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Activity of Kelakai Leaf Extract (*Stenochlaena palustris*) as an Antioxidant and Antidiabetic: an in vitro and in silico study

Aktivitas Esktrak Daun Kelakai (Stenochlaena palustris) Sebagai Antioksidan dan Antidiabetes: Studi In Vitro dan In Silico

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ABSTRACT

Background: Indonesia is among the countries with a high prevelance of diabetes mellitus and the number of cases continues to increase globally. The way to overcome diabetes mellitus is by using drugs that can increase antioxidants and inhibit the activity of α-glucosidase, but it may cause gastrointestinal side effects. Therefore, alternative treatments from natural sources are needed. One of the natural ingredients predicted to have antioxidant and antidiabetic activity is kelakai leaves (*Stenochlaena palustris*). This study aimed to evaluate the antioxidant and antidiabetic activities of kelakai leaf extract using in vitro and in silico approaches. **Methods:** Kelakai leaves were extracted using ethanol by maceration. Phytochemical contents were analyzed using spectrophotometric and gravimetric methods. Antioxidant activity was evaluated using the DPPH assay to determine IC_{50} values. Antidiabetic potential was assessed through molecular docking of selected bioactive compounds against α-glucosidase enzyme (PDB ID: 2QMJ) using Autodock Vina. **Results:** Kelakai leaves extract showed potential as a source of bioactive compounds, particularly in terms of antioxidant activity and potential inhibition of α-glucosidase. This is indicated by the very strong IC_{50} value of 9.384 ppm. Molecular docking analysis revealed that nictoflorin exhibited the best binding affinity (-8.9 kcal/mol), comparable to the native ligand, and formed hydrogen bonds with key amino acid residues, including Asp203, Thr205, Arg526, Thr544, and His600, indicating strong interaction with α-glucosidase active site. **Conclusions:** The potential of kelakai leaf extract as an antidiabetic is indicated by the interaction between α-glucosidase and all test compounds, as seen from the bond energy reflecting the level of affinity.

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INTRODUCTION

Diabetes mellitus (DM) is a metabolic disorder characterized by pancreatic dysfunction, which results in elevated blood glucose levels that exceed normal limits. According to the International Diabetes Federation (IDF), diabetes mellitus causes approximately 6.7 million deaths worldwide, and Indonesia has the highest number of diabetics in Southeast Asia and ranks

among the countries with the highest prevalence of diabetes worldwide. In 2021, the prevalence of diabetes in Indonesia reached 10.6%, and it is projected that this figure will continue to rise, reaching 578 million by 2030 and 700 million by 2045 (IDF Diabetes Atlas, 2021). Elevated blood glucose levels can lead to increased production of Reactive Oxygen Species (ROS) and Reactive Nitrogen Species (RNS), which may induce apoptosis in pancreatic cells, resulting in the loss of β-cells (Rajlic et al., 2023)

In medicine, diabetes mellitus can be treated with insulin injections and oral hypoglycemic agents. However, traditional medicine is often considered a viable option to minimize side effects and reduce treatment costs. Indonesia is rich in herbal plants that contain secondary metabolite compounds with potential as traditional medicines due to their biological activity. A drug target strategy that can effectively lower blood sugar levels involves the α-glucosidase drug class. Alpha-glucosidase is an enzyme that plays a crucial role in the absorption of glucose from consumed food (Dirir et al., 2022) . One plant recognized for its antidiabetic properties is the kelakai plant, which possesses antioxidant capabilities that help neutralize oxidative stress in individuals with diabetes (Hendra et al., 2022).

Kelakai leaves (*Stenochlaena palustris*) are a type of fern that possesses a crunchy texture and are commonly used in vegetable dishes in the Kalimantan region (Chear et al., 2016). Kelakai grows wild and spreads across Central Kalimantan. Traditionally, the decoction of kelakai leaves is utilized to treat various ailments, including fever, diarrhea, skin diseases, skin disorders, and stomach ulcers. Several studies on kelakai leaf extracts have demonstrated antifungal, antibacterial, antimalarial, antioxidant, and anticancer properties (Bajracharya & Bajracharya, 2022). Phytochemical analyses of kelakai leaves reveal the presence of secondary metabolites, such as polyphenols, flavonoids, hydroxycinnamic acids, tannins, alkaloids, phenols, and beta-carotene (Kusmardiyani et al., 2016; Fatmawati et al., 2022). These metabolite compounds in kelakai leaves can neutralize free radicals and inhibit the production of TNF-α (Kusmardiyani et al., 2016). However, studies evaluating the antioxidant activity of kelakai leaves together with their potential interaction with α-glucosidase using molecular docking are still limited. Therefore, this study was conducted to evaluate the antioxidant activity and molecular docking analysis of kelakai leaf extract.

