JJBS

Volume 17, Number 2,June 2024
ISSN 1995-6673
Pages 293 – 299
https://doi.org/10.54319/jjbs/170209

Jordan Journal of Biological Sciences

An Insight on Potential Role of Glucomannan, Mannan, and Flavonoids in Porang Tubers (*Amorphophallus muelleri* Blume) as Anti-Diabetic Through the Alpha-amylase Inhibition

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Received: December 31, 2022; Revised: September 30, 2023; Accepted: January 2, 2023

Abstract

Exploring the natural product as a potential alpha-amylase inhibitor with no adverse side effects is still being concerned in developing diabetes mellitus drugs. This study aims to identify the phytochemical components of porang tuber extract. We also investigated bioactivity and predicted the biological function of the porang tubers bioactive in inhibiting human pancreatic alpha-amylase. The phytochemical components were analyzed by Liquid Chromatography Mass Spectrophotometry (LCMS). We investigated the biological activity of compounds as anti-diabetic agents by the PASS server. The 3D structure of the active compound that was showing anti-diabetic activity was prepared using PyRx software. Docking simulations were analyzed using Hex 8.0 software, while for visualization Discovery Studio Client 4.1 software was used. Our study found the presence of 67 phytochemical constituents in porang tuber extract. The five largest compounds with anti-diabetic activity are glucomannan, mannan, and flavonoids (quercetin, orientin, and hyperoside). PASS analysis showed that glucomannan and mannan had the most potential as anti-diabetic. It was determined by the Pa value of 0.77. The active sites of alpha-amylase (ASP197, GLU233, and ASP300 residues) were bound by glucomannan, mannan, orientin, and hyperoside, which plays an essential role in catalytic activity. Glucomannan showed the strongest interaction with the pancreatic alpha-amylase domain (lowest binding energy -394.1 kcal/mol). This result indicates that porang tubers are very potential as an alpha-amylase inhibitor. Further research is needed to validate this finding.

Keywords: anti-diabetic, alpha-amylase inhibitor, functional food, natural remedies.

1. Introduction

Currently, natural bioactive compounds from plants are studied since their therapeutic properties can be commercialized (Aldayel *et al*., 2020). Porang (*Amorphophallus muelleri* Blume) is an herbaceous plant-producing tuber with nutraceuticals that promise to treat health problems, such as hyperglycemia and hyperlipidemia (Chen *et al*., 2019; Harijati *et al*., 2012). Porang tubers are rich in macronutrients, including carbohydrates (80.16%), starch (54.23%), fiber (4.96%), protein (5.77%), fat (4.96%), and glucomannan (Wulan *et al*., 2019).

Glucomannan is the main carbohydrate component in porang tuber with 99,436.85 µg/g concentration shown by LCMS analysis (Gusmalawati *et al*., 2019). Besides glucomannan, other carbohydrate compounds such as mannan, trehalose, mannose, galactose, glucose, rhamnose, arabinose, and xylose were detected in postharvest porang tubers (Gusmalawati *et al*., 2021). In addition, several secondary metabolite including flavonoids, alkaloids, saponins, coumarins, tannins, steroids, and quinones were also detected in porang tuber. Traditionally, *Amorphophallus* species tubers were beneficial to treat hemorrhoids, stomach pain, tumors, asthma, and rheumatism. Furthermore, the extract of *Amorphophallus* sp. tuber was also reported to have anti-diabetic, antioxidant, anti-cholesterol, and anti-bacterial properties (Firdouse and Alam, 2011; Shete *et al*., 2015; Van *et al*., 2020).

Foods rich in phytochemicals such as polysaccharides and polyphenols are suitable for medicinal and dietary supplements. Several plant polysaccharides have been confirmed to have secure anti-diabetic activity through various mechanisms. One potential mechanism is by inhibiting alpha-amylase activity (Chen *et al*., 2019). Diabetes mellitus is a chronic metabolic disease with high blood glucose levels due to insufficient insulin secretion or insulin tolerance, or a combination of both. Hyperglycemia

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conditions can trigger health complications such as cardiovascular disease, ischemic heart disease, and other diabetes complications (Quek *et al*., 2020).

