: 5NN8) was retrieved from the RCSB Protein Data Bank (https://www.rcsb.org/) in PDB format (*.pdb*). Test ligands comprised secondary metabolites from *Caesalpinia sappan* L. bark: brazilin (CID 73384), protosappanin A (CID 128001), protosappanin B (CID 13846689), and sappanchalcone (CID 5319493), downloaded from PubChem (https://pubchem.ncbi.nlm.nih.gov/) in SDF format (*.sdf*). Ligand energy minimization was performed using PyRx 0.8, and the resulting structures were saved as PDBQT (*.pdbqt) files. Acarbose acted as the reference or control ligand, exemplifying a clinically approved α-glucosidase inhibitor.

Study Design

This research employed an integrated *in silico* and *in vitro* approach. Computational docking was employed to evaluate the molecular interactions between *C. sappan* metabolites and the α-glucosidase target. Docking analysis parameters included binding affinity energy (ΔG), inhibition constant (IC), and intermolecular interactions (hydrogen bonds or hydrophobic contacts). *In vitro* testing will measure the inhibitory potency (IC_{50}) of *C. sappan* bark extract against the α-glycosidase enzyme using the DPPH method.

In Silico Molecular Docking

Ligand Selection and Preparation

Test ligands were selected based on prior phytochemical reports of *C. sappan* bark. Structures were validated using SwissADME for Lipinski's Rule of Five compliance (MW <500 Da, HBD ≤5, HBA ≤10, logP -0.4 to 5.0) and bioavailability score (>0.5). Ligands were converted from SDF to PDB format using Discovery Studio 2016, energy-minimized in PyRx 0.8, and exported as PDBQT files.

Receptor Preparation

The α-glucosidase structure (PDB: 5NN8, resolution 1,99 Å) was prepared in Discovery Studio 2016 to remove water molecules, ions, and non-native ligands, and then converted to PDBQT format using AutoDock Tools 1.5.6. Protein stability was verified using Ramachandran plot analysis (PROCHECK), confirming that more than 90% of residues are in favored regions.

Docking Validation and Execution

Docking validation involved re-docking the native ligand (acarbose) to confirm root-mean-square deviation (RMSD) < 2Å. Molecular docking was performed using PyRx 0.8 with AutoDock Vina algorithm. Grid box coordinates were centered on the active site (based on the native ligand position), with dimensions of 30×30×30 Å and exhaustiveness of 8. The 9 best binding poses per ligand were analyzed for binding energy (kcal/mol), Ki (μM), and interactions with catalytic residues (Asp518 and Asp616) using Discovery Studio Visualizer.

In Vitro Antioxidant Assay (DPPH Method)

C. sappan bark extract was serially diluted (50 - 0,78125 ppm) in 7 concentrations. Each 40 µL sample (n = 3) was mixed with 120 µL of 0,1 mM DPPH in methanol in 96-well plates, incubated in the dark for 30 min, and the absorbance was measured at 517nm using a SPECTROstar Nano (BMG LABTECH). Inhibition percentage was calculated as:

$$\%\,\text{Inhibition} = \frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}} \times 100$$

IC_{50} was determined via linear regression against ascorbic acid standards. Antioxidant strength was classified as: very strong (<50 ppm), strong (50-100 ppm), moderate (101-150 ppm), weak (>150 ppm).

Data Analysis

The best ligand was selected based on the lowest ΔG, favorable constanta inhibition, and interactions with catalytic residues. Results were presented descriptively, accompanied by visualizations (2D/3D interaction maps, Ramachandran plots, and regression curves.

RESULTS AND DISCUSSION

The α-glucosidase structure (PDB ID: 5NN8) met crystallographic quality criteria, with 91,1% of residues in the most favored regions of the Ramachandran plot, indicating high structural stability and suitability for molecular docking analysis (Table 1). Drug-likeness evaluation showed that all tested *Caesalpinia sappan* bark compounds generally complied with Lipinski's Rule of Five and exhibited acceptable bioavailability scores, supporting their potential as oral drug candidates.

The Ramachandran plot of the 5NN8 receptor is divided into four areas (Figure 1). The most active area is red, the additional allowed area is brown, the area that can be considered is yellow, and the area that is not allowed is light yellow.

Protein quality depends on the number of non-glycine residues in the most active residue area and the prohibited residue area. This protein has high quality and structural stability because it meets the requirements of more than 90% in the recommended area or in the active residue area (Ting et al., 2020).