MATERIAL AND METHODS

Materials

96% ethanol, kelakai leaf simplisia, 5% $NaNO_2$, 10% $AlCl_3$, 4% NaOH, chloroform, H_2SO_4, 95% methanol, 10% acetic acid, Ammonium hydroxide, n-butanol, 5% NaCl, Folin Ciocalteau reagent, 70% Na_2CO_3, distilled water, DPPH solution, Vitamin C, and α-glucosidase receptor with PDB code 2QMJ, while the natural ligand or native ligand is akarbose.

Methods

Phytochemical Analysis and Extract Preparation

Kelakai leaves (*Stenochlaena palustris*) were air-dried at room temperature away from direct sunlight and ground into powder. The dried powder (373 g) was macerated with 96% ethanol for 24 h, and the extraction was repeated until a clear filtrate was obtained. The combined filtrates were concentrated using a rotary evaporator, followed by further evaporation in a water bath at 50^{o}C to obtain a thick extract, which was stored at low temperature until further analysis (Fitriyanti *et al*., 2023).

The determination of phytochemical contents was carried out using both spectrophotometric and gravimetric methods. Total flavonoid content was determined using the $AlCl_3$ colorimetric method, while total phenolic and tannin contents were measured using Folin-Ciocalteu and Folin–Denis methods, respectively. Total terpenoid content was also analyzed using a colorimetric assay. All absorbance measurements were performed using a UV-Vis spectrophotometer at specific wavelengths according to each method, with slight modifications from standard procedures (Perez *et al.*, 2023) (Nicolescu *et al.*, 2025) (Elgailani and Ishak, 2014). Total alkaloid and saponin contents were determined using gravimetric methods involving extraction, precipitation, filtration, drying, and weighing until constant mass was obtained, following standard phytochemical procedures (Nimyel and Lori, 2023).

Antioxidant Assay

Preparation of 0.1 mM DPPH Solution

A total of 10 mg of DPPH powder was weighed and dissolved in 96% ethanol, then transferred into a 100 ml dark volumetric flask, and the solvent was added to the mark. The mixture was shaken until homogeneous. A total of 2 ml of 0.1 mM DPPH solution was transferred into a test tube and 2 ml of 96% ethanol was added. The mixture was vortexed until homogeneous and incubated at room temperature in the dark for 30 minutes. The absorbance of the test solution was then measured using a spectrophotometer at a wavelength of 517 nm (Baliyan *et al.*, 2022).

DPPH Wavelength Optimization

A total of 2 mL of 0.1 mM DPPH solution was transferred into a test tube and 2 ml of 96% ethanol was added. The mixture was vortexed until homogeneous and incubated at room temperature in the dark for 30 minutes. The absorption spectrum was determined using a spectrophotometer at a wavelength of 517 nm (Baliyan *et al.,* 2022).

Preparation of Vitamin C Solution

A stock solution of vitamin C with a concentration of 1000 ppm was prepared by weighing 10 mg of vitamin C and dissolving it in 96% ethanol. The solution was transferred into a 10 ml volumetric flask and the solvent was added to the mark. Serial concentrations of 2, 4, 6, 8, and 10 ppm were prepared. Each concentration was transferred into a volumetric flask, and 96% ethanol was added to the mark. Two milliliters of each test solution was pipetted into a test tube, 2 ml of 0.1 mM DPPH solution was added, and the mixture was vortexed until homogeneous. The solution was incubated at room temperature for 30 minutes, and the absorbance was measured using a spectrophotometer at a wavelength of 517 nm (Baliyan *et al.,* 2022).

Preparation of Kelakai Leaf Extract Solution

A stock solution of kelakai leaf extract with a concentration of 1000 ppm was prepared by weighing 10 mg of the extract and dissolving it in 96% ethanol. The solution was transferred into a 10 mL volumetric flask, and the solvent was added to the mark. Serial concentrations of 2, 4, 6, 8, and 10 ppm were prepared. Each concentration was transferred into a test tube, and 96% ethanol was added to the mark. Two milliliters of each test solution was pipetted into a test tube, 2 ml of 0.1 mM DPPH solution was added, and the mixture was vortexed until homogeneous. The solution was incubated at room temperature for 30 minutes, and the absorbance was measured using a spectrophotometer at a wavelength of 517 nm (Baliyan *et al.,* 2022).