One of the therapeutic approaches to overcome metabolic disorders is drugs that inhibit the digestion and absorption of carbohydrates. Pancreatic alpha-amylase is the main enzyme that plays a role in breaking down starch into simple sugars (glucose, maltose, maltotriose, and dextrin) through hydrolytic activity. High intake of simple carbohydrates in the human body can lead to weight gain and escalated postprandial glycemia. Reduction of postprandial hyperglycemia can be achieved by inhibition of alpha-amylase (Quek *et al*., 2020; Xie *et al*., 2020). Acarbose is a synthetic drug to treat diabetes mellitus, which inhibits the enzyme alpha-amylase. However, the use of acarbose causes some side effects, such as gastrointestinal disorders (Alqahtani *et al*., 2019). Therefore, alpha-amylase inhibitors that did not generate adverse side effects from natural sources are needed.

This study aims to determine the phytochemical content of porang tubers by LCMS analysis. An in-silico study of biological function was carried out to predict the potential role of porang tuber bioactive compounds as inhibitors of human pancreatic alpha-amylase.

2. Materials And Methods

2.1. Extraction of Porang Tubers

The specimens used in this study were fresh porang tubers for the third growing period obtained from East Java. The mashed porang tubers were extracted using 95% methanol as solvent by the maceration. Briefly, maceration was carried out for 24 hours at room temperature. The homogenate was filtered with the Erlenmeyer vacuum. A rotary evaporator at a temperature of 40°C was used for generating concentrated filtrate. The concentrated extract was centrifuged for 20 minutes at 5000 rpm. The supernatant obtained was then stored at 4°C for further analysis (Gusmalawati *et al*., 2019; Babu & Radhamany, 2021).

2.2. LCMS Analysis of Methanol Extract of Porang Tubers

A Shim Pack FC-ODS column (2 mm x 150 mm, 3 m) with a column temperature of 35°C was used for liquid chromatography separation (Zhao *et al*., 2015). The eluent used was 95% methanol with 0.8 mL/minute flow rate and a maximum pump pressure of 15 kgf/cm^2. The wavelengths used are 254 nm and 190 nm with polarity (+) and AUX range (2AU/V). Furthermore, liquid chromatography runs for 80 minutes followed by mass spectrophotometry analysis with the following parameters: probe temperature= 250°C, CDL temperature= 250°C, nebulizer gas flow= 1.5 mL/minute, and block temperature= 400°C. The experiment was set up as follows: acquisition mode= scan +, interval= 0.5 sec, detector gain= 1.5 kV, start m/z= 50, stop m/z= 500, scan speed= 1000, probe voltage= +4 .5 kV, and CDL voltage= 25 V. Chromatograms were displayed using the LCMS PostRun program.

2.3. Ligand and Receptor Preparation

The 3D chemical structures of glucomannan (CID: 24892726), mannan (CID: 25147451), quercetin (CID: 5280343), orientin (CID: 5281675), and hyperoside (CID: 5281643) were obtained from NCBI PubChem database (https://www. ncbi.nlm.nih.gov/) in sdf format. RSCB PDB (https://www.rcsb.org/) was used as a database to derive the 3D structure of pancreatic alpha-amylase (PDB ID: 4W93). Ligands (glucomannan, mannan, quercetin, orientin, and hyperoside) were prepared using PyRx software. Receptor preparation (pancreatic α-amylase) was via eliminating water molecules or ligands bound to receptors using Discovery studio software. Then, the receptor was saved in .pdb format using open babel (Agustin *et al*., 2020).

2.4. Biological Activity Prediction

Bioactivity analysis was carried out to identify compounds from porang tubers that have the potential as anti-diabetic. This study employs a PASS server (http://www.pharmaexpert.ru/passonline/). PASS is a database containing compound activity data (Krieger *et al*., 2015).