Lipinski's rules were used as a reference for ligands in selection as a potential drug. A ligand is considered stable if it meets these criteria (Table 2). The rules are as follows: molecular weight <500 Da, hydrogen bond donors <5, hydrogen bond acceptors <10, logP value between -0,4 and 5, and molar refractive index between 40 and 130 (Lipinski, 2004)

Four test ligands satisfy Lipinski's rules: brazilin, protosappanin A, sappanchalcone, (Z,Z,Z)-9,12,15-octadecatrienoic acid, methyl ester, and phytol. Protosappanin B and sappanchalcone each meet only one Lipinski criterion, so they remain considered safe. According to ADMETSAR bioavailability analysis, these four ligands have a bioavailability score of 0,55, indicating that all are suitable candidates for development as single oral medications.

Molecular docking analysis demonstrated that all ligands interacted with the catalytic site of α-glucosidase, particularly residues Asp518 and Asp616. Among the tested compounds, brazilin showed the most favorable binding affinity (ΔG = −7,1 kcal/mol), approaching that of the reference inhibitor, acarbose (ΔG = -7,6 kcal/mol) (Table 3). Hydrogen bond formation and hydrophobic interactions indicated a

Table 1. Ramachandran Plot Analysis of Receptor (PDB ID : 5NN8)

Plot statistics	Number of Residues	Percentage (%)
Residues in most favoured regions	646	91,1
Residues in additional allowed regions	63	8,9
Residues in generously allowed regions	0	0
Residues in disallowed regions	0	0
Number of non-glycine and non-proline residues	709	100
Number of glycine residues	64	
Number of proline residues	65	
Total number of residues	845	

competitive inhibition mechanism, suggesting that brazilin may effectively interfere with carbohydrate hydrolysis and postprandial glucose release.

Another parameter observed in the molecular docking study in this research is chemical bonding. The chemical bonds observed were hydrogen bonds and hydrophobic interactions, which contribute to the stability of the receptor-ligand bond. These bonds were identified through visual inspection of the molecular docking results. The chemical bonds formed upon molecular docking with the α-glucosidase enzyme are shown in Table 4. The chemical bonds observed were hydrogen bonds and hydrophobic interactions, which contribute to the stability of the receptor-ligand bond. These bonds were obtained after visual inspection of the molecular docking results.

The type of receptor-ligand interaction inferred from molecular docking results can be determined by visual inspection, either 2D or 3D. Hydrogen bonds, hydrophobic interactions, and the distance between hydrogen bonds are parameters for determining the strength of receptor-ligand interactions. Hydrogen bonds play an important role in stabilizing the bond energy between the receptor and the ligand. Hydrophobic interactions can increase the binding affinity of the target protein for the drug (Purwono et al., 2024; Purwono et al., 2025; Varma et al., 2010).

Based on the visualization results, all ligands can interact with the catalytic sites of the α-glucosidase enzyme, specifically Asp518 and Asp616 (Rodhi et al., 2023). The presence of hydrogen bonds in the interaction between Brazillin and the active catalytic site suggests a competitive inhibitor mechanism against the α-glucosidase enzyme. In this study, all tested ligands interacted with the enzyme's catalytic site. Based on the affinity energy and chemical bond values, brazilin inhibited the α-glucosidase enzyme with a value similar to that of acarbose, although it did not exceed its affinity energy. Visualization interaction between ligands and protein target are shown in Figure 3.

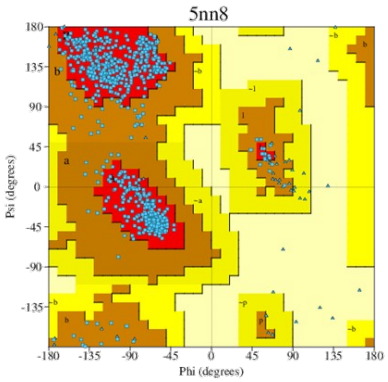

Figure 1. The Ramachandran plot of the 5NN8 receptor

Table 2. Stability analysis of acarbose and test ligands of secondary metabolite from secang wood (*Caesalpinia sappan* L.) using Lipinski's rule

Ligands	MW (Da)	HBD	HBA	Log P	Molar Refractivity	Bioavaibility score
Brazilin	286	4	5	1,3822	72,937	0,55
Protosappanin A	272	3	5	0,783	63,000	0,55
Protosappanin B	304	6	5	1,1287	78,107	0,55
Sappanchalcone	286	5	3	2,7080	77,794	0,55