Antioxidant Activity Assay (DPPH Method)

The antioxidant activity of kelakai leaves was evaluated using the DPPH method. The free radical used as a model to measure the radical scavenging ability was 1,1-diphenyl-2-picrylhydrazyl (DPPH). A series sample concentrations were prepared, and each solution was mixed with DPPH solution. The mixture was vortexed until homogenous and incubated at room temperature for 30 minutes in the dark. The absorbance was then measured using a spectrophotometer at a wavelength of 517 nm. Assorbic acid was used as a positive control to compare the antioxidant activity of the samples (Baliyan *et al.,* 2022).

Data Analysis of antioxidant Activity

Antioxidant activity was expressed as the percentage of inhibition. The inhibition percentage was calculated by subtracting the absorbance of the sample from the absorbance of the blank, dividing the result by the absorbance of the blank, and then multiplying by 100 percent. The relationship beetwen sample concentration and percentage of inhibition was analyzed using a linier regression equatin. The IC_{50} value, defind as the concentration required to inhibit 50 percent of DPPH radicals, was determined by substituting the inhibition value of 50 percent into the regression equation. The Antioxidant Activity Index (AAI) was calculated by dividing the concentration of DPPH (in ppm) by the IC_{50} value of the sample (in ppm) (Baliyan *et al.*, 2022).

Molecular Docking

The molecular docking in this study was conducted using the α-glucosidase receptor with PDB code 2QMJ, while the native ligand was acarbose. The hardware used for the docking process was an Intel Inside CORE i3 CPU 2.00 GHz with 4 GB RAM, and the software included Autodock Vina, Autodock Tools 1.5.7, Discovery Studio 2021, and Marvin View. The test compounds were flavonoid derivatives (trifolin, chlorogenic acid, epicatechin, kaempferol, and nictoflorin) and an alkaloid derivative (N-butylbenzenesulfonamide). These compounds were reported to be present in *Stenochlaena palustris* (kelakai) and were selected based on their phytochemical constituents (Fatmawati *et al*., 2022). The compound structure were downloaded in 2D format from the website PubChem. The 2D compounds were then converted into 3D using Marvin View and saved in pdb format. The α-glucosidase receptor was downloaded from RCSB PDB and saved in pdb format.

Preparation of Test Ligands

The test ligand preparation was performed using Discovery Studio 2021. The 2QMJ receptor, which is bound to the native ligand acarbose, first needed to be separated by removing water molecules and proteins. The remaining acarbose ligand was then saved in pdb format and used for validation or redocking. Preparation of both the native ligand and test ligands continued with Autodock Tools 1.5.7 by adding hydrogen atoms and merging non-polar hydrogens. In the ligand option, "torsion tree" was selected to remove the root; then "choose torsion" was selected, followed by "done." The torsion tree was selected again to set the number of torsions, and "dismiss" was selected. The prepared ligands were then saved in pdbqt format (Morris *et al.*, 2009) (Putri *et al.*, 2024).

Preparation of the Receptor

The α-glucosidase receptor preparation was conducted using Discovery Studio 2021. The 2QMJ receptor, initially bound to the native ligand acarbose, was separated by removing water molecules and ligands. The remaining receptor was saved in pdb format. The α-glucosidase receptor preparation continued with Autodock Tools 1.5.7 by adding hydrogen atoms, computing Gasteiger charges, and merging non-polar hydrogens. The prepared receptor was saved in pdbqt format (Morris 2009) (Putri *et al.,* 2024).

Validation and Visualization of Molecular Docking (Redocking)

The molecular docking validation was performed using Autodock Tools 1.5.7 to ensure that the docking parameters were valid before docking the α-glucosidase receptor with several test ligands. Validation was conducted by redocking the α-glucosidase receptor with the native ligand acarbose using Autodock Vina. It is essential to consider the Root Mean Square Deviation (RMSD) value, where an RMSD ≤ 2 Å indicates that the docking parameters are valid and that docking of the test compounds can proceed. Visualization was performed using Discovery Studio 2021. The parameters analyzed during visualization included amino acid residues, hydrogen bonds, and binding energy. The test ligands, namely Nicotiflorin, Chlorogenic Acid, Trifolin, Epicatechin, Kaempferol, and N-Butylbenzenesulfonamide, were subsequently docked using a validated docking protocol with a defined grid box (Morris 2009) (Putri *et al.*, 2024).

