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In Vitro and *In Silico* Assessment of Methanol Extract from *Moringa oleifera* Seeds as α-Amylase Inhibitor

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Abstract

Diabetes mellitus (DM) is a metabolic disorder characterized by hyperglycemia, posing a global health threat. Current diabetes treatments often rely on synthetic drugs with side effects, prompting the search for safer natural alternatives. This study **i**nvestigated the secondary metabolic compounds in methanol extract from *M. oleifera* seeds and evaluated their antidiabetic activity. The α-amylase enzyme was analyzed to determine optimal incubation time, pH, and temperature. The antidiabetic activity was assessed via α-amylase inhibition using in vitro and in silico methods. Phytochemical screening revealed the presence of flavonoids, alkaloids, and tannins in the extract. Optimal enzyme conditions were 30 minutes of incubation, pH 6.9, and 25 °C. The extract showed the highest activity at 15% concentration with 67.94% inhibition and an IC50 of 15.38%, compared to acarbose with 41.76% inhibition and an IC50 of 17.89%. In silico analysis indicated that 9-octadecanoic acid (Z) methyl ester had a lower inhibition constant and binding energy (2.67 mM and -3.51 kcal/mol) than acarbose (15.72 mM and -2.46 kcal/mol), suggesting a higher enzyme affinity. These findings suggest that *M. oleifera* seeds contain compounds with potential as antidiabetic agents.

Keywords: Antidiabetic, Methanol Extract, Moringa oleifera Seeds, α-Amylase

INTRODUCTION

Diabetes mellitus (DM) is a disorder that occurs when the pancreas produces insulin in small or insufficient amounts. This condition causes an increase in blood glucose levels and is often called hyperglycemia. The disease affected up to 23.33 million people in Indonesia in 2021 and is projected to affect 28.57 million people by 2030 (Nugrahani, 2024). Appropriate treatment of this disease is necessary to avoid organ dysfunction and failure, which in diabetes patients causes several secondary complications such as neurodegenerative diseases, neuropathy, nephropathy, retinopathy, and cardiovascular disease (Ogunyemi et al., 2022).

One strategy for treating diabetes is to limit the activity of digestive enzymes in the intestinal tract. Alpha-amylase is an enzyme that hydrolyzes α-D-(1,4)-glycoside bonds in polysaccharide carbohydrates (starch) to produce maltose, which is then further hydrolyzed by the α-glycosidase enzyme to produce glucose, which is absorbed into the hepatic portal vein through the small intestine (Li et al., 2021). The use of α-amylase inhibitors to slow the final stages of carbohydrate metabolism is considered a safe treatment for hyperglycemia. However, synthetic and chemical inhibitors have side effects such as stomachaches, diarrhea, bloating, and other digestive problems (Gong et al., 2020). Due to its milder side effects, natural α-amylase inhibitors are considered a better option for treating diabetes.

The *M. oleifera* plant has been documented as one of the most important herbal plants because of its enormous medicinal and non-medicinal benefits. Traditionally, this plant has been used to heal wounds, inflammation, gastritis, liver disease, heart disease, and cancer (Pareek et al., 2023). Almost all parts of this plant, including the seeds, pods, flowers, and leaves, have medicinal benefits, so they have been widely researched for medicinal purposes. According to a study, the leaves have antimicrobial and hypoglycemic activity, the flowers have anti-inflammatory activity, and the seeds have antihypertensive and liver-protective properties(Al-Malki & El Rabey, 2015; Salimi, Bialangi, Abdulkadir, & Parulian, 2019).

Moringa oleifera leaf extract is widely studied for its anti-diabetic properties. For example, Anwer et al. (2021) found that treatment with *M. oleifera* leaf extract at doses of 100, 200, and 400 mg/kg significantly reduced blood sugar levels in male Wistar rats. Additionally, Ogundipe et al. (2022) have also reported α-amylase inhibitory activity from *M. oleifera* leaf extract with an IC_{50} value of 0.1802 mg/mL. The compounds contained in *M. oleifera* leaf extract that enable this effect to occur are saponins, flavonoids, phenols, tannins, and alkaloids.