2.5. Molecular Docking and Visualizations

Molecular docking between the receptor and the ligand was performed using Hex 8.0 software. Further, the docking was set in Shape+Electro+DARS mode. Then, docking results are saved with the .pdb extension. Analysis and visualization of binding interactions were established using the Discovery studio 2016 software (Agustin *et al*., 2022).

2.6. Molecular Dynamics Simulations

Our study used YASARA structural software for molecular dynamics simulation with a run time of 20,000 ps and autosaved every 25 ps. The following cell physiology conditions are performed when running the software: temperature 37°C, pH 7.4, salt content 0.9%, and pressure 1 atm. The simulation is conducted utilizing the macro program md_runfas. Furthermore, root-mean-square deviation (RMSD) protein backbone was performed using the md_analyze macro, and root-mean-square (RMSF) fluctuation analysis was performed using the md_analyzeres macro (Krieger *et al*., 2015).

3. Results

3.1. LCMS Analysis of Porang Tubers

Identification of phytochemical components of fresh porang tubers using LCMS analysis showed 67 compounds (Figure 1). Interestingly, our study found the main compounds identified included glucomannan (RT = 54,184), mannan (RT = 46,166), quercetin (RT = 11,427), orientin (RT = 22,172), and hyperoside (RT = 24,02) (Table 1). Glucomannan has the chemical formula $C_{30}H_{52}O_{26}$ defined by m/z 828; mannan with chemical formula $C_{24}H_{42}O_{21}$ has an m/z value of 66; the chemical formula for quercetin is $C_{15}H_{10}O_7$ with m/z 302. Orientin and hyperoside have the chemical formulas $C_{21}H_{20}O_{11}$ and $C_{21}H_{20}O_{12}$ with m/z 448 and 464, respectively.