Table 1. Validated GridBox of 2QMJ

Protein	Center	Size
2QMJ	X -21.782	X 30
	Y -6.01	Y 30
	Z -5.665	Z 46

RESULTS AND DISCUSSION

The extract of kelakai leaves (*Stenochlaena palustris*) obtained using 96% ethanol as a solvent in this study had a yield value of 16.98% (Table 2). A high yield value indicates that the active compounds in the extract are also high (Hasnaeni, Wisdawati, & Asman., 2019). According to Esati et al., 2022, a yield value is considered good if it exceeds 10% (Esati et al., 2022). This indicates that the active compounds in the kelakai leaf extract were effectively extracted by the solvent. The use of 96% ethanol as a solvent in the maceration method is due to its polarity,

which allows for the extraction of more active compounds from plants compared to water and methanol. Additionally, 96% ethanol has a higher solvent capacity than other concentrations and can penetrate plant cell membranes (Dewatisari, 2020).

Table 2**.** Results of kelakai leaf (*Stenochlaena palustris*) extraction

Initial weight	Dry Weight	Simplisia Weight	Filtrate Result	Concentrated Extract	Extraction Yield
5 kg	2,05 kg	373 g	500 ml	63,35 g	16,98%

The quantitative test of active compound content in kelakai leaf extract can be seen in Table 3, which shows that the extract contains alkaloids, saponins, flavonoids, terpenoids, phenolics, and tannins, with terpenoids having the highest concentration at 40,38%. This result aligns with the research by Syamsul et al., 2019, which also found alkaloids, flavonoids, tannins, saponins, and terpenoids in kelakai leaf extract (Syamsul et al., 2019). Furthermore, this study is consistent with Fatmawati et al., 2022, which showed positive results for flavonoid, alkaloid, and terpenoid content in kelakai leaf ethanol extract (Fatmawati et al., 2022).

Table 3. Quantitative analysis results of kelakai leaf (*Stenochlaena palustris*)

No.	**Compounds (mg/ml)**	**Percentage (%)**
1.	Alkaloids	11,34
2.	Saponins	11,81
3.	Flavonoids	6,80
4.	Triterpenoids	40,38
5.	Phenolics	1,39
6.	Tannins	0,05

The antioxidant activity of kelakai leaf extract was measured using the DPPH (1,1-diphenyl-2-picrylhydrazyl) method at a wavelength of 517 nm. The presence of antioxidant activity in the sample caused a color change from deep violet to yellow. The antioxidant activity using the DPPH method is expressed as the 50% Inhibition Concentration or IC_{50}. A smaller IC_{50} value indicates higher antioxidant activity (Retno Sari, 2023). The calculated IC_{50} value showed that kelakai leaf extract had the highest and very strong antioxidant activity with an IC_{50} value of 9.384 ppm, while the IC_{50} value of vitamin C used as a comparison was 3.805 ppm (Table 4). Based on this data, kelakai leaf extract demonstrates antioxidant activity, although it is still lower than the standard (vitamin C). According to Simorangkir et al., 2019, antioxidant activity with an IC_{50} value of less than 50 ppm is categorized as very strong (Moniung et al., 2022).

Table 4. Antioxidant activity assay results of kelakai leaf extract (*Stenochlaena palustris*)

Sample	Concentration (ppm)	Abs.	%Inhibition	IC_{50} (ppm)	AAI
Vitamin C	2	0.361	30.04	3.805	26.28
	4	0.238	53.88		
	6	0.149	71.12		
	8	0.04	92.25		
	10	0.028	94.57		
Extract *Stenochlaena palustris*	2	0.435	15.70	9.384	10.65
	4	0.42	18.60		
	6	0.31	39.92		
	8	0.271	47.48		
	10	0.266	48.45		

The antioxidant activity of kelakai leaf extract is related to the secondary metabolites contained within the extract. The alkaloids, saponins, flavonoids, and triterpenoids content, in that order, are secondary metabolites with the highest antioxidant activity in the kelakai leaf extract (Table 3). Alkaloids can act as antioxidants by reducing the activity of H_2O_2. Additionally, alkaloids can inhibit protein synthesis from NADPH-oxidase (NOX) and p47phox or p40phox. Some alkaloid derivatives can also inhibit protein kinase C, though not all derivatives have this activity. Alkaloids also promote the translocation of the transcription factor Nrf2, associated with increased FOXOs and PPARs. FOXOs increase the expression of mitochondrial superoxide dismutase (SOD2) and catalase, while PPARα induces the expression of cytosolic Cu/Zn superoxide dismutase (SOD1), which reduces NADPH-oxidase activity. PPARγ also increases the expression of SOD1/SOD2 and catalase (Macáková et al., 2019).

