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In Silico Study of Gamma-Mangostin Compound from Garcinia Mangostana L. Fruit Skin and Activity Test as an Alpha Amylase Inhibitor

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Abstract

The aim of this study is to determine the interaction between the gamma-mangostin compound and the α-amylase enzyme using molecular docking modeling and in vitro. The results of molecular docking showed that all compounds could interact with the active site of the α-amylase enzyme. The gamma-mangostin compound has a fairly negative binding affinity. The binding affinity score of the compound was then compared with the control, namely the carbohydrate inhibitor acarbose. The results showed that the binding affinity quite close to the control, namely -9.1 kcal/mol, while the control was -16.4 kcal/mol, but the score was not negative enough. Interaction of the target protein with the alpha amylase compound to determine the amino acid residues on the active side of the protein. The results showed that there were hydrogen, alkyl and van der waals bonds on the active side of the protein that were the same as the control. In silico and in vitro, the gamma-mangostin compound has the potential to reduce blood sugar levels with an α-amylase inhibitor, in vitro with percentage of 43.33% approaching the positive control, namely acarbose, which is 56.25% at a concentration of 200 ppm and an IC50 value of 166.06 ppm.

Introduction

Diabetes Mellitus (DM) is one of the chronic metabolic disorders characterized by increased blood glucose levels > 200 mg / dL. This disease is often known as the "silent killer and "mother of disease", because diabetes is the parent of other diseases including hypertension, heart disease, blood vessels, stroke, blindness and kidney failure (Kemenkes RI, 2014). The many negative impacts of diabetes mellitus on health require serious treatment, so therapy is needed to overcome it (Saeedi et al., 2019). Pharmacological therapy that is often used in the treatment of DM so far is still based on chemical therapy and it is known that there are still many side effects reported (Spronk et al., 2018). Nowadays, natural medicine including its extracts have been widely used, one type of plant that has the potential to lower blood glucose levels is the skin of Garcinia mangostana L fruit (Godavari & Amutha, 2017; Sumarmin, 2018). Diabetes is a chronic metabolic disorder caused by the pancreas not being able to produce enough insulin so that the body cannot use insulin 1 DM and type 2 DM. Type 2 diabetes occurs in the majority of diabetes cases and is mainly caused by lifestyle, environmental factors, and genetics associated with diabetes (Dinnar, 2022; Laakso & Fernandes Silva, 2022).

One of the therapeutic methods in treating type 2 diabetes is to slow down glucose absorption by inhibiting the α-amylase enzyme (Kurnia, 2020; Rijai et al., 2018; Filimonov et al., 2018). Inhibition of this enzyme slows down carbohydrate digestion, increases the overall carbohydrate digestion time and causes a decrease in the rate of glucose absorption and thus a decrease in postprandial plasma glucose levels (Amrulloh et al., 2024). α-Amylase (α-1,4-

glucan-4-glucanohydrolase) is a catalyst that hydrolyzes α-1,4-glycosidic polysaccharides from starch into oligosaccharides which are then digested to produce glucose. For absorption in the body, starch is hydrolyzed into simple oligosaccharides. With the activity of the α-amylase enzyme and insulin deficiency or insulin resistance, blood glucose levels continue to increase, leading to type 2 diabetes (Nursamsiar et al., 2020).

Inhibition of this enzyme slows down and prolongs the overall carbohydrate digestion time, resulting in a decrease in the rate of glucose absorption, and thus a decrease in postprandial plasma glucose levels (Kartini et al., 2023). Research on gamma-mangostin compounds has proven that this compound has good inhibitory activity against the α-amylase enzyme in silico. Gamma-mangostin compounds were tested for their inhibitory activity against α-amylase, and can interact with the active site of the α-amylase enzyme through hydrogen bonds with amino acid residues (Gaspersz & Sohilait, 2019). The results of the study reported that a dose of 150 mg/kgBW of Garcinia mangostana L. fruit skin extract effectively reduced glucose in white mice (Maliangkay et al., 2018).

Other studies have shown that α-mangostin compounds at doses of (50;100;150) mg/kgBW can reduce blood glucose levels (Santoso & Wulandari, 2020). The formulation of the study was to determine the potential for diabetes activity of the γ-mangostin compound in silico, which aims to model the interaction between the gamma-mangostin compound and the α-amylase enzyme. The molecular docking method is used because it can provide an initial picture of the gamma-mangostin compound candidate that has the potential as an α-amylase enzyme inhibitor and to determine its activity in vitro against the inhibition of the α-amylase enzyme. This study is important to contribute to the discovery of the γ-mangostin compound which is one of the new paradigms for the development of antidiabetic drugs. This study was conducted from an in silico study of γ-mangostin, phytochemical screening of antidiabetic, extraction and identification of γ-mangostin compounds and testing the activity of γ-mangostin as an α-amylase enzyme inhibitor.