Note: Molecule Weight (MW), *hydrogen bound donor* (HBD), *hydrogen-bound acceptor* (HBA), unfulfilled the criteria of Lipinski

Antioxidant capacity measurement was conducted using the DPPH (1,1-diphenyl-2-picrylhydrazyl) method, based on the principle that DPPH radicals interact with antioxidant compounds. Antioxidant compounds donate a hydrogen atom to DPPH radicals, reducing DPPH to a non-radical form. This change is accompanied by a color shift, specifically the loss of the purple-colored DPPH dye. The fading of this color results in a decrease in absorbance at the maximum wavelength, measured using a UV-VIS spectrophotometer (Jannah et al., 2024).

Antioxidant activity is indicated by the IC_{50} value, which is the sample solution concentration required to inhibit 50% of DPPH free radicals. The IC_{50} value of secang wood bark extract was measured to assess its antioxidant potential. Inhibition percentage was calculated using the formula based on the absorbance values of the sample and the blank or control. Based on

Table 3. Energy affinity value (ΔG) resulting from the interaction between *Caesalpinia sappan* secondary metabolite compounds and the α-glucosidase enzyme

Test Ligands	α-Glukosidase (Ccal/mol)	Constanta Inhibition (CI) (μM)
Acarbosa (Ligand co-crystal)	-7,60	2,69
Brazilin	-7,10	6,25
Protosappanin A	-6,40	10,36
Protosappanin B	-6,80	20,36
Sappanchalcone	-6,11	33,78

Table 4. Chemical interactions between α-glucosidase enzyme and secondary metabolites of sappan wood

Ligands name	Hydrogen-bonding amino acid residues	Bond Length (Å)	Hydrophobic amino acid residues
Akarbosa (ligan ko-kristal)	Asp282	2,62	Ala284, Trp376, Leu405, Ile441, Trp481, Trp516, **Asp518**, Met519, Trp613, Phe649, Leu650
	Asp404	3,00	
	Arg600	2,95	
	Asp616	3,03	
		2,88	
		2,95	
	Trp618	2,84	
	His674	3,19	
		3,34	
Brazilin	**Asp518**	2,84	Trp376, Trp481, Met519, Phe525, **Asp616**, Phe649, Leu650
		3,15	
	Arg600	2,81	
Protosappanin A	Asp282	2,77	Trp376, Trp481, Trp516, **Asp518**, Met519, Phe525, Arg600, **Asp616**, Phe649
	Asp404	3,08	
	His674	3,26	
Protosappanin B	Asp282	2,81	Trp481, **Asp518**
	Ser523	3,20	
	Asp616	2,99	
Sappanchalcone	**Asp518**	2,93	Ala284, Trp376, Trp481, Trp516, Phe525, Ala555, **Asp616**, Phe649
	Arg600	2,97	

the calculations, the inhibition percentages for secang wood bark extract and Vitamin C are shown in Table 5.

The average linear regression equation was derived from the IC50 R values, and the resulting regression graph is shown in Figure 4. The antioxidant capacity of secang wood bark extract was measured, yielding an IC_{50} value of 16,62 ppm. The IC_{50} value for Vitamin C, used as a control, was 6,93 ppm. Both Vitamin C and secang wood bark extract exhibit very strong antioxidant activity, as their IC_{50} values are < 50 ppm (Molyneux et al., 2004). IC_{50} was determined by linear regression of sample concentration versus the percentage of DPPH radical inhibition. The average linear regression equation for secang wood bark extract was $y = 2.98x + 0.44$, with a coefficient of determination (R^2) > 0.99.

The treatment of degenerative diseases requires the administration of antioxidants as free radical scavengers. The synergistic effect of α-glucosidase enzyme inhibition and antioxidant capacity is expected to be an alternative candidate for overcoming hyperglycemia, particularly postprandial hyperglycemia, which contributes to the development of insulin resistance. Antioxidant capacity testing using the DPPH method measures the capacity of extracts to capture nitrogen-free radicals. Based on the classification, if the IC_{50} value is below 50 ppm, it is classified as a very strong antioxidant. Meanwhile, values between 50-100 ppm indicate strong antioxidant activity (Molyneux et al., 2004). The results of the antioxidant testing of *Caesalpinia sappan* bark extract show that it belongs to the strong antioxidant group.