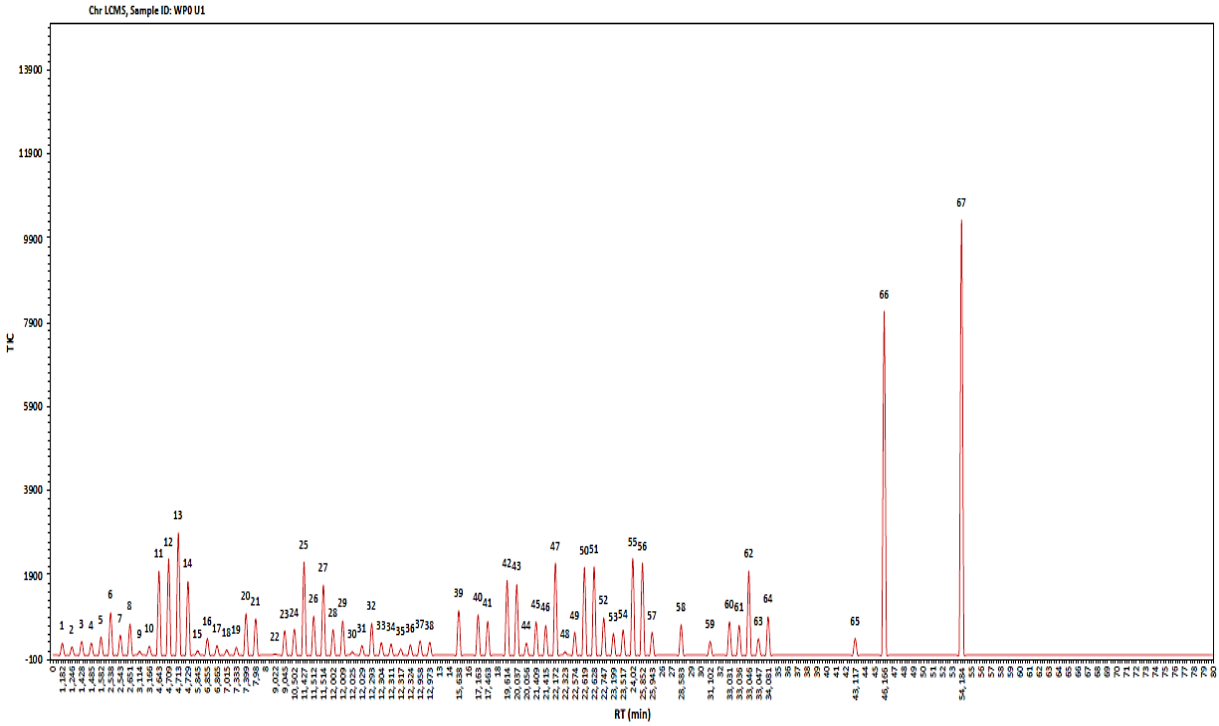

Figure 1. Chromatogram of porang tuber extract by LC-MS analysis. 25. Quercetin, 47. Orientin, 55. Hyperoside 66. Mannan, 67. Glucomannan.

Evaluation of biological activity based on PASS server showed that glucomannan, mannan, quercetin, orientin, and hyperoside from porang tubers were bioactive compounds that have the potential as anti-diabetic. It was indicated by the Pa value of >0.2 in quercetin; besides, glucomannan, mannan, and hyperoside have a Pa value (>0.6) (Table 1). The PASS server was a structural approach by comparing the inputted compounds with fixed compounds to have certain activities. The assessment is based on the Probability to be active (Pa) value of the bioactive compound. The current study showed that glucomannan and mannan occupied the highest Pa value of 0.77. It was indicated as the most potent active compound as anti-diabetic.

Table 1. Results of LC-MS analysis of methanol extract from porang tubers and predictions of their biological activity represent an anti-diabetic role.

Peak number	Compound	Chemical formula	RT (min)	m/z	Composition (%)	Pa value
67	Glucomanan	$C_{30}H_{52}O_{26}$	54,184	828	14,24644	0,777
66	Mannan	$C_{24}H_{42}O_{21}$	46,166	66	11,25441	0,777
25	Quercetin	$C_{15}H_{10}O_7$	11,427	302	3,03861	0,273
47	Orientin	$C_{21}H_{20}O_{11}$	22,172	448	3,01088	0,774
55	Hyperoside	$C_{21}H_{20}O_{12}$	24,02	464	3,15940	0,661

3.2. Molecular Docking Analysis of Pancreatic Alpha-amylase with Glucomannan, Mannan, Quercetin, Orientin, and Hyperoside

The receptor-ligand interactions were indicated by the binding site of the amino acid residue, chemical bond types, and the binding affinity (Table 2). The molecular mechanism of inhibition of pancreatic alpha-amylase through the interaction of bioactive compounds has been established. Five bioactive compounds of porang tuber can bind to the active site of pancreatic alpha-amylase in several residues.

Six amino acid residues of domain A of pancreatic alpha-amylase interact with glucomannan, including Gln63, Arg195, Trp59, His299, Asn53, and Asp197. Mannan compounds can bind amino acid residues Gln63, Thr163, Arg195, Glu233, Tyr62. The Asp 197 amino acid residue acts as a nucleophile in the alpha-amylase catalytic mechanism, whereas Glu 233 plays as an acid-base catalyst during the starch hydrolysis reaction. Asp 300 was crucial in optimizing substrate orientation at the S1 subsite through multiple hydrogen bonds. Our study showed that glucomannan and mannan could bind amino acid residues (Asp197 and Glu233), critical in catalytic activity through hydrogen interactions.

The interaction of quercetin with the binding site of the pancreatic alpha-amylase complex involves residues Thr264, Gly308, Ala260, Leu237, Lys257, and Lys261. Hydrogen and hydrophobic binding stabilized these interactions (Thr264, Gly308, Ala260, Leu237, Lys257, and Lys261) (Figure 2). Molecular docking results showed that eight amino acid residues in domains A and B of pancreatic alpha-amylase were able to bind to orientin, including Gln63, His299, Glu233, Asp197, Asp300, Leu165, His305, and Trp59 residues. This binding site is stabilized by hydrogen bonding, hydrophobic, and electrostatic interactions. The interaction of pancreatic alpha-amylase complex and hyperoside occurs in domain A. Acarbose is a commercial alpha-amylase inhibitor used to treat type 2 diabetes mellitus. In silico studies of acarbose-pancreatic α-amylase complexes show only one Trp59 residue bound to acarbose via hydrogen interactions.