The majority of research on M. oleifera plants currently focuses only on the leaves, although *M. oleifera* seeds contain a metabolite composition that is similar to that of the leaves in terms of both quantity and quality (Arora & Arora, 2021). Therefore, the objective of this study is to identify secondary metabolite compounds of methanol extract from *M. oleifera* seeds and evaluate their inhibitory activity against α-amylase using in vitro and in silico methods.

METHODOLOGY

Materials and Instrumentals

The materials used in this study were *M. oleifera* seeds, methanol (Merck), H_2SO_4 (Merck), NaOH (Merck), HCl (Merck), enzyme α-amylase (Sigma-Aldrich), DNS powder (Merck), Na_2HPO_4 (Merck), aquades, phosphate buffer, DMSO, starch, and acarbose. The instruments used in this study were a rotary evaporator, oven, desiccator, T60 UV-Vis spectrophotometer (Shimadzu-Japan), and GC-MS QP2010S (Shimadzu-Japan).

Methods

The research methodology includes sample preparation and extraction, phytochemical screening, GCMS analysis, α-amylase enzyme activity test, incubation time determination, pH and optimal temperature of α-amylase enzyme, antidiabetic activity test of methanol extract from *M. oleifera* seeds with in vitro test and in silico test.

EXPERIMENT

Sample Preparation and Extraction

The seeds were ground and sieved using a 60-mesh sieve after being dried in the oven for 6 hours at 30 ºC. Seed powder (50 g) was macerated in methanol with a ratio of 1:10 (w/v), then allowed to stand and stir for 2x72 hours at room temperature. The mixture was filtered, and the solvent was evaporated using a rotary evaporator to obtain a thick methanol extract (Olivia, Umeh, & Ogugofor, 2021).

Phytochemical Screening

Phytochemical screening was carried out to determine the presence of secondary metabolites such as alkaloids, flavonoids, saponins, and terpenoids in methanol extracts of *M. oleifera* seeds using procedures as described (Hasanela, Bandjar, Yanyaan, & Hitijahubessy, 2023; Kafelau et al., 2022; Purwaningsih, Fathiah, Amaliyah, & Kuswiyanto, 2023).

GCMS Analysis

Methanol extract of *M. oleifera* seeds was analyzed using GC-MS under conditions (oven temperature 80–250 °C, pressure 60 kPa, total rate 6.0 mL/min, column rate 0.94 mL/min, linear velocity 35.7 cm/second, purge flow 3.0 mL/minute, and split ratio - 1.0 following the standard working parameters of the instrument). Compound determination was carried out by matching the molecular weights and fragmentation patterns of the compounds in the methanol extract with the compounds in the GC-MS system library (Taiyeb et al., 2024).

The α-Amylase Enzyme Activity Test

The procedure for measuring α-amylase enzyme activity has been modified from that described by Dike et al. (2022). A reaction tube containing 120 µL of phosphate buffer pH 6.8 and 180 µL of enzyme was vortexed and incubated for ten minutes at 20 ºC. The mixture was added with 1% starch substrate (300 µL) and incubated again for 10 minutes, then 300 µL of DNS was added to end the reaction. The mixture was vortexed and soaked for 10 minutes in boiling water. After the mixture reached room temperature, 2700 µL of distilled water was added, and the absorbance was measured using a UV-Vis spectrophotometer at a wavelength of 540 nm. Blanks were prepared without the addition of the α-amylase enzyme.

Determination of Incubation Time, pH, and Optimum Temperature of the α-Amylase Enzyme

The procedure for determining optimum enzyme activity has been modified from that described by (Hasnah Natsir et al., 2022). Enzyme activity was measured at various incubation times (10; 20; 30; and 40 minutes), pHs (6.4; 6.8; 6.9; 7; and 7.4), and temperatures (20, 25, 30, 37, and 40 °C).