Methods

ChemOffice 8.0 (www.cambridgesoft.com) and HyperChem 8.07 release for Windows (Hypercube Inc.). To test the interaction of ligand and protein, AutoDock 4.2 and Discover Studio Visualizer v4.5.0 were used. The hardware specifications used were Windows 7, 64 bits as OS; Intel Core i3-2330 2.2 Ghz processor, 2GB RAM 1067 MHz DDR3 (Gaspersz & Sohilait, 2019).

The materials used were the 3D structure of the ligand binding domain (LBD) of the α-amylase enzyme (PDB ID: 1B2Y) obtained from the online database Protein Data Bank (www.pdb.org) and the compound gamma-mangostin (Gaspersz & Sohilait, 2019). The ligand used was the compound gamma-mangostin. Canonical SMILE notation and 3D structure (sdf. Format) of gamma-mangostin compound were collected from PubChem database and used as test sample. SMILE notation is an algorithm for chemical compound nomenclature that can be read well by computer (Rollando, 2018). Through SMILE notation, various analysis with webserver and software can be done.

Human pancreatic α-amylase macromolecule containing 6-methyl-5-(4,5,6-trihydroxy-3-hydroxymethyl-cyclohex-2-enylamino)-tetrahydropyran-2,3,4-triol as natural ligand (PDB: 1B2Y) was downloaded from Protein Data Bank (PDB) website at www rcsb.org. Protein structure was viewed with Discover Studio Visualizer v4.5 software package, separated from water molecules and its natural ligand, saved in *.pdb file format, and treated as receptor (Wardani et al., 2022). Analysis of potential activity of gamma-mangostin compound gamma-mangostin compound is predicted using PASS Online webserver. The values of Probability of activity (Pa) and Probability of inactivity (Pi) are used as parameters in various biological functions related to anti-diabetes.

Analysis of the toxicity level of gamma-mangostin compound is predicted using Protox-3 webserver. The analysis aims to determine the toxicity classification of the compound used as a candidate compound of interest Analysis of the drug feasibility of gamma-mangostin compound is analyzed using Lipinski's Rule of 5 principle, namely using the parameters of molecular size (MW), hydrogen donor, hydrogen acceptor, and LogP. All analyses were performed using the SWISS ADME webserver. Molecular docking simulation analysis Identification of the binding activity between the gamma-mangostin compound and the target protein was performed through molecular docking simulation using the VinaWizard plugin in PyRx 1.1 software. The results of the analysis were then visualized using PyMOL) software (Gaspersz & Sohilait, 2019).

The complex resulting from the docking simulation between the gamma-mangostin compound and the target protein was then further analyzed using Discovery Studio software. This aims to determine the amino acid residues around the gamma-mangostin compound on the active side of the target protein (Gaspersz & Sohilait, 2019). The extraction method used is maceration, the method is to take 500 grams of Garcinia mangostana L. powder plus 2L of technical methanol and leave it for three days, the extract is filtered 3 times and evaporated at a temperature of 70℃ with a rotary evaporator until a thick extract is obtained

The methanol extract obtained is fractionated with a separating funnel with solvents, namely water and ethyl acetate. Then the separating funnel extract is taken from the separated extract. Next, column chromatography is carried out. The gravity column has a diameter size specification of 1-3 cm and a column length of 50 cm, with a stationary phase of silica gel (merck Sie-gel 60 GF254) weighing 100 g with a silica height of ± 10 cm when inserted into the column and the sample is mixed with 5 g of impregnated silica gel (merck kieselgel 60 GF254 0.2-0.5 mm). The mobile phase used is adjusted to each volume of 400 ml. Then the fractionation results are collected approximately 50 ml until all the solvent is used up (Rosalina & Mahendra, 2021). The solvent collection results are evaporated until there are no water droplets, transferred to a flacon and evaporated until dry. The results of each separation are collected in a glass bottle container and concentrated and the separation profile is checked with the appropriate mobile phase. The results of each fraction were also evaporated and weighed.

The results of the TLC profile showed that the fraction purified using KCV column chromatography was fraction B with a mass of ±2.2 g. The column characteristics are as follows: length 50 cm, width 3 cm, and stationary phase height 25. Methanol was used as the mobile phase and the column was washed with methanol. Separation was carried out by observing the color separation in the column and accommodating each separation in a container (Ladeska et al., 2017). The results of the sub-fractions were checked for TLC profiles and viewed under UV lamps at 254 nm and 365 nm. Fraction solutions that had the same stain or Rf profiles were combined. The results of the fraction solutions were evaporated and weighed. The results of the separated compounds are checked with the Rf value that matches the identification of the standard Rf standard The α-amylase enzyme inhibition test is a test to determine the decrease in α-amylase enzyme activity in breaking down starch, so that the results of the decrease in starch digestibility are obtained. The lower the starch digestibility, the less starch can be hydrolyzed in a certain time (Abbas et al., 2019). Starch can react with iodine reagent so that the residual starch content can be measured spectrophotometrically

Preparation

Phosphate buffer solution is made by mixing 125 ml of 0.2 M potassium dihydrogen phosphate KH2PO4 solution (4.0827 grams into 150 ml of distilled water) using 56 ml of 0.2 M sodium hydroxide (NaOH) solution (0.8 grams in 100 ml of distilled water) then diluted using 500 ml of distilled water. Measurements are made using a pH meter until it reaches pH 6.8. (Muchtaridi et al., 2017).