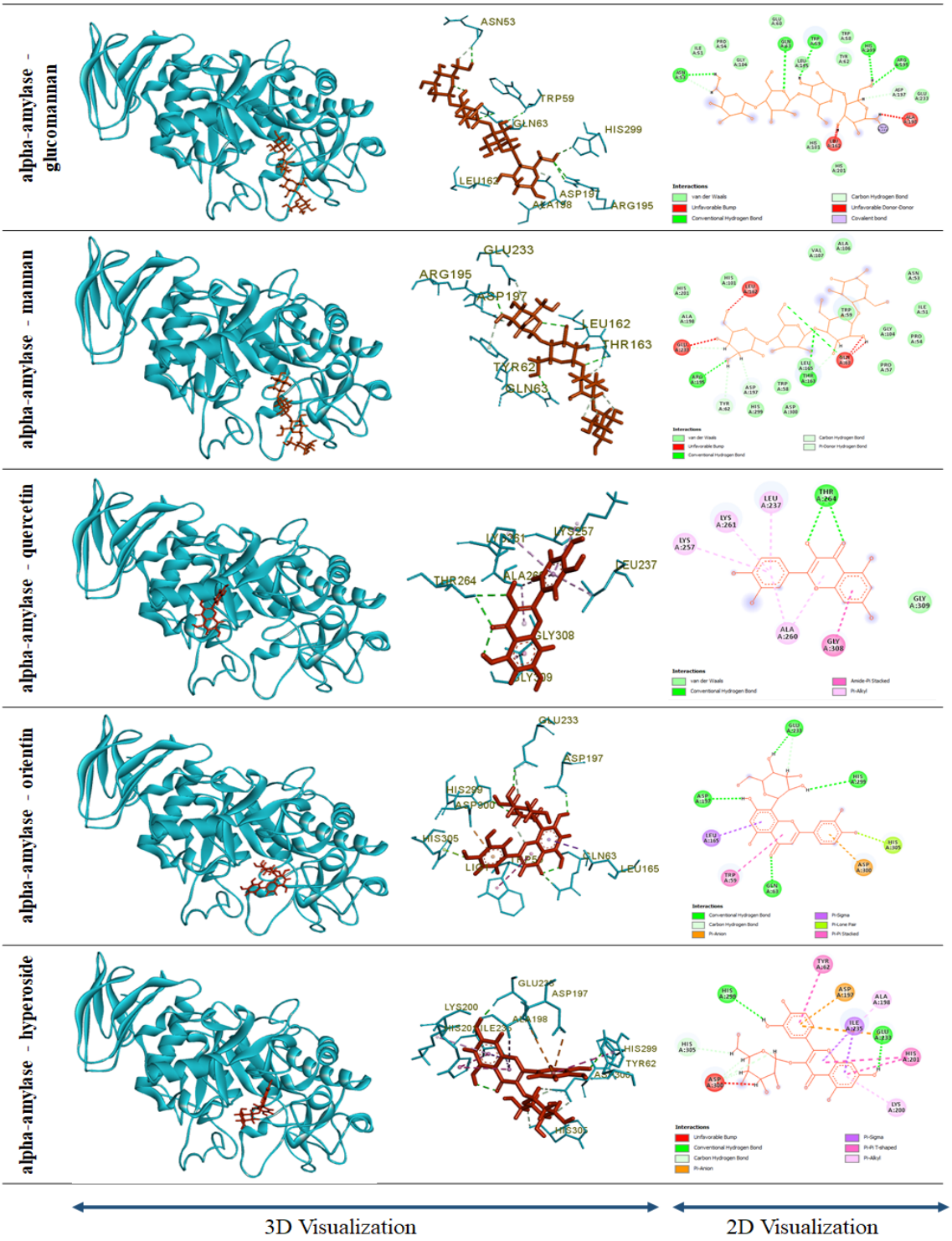

Figure 2. Interaction of pancreatic alpha-amylase with glucomannan, mannan, quercetin, orientin, and hyperoside.