Antidiabetic Activity Test of Methanol Extract from *M. oleifera* seeds

In Vitro Test

The procedure for the *in vitro* test had been modified from that described by Ahmed et al. (2020).

Extracts were prepared at concentrations of 5%, 10%, and 15% using DMSO solvent. Extracts with various concentrations were added to each reaction tube containing 0.5 mL of phosphate buffer pH 6.8 and 0.5 mL of enzyme, then vortexed and incubated at 25 ºC for 30 minutes. The mixture was added with 1% starch substrate (0.5 mL) and incubated again at 25 ºC for 30 minutes, then 1 mL of DNS was added to end the reaction. The mixture was vortexed and soaked for 10 minutes in boiling water. After the mixture reached room temperature, 1 mL of distilled water was added, and the absorbance was measured using a UV-Vis spectrophotometer at a wavelength of 540 nm. Blanks were prepared without the addition of the α-amylase enzyme. Positive controls (acarbose) and negative controls were prepared using the same procedure by replacing the extract with DMSO. Calculation of % enzyme inhibition using equation (1).

$$\% \text{ inhibition} = \frac{\text{blank absorbance-sample absorbance}}{\text{blank absorbance}} \text{ X } 100\ \% \quad (1)$$

The IC_{50} value was calculated using the linear regression equation, with the sample concentration as the x-axis and % inhibition as the y-axis. Based on the equation y=ax+b, the IC_{50} value can be calculated using equation (2).

$$IC_{50} = \frac{50-b}{a} \quad (2)$$

***In Silico* Test**

Chemical compounds that show inhibitory activity against the α-amylase enzyme were selected from the data screening of the GC-MS results. The next step is to download the α-amylase macromolecule in pdb format from the Protein Data Bank and the ligand in pdb format from PubChem (https://pubchem.ncbi.nlm.nih.gov) to prepare the protein structure and ligand structure. Grid box determination was carried out between the active site of the α-amylase enzyme which interacts with the ligand using AutoDock Tools (Danova et al., 2023). Molecular docking in AutoDock Vina was performed after saving the ligands and proteins in pdbqt format. The 3D structure was visualized using the Discovery Studio Visualizer (DSV) (Sulfahri, Arif, Iskandar, & Wardhani, 2019).

RESULTS AND DISCUSSION

Phytochemical Screening

Moringa oleifera seeds were prepared into powder as presented in Figure 1 to optimize the solvent's contact with the sample during the extraction process and facilitate the solvent's easier trapping of the chemicals present in the sample.

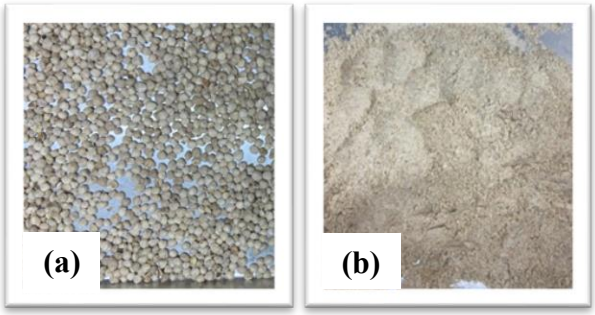


Figure 1. (a) *M. oleifera* seeds and (b) *M. oleifera* seed powder

The maceration extraction method was chosen in this study because it preserves active substances that are not heat-resistant, stabilizes the concentration of compounds in Moringa seeds, and prevents damage due to extraction procedures involving heat. Methanol was selected as a solvent because of its ability to dissolve almost all organic compounds, including polar and semi-polar compounds. Methanol attracts polar and non-polar substances due to its hydroxyl and methyl groups. In addition, the volatile properties of methanol allow the solvents in the extract to evaporate easily without damaging the chemical components it contains (Mohd Daud et al., 2021).