Weigh 0.5 grams of starch solani and dissolve it with 20 ml of distilled water while heating and stirring for 30 minutes (Rivero & Garibay, 2019). Iodine solution is made by dissolving 0.5 grams of iodine with 50 ml of distilled water and stirring until homogeneous (Anugrahini & Wahyuni, 2021). Weigh 20 mg of α-amylase enzyme powder. then dissolve it with 100 ml of pH 6.8 phosphate buffer and stir it until homogeneous (Prahesti et al., 2018). Acarbose solution is made by weighing 500 mg of acarbose and dissolving it using 10 ml of pH 6.8 phosphate buffer in a measuring flask (Pujiyanto et al., 2019). Then the parent solution was diluted to obtain several concentrations of acarbose solution, namely 200 ppm, 150 ppm, 100 ppm, and 50 ppm. A total of 10 mg of gamma-mangostin sample was dissolved in 2 ml of DMSO and phosphate buffer pH 6.8 was added to a 50 ml measuring flask to obtain a sample solution concentration of 1000 µg/ml (Nugraha & Hasanah, 2018). Then, the stock solution was diluted to obtain several concentrations of acarbose solution, namely 200 ppm, 150 ppm, 100 ppm, and 50 ppm

Testing

This test was carried out by adding 10 µl of dimethyl sulfoxide (DMSO) with 120 µL of phosphate buffer pH 6.8 and 20 µL of 0.1 U/mL α-amylase enzyme solution in a cuvette. Then incubated for 10 minutes at 37oC and added 20 µL of starch substrate and incubated again at 37oC for 10 minutes (Alfiani, 2022). The reaction was stopped by adding 80 µL of iodine solution. The absorbance of the para-nitrophenol produced was read at 452 nm The positive control in this study was acarbose. A total of 10 µL of acarbose solution was added with 120 µL of phosphate buffer solution pH 6.8 and 20 µL of 0.1 U/ml α-amylase enzyme solution into a cuvette (Wirasti et al., 2021). Sample testing was carried out by adding 10 µL of sample solution with 120 µL of phosphate buffer solution pH 6.8 and 20 µL of 0.1 U/ml α-amylase enzyme solution into a cuvette. Furthermore, incubation was carried out for 10 minutes at a temperature of 37oC. The reaction was stopped by adding 80 µL of iodine solution. The resulting para-nitrophenol was read with an absorbance of 452 nm. The test was carried out 3 times in replication (Kusmiyati et al., 2023).

Result and Discussion

SMILE notation of the compound of interest, namely gamma-mangostin compound, was collected from the PubChem database (Table 1). SMILE notation is an algorithm for the nomenclature of chemical compounds that can be read well by computers. Through SMILE notation, various analyses with web servers and software can be carried out.

Table 1. SMILE notation of the compounds of interest

	Compound	PubChem ID	SMILES
1	Gamma-mangostin	5464078	CC(=CCC1=C(C2=C(C=C1O)OC3=C(C2=O)C(=C(C(=C3)O)O)CC=C(C)C)O)C

The compounds were further identified through biological activity analysis. The results of this biological activity analysis are based on the Probability activity (Pa) and Probability inactivity (Pi) values. Pa values approaching 1 indicate that the biological activity of the compound has been validated based on in vitro studies to in vivo scales (Saudale et al., 2020). The results show that the compound has various activities related to anti-diabetic potential, including antioxidant, antiulcerative, and antidiabetic symptomatic. This is indicated based on the Pa score of each compound. However, the pomegraniin A compound could not be analyzed because its molecular size was too large (Table 2)

Table 2. Prediction results of biological activity of the compounds of interest

Compound	Pa score	Pi score	Biology Activities
Gamma-mangostin	0.826	0.003	*Antioxidant*

	0.753	0.004	*Antiulcerative*
	0.190	0.141	*Antidiabetic symptomatic*

Next, the compound of interest is further analyzed to predict its toxicity level. This toxicity level prediction aims to determine the safe dose level when the drug/compound is consumed orally. This safety is based on the LD50 level (the amount of a material that can cause 50% death in a group of test animals). The smaller the amount of drug/compound (mg/kg) that can cause LD50, the higher the toxicity or the more dangerous the drug/compound is for consumption (Zhu et al., 2009). Although the PASS online prediction results that gamma mangosteen shows a low Pa value, it is still supported by the antioxidant and wound healing or ulcerative abilities that have high Pa values. The results of this study are also supported by a study published by Chen (2021) showing that γ-mangostin, a xanthone compound from mangosteen (Garcinia mangostana), has the effect of lowering blood glucose and increasing insulin sensitivity through the mediation of the AMPK/PPARγ pathway. This study found that γ-mangostin has antihyperglycemic properties in diabetic-induced mice, without showing hepatotoxicity or nephrotoxicity. In addition, γ-mangostin interacts with α-amylase and α-glucosidase, enzymes involved in carbohydrate metabolism, and increases glucose uptake by cells by increasing insulin sensitivity. These findings suggest that γ-mangostin may be a potential candidate for the development of antidiabetic drugs.