The α-glucosidase enzyme plays a crucial role in carbohydrate metabolism, converting them into glucose and other monosaccharides. Activation of this enzyme in the small intestine after eating causes postprandial hyperglycemia. This prolonged condition causes oxidative stress, leading to long-term damage to various organs. Oxidative stress that exceeds the capacity of endogenous antioxidant defenses can cause cellular dysfunction, resulting in organ complications due to diabetes. Excessive ROS can stimulate the inflammatory process by releasing various proinflammatory mediators (Gonzales et al., 2023; Itam et al., 2021).

Hyperglycemia can increase the production of proinflammatory cytokines, such as TNF-alpha and IL-6, and, in severe cases, lead to organ damage. The presence of various inflammatory mediators reduces the ability of insulin receptors to transduce insulin

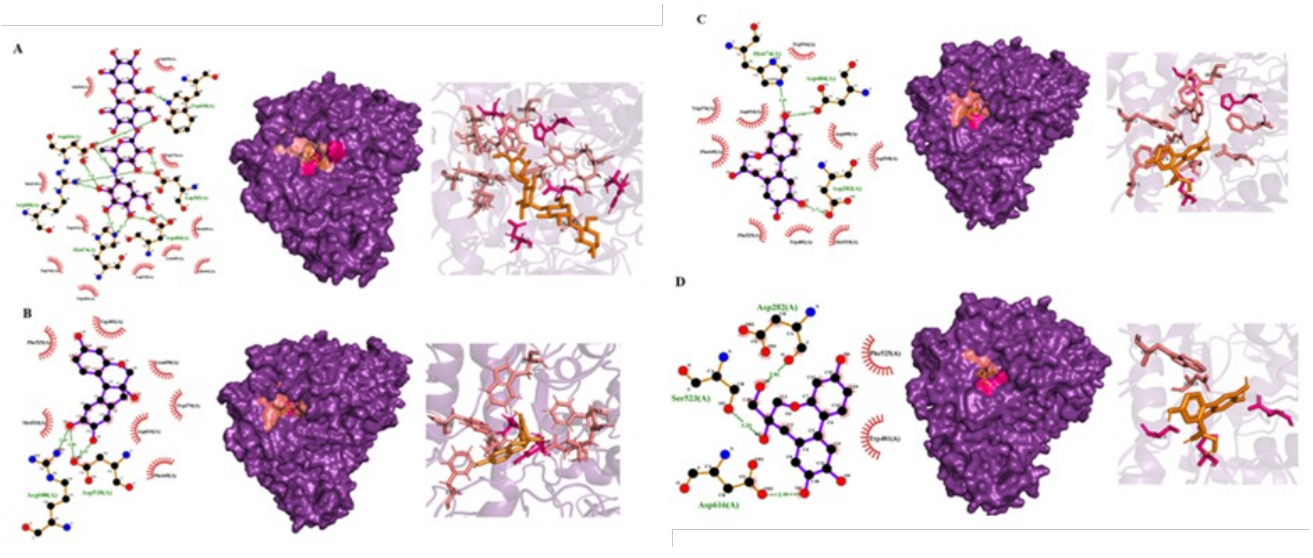

Figure 3. Visualization of interaction between ligands and the targeted receptor of enzyme α-glukosidase (PDB ID: 5NN8) against Acarbosa (A), Brazilin (B), Protosappanin D (C), and *Sappanchalcon* (D).

signals in several organs, including muscle, liver, and adipose tissue. The presence of IL-6 also increases SOCS-3 (Suppressor of Cytokine Signaling-3) expression, thereby inhibiting insulin receptor phosphorylation and leading to insulin resistance. Prolonged hyperglycemia triggers systemic inflammation and oxidative stress, which damage pancreatic β cells, leading to decreased insulin production and pancreatic tissue damage. This ongoing inflammation worsens pancreatic function and accelerates the development of diabetes mellitus complications. Controlling blood glucose levels and inflammation is crucial to preventing further pancreatic damage (Shoelson et al., 2006; González et al., 2023).

The administration of several antioxidant preparations, such as vitamin E, vitamin A, and flavonoids, helps prevent oxidative stress conditions that have a hyperglycemic effect by scavenging ROS and activating Nrf2 (Nuclear factor erythroid-derived 2-like 2). Nrf2 is the primary regulator of the antioxidant response, controlling the expression of proteins involved in inflammation through antioxidant enzymes to reduce ROS (Gonzales et al., 2023; Baird et al., 2020). Additionally, Nrf2 plays a role in cellular protection, thereby preventing chronic diseases such as atherosclerosis, diabetes mellitus, and cancer (Gupte et al., 2023).