Hyperoside can bind ten amino acid residues, including Glu233, His299, Asp300, His305, Asp197, Ile235, Tyr62, His201, Ala198, and Lys200 through hydrophobic interactions and hydrogen bonds. Interestingly, orientin and hyperoside could inhibit all three catalytic residues of pancreatic alpha-amylase (Glu233, Asp197, and Asp300). Thus, orientin and hyperoside from porang tubers were indicated to have a potential anti-diabetic role through inhibition of the active site of pancreatic alpha-amylase (Figure 2).

Glucomannan showed the maximum interaction with a binding energy of -394.1 kcal/mol lowest among five main compounds docked. The binding energy of mannan, quercetin, orientin, and hyperoside was -338.0 kcal/mol, -267.6 kcal/mol, -298.4 kcal/mol, and -304.2 kcal/mol, respectively (Table 2).

Table 2. Molecular docking results of the interaction between pancreatic alpha-amylase with glucomannan, mannan, quercetin, orientin, and hyperoside.

Compounds	Point interaction	Chemistry bond	Type	Binding energy (kcal/mol)
	A:GLN63:HE21 - :LIG1:O	Hydrogen Bond	Conventional Hydrogen Bond	
	A:ARG195:HH11 - :LIG1:O	Hydrogen Bond	Conventional Hydrogen Bond	
	A:ARG195:HH21 - :LIG1:O	Hydrogen Bond	Conventional Hydrogen Bond	
	:LIG1:H - A:TRP59:O	Hydrogen Bond	Conventional Hydrogen Bond	
Glucomannan	:LIG1:H - :LIG1:O	Hydrogen Bond	Conventional Hydrogen Bond	-394.1
	:LIG1:H - A:HIS299:NE2	Hydrogen Bond	Conventional Hydrogen Bond	
	:LIG1:H - A:ASN53:O	Hydrogen Bond	Conventional Hydrogen Bond	
	:LIG1:H - A:ASP197:OD1	Hydrogen Bond	Carbon Hydrogen Bond	
	:LIG1:H - A:ASN53:O	Hydrogen Bond	Carbon Hydrogen Bond	
	A:GLN63:HE21 - :LIG1:O	Hydrogen Bond	Conventional Hydrogen Bond	
	A:THR163:HG1 - :LIG1:O	Hydrogen Bond	Conventional Hydrogen Bond	
	A:ARG195:HH21 - :LIG1:O	Hydrogen Bond	Conventional Hydrogen Bond	
	:LIG1:H - :LIG1:O	Hydrogen Bond	Conventional Hydrogen Bond	
	:LIG1:H - A:THR163:O	Hydrogen Bond	Carbon Hydrogen Bond	
Mannan	:LIG1:H - :LIG1:O	Hydrogen Bond	Carbon Hydrogen Bond	-338.0
	:LIG1:H - A:ASP197:OD1	Hydrogen Bond	Carbon Hydrogen Bond	
	:LIG1:H - A:GLU233:OE2	Hydrogen Bond	Carbon Hydrogen Bond	
	:LIG1:H - :LIG1:O	Hydrogen Bond	Carbon Hydrogen Bond	
	:LIG1:H - :LIG1:O	Hydrogen Bond	Carbon Hydrogen Bond	
	:LIG1:H - A:TYR62	Hydrogen Bond	Pi-Donor Hydrogen Bond	
	A:THR264:HG1 - :LIG1:O	Hydrogen Bond	Conventional Hydrogen Bond	
	A:THR264:HG1 - :LIG1:O	Hydrogen Bond	Conventional Hydrogen Bond	
	:LIG1:H - :LIG1:O	Hydrogen Bond	Conventional Hydrogen Bond	
	A:GLY308:C,O;GLY309:N - :LIG1	Hydrophobic	Amide-Pi Stacked	
Quercetin	:LIG1 - A:ALA260	Hydrophobic	Pi-Alkyl	-267.6
	:LIG1 - A:LEU237	Hydrophobic	Pi-Alkyl	
	:LIG1 - A:LYS257	Hydrophobic	Pi-Alkyl	
	:LIG1 - A:ALA260	Hydrophobic	Pi-Alkyl	
	:LIG1 - A:LYS261	Hydrophobic	Pi-Alkyl	
	A:GLN63:HE21 - :LIG1:O	Hydrogen Bond	Conventional Hydrogen Bond	
	:LIG1:H - A:HIS299:NE2	Hydrogen Bond	Conventional Hydrogen Bond	
	:LIG1:H - A:GLU233:OE2	Hydrogen Bond	Conventional Hydrogen Bond	
	:LIG1:H - A:ASP197:OD2	Hydrogen Bond	Conventional Hydrogen Bond	
	:LIG1:H - :LIG1:O	Hydrogen Bond	Conventional Hydrogen Bond	
Orientin	:LIG1:H - :LIG1:O	Hydrogen Bond	Carbon Hydrogen Bond	-298.4
	:LIG1:H - A:GLU233:OE2	Hydrogen Bond	Carbon Hydrogen Bond	
	A:ASP300:OD1 - :LIG1	Electrostatic	Pi-Anion	
	A:LEU165:CD1 - :LIG1	Hydrophobic	Pi-Sigma	
	:LIG1:O - A:HIS305	Other	Pi-Lone Pair	
	A:TRP59 - :LIG1	Hydrophobic	Pi-Pi Stacked	