The results of the analysis in Table 3 show that the compound of interest has a toxicity level within a safe range, namely the toxicity class 3-5 range. This shows that the compound can be consumed orally according to the safe dose threshold. In addition, consumption of the compound is also predicted to be safe because it does not cause toxic effects on cells and the liver.

Table 3. Prediction results of the toxicity level of the compound of interest

Compound	**Toxicity class**	**LD50 (mg/kg)**	***Accuracy (%)***	***Probability***	
				Hepatotoxicity	***Cytotoxicity***
Gamma-mangostin	5	3200	67.38	0.70 (*Inactive*)	0.82 (*Inactive*)

Toxicity class grouping:

Class I: fatal if swallowed (LD50 ≤ 5)
Class II: fatal if swallowed (5 < LD50 ≤ 50)
Class III: toxic if swallowed (50 < LD50 ≤ 300)
Class IV: harmful if swallowed (300 < LD50 ≤ 2000)
Class V: may be harmful if swallowed (2000 < LD50 ≤ 5000)
Class VI: non-toxic (LD50 > 5000)

The next analysis, namely the drug feasibility analysis, is carried out based on the Lipinski's rule of 5 principle. This principle is a parameter to test whether a compound is suitable to be used as a drug, especially oral drugs. This is very important to determine the ability of a compound when entering cells or the human body. Several parameters used are molecular weight which indicates that compounds with small sizes (≤ 500 g / mol) can more easily enter the cell membrane. While hydrogen donors and hydrogen acceptors affect the permeation and absorption of compounds. The LogP parameter is related to the lipophilicity ability which has an ideal value of <5 so that it is good in the absorption process (Fadlan et al., 2022).

Table 4. Results of drug feasibility predictions from compounds of interest

Compound	***Lipinski's Rule of five***				
	Molecular Weight (≤ 500 g/mol)	***Hydrogen Donor (≤ 5)***	***Hydrogen Acceptor (≤ 10)***	***LogP (<5)***	***Violation***
Gamma-mangostin	396.43	4	6	1.98	0

The results of the analysis in Table 4 show that there are sample compounds that do not show any violations of the Lipinski rule of five parameters (Pangemanan et al., 2022). In general, a maximum of 1 violation can be tolerated in the Lipinski rule of five. It can be predicted that the gamma-mangostin compound will be easily absorbed by cells. After analyzing biological activity, toxicity and drug suitability, the compound of interest was then analyzed for its binding strength to the target protein, namely the alpha-amylase protein (PDB ID 1B2Y). The 3D structure of the protein was downloaded from the PDB database.

In diabetes, the case is indicated by increased blood sugar levels due to poor or excessive diet. High carbohydrate consumption is one factor that can affect high blood sugar levels. The alpha amylase enzyme is a protein that has a primary function in digesting carbohydrates. This enzyme works by hydrolyzing starch or carbohydrates into simple sugars, such as glucose and maltose, which can be absorbed through the bloodstream. Therefore, to reduce the condition of diabetes, the expression of the alpha amylase protein needs to be minimized. High blood sugar levels can also cause an increase in the level of oxidative stress (ROS) reactions in the sufferer's body. In addition, people with diabetes often have diabetic wounds that are difficult to heal or commonly called ulcers (Fandinata & Ernawati, 2020). The first interaction analysis is a simulation of the interaction between the compound of interest and control of the alpha-amylase protein (PDB ID 1B2Y). Native ligand is a ligand that attaches to the active site of the protein so that the molecule can be used as a control. In this case, the native ligand, namely the carbohydrate inhibitor acarbose, is used as a template to determine the location of the active site (Putri, 2022). Molecular docking analysis was carried out using the principle of specific docking. The grid positions used were: CENTER_X = 17.9024 CENTER_Y = 5.3150 CENTER_Z = 46.7524

Table 5. Molecular docking simulation results of the gamma-mangostin compound and acarbose control.