Figure 2. Methanol extract of *M. oleifera* seeds

The maceration process was carried out for 2 x 72 hours while stirring to accelerate the contact between the sample and the solvent. The process of maceration by repetition (re-maceration) will be more efficient compared to single maceration. This is possible because a large number of active compounds in the sample are still left behind from the first maceration process, so the extraction result obtained is more optimal. The filtrate from the maceration process is concentrated using a rotary evaporator to produce a thick extract (Figure 2). In this study, the yield of the extract obtained was 26.4%. This result is higher when compared to other studies in which (Kiswandono,

2011) obtained a methanol extract marinade from moringa seeds of 5.26% by maceration method and 6.16% by reflux method. The difference in extract yield values is most likely influenced by the type of solvent and the extraction method used.

Phytochemical Screening

The results of phytochemical screening revealed the presence of alkaloid compounds, flavonoids, and tannins in the methanol extract of *M. oleifera* seeds (Table 1). The presence of various secondary metabolites in the plant plays a role as a defense mechanism against many diseases by microorganisms and affects the medicinal properties of herbs. H. Natsir et al., (2019) have studied the health benefits of this plant and found that methanol extract from its leaves has antidiabetic properties and contains compounds that are secondary metabolites, including flavonoids, saponins, tannins, and alkaloids. The majority of these compounds were also present in the extract from *Moringa* seeds. This suggests that there are similarities between the compounds present in the *Moringa* leaves and seeds.

The results obtained from the research carried out are shown in Table 1.

Table 1. Phytochemical Screening

Compounds	Reagent	Results of Observations
Alkaloids	Dragendroff Mayer	Positive
Flavonoids	Mg powder + HCl	Positive
Steroids/terpenoids	Lieberman Buchard	Negative
Tannins	$FeCl_3$	Positive
Saponin	Aquadest	Positive

GC-MS Analysis

Identification of compounds from methanol extracts of *M. oleifera* seeds was performed using mass spectrometric gas chromatography. This method was used to identify single-component and mixed components. Mass spectrophotometers were used to determine fragmentation, and molecules, and identify components in small quantities. The chromatogram of the methanol extract in Figure 3 showed 47 peaks, which represented the presence of 47 metabolite compounds. The diversity of phytochemical compounds in plant extracts is closely related to their bioactivity. The main compounds with the highest concentrations play an important role in the activity of medicinal plants (Hasnah Natsir et al., 2022).

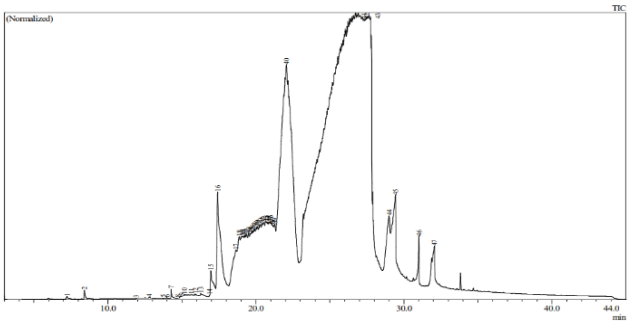


Figure 3. GC-MS chromatogram of methanol extract of *M. oleifera* seeds

The chromatogram of the methanol extract gives the three highest peaks, where each peak represents the presence of a particular compound. For example, peak 41 with a retention time of 26.736 minutes and a concentration of 44.98% represents the compound *9-Octadecenoic Acid (Z) 9-Hexadecenyl Ester*. Peak 40, with a retention time of 22.065 minutes and a concentration of 13.92%, represents the compound *9-Octadecenoic Acid (Z), Methyl Ester*. Further at the peak 43, with a retention time of 27.653 minutes and a concentration of 11.72% represents the compound *9-Octadecenoic Acid (E).*

The compound *9-octadecenoic acid, methyl ester* was found in methanol extract of black betel leaves at a concentration of 7.02%. This compound is an important compound useful as a perfume ingredient, and it has antioxidant, anti-cancer, and antidiabetic properties (Maharani & Fernandes, 2021). Figure 4 presents the mass spectrum of *9-octadecenoic acid, a methyl ester* compound that was identified in the methanol extract of *M. oleifera* seeds.

