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IN SILICO STUDY OF TRITERPENOID IDENTIFIED FROM *Ceriops decandra* LEAVES AS INHIBITORS OF α-AMYLASE

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ABSTRACT

α-amylase has a pivotal role in catalyzing the cleavage of α-1,4-glycosidic bonds of polysaccharides to produce oligosaccharides. The inhibition of α-amylase delays the breakdown of carbohydrates, causing a reduction of blood glucose levels absorption in diabetes patients. The exploration of α-amylase inhibitors has attracted because society assumed that utilizing herbal medicine reduced the side effect of prescribed drugs. Mangrove from genus Ceriops have been used as antidiabetic, but the mechanism as α-amylase inhibitors has not been reported. Consumption of leaves extract of *C.decandra* reduced blood glucose level in diabetic rats, and triterpenoids have been identified from the leaves. With this in mind, this study aims to predict the molecular interactions between α-amylase (PDB ID: 4GQR) and the inhibitors, triterpenoid identified in *C.decandra* leaves, and to evaluate the potency of triterpenoid as α-amylase inhibitor. There are five triterpenoids identified in *C.decandra* leaves used as ligand tests, including lupenone, betulin, betulonic acid, betulinic acid, and lupeol. The descriptive method was applied in this investigation. This study was carried out from June to September 2022. Based on the molecular interactions, the binding affinity of triterpenoids was lower than the native ligand and control ligand. Lupenone, lupeol, betalonic acid, and betulinic acid inhibited α-amylase activity by non-competitive inhibition. It was predicted that betulin inhibited α-amylase activity through competitive inhibition.

Keywords: α-amylase, triterpenoid, *Ceriops decandra*.

I. INTRODUCTION

Mangroves grow in an intertidal area with high salinity. Mangrove has an ecological function by protecting shorelines from erosion and tsunami; and providing nursery area for some marine organisms such as fish, *crustacean, reptile*, and birds. Mangrove resources have been used by mangrove societies as a food and herbal medicine [1]. Therefore, it is vital to keep mangrove sustainable. *Ceriops decandra* known as spurred mangrove, is one of mangrove species, have been used as a food or traditional medicine [2]. Genus *Ceriops* have five species, including *C.autralis, C.decandra, C.pseudodecandra, C.tagal* and *C.zippeliana* [1].

The bark of *C.tagal* utilizes to cure diabetes and to stop bleeding [3], [4]. All of the plant parts from *C. roxburghiana* uses as a traditional medicine for antiulcer and antidiabetes. The stem, fruit and leaves of *C.decandra* also used by mangrove society to treat hepatitis and ulcer [3]. *C.tagal* leaves inhibited α-glucosidase with an IC_{50} value of 0,07±0,001 mg/mL[5] and α-

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amylase activity with an IC_{50} value of 2,576 ± 0,029 mg/mL [6]. In the previous study, betulinic acid, and lupeol extracted from the bark of *C.tagal* inhibited α-glucosidase with IC_{50} values of 5,31µM and 55,84 µM, respectively [7]. The administration of *C decandra* leaves extract 120 mg/kg decrease diabetic rats for 30 days [8]. Based on the previous work, phytochemical compounds of genus Cecropia are potent as antidiabetic by inhibiting α-glucosidase and α-amylase. However, the study of phytochemical compounds identified from *C.decandra* as inhibitors α-glucosidase and α-amylase has not been conducted.

α-amylase has a pivotal role in carbohydrate metabolism to breakdown starch at α-1,4 glycosidic bond into oligosaccharide. an α-amylase inhibitor is one of the targets in the antidiabetic development. Inhibiting α-amylase activity during carbohydrate metabolism will delay carbohydrate breakdown into small molecules such as glucose. Hence, its control blood glucose fluctuation in the human blood [9].

Inhibitor of α-glucosidase and α-amylase were used to control postprandial hyperglycemic. Commercial drugs such as acarbose, miglitol and voglibose were consumed as α-glucosidase inhibitors [10]. However, consuming commercial drugs also triggered adverse effect such as flatulence, diarrhea and other digestive disorders. Thus, several studies were conducted to evaluate the natural product from terrestrial and marine plants as α-glucosidase inhibitor.