Compound	Binding Affinity Score (Kcal/mol)
Acarbose (Control)	-16.4
Gamma-mangostin	-9.1

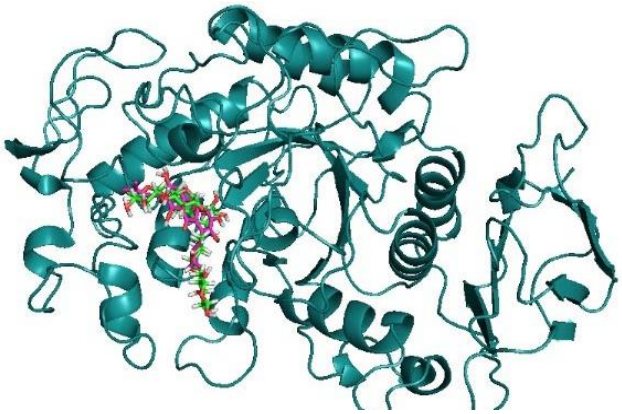

Figure 1. In silico or Molecular Docking Result

Description: Alpha-amylase (dark green color), gamma mangostin compound (pink color), acarbose (light green color)

The results showed that the gamma-mangostin compound has a fairly negative binding affinity. The binding affinity score of the compound was then compared with the control, namely the carbohydrate inhibitor acarbose. The results showed that the gamma-mangostin compound has a binding affinity that is quite close to the control, but the score is not negative enough (Table 5). This is indicated by the compound's binding affinity score indicator, which is -9.1 kcal/mol, while the control is -16.4 kcal/mol. In general, the more negative the binding affinity value of

a compound, the stronger the binding interaction of the compound to the target protein (Gaspersz & Sohilait, 2019).

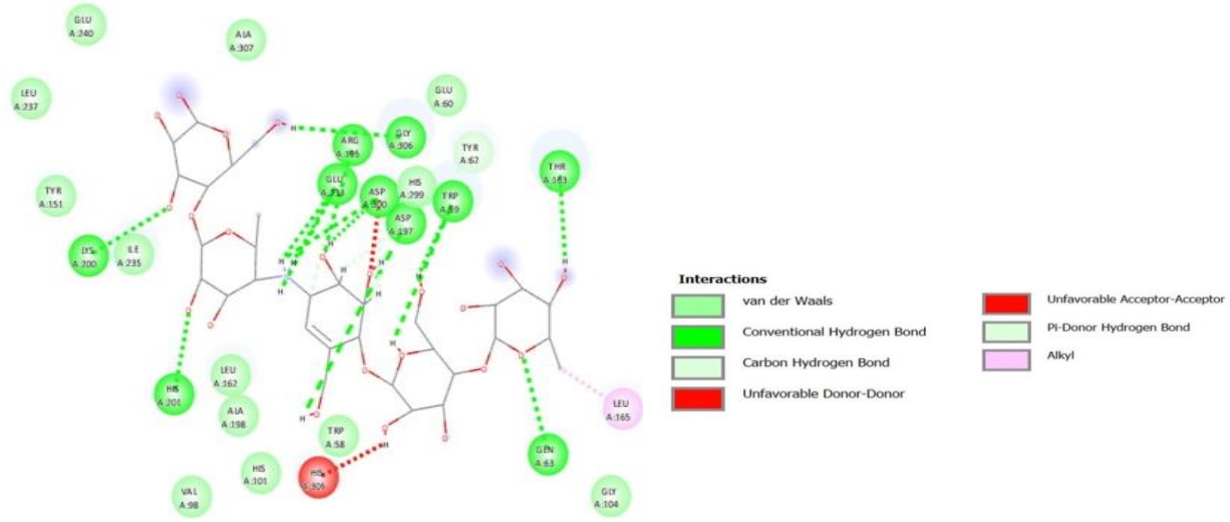

Figure 2. Amino acid residues in the active site of the alpha-amylase protein that interact with acarbose control

The potential of gamma-mangostin as an alpha-amylase inhibitor, it is necessary to conduct an in-depth analysis of its molecular interactions with the active residues of the enzyme. Some of the main factors that must be considered include: Hydrogen bonding is an important interaction that can increase the stability of the inhibitor-enzyme complex. Docking analysis needs to identify specific residues on alpha-amylase that participate in the formation of hydrogen bonds with gamma-mangostin. In Figure 2, gamma-mangostin forms several hydrogen bonds with the active residues of the enzyme (e.g. *Asp197*, *Glu233,* or *His299* which are often involved in catalysis), so this can increase the affinity and inhibitory potential as an alpha amylase inhibitor.

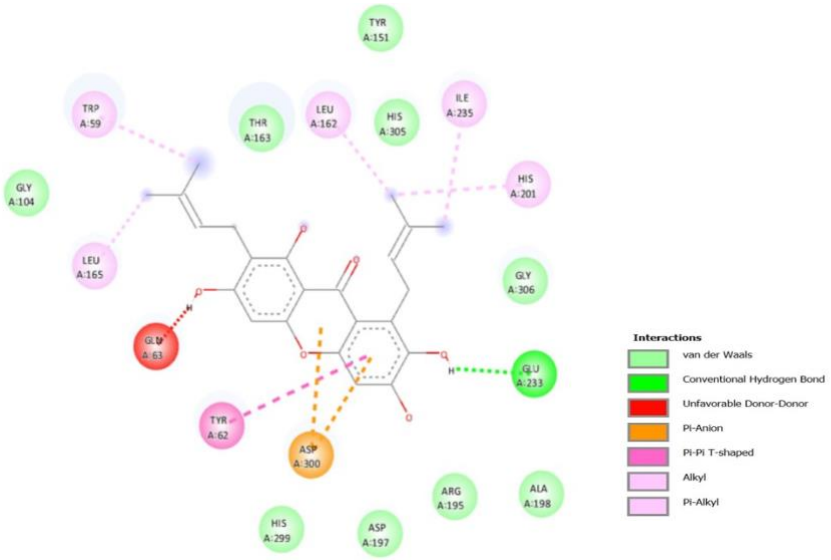

Figure 3. Amino acid residues in the active site of the alpha-amylase protein that interact with alpha-mangostin compound