Terpenoids, another secondary metabolite, can increase the expression of antioxidant enzymes such as glutathione peroxidase, which helps protect cells from oxidative damage. Terpenoids also act as antioxidants by improving the function of pancreatic β-cells to secrete insulin and reducing oxidative stress through the regulation of Nrf2 signaling (Song et al., 2022). According to the study by Song et al., 2022, the Terpenoid-Rich Extract of Dillenia indica (TRDI) showed an IC_{50} value of 9.76 ± 0.50 µg/mL, demonstrating that terpenoids exhibit antioxidant activity through hydrogen atom transfer and single-electron transfer mechanisms. Other research has shown that the antioxidant activity of terpenoids can prevent oxidative stress by increasing SOD, GST, non-enzymatic (GSH), decreasing lipid peroxidation, and increasing ATPase activity (Agliassa & Maffei, 2018).

Another secondary metabolite in kelakai leaf extract is flavonoids. Flavonoids function as antioxidants by increasing glutathione, GPx, GST, and SOD activity, and reducing lipid

peroxidation in the liver (Yi et al., 2023). According to Sharma et al., 2019, one flavonoid derivative, kaempferol, reduces nephropathy in diabetes through antioxidant activity by inhibiting RhoA/Rho Kinase (Sharma et al., 2019). Additionally, saponins also possess antioxidant activity by inhibiting Nitric Oxide (NO) radicals. Previous study showed that the saponin content in D. basuticus extract had strong scavenging activity in inhibiting NO activity, as indicated by an IC_{50} value of 3.31 mg/ml compared to the standard (quercetin) IC_{50} of 3.67 mg/ml (Chester et al., 2019).

Molecular docking was performed to predict the active compounds in kelakai leaf extract that may inhibit α-glucosidase in diabetic patients. α-glucosidase is an enzyme in the digestive tract that breaks down carbohydrates into glucose. One approach to managing diabetes mellitus is using drugs that inhibit α-glucosidase activity, but this can cause side effects, particularly in the digestive tract. Therefore, alternative treatments are needed to lower blood glucose levels in diabetes mellitus, and natural compounds are being explored as potential inhibitors of α-glucosidase. One natural compound predicted to inhibit α-glucosidase is kelakai leaf extract. The α-glucosidase structure used in this study has a PDB code of 2QMJ, selected due to the absence of mutations and its crystal structure from X-ray diffraction with a resolution of 1.9Å and a good resolution is less than 2Å (Assefa et al., 2020). Protein preparation was carried out using BIOVIA Discovery Studio 2021 software to separate α-glucosidase from acarbose, its native ligand. The separation of acarbose from α-glucosidase was necessary to obtain the active site pocket for the docking simulation. Additionally, water molecules were removed to ensure that only amino acid molecules remained, allowing for stronger interactions between the test ligand and the protein. The 3D structure of the separated acarbose and α-glucosidase ligands is shown in Figure 1.

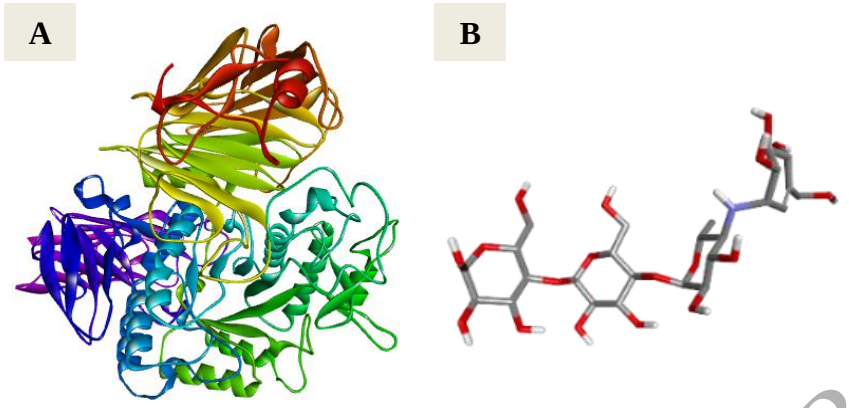

Figure 1. α-glucosidase receptor (A) and Acarbose ligand (B)