Biological activity of *Ceriops* sp. is affected by the phytochemical composition. Triterpenoid was a prominent compound from *Ceriops* sp. Triterpenoid is a secondary metabolite derivated from precursor 2,3-oxidosqualene and consists of 30 carbons. This molecule was generated by mevalonate pathway [11],[12]. It had been identified the phytochemical compounds of *C decandra* leaves including 3β-E-coumaroylbetulinic acid, lupeol, betulinic acid, betulin, betulinic acid, lupenone, 3β-E-feruloyllupeol acid, 3β-Z-feruloyllupeol acid [13]; lupenone, lupeol, betulinaldehyde, 3β-Z-coumaroyllupeol, 3β-E-coumaroyllupeol, 3-epi-betulinic acid, betulin, betulinic acid, 3β-E-feruloylbetulin, 30-nor-lup-3β-ol-20-one 12,3β-E-caffeoyllupeol, lup-20(29)-en-3β,30-diol, 3β-hydroxylupan-29-oic acid, 3β,20-dihydroxylupane, oleanolic acid and ursolic acid [14].

In this study, triterpenoids, namely lupenone, lupeol, betalonic acid, betulinic acid and betulin identified from *C.decandra* leaves were used as α-amylase inhibitors. This study aimed to predict molecular interaction between α-amylase as a receptor (PDB ID: 4GQR) and the selected inhibitor, triterpenoids identified from *C.decandra* leaves

2. RESEARCH METHOD

Time and Place

This study was conducted from August to October 2022 at Fish Product Technology Laboratory, Fisheries and Marine Sciences Faculty, Universitas Brawijaya, Malang.

Research Method

Ligands Preparation

Five triterpenoid compounds of *C.decandra* leaves, namely lupenone, lupeol, betulin, betulinic acid and betulonic acid. The compounds were chosen based on compounds identified from the earlier study. The reference compounds or positive control as a α-glucosidase inhibitor used for this study was acarbose and voglibose. The chemical structure (3D), namely triterpenoid and reference compounds (3D) were retrieved from https://pubchem.ncbi.nlm.nih.gov/ in SDF format [15]. In addition, native ligand NAG (2-acetamido-2-deoxy-beta-D-glucopyranose) and MYC (3,5,7-

trihidroxy-2-(3,4,5-trihydroxyphenyl)-4H-chromen-4-one) were downloaded from https://www.rcsb.org/structure/4GQR.

Druglikeness and Toxicity Analysis

Druglikeness and toxicity analysis were conducted according to the previous study [16]. Druglikeness analysis was carried out by the online website http://www.swissadme.ch/index.php [17]–[19]. Toxicity was examined using Protox tool https://tox-new.charite.de/protox_II/index.php?site=home [20]–[22].

Protein Preparation

The protein structure α-amylase (PDB ID: 4GQR) was downloaded from https://www.rcsb.org/structure/4GQR in SDF format. The receptor was prepared by removing ligands and water molecules by BIOVIA Discovery Studio 2019. Hydrogen polar was added and saved in PDB format.

Molecular Docking Analysis

Molecular docking analysis was performed by PyRx-Virtual Screening Tool (AutoDock Vina) [23]. The receptor was inputted as a macromolecule. Ligands such as triterpenoid positive control and native ligand were inputted as SDF format by Open Babel (PyRx-Virtual Screening Tool). The energy was minimized, and Open Babel converted the SDF format into PDBQT format. Grid box was set up *center* x=8.4474; y=27.9862; z=49.1350 and dimension x=58.9736; y= 73.7796; and z=58.5527. The result of the binding affinity value was saved in CSV format. The docked molecule was saved in PDB format, and Discovery Biovia Studio 2019 visualized it.