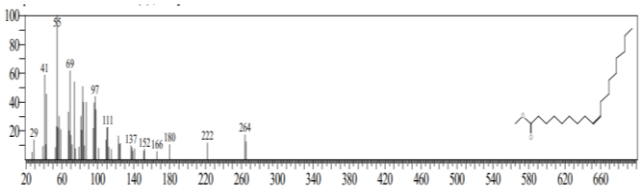


Figure 4. Chromatogram of 9-octadecenoic acid (Z)-methyl ester compound

The α-Amylase Enzyme Activity

Effect of Incubation Time on α-Amylase Enzyme Activity

The activity of the α-amylase enzyme was measured at variations in incubation time (10; 20; 30; and 40 min) to determine its optimal incubation time. In this study, the optimal incubation time of the enzyme was achieved after incubation for 30 min with an activity of 2.56 U/ml as presented in Figure 5. These

results were consistent with a study by Paul et al. (2017), who found that the enzyme α-amylase worked optimally after an incubation time of 30 minutes.

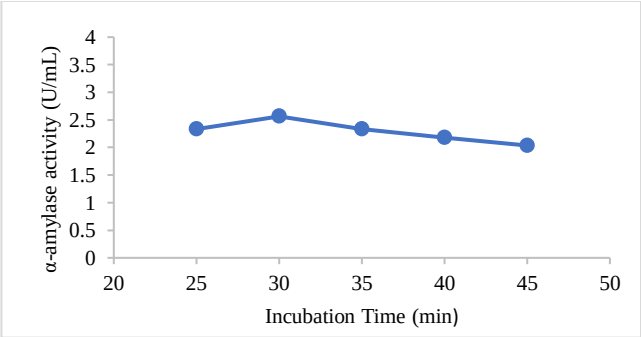


Figure 5. Effect of incubation time on α-amylase activity (temperature 25 °C, pH 6.8, 1% substrate)

Effect of pH on α-amylase Enzyme Activity

The condition of environmental pH strongly influences enzyme activity because charge distribution on enzyme molecules and substrates depends on substrate binding and catalyst The results of measurements of α-amylase enzyme activity at various pHs (6.4; 6.8; 6.9; 7 and 7.4) were presented in Figure 6.

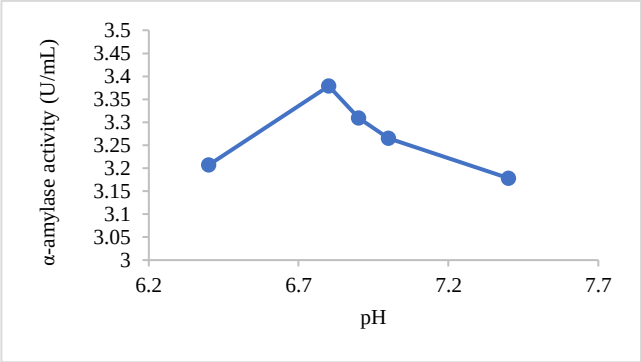


Figure 6. Effect of pH on α-amylase activity (temperature 25 °C, incubation time 30 min, 1% substrate)

The highest activity of the α-amylase enzyme is achieved at pH 6.8, with a value of 3.38 U/ml. The specific activity of α-amylase increases drastically at pH 6.8 and then decreases at pH 6.9–7.4. According to a study by Sharif et al. (2023), α-amylase has an optimal pH of 6.9 and enzyme activity of 4.4 U/ml. These findings are in line with that study. Activity increases more when approaching a neutral state. Enzyme activity will be optimal when its two charges are in balance and denatured when it is far from its optimum pH, since changes in enzyme structure affect enzyme activity.

Effect of Temperature on α-Amylase Enzyme Activity

The activity of the α-amylase enzyme was measured at various temperatures (20, 25, 30, 37, and 40 °C) to determine its optimum temperature. In this study, the enzyme α-amylase worked optimally at 25 °C with an activity of 5.89 U/ml. The enzyme however shows its catalytic ability above 25 °C, although it decreases slightly as presented in Figure 7.