In alkyl and hydrophobic bonds, gamma-mangostin has a polyphenol structure with methoxy groups that can interact through hydrophobic forces or alkyl bonds with hydrophobic residues in the active pathway of alpha-amylase. In Figure 3 it can be seen that gamma-mangostin forms strong hydrophobic interactions with residues such as *Trp59* or *Tyr151*, this can stabilize binding and increase affinity for the enzyme. So this supports the ability of gamma mangostin

as an alpha amylase inhibitor. In addition to the factors mentioned above, there is another factor that needs to be considered, namely Van der Waals forces play an important role in non-specific binding and stabilization of the enzyme-inhibitor complex. Gamma-mangostin may interact with other residues through Van der Waals forces, which can help in fitting the molecule to the active pocket of the enzyme.. From Figure 2 and 3, it can be seen that the complex interaction of the target protein with the alpha-amylase compound was further analyzed to determine the amino acid residues on the active side of the protein. The results showed that there were hydrogen, alkyl and van der waals bonds on the active side of the protein (Dewi et al., 2022). The same amino acid residues in the binding of the control and target compounds indicate that the gamma-mangostin compound binds to the same active side as acarbose.

Although gamma-mangostin exhibits lower affinity than acarbose (-9.1 kcal/mol vs -16.4 kcal/mol), a deeper understanding of this interaction pattern may aid in structural modification of the compound to enhance its inhibitory potential. For example, optimization of certain functional groups to enhance hydrogen bonding or increase hydrophobic interactions may enhance its effectiveness as an alpha-amylase inhibitor. Further molecular dynamics analysis may provide additional insights into the stability of this complex in biological environments.

Extract Preparation

The materials used in this study were mangosteen peel powder from Purworejo, then determination was carried out at the UNIDA Gontor Biology Laboratory.

Table 6. Results of methanol extraction of mangosteen peel

Keterangan	Hasil
Dry Simplisia	500 gram
Dry Exstrac	42 gram
Rendemen	8,4 %

These results show that gamma-mangostin has the potential to reduce blood sugar levels. Other studies have shown that γ-mangostin ligands have the potential to act as inhibitors of the α-amylase enzyme compared to α- and β-mangostin.

Liquid-Liquid Fractionation

A 42g methanol extract was fractionated by adding 50ml of water and 50ml of ethyl acetate 3 times. Then the separated extract was taken using a separating funnel. A fraction of 14.5g was obtained

Column Chromatography

A total of 10g was collected, inserted into a KCV column with a size specification of 15 x 4 cm, and silica gel fixation phase (MERCK SIEL 60 GF254) was added. Weighing was carried out. The column and sample were mixed with 5g of impregnated silica gel (Merck Kieselgel 60 GF254 0.2-0.5 mm). The results of the TLC profile verification showed that the purified paint was based on weight ± 2.2g using column chromatography. The column specifications are 50 cm long, 3 cm wide, and 25 stationary phase height. The mobile phase used was methanol and the column was rinsed using methanol. Separation was carried out by observing the color separation in the column and the adaptation of each separation in the container. The results of each separation were collected in glass bottles and concentrated bottles, and the separation profile was verified using a mobile phase of hexane: ethyl acetate (2: 3). The results of each fraction were also evaporated and weighed. Fractions A, B and C were obtained. The elution results were observed at a wavelength of 254 nm.

Preparative Chromatography

The results of the sub-fractions were checked for TLC profiles and viewed under UV lamps at 254 nm and 365 nm. Fraction solutions that had the same spots or Rf were combined. The results of the fraction solutions were evaporated and weighed. The results of the sub-fractions from sephadex were further purified using preparative TLC with a 0.25 mm silica gel GF254 stationary phase and an optimized mobile phase. The results of the compound separation were scraped and separated from the stationary phase using a hexane: ethyl acetate (2: 3) solvent on the separation column. The purity of the compound was checked using normal phase TLC silica gel GF254 0.25 mm and reversed phase TLC Silica gel 60 RP-18 F_{254}.