3. RESULT AND DISCUSSION

Druglikeness and Toxicity Analysis

Triterpenoid was a prominent compound of *Ceriops* sp. Triterpenoid compounds were identified from *C.decandra*, but the information related to their biological activity as antidiabetic is limited. Based on the previous study, five triterpenoids were identified from *C.decandra* leaves such as lupenone, lupeol, betulin, betulinic acid, and betulonic acid [13], [14] and the compounds were selected as α-amylase inhibitors. Druglikeness and toxicity class were evaluated to predict the potency of the compounds as oral drugs, as presented by Table 1.

Table 1. Investigations of druglikeness and toxicity of triterpenoid identified from *C.decandra* leaves

Compound	***Druglikeness* : ADME**			**Toxicity**		**Organ**	
		Lipinski	**Bio-availability**	**LD_{50} (mg/kg)**	**Level**	**Hepato-toxicity**	**Probability**
Lupenone	Yes	1:LOGP>4.15	0.55	5,000	5	Inactive	0.74
Lupeol	Yes	1:LOGP>4.15	0.55	2,000	5	Inactive	0.91
Betulin	Yes	1: OGP>4.15	0.55	2,000	4	Inactive	0.88
Betulinic acid	Yes	1:LOGP>4.15	0.85	2,610	5	Inactive	0.54
Betulonic acid	Yes	1: OGP>4.15	0.85	2,610	5	Inactive	0.70

The SwissADME database http://www.swissadme.ch/ was used to obtain information related to the Lipinski properties of the compounds. There are five rules of Lipinski to determine the druglikeness of its compounds such as (1) molecular weight < 500 Da, (2) MLog P < 5 to show the lipophilicity, (3) hydrogen donor bond < 5, (4) hydrogen acceptor < 10 and (5) molar refractivity 40-130 [17], [19]. According to Lipinski's rule, it appears that all of the triterpenoids fulfilled the rules; therefore, it was expected to be well absorbed and permeable in the human body. In addition, the bioavailability of triterpenoids was 0.55-0.85.

Toxicity analysis used ProTox database https://tox-new.charite.de [20]. Level 1 is the most toxic, and level 6 is the least toxic. As presented by Table 1, none of the triterpenoid compounds exhibited any toxicity. Based on hepatotoxicity analysis, the triterpenoids compounds do not have hepatotoxicity properties or are inactive. It indicated that triterpenoids identified from *C. decandra* leaves do not have toxicity for human consumption

Molecular Interaction Analysis

Molecular interaction analysis between triterpenoid compounds with protein α-amylase, are provided in Table 2. Binding affinity of molecular interaction ligand and α-amylase (PDB ID: 4GQR); and molecular interaction between ligand and amino acid of α-amylase (PDB ID: 4GQR) are presented in Table 3. The 3D (a) and 2D (b) plots of α-amylase (PDB ID: 4GQR) interactions with the triterpenoid compounds were visualized in Figure 1-9.

Table 2. Molecular interaction between ligand and amino acid of α-amylase (PDB ID: 4GQR)