The separated native ligand was then prepared for use in the validation or redocking process. The ligand preparation involved the addition of hydrogen atoms to provide partial charges to the ligand molecule (Hartanti et al., 2022). The hydrogen-added compounds were saved in a. pdbqt file and determined based on the number of rotatable bonds. The more rotatable bonds a ligand has, the more flexible it becomes. The. pdbqt file shows that the ligand molecule already has partial charges on each of its atoms. The test ligands used in this study were trifolin, chlorogenic acid, epicatechin, kaempferol, nictoflorin, and n-butylbenzenesulfonamide (Fatmawati et al., 2022). These test ligands were selected based on previous research by Fatmawati et al., who analyzed the ethanol extract of *Stenochlaena palustris* leaves using LCMS. The selection of test compounds was based on the prepared ligand structures, as shown in Figure 2.

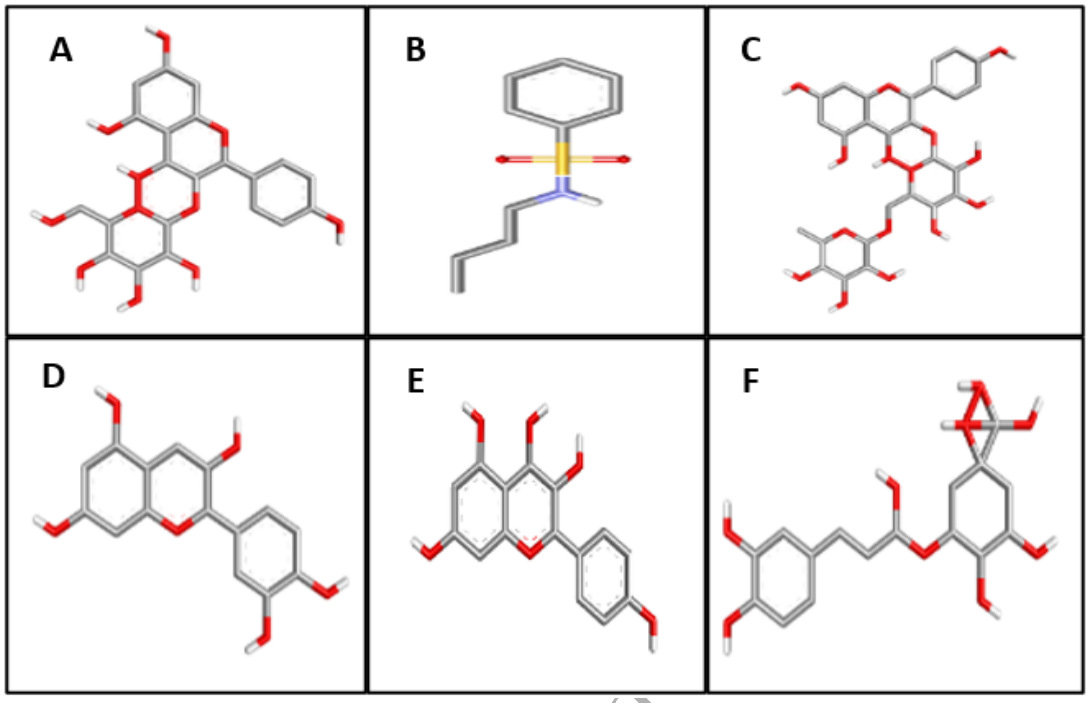

Figure 2. Nictoflorin (A), N-Butylbenzenesulfonamide (B),Trifolin (C), Epicatechin (D), Kaempferol (E), Chlorogenic Acid (F)

Validation or redocking was conducted to ensure that the method used was accurate before proceeding to the next stage, the docking process with the test ligands. The grid box position used for redocking with α-glucosidase was x center=-21.764, y center=-6.546, and z center=-5.222, while the grid box dimensions were size_x=15, size_y=15, and size_z=17.25, centered on the enzyme's active site. The grid box parameters were set to allow the ligand enough space to rotate and find the active site. The success of this validation was determined by the Root Mean Square Deviation (RMSD) value, which measures how close the docking results are to the known experimental or reference structure. The lower the RMSD value, the more accurate the docking prediction. The validation was considered successful if the RMSD value was <2Å (Assefa et al., 2020). In this experiment, the RMSD value obtained was 1.7838 Å, indicating that the method used was valid and could proceed with the test ligand experiments. The validation results showed that the inhibitor's binding position did not significantly change compared to the position before the validation process. The 2D and 3D visualizations of the interactions between acarbose and α-glucosidase are shown in Figure 3.