Identification of TLC

The results of the separated compounds were checked with the Rf value which corresponded to the identification value of Rf = 0.89

Figure 4. TLC identification

α-Amylase Enzyme Inhibition Activity Test with UV-Vis Spectrophotometer

Percentage of α-Amylase Enzyme Inhibition from acarbose

Table 7. Percentage of α-Amylase Enzyme Inhibition from acarbose

Number	Concentration (ppm)	Blank Absorbance	Absorbance of the sample	% Inhibition
1	200	0,240	0,105	56,25
2	150		0,108	55,00
3	100		0,125	47,91
4	50		0,141	41,25

Curve for Calculating the IC50 Value of Acarbose

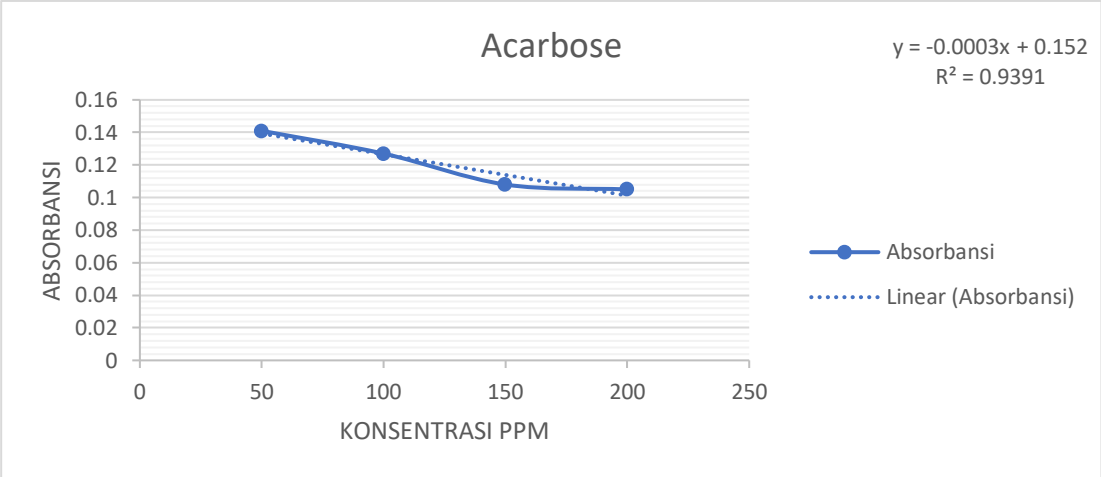

Figure 5. Curve for Calculating the IC50 Value of Acarbose

y = 50 y = 0,0003x + 0,152

a = 0,152

b = 0,0003x

y = ax + b

$x = \frac{y-a}{b}$

$= \frac{50-0,152}{0,0003}$

= 166,16 ppm

Percentage of α-Amylase Enzyme Inhibition from gamma-mangostin

Table 8. Percentage of α-Amylase Enzyme Inhibition from gamma-mangostin

Number	Concentration (ppm)	Blank Absorbance	Absorbance of the sample	% Inhibition
1	200	0,240	0,136	43,33
2	150		0,139	42,08
3	100		0,162	32,50
4	50		0,176	26,67

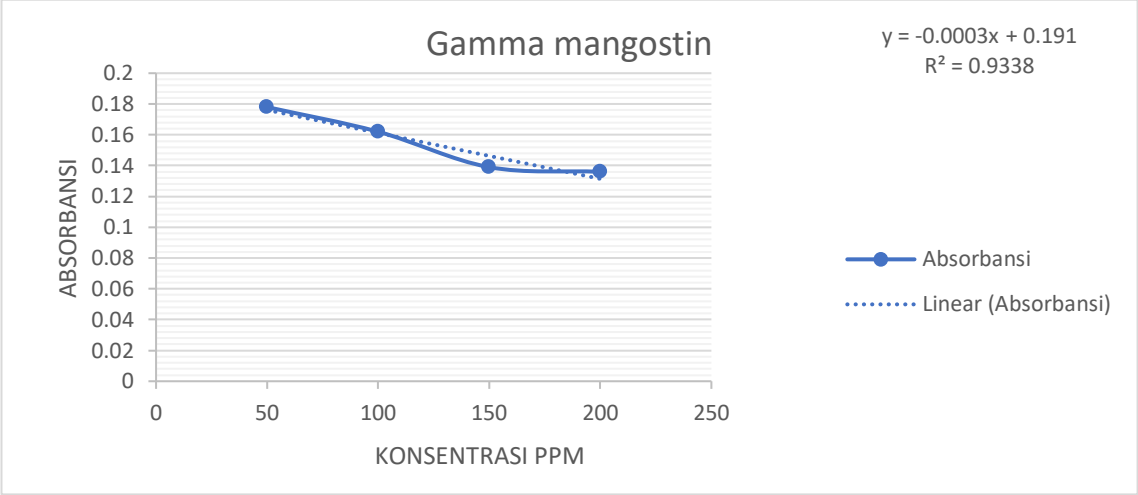

Figure 5. Curve for calculating IC50 value of gamma-mangostin

y = 50 y = 0,0003x + 0,191

a = 0,191

b = 0,0003x

y = ax + b

$x = \frac{y-a}{b}$

$= \frac{50-0,191}{0,0003}$

= 166,03 ppm

In this test, several groups are tested, including blank solution, control solution, standard acarbose solution and acarbose control, as well as test compound and test control solution.