Name	Interaction	Distance (Å)	Category	Type
Lupenone	A:ILE235 - N:UNK1	5.25612	Hydrophobic	Alkyl
	N:UNK1:C - A:LEU162	3.94027	Hydrophobic	Alkyl
	N:UNK1:C - A:ILE235	5.32713	Hydrophobic	Alkyl
	N:UNK1:C - A:ILE235	5.0739	Hydrophobic	Alkyl
	N:UNK1:C - A:ILE235	3.78289	Hydrophobic	Alkyl
	A:TRP58 - N:UNK1	4.84864	Hydrophobic	Pi-Alkyl
	A:TRP58 - N:UNK1:C	5.42056	Hydrophobic	Pi-Alkyl
	A:TRP58 - N:UNK1:C	4.4967	Hydrophobic	Pi-Alkyl
	A:TRP59 - N:UNK1:C	4.35372	Hydrophobic	Pi-Alkyl
	A:TRP59 - N:UNK1:C	4.40448	Hydrophobic	Pi-Alkyl
	A:TRP59 - N:UNK1:C	5.15932	Hydrophobic	Pi-Alkyl
	A:TYR62 - N:UNK1	5.35947	Hydrophobic	Pi-Alkyl
	A:TYR62 - N:UNK1:C	4.41284	Hydrophobic	Pi-Alkyl
	A:TYR151 - N:UNK1:C	4.06934	Hydrophobic	Pi-Alkyl
Lupeol	A:LEU162 - N:UNK1	5.4469	Hydrophobic	Alkil
	A:ILE235 - N:UNK1	5.21762	Hydrophobic	Alkyl
	N:UNK1:C - A:ILE235	5.37845	Hydrophobic	Alkyl
	N:UNK1:C - A:LEU162	3.97689	Hydrophobic	Alkyl
	N:UNK1:C - A:ILE235	5.06708	Hydrophobic	Alkyl
	N:UNK1:C - A:ILE235	3.88604	Hydrophobic	Alkyl
	A:TRP58 - N:UNK1	5.01972	Hydrophobic	Pi-Alkyl
	A:TRP58 - N:UNK1:C	4.46766	Hydrophobic	Pi-Alkyl
	A:TRP59 - N:UNK1:C	4.28325	Hydrophobic	Pi-Alkyl
	A:TRP59 - N:UNK1:C	4.42381	Hydrophobic	Pi-Alkyl
	A:TRP59 - N:UNK1:C	5.14286	Hydrophobic	Pi-Alkyl
	A:TYR62 - N:UNK1	5.41874	Hydrophobic	Pi-Alkyl
	A:TYR62 - N:UNK1:C	4.41489	Hydrophobic	Pi-Alkyl
	A:TYR151 - N:UNK1:C	4.19422	Hydrophobic	Pi-Alkyl
Betulin	N:UNK1:H - A:ASP197:OD2	2.39405	Hydrogen Bond	Conventional Hydrogen Bond
	A:LEU162 - N:UNK1	5.39669	Hydrophobic	Alkyl
	A:ALA198 - N:UNK1	4.75849	Hydrophobic	Alkyl
	N:UNK1 - A:LEU162	5.34792	Hydrophobic	Alkyl
	N:UNK1:C - A:LEU165	4.67328	Hydrophobic	Alkyl
	N:UNK1:C - A:LEU162	4.29089	Hydrophobic	Alkyl
	A:TRP58 - N:UNK1:C	4.90251	Hydrophobic	Pi-Alkyl
	A:TRP59 - N:UNK1	4.9075	Hydrophobic	Pi-Alkyl
	A:TRP59 - N:UNK1:C	4.17546	Hydrophobic	Pi-Alkyl
	A:TRP59 - N:UNK1	5.29223	Hydrophobic	Pi-Alkyl
	A:TRP59 - N:UNK1:C	4.17335	Hydrophobic	Pi-Alkyl
	A:TRP59 - N:UNK1:C	4.51596	Hydrophobic	Pi-Alkyl
	A:TYR62 - N:UNK1	5.34517	Hydrophobic	Pi-Alkyl