Brazilin is a homoisoflavonoid because its structure is similar to flavonoids, but it has one additional carbon atom in the middle chain (forming a 16-carbon structure, not 15-carbon like regular flavonoids). Flavonoids can directly capture free radicals by donating hydrogen atoms, thereby deactivating them (Adnan et al., 2022). This is supported by the results of antioxidant capacity testing of this extract, which is classified as a very strong antioxidant. The search for candidate agents for diabetes mellitus could identify this compound as a new candidate, which could be developed into a phytopharmaceutical preparation. The synergistic mechanism of *Caesalpinia sappan* bark extract in inhibiting the α-glucosidase enzyme and scavenging free radicals generated by oxidative stress can suppress the progression of diabetes mellitus.

When compared with recent *in silico* studies published between 2023 and 2025, the binding affinity of brazilin against α-glucosidase observed in this study

Table 5. IC_{50} Antioxidant Capacity Percent Values for Secang Wood Bark Extract and Vitamin C

Sample	Average Regression Equation	IC_{50} (ppm)	Category
Secang Wood Bark Extract	Y = 28X + 0.44	16.62	Very Strong
Vitamin C	Y = 6.77X + 3.08	6.93	Very Strong

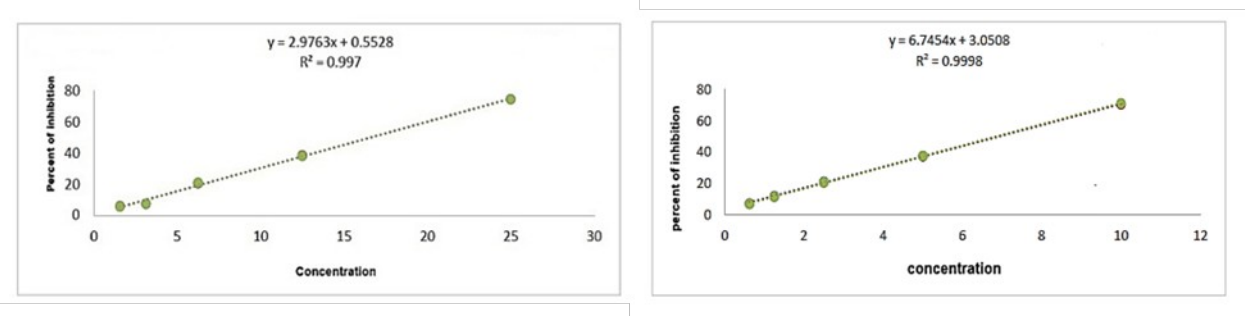

Figure 4. Linear regression graph for Vitamin C (above) and secang wood bark extract (below), using the average equation from the three obtained equations based on inhibition percentage.

is comparable to several plant-derived flavonoids and phenolic compounds reported as potential antihyperglycemic agents (Klara et al., 2023). Previous studies have shown that natural α-glucosidase inhibitors generally exhibit binding energies ranging from –6.0 to –8.0 kcal/mol, with interaction patterns dominated by hydrogen bonding to catalytic aspartate residues (Gholami et al., 2024, Wang et al., 2024). The binding profile of brazilin in the present study falls within this optimal range, supporting its relevance as a promising lead compound. Moreover, the dual activity as an α-glucosidase inhibitor and an antioxidant aligns with current therapeutic strategies that emphasize multitarget approaches for diabetes management.

CONCLUSION

Caesalpinia sappan bark extract, particularly brazilin, demonstrated promising α-glucosidase inhibitory activity and strong antioxidant capacity. Further in vitro and in vivo studies are recommended.

AUTHORS CONTRIBUTION

I.K.K. contributed to the conceptualisation of the study, literature review, molecular docking analysis, antioxidant assay, data analysis, and manuscript drafting. R.M.P. served as the principal investigator and main supervisor, contributing to the study design, research supervision, data interpretation, and critical revision of the manuscript. S.D.W. contributed to the study design, research implementation, laboratory analysis, and data validation. W.A. contributed to computational analysis, molecular docking methodology, and data interpretation. All authors read, reviewed, and approved the final manuscript.

"The authors declare that there is no conflict of interest with any party involved in this study."

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