*Bold letter indicates donor residue

Analysis of the structural stability and fluctuation of pancreatic alpha-amylase residues bound to glucomannan, mannan, quercetin, and orientin was carried out. Molecular dynamics simulation results show the interaction of the pancreatic alpha-amylase protein with the four compounds (glucomannan, mannan, quercetin, and orientin) as stable as indicated by an RMSD value of less than 2.5 from beginning to end (Figure 3a). Ligand movement and ligand configuration from beginning to end showed RMSD values of about 2 – 10 and 0.5 – 2, respectively (Figure 3b-

c). Molecular dynamics analysis showed the RMSF value of less than three from the beginning to the end, which indicated the stable particle position (Figure 3d).

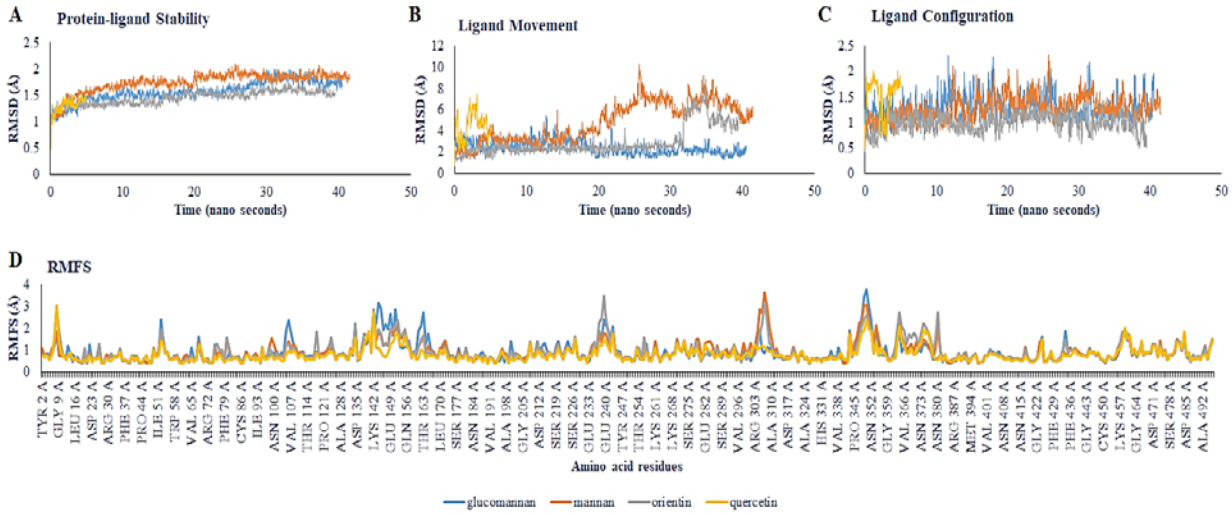

Figure 3. The molecular dynamics of the complex structure between pancreatic alpha-amylase with glucomannan, mannan, quercetin, and orientin interactions during simulation. A. The stability of protein-ligand complex can be seen from the RMSD value. B. Ligand movement. C. Ligand configuration. D. RMSF value represents the stability of amino acid residues.