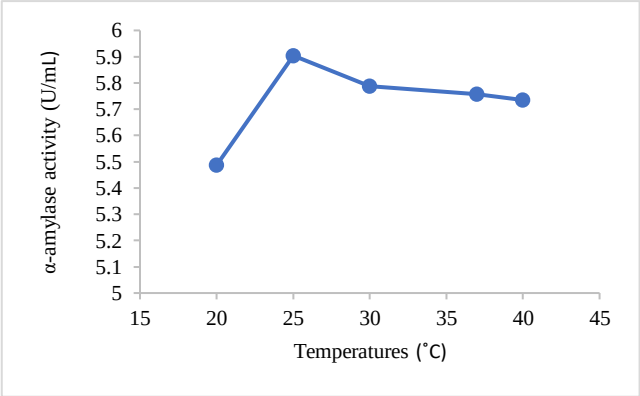


Figure 7. Effect of temperature on α-amylase activity (pH 6.9, incubation time 30 min, 1% substrate)

In Vitro α-Amylase Enzyme Inhibitory Activity

The most active extracts were shown to have the highest percent of inhibition and the lowest IC_{50} values. Sample testing was started by calculation of the inhibitory percent of each concentration of methanol extracts and acarbose as positive controls. The value of percent inhibition indicates the ability of the extract in a given concentration to inhibit the activity of the enzyme α-amylase and for the IC_{50} value was determined through linear regression, where the x-axis represents the sample concentration and the y-axis represents the % inhibition. The results of measurements with the UV-Vis Spectrophotometer at 540 nm wave was shown the absorbance of extracts and acarbose in Table 2.

Table 2. UV-Vis Spectrometer Absorbance Results, % Inhibition, and IC_{50} Value of Methanol Extracts and Acarbose

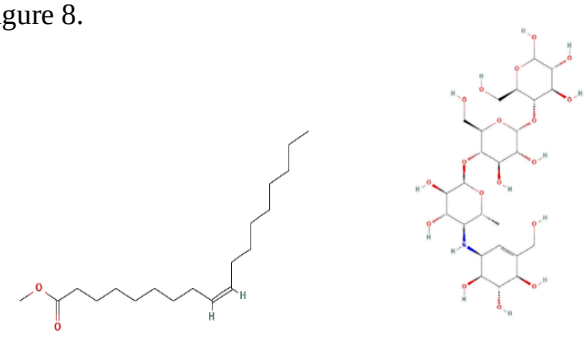
Concentration (%)	Methanol Extract of *M. Oleifera* Seeds			Acarbose		
	Absorbance	% Inhibition	IC_{50} (%)	Absorbance	% Inhibition	IC_{50} (%)
5	0.219	35.58		0.293	13.82	
10	0.152	55.29	15.38	0.251	26.17	17.89
15	0.109	67.94		0.198	41,76	

The results of the calculation of the percent inhibition showed that methanol extract at a concentration of 15% had a higher value of inhibition

percentage (67.94%) than acarbose (41.76%) at the same concentration. The IC_{50} values for methanol and acarbose extracts were 15.38% and 17.89%, respectively. Based on these results, *M. oleifera* seed methanol extract had α-amylase enzyme inhibitory activity, which indicated its potential to reduce blood sugar levels. (Shanak et al., 2021). Despite acarbose possessing more active functional groups, which theoretically should enhance its binding and inhibition of α-amylase through extensive hydrogen bonding and interactions, 9-octadecanoic acid (Z) methyl ester exhibits higher inhibition efficiency. This can be explained by the significant role of hydrophobic interactions and molecular flexibility. The long hydrophobic tail of 9-octadecanoic acid (Z) methyl ester interacts favorably with the hydrophobic regions of the enzyme, providing a more stable inhibitor-enzyme complex. Additionally, its simpler structure allows for better conformational adaptation to the enzyme's active site. These factors, along with favorable inhibition kinetics, contribute to its higher inhibition percentage, demonstrating that effective inhibition can arise not only from the number of active functional groups but also from the overall molecular dynamics and interactions within the enzyme's active site (Badaya & Sasidhar, 2020).