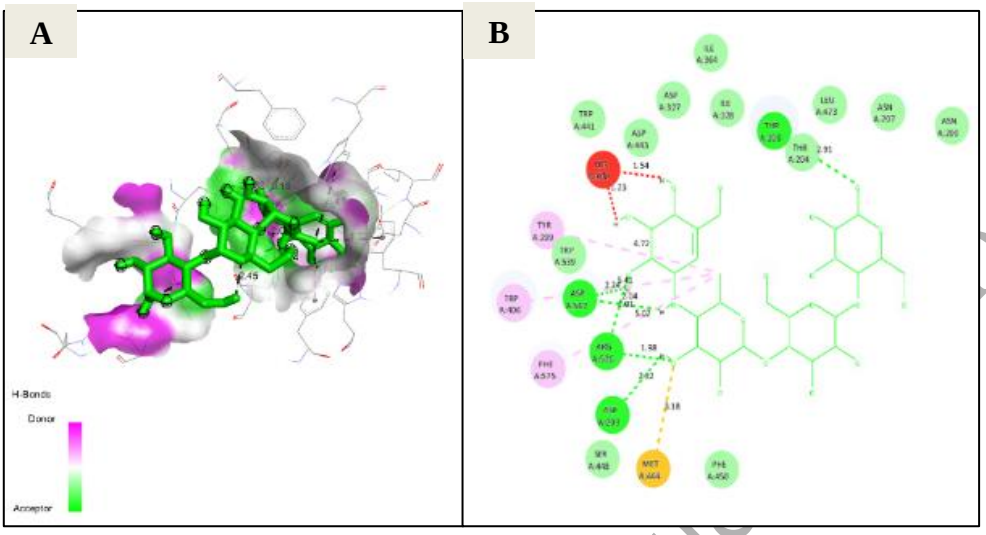

Figure 3. 2D visualization of the interaction between the α-glucosidase receptor and acarbose (A), and 3D visualization of the interaction between the α-glucosidase receptor and acarbose (B)

The redocking results revealed hydrogen bonds with the involved amino acids, including Asp203, Asp327, Asp443, Asp542, Met444, Arg526, Thr205, Thr544, His600, and Trp406. Subsequently, docking with the test ligands was performed using the coordinates or grid box validated earlier, as these coordinates represent the location of native ligand interaction with α-glucosidase. The docking results for the test ligands and α-glucosidase receptor yielded binding energies and hydrogen bonds. The binding energy reflects the affinity between the test compound and the α-glucosidase receptor. The lower the binding energy, the more stable the interaction between the protein and the ligand (Assefa et al., 2020). According to the docking data, none of the test compounds demonstrated a lower binding energy than the native ligand (Table 5). However, the nictoflorin compound showed a binding energy close to that of the native ligand, at -7.7 Kcal/mol. This result suggests that nictoflorin has the highest affinity for binding to the active site of the α-glucosidase receptor compared to the other test ligands.

Table 5. Docking results of the test compounds from kelakai leaf extract (*Stenochlaena palustris*) against α-glucosidase

No	Compounds	ΔG (Kcal/mol)	Hydrogen Bod	Distance of H-Bond	Other Bond Residues
1.	Akarbose	-7.7	Asp203 Asp 327 Met 444 Arg 526 Asp 542	2.31 Å 1.65 & 1.69 Å 3.07 Å 2.86 Å 1.94 & 1.87 Å	Thr 205, Tyr 299, Asp 443, Trp539, Phe 575
2.	Nicotiflorin	-8.9	Asp203, Thr205, Arg526, Thr544, His600	2.36 Å 2.48 & 2.98 Å 3.09 Å 2.69 Å 2.34 Å	Trp441, Trp539, Asp542, Phe575, Ala576
3.	Chlorogenic Acid	-7.4	Asp203, Asp443, Asp542.	2,55 & 2.56 Å 2.07 Å 2.05 & 2.38 Å	Tyr 299, Phe 575
4.	Trifolin	-7.4	Asp203, Asp443, Met444 Arg526.	2.84 Å 2.23 Å 2.77 Å 1.77 Å	Tyr 299, Trp406, Asp 542, Phe 575,
5.	Epicatechin	-7.0	Asp443, His600.	2.35 Å 2.54 Å	Tyr299, Trp 406, Asp 443, Phe450, Asp542, Phe575
6.	Kaempferol	-7.1	Asp203, Asp327.	2.54 Å 1.87 Å	Trp406, Asp443, Met444, Asp542, Phe 575.
7.	N-Butylbenzenesulfonamide	-5.3	Asp327, Trp 406.	2.11 Å 2.36 Å	Tyr 299, Trp 406, Trp441, Asp443, Met444, Trp539, Asp 542, Phe575, His600

The variation in binding energies can be attributed to the different types and numbers of bonds formed between the ligand and receptor, including van der Waals interactions, hydrogen bonds, electrostatic interactions, and hydrophobic interactions (Nayeem et al., 2021). The interaction between the nictoflorin ligand and the receptor is visualized in Figure 4.