4. Discussion

This study found the chemical components to be similar to the previous study, which reported 67 compounds contained in porang tuber extract, both from the second and third growing periods (Gusmalawati *et al*., 2019). Exploration of natural bioactive compounds within plant is critical to predicting the pharmacological activity of plants. Glucomannan and mannan are polysaccharides with nutraceutical roles, such as relieving constipation and reducing weight and cholesterol. In addition, quercetin, orientin, and hyperoside are flavonoids known to have antioxidant, anti-inflammatory, antiapoptotic, and anti-diabetic activities (Harijati *et al*., 2012; Shi *et al*., 2019). Previous studies reported that Moringa oleifera was effective as an anti-diabetic through the inhibition of TNF-α (tumor necrosis factor) and IFN-γ (interferon-gamma) in male BALB/c mice (Lestari *et al*., 2022). This effectiveness is likely due to bioactive phenolic compounds, flavonoids, tannins, terpenoids, and alkaloids, which have anti-diabetic activity. The assessment based on the Probability to be active (Pa) value of the bioactive compound. The Pa value reflects the ability of an active compound in particular biological processes. The higher the value of Pa means the more potential (Syamsul *et al*., 2019; Setiawan *et al*., 2018). The current study showed that glucomannan and mannan occupied the highest Pa value of 0.77. In conclusion, bioactive in porang tuber extract was indicated as the most potent active compound as anti-diabetic.

The result in this study ensures that the compounds contained in porang tuber extract have many health benefits as herbal medicine. The main compounds (glucomannan, mannan, quercetin, orientin, and hyperoside) identified in porang tubers were targeted for molecular docking to investigate their potential role as anti-diabetic. Three amino acid residues, Asp197, Glu233, and Asp300 of domain A, revealed in this study were identified as active sites of pancreatic alpha-amylase (Kikiowo *et al*., 2020). Here, the presence of hydrogen bonding plays a vital role in stabilizing the structure of biomolecules and catalyzing enzymes (Alqahtani *et al*., 2019; Kikiowo *et al*., 2020). The binding of glucomannan and mannan to Asp197 and Glu233 amino acid residues were critical in catalytic activity through hydrogen interactions. As a result, alpha-amylase activity can be inhibited and then slows down the digestion of carbohydrates, so glucose absorption into the bloodstream will be reduced (Quek *et al*., 2020).

A previous study correlated the compound structure with its activity. It showed that the interaction of flavonoids and pancreatic alpha-amylase inhibit the activation of one of the enzyme conformations by shifting the equilibrium to the most stable conformation. As a result, enzymatic activity will be decreased (Martinez-Gonzalez *et al*., 2019). The type of interaction in the binding of the ligand-receptor complex is affected by the binding energy. The lower binding energy indicated the docked compound is easier binding to the receptor-binding domain (RBD) (Matthew *et al*., 2021).

5. Conclusion

The present study is a novel discovery investigating phytochemical components, and in silico approach revealed the pancreatic alpha-amylase inhibitory of porang tubers grown in East Java, Indonesia. LCMS analysis showed 67 compounds, and it was dominated by glucomannan, mannan, and flavonoids. The main flavonoid components in porang tubers were represented by quercetin, orientin, and hyperoside. Our finding reveals that porang tuber extract can be employed as a natural medicine for diabetes mellitus by inhibiting the pancreatic alpha-amylase enzyme. Further study regarding the function of primary and secondary metabolite of porang as alpha-amylase inhibitor both in vitro and in vivo is required to confirm this result.

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