Name	Interaction	Distance (Å)	Category	Type
	A:HIS201 - N:UNK1:C	5.02659	Hydrophobic	Pi-Alkyl
Betulinic acid	A:ALA198 - N:UNK1	4.77826	Hydrophobic	Alkyl
	N:UNK1:C - A:LEU165	4.87105	Hydrophobic	Alkyl
	N:UNK1:C - A:ILE235	5.42345	Hydrophobic	Alkyl
	N:UNK1:C - A:LEU162	4.28617	Hydrophobic	Alkyl
	A:TRP58 - N:UNK1:C	4.86746	Hydrophobic	Pi-Alkyl
	A:TRP59 - N:UNK1	4.9317	Hydrophobic	Pi-Alkyl
	A:TRP59 - N:UNK1:C	4.10172	Hydrophobic	Pi-Alkyl
	A:TRP59 - N:UNK1	5.42305	Hydrophobic	Pi-Alkyl
	A:TRP59 - N:UNK1:C	4.1589	Hydrophobic	Pi-Alkyl
	A:TYR62 - N:UNK1	5.22126	Hydrophobic	Pi-Alkyl
	A:HIS201 - N:UNK1:C	4.83568	Hydrophobic	Pi-Alkyl
Betulonic acid	N:UNK1:H - A:ASP300:OD2	2.64939	Hydrogen Bond	Conventional Hydrogen Bond
	A:ILE235 - N:UNK1	5.16322	Hydrophobic	Alkyl
	N:UNK1 - A:LEU162	5.32205	Hydrophobic	Alkyl
	N:UNK1:C - A:LEU162	3.9567	Hydrophobic	Alkyl
	N:UNK1:C - A:ILE235	5.15545	Hydrophobic	Alkyl
	N:UNK1:C - A:ILE235	5.05929	Hydrophobic	Alkyl
	N:UNK1:C - A:ILE235	3.85594	Hydrophobic	Alkyl
	A:TRP58 - N:UNK1	4.81662	Hydrophobic	Pi-Alkyl
	A:TRP58 - N:UNK1:C	4.57727	Hydrophobic	Pi-Alkyl
	A:TRP59 - N:UNK1:C	4.44346	Hydrophobic	Pi-Alkyl
	A:TRP59 - N:UNK1:C	4.59507	Hydrophobic	Pi-Alkyl
	A:TYR62 - N:UNK1	5.44969	Hydrophobic	Pi-Alkyl
	A:TYR151 - N:UNK1:C	4.03258	Hydrophobic	Pi-Alkyl
Acarbose	A:GLY283:N - N:UNK1:O	2.80933	Hydrogen Bond	Conventional Hydrogen Bond
	A:GLY334:N - N:UNK1:O	3.25493	Hydrogen Bond	Conventional Hydrogen Bond
	A:GLY403:N - N:UNK1:O	2.84327	Hydrogen Bond	Conventional Hydrogen Bond
	N:UNK1:H - N:UNK1:O	2.24725	Hydrogen Bond	Conventional Hydrogen Bond
	N:UNK1:H - A:TRP280:O	2.1747	Hydrogen Bond	Conventional Hydrogen Bond
	N:UNK1:H - A:TRP280:O	2.68066	Hydrogen Bond	Conventional Hydrogen Bond
	N:UNK1:H - A:ASP402:OD1	2.58024	Hydrogen Bond	Conventional Hydrogen Bond
	N:UNK1:H - A:PRO332:O	2.457	Hydrogen Bond	Conventional Hydrogen Bond
	N:UNK1:H - N:UNK1:O	2.75418	Hydrogen Bond	Conventional Hydrogen Bond
	N:UNK1:H - N:UNK1:O	2.61289	Hydrogen Bond	Carbon Hydrogen Bond
	A:ASP402:CA - N:UNK1:O	3.58982	Hydrogen Bond	Carbon Hydrogen Bond
	N:UNK1:C - A:GLU282:OE1	3.63178	Hydrogen Bond	Carbon Hydrogen Bond
Voglibose	A:ARG252:NH1 - N:UNK1:O	3.29071	Hydrogen Bond	Conventional Hydrogen Bond
	A:SER289:OG - N:UNK1:O	2.93084	Hydrogen Bond	Conventional Hydrogen Bond
	A:GLY403:N - N:UNK1:O	3.02665	Hydrogen Bond	Conventional Hydrogen Bond
	A:ARG421:NH2 - N:UNK1:O	2.93078	Hydrogen Bond	Conventional Hydrogen Bond
	N:UNK1:H - N:UNK1:O	2.41822	Hydrogen Bond	Conventional Hydrogen Bond
	N:UNK1:H - A:PRO332:O	2.3602	Hydrogen Bond	Conventional Hydrogen Bond
	N:UNK1:H - A:GLY334:O	2.4605	Hydrogen Bond	Conventional Hydrogen Bond
	N:UNK1:H - A:GLY334:O	2.07063	Hydrogen Bond	Conventional Hydrogen Bond
	N:UNK1:C - A:ASP402:OD1	3.12715	Hydrogen Bond	Carbon Hydrogen Bond
NAG	A:ARG195:NH2 - N:UNK1:O	3.13781	Hydrogen Bond	Conventional Hydrogen Bond
	N:UNK1:H - A:ASP197:OD1	2.59064	Hydrogen Bond	Conventional Hydrogen Bond
	N:UNK1:H - A:ASP197:OD1	2.50056	Hydrogen Bond	Conventional Hydrogen Bond
	N:UNK1:H - A:GLU233:OE1	2.87847	Hydrogen Bond	Conventional Hydrogen Bond
MYC	A:THR6:OG1 - N:UNK1:O	2.72055	Hydrogen Bond	Conventional Hydrogen Bond
	A:ARG398:NH2 - N:UNK1:O	3.26299	Hydrogen Bond	Conventional Hydrogen Bond
	N:UNK1:H - A:ASP402:OD1	2.69389	Hydrogen Bond	Conventional Hydrogen Bond
	N:UNK1:H - A:PRO332:O	2.60037	Hydrogen Bond	Conventional Hydrogen Bond
	N:UNK1:H - A:ARG10:O	2.49026	Hydrogen Bond	Conventional Hydrogen Bond
	N:UNK1:H - A:THR6:O	2.79867	Hydrogen Bond	Conventional Hydrogen Bond
	N:UNK1:H - N:UNK1:O	2.02014	Hydrogen Bond	Conventional Hydrogen Bond
	N:UNK1:H - A:SER3:OG	2.61738	Hydrogen Bond	Conventional Hydrogen Bond
	N:UNK1 - A:PRO4	4.60301	Hydrophobic	Pi-Alkyl
	N:UNK1 - A:PRO4	4.0107	Hydrophobic	Pi-Alkyl