In Silico α-Amylase Enzyme Inhibitory Activity

In this study, the methanol extract of *M. oleifera* seeds was indicated as an antiabetic by inhibiting the enzyme α-amylase. A study reported that 9-octadecanoic acid (Z) methyl ester was identified as the main compound in moringa. It was then used as a ligand molecule. The chemical structure of the ligand was taken from the PubChem database (https://pubchem.ncbi.nlm.nih.gov), as presented in Figure 8.



(a) **(b)**

Figure 8 (a) Chemical structure of 9 octadecanoic acid compounds (Z) methyl ester and (b) Chemical structure of acarbose

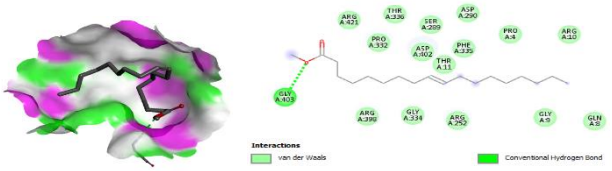
All ligands were downloaded and stored as sdf extensions. then the AutoDock Tools program was applied to convert the sdf file to pdbqt. As a target molecule, the enzyme α-amylase was assigned as a receptor because of its role in glucose inhibition and preventing diabetes. Receptor structures were obtained from protein data banks (PDB ID: 3OLE). Polar hydrogen and Kollman's combined atomic charge were added to the receptor using *AutoDock Tools 1.5.6*, and then α-amylase enzymes were stored in pdbqt format.

Table 3. Conformation Results and RMSD Values

Conformation	RMSD (Å)
1	1,66
2	1,35
3	1,35
4	1,67
5	1,38
6	1,36
7	1,65
8	1,68
9	1,69
10	1,35

Standard ligands (PCA) are inserted into proteins (PDB: 3OLE) to find protein binding sites. Results from docking simulations give 10 conformations which are presented in Table 3. All conformations had RMSD values below 2 Å. Successful redocking analysis can be seen from RMSD values and match patterns in hydrogen bond interactions compared to experimental results. Conformations 2,3 and 10 show the lowest RMSD values (1.35 Å) among all cluster ratings and agreement in hydrogen bond interactions, since the matching structure orientation between the two signifies this conformation under stable conditions when binding to protein receptors. The overlapping structure between the ligand prior to docking and the conformational structure 10 was presented in Figure 9.

Figure 9. PCA overlap structure

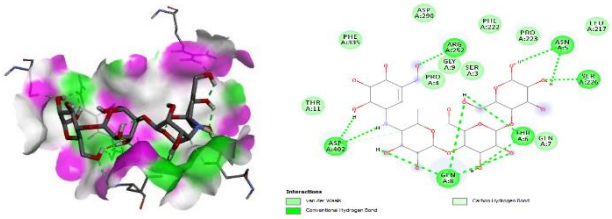


(a) (b)

Figure 10. (a) Interaction of α-amylase receptor with ligand 9-Octadecanoic acid (Z) methyl ester in 3D structure and (b) 2D structure indicates the type of amino acid residue formed between ligand and receptor

The results of the molecular docking analysis in Figure 10 show an interaction between the α-amylase receptor and the 9-octadecanoic acid (Z) methyl ester compound with the amino acid residue Gly-A:403. The bonds formed from this interaction are hydrogen bonds. In visualization, ligands have different bond types characterized by different colors of each type of residue such as van der Waals interactions (light green color without dotted lines), conventional hydrogen bonds (dark green color with dotted lines), pi-sigma bonds (purple color with dotted lines), alkyl and pi-alkyl bonds (pink color with dotted lines) (Gyebi et al., 2022).