These groups have different purposes. Blank solution assignment to identify chemical movements independently without any test that functions as an inhibitor. Blank control solution to overcome the use of solvents without extracts or proteins included in the reaction. The use of test collection points to determine the effect of gamma-mangostin on α-amylase protein inhibitors, while the test control collection is used as a control to see sample movement without protein mediation or as a reference in the investigation of the test group. The same treatment is given to the acarbose comparator as done in the compound test.

The α-amylase inhibitor activity test against the γ-mangostin compound is carried out using starch as a substrate that reacts with the active compound to produce substances and acarbose is used as a positive control. Acarbose is used because it is easy to obtain and is often used as a comparison in various studies. Acarbose is an antidiabetic drug that functions to lower blood sugar levels. In this study, different concentration levels were used to test the effect of concentration on increasing α-amylase enzyme activity (Pujiyanto et al., 2019). The higher the inhibition power, the better the effect produced, namely if the low, the effect produced is more pronounced (Rahmadi et al., 2016).

The results obtained from this test are the absorbance value used to determine the percentage (%) of the inhibition power of α-amylase enzyme activity, which can then be used to create a linear regression equation to calculate IC50 (Wahyuningsih, 2019). The concentration of the sample that is able to inhibit α-amylase enzyme activity by 50%. Measurement of the IC50 value allows the inhibitory potential of each extract against the enzyme to be determined. IC50 is obtained from measuring the % inhibition. The IC50 value is obtained using a linear regression equation that shows the relationship between the variable x, which represents the concentration of the extract, and the variable y, which represents the percentage of inhibition (Nursamsiar et al., 2020). This table shows that the more concentrated the solution, the higher the value of the inhibition activity or α-amylase. Conversely, the lower the concentration of the solution, the lower the percentage of inhibition or α-amylase. The highest α-amylase inhibitory activity value was 56.25% in acarbose at a dilute concentration of 200 ppm, which means it has the best ability to inhibit the amylase enzyme at dilute concentrations of 150, 100, and 50 ppm.

IC50 value is the α-amylase inhibitor activity value of mangosteen gamma is 43.33%, meaning that at a dilute concentration of 200 ppm, this inhibitor has the ability to inhibit the amylase enzyme that is close to the inhibitory value of acarbose. The higher the concentration of the sample used, the higher the enzyme inhibition activity (Alfiani, 2022). The results of the α-amylase inhibition activity test showed an IC50 value in the acarbose control of 166.16 ppm, while the IC50 value of gamma-mangostin was 166.06 ppm. Meanwhile, the percentage of α-amylase inhibitor in the positive control, namely acarbose, was 56.25% higher than the gamma-mangostin compound of 43.33%.

These results show that gamma-mangostin has the potential to reduce blood sugar levels. Other studies have shown that γ-mangostin ligands have the potential to act as inhibitors of the α-amylase enzyme compared to α- and β-mangostin. The γ-mangostin ligand forms a more stable complex with the α-amylase enzyme with a low binding affinity of -7.4 kcal/mol (Gaspersz & Sohilait, 2019). In this study, the value was -9.1 kcal/mol. This is a negative value, meaning that the more negative the binding affinity value of a compound, the stronger the binding interaction of the compound with the target protein. In vitro, gamma-mangostin has the potential to lower blood sugar levels with an α-amylase inhibitor percentage of 43.33% approaching the positive control, namely acarbose, which is 56.25% at a concentration of 200 ppm.

Conclusion

New scientific evidence demonstrates that gamma-mangostin extracted from Garcinia mangostana L. peel shows strong potential to function as an antidiabetic agent through its inhibitory effect on α-amylase enzyme activity. The in silico study showed gamma-mangostin establishes a -9.1 kcal/mol binding affinity with the α-amylase enzyme by creating hydrogen, alkyl and Van der Waals contact with essential active site residues. The drug affinity of gamma-mangostin was -9.1 kcal/mol yet this value remained less powerful than acarbose's (-16.4 kcal/mol) while the active site chemical bonding was found to be similar. The in vitro experiments confirmed its inhibitory power by reaching a 43.33% inhibition rate at 200 ppm concentration which reached near the 56.25% acarbose level. Gamma-mangostin showed enzyme inhibition ability that closely matched standard drug characteristics with an IC_{50} value of 166.06 ppm and standard drug value of 166.16 ppm. The benefits of gamma-mangostin extend to satisfying drug-likeness requirements according to Lipinski's Rule of Five while achieving a toxicity class of 5 accompanied by an LD_{50} of 3200 mg/kg as well as predicted effects against ulcers and antioxidant processes. The combination of various pharmacological properties makes this natural therapeutic agent more desirable. The combination of computational and laboratory findings concludes that gamma-mangostin demonstrates promising potential to advance as an α-amylase inhibiting antidiabetic medication. In vivo studies combined with structural modifications for stronger binding performance should be completed together with multiple safety tests to prove its clinical potential and therapeutic dependability.

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