Table 3. Binding affinity of molecular interaction ligand and α-amylase (PDB ID: 4GQR)

Compounds	Binding Affinity (kcal/mol)	rmsd/ub	rmsd/lb
Lupenone	-9.3	0	0
Lupeol	-9.1	0	0
Betulin	-8.8	0	0
Betulinic acid	-8.9	0	0
Betulonic acid	-9.2	0	0
Acarbose (control)	-8.5	0	0
Voglibose (control)	-6.3	0	0
NAG (native ligand)	-5.6	0	0
MYC (native ligand)	-8	0	0

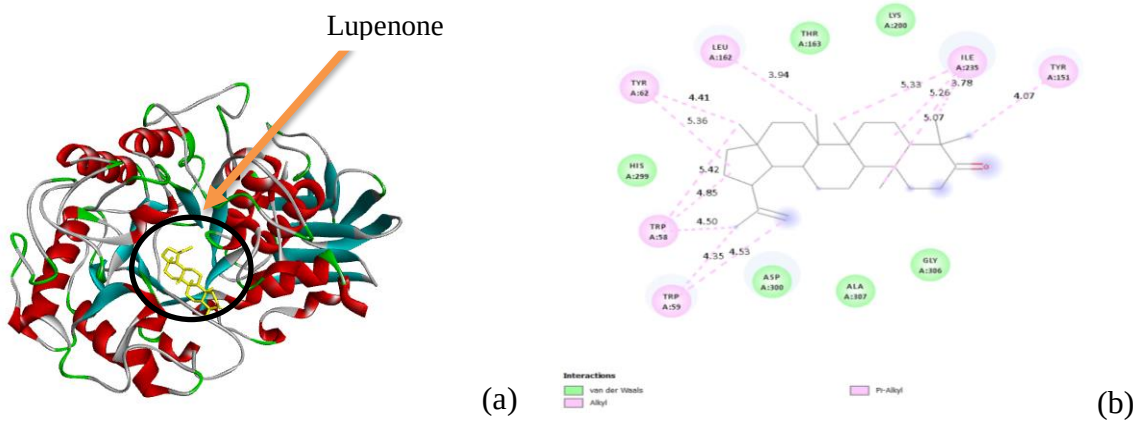

Figure 1. The 3D (a) and 2D (b) plot of α-amylase (PDB ID: 4GQR) interactions with lupenone