Although 9-octadecanoic acid (Z) methyl ester had only one hydrogen bonding interaction with α-amylase compared to six hydrogen bonds in acarbose, it showed a lower bonding energy, indicating easier bonding. This was also likely due to the nature of hydrophobic interactions and the flexibility of the 9-octadecanoic acid (Z) methyl ester molecule. The long hydrophobic tail of 9-octadecanoic acid (Z) methyl ester enhanced its interaction with the hydrophobic pocket of the enzyme, which significantly contributed to the binding affinity and stability of the inhibitor-enzyme complex. These hydrophobic interactions, although non-covalent, provided a large stabilization energy that compensates for the smaller number of hydrogen bonds (Adhav & Saikrishnan, 2023). In addition, the molecular flexibility of the 9-octadecanoic acid (Z) methyl ester allowed it to more easily adapt to the binding site, optimizing van der Waals interactions and the overall binding conformation.



(a) (b)

Figure 11. (a) The interaction of α-amylase receptors with acarbose in a 3D structure and (b) a 2D structure shows the type of amino acid residue formed between the ligand and the receptor

The results of amino acid residues are seen in 2D and 3D structures that show the precision of binding between ligands and amino acids in macromolecules. According to the results of the molecular docking analysis in Figure 11, there are interactions between the receptor (α-amylase) and the ligand (acarbose). The significance of hydrogen bonding and van der Waals interactions in determining the bonding energy and inhibition constant. The 3D structure (Figure 11a) highlights the spatial arrangement and the extent of interaction between the ligand and the receptor, showing a comprehensive network of interactions that stabilize the ligand within the active site. The 2D structure (Figure 11b) further details specific amino acid residues involved in conventional hydrogen bonds, contributing to the binding affinity. The strength and number of these interactions are directly proportional to the negative binding energy and lower inhibition constant. Specifically, acarbose forms multiple hydrogen bonds with residues like Asp A:402, Gln A:8, Thr A:6, Ser A:226, Asn A:5, and Arg A:252, which significantly contribute to a stable inhibitor-enzyme complex, thereby resulting in a low inhibition constant and high binding energy. This indicates that the extensive hydrogen bonding network reduces the free energy of binding, thereby enhancing acarbose's inhibitory effectiveness against α-amylase.

Table 8. Docking results of α-amylase enzyme inhibitor compounds

Compound	Inhibition constant (mM)	Bonding energy (kcal/mol)	H-bond interaction residues	Van der Waals interaction residues
9-octadecanoic acid (Z) methyl ester	2,67	-3.51	GlyA:403	Pro A:4, Gln A:8, Gly A9, Arg A:10, Thr A:11, Arg A:252, Ser A: 289, Asp A:290, Pro A:332, Gly A:334, Phe A:335, Thr A:336, Arg A:398, Asp A:402, Arg A:421
Acarbose	15,72	-2,46	Asn A:5, Thr A:6, Gln A:8, Ser A:226, Arg A:252, Asp A:402	Ser A:3, Pro A:4, Asn A:5, Thr A:6, Gln A:7, Gln A:8, Gly A:9, Thr A:11, Leu A:217, Phe A:222, Pro A:223, Ser A:226, Asp A:290, Phe A:335

CONCLUSION

Methanol extract of *M. oleifera* seeds contained flavonoid, alkaloid, and tannin compounds based on phytochemical screening results. Methanol extract demonstrated α-amylase enzyme inhibition activity in vitro, where the highest inhibitory activity was observed at a concentration of 15%, with an inhibitory value of 67.94% and an IC_{50} of 15.38%. In contrast, acarbose at the same concentration had an inhibitory value of 41.76% and an IC_{50} of 17.89%. The inhibitory activity of the methanol extract *was* higher than that of acarbose. Based on the results of in silico analysis, ligands of 9-octadecanoic acid (Z) methyl ester compounds had inhibition constants and bond energies of 2.67 mM and -3.51 kcal/mol, respectively, while acarbose had 15.72 mM and -2.46 kcal/mol. These results suggest that the ligands could bind to receptors, form ligand-receptor complexes, and demonstrate inhibitory potential against α-amylase proteins.

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