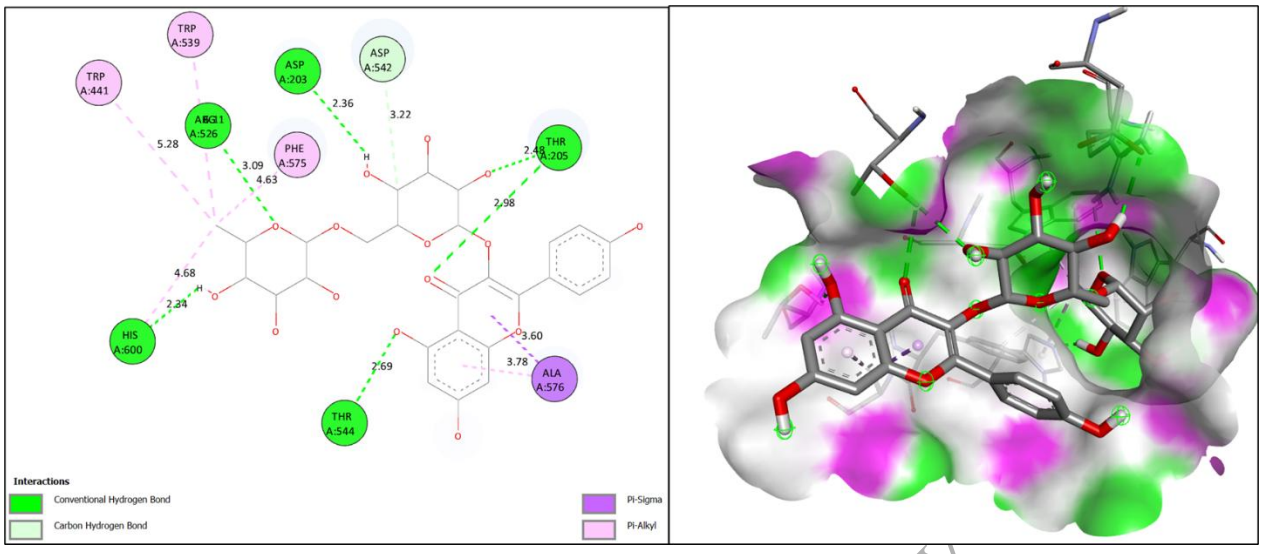

Figure 4. 2D visualization of the interaction between the α-glucosidase receptor and nictoflorin (A), and 3D visualization of the interaction between the α-glucosidase receptor and nictoflorin (B)

Furthermore, Table 5 shows that nictoflorin interacts with α-glucosidase through bonds with amino acids, including Asp 203, Thr 205, Arg 526, Thr 544, and His 600. These interactions are similar to those between α-glucosidase and acarbose. This indicates that the binding pocket or active site of both the native ligand and nictoflorin to α-glucosidase is similar, suggesting a comparable affinity for inhibiting α-glucosidase activity. Nictoflorin is a derivative of flavonoid compounds. The docking results in this study are consistent with previous research indicating that flavonoids have an inhibitory effect on α-glucosidase activity (Nayeem et al., 2021).

CONCLUSION

The extract of kelakai leaves (*Stenochlaena palustris*) demonstrates potential as a source of bioactive compounds, particularly in the context of antioxidant activity and α-glucosidase inhibition potential. This is evidenced by a strong IC_{50} value of 9.384 ppm. The antidiabetic potential of kelakai leaf extract is shown through the interaction between α-glucosidase and all tested compounds, as reflected by binding energy values, which indicate the level of affinity. The best result was found with the compound nictoflorin, which had a binding energy value close to that of the native ligand, at -7.7 Kcal/mol. Nictoflorin also exhibited interactions with the same amino acids as α-glucosidase, including Asp 542, Phe 575, Tyr 299, Trp 406, His 600, and Met 444.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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