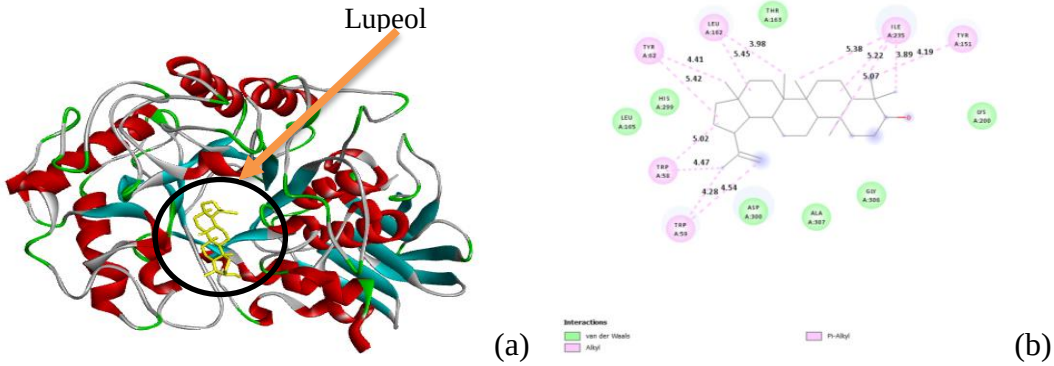

Figure 2. The 3D (a) and 2D (b) plot of α-amylase (PDB ID: 4GQR) interactions with lupeol

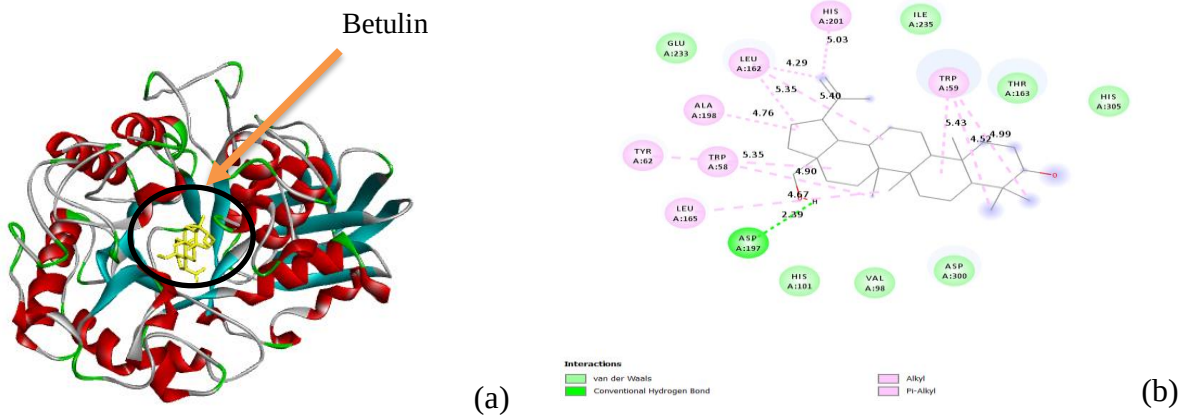

Figure 3. The 3D (a) and 2D (b) plot of α-amylase (PDB ID: 4GQR) interactions with betulin

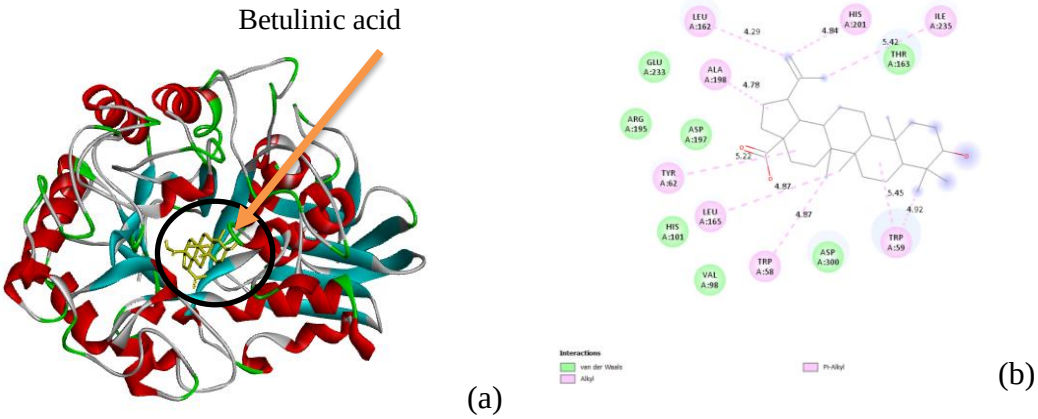

Figure 4. The 3D (a) and 2D (b) plot of α-amylase (PDB ID: 4GQR) interactions with betulinic acid

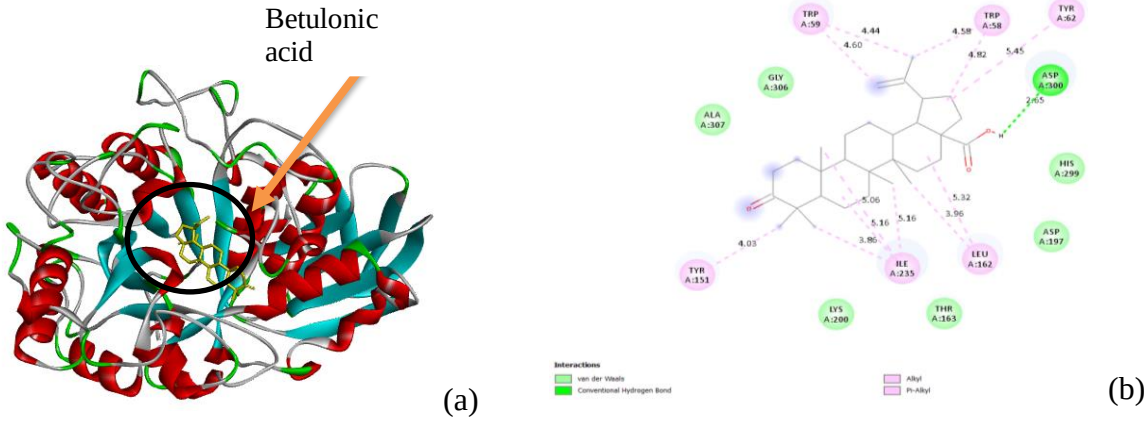

Figure 5. The 3D (a) and 2D (b) plot of α-amylase (PDB ID: 4GQR) interactions with betulonic acid

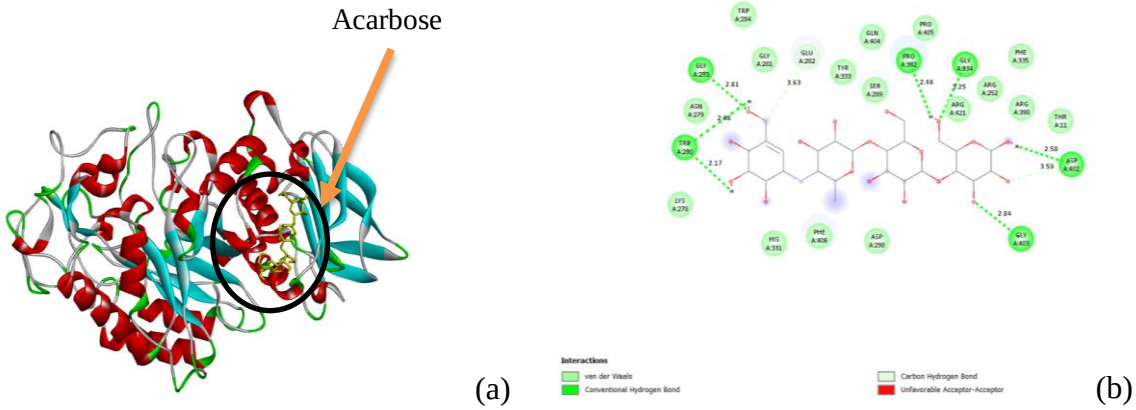

Figure 6. The 3D (a) and 2D (b) plot of α-amylase (PDB ID: 4GQR) interactions with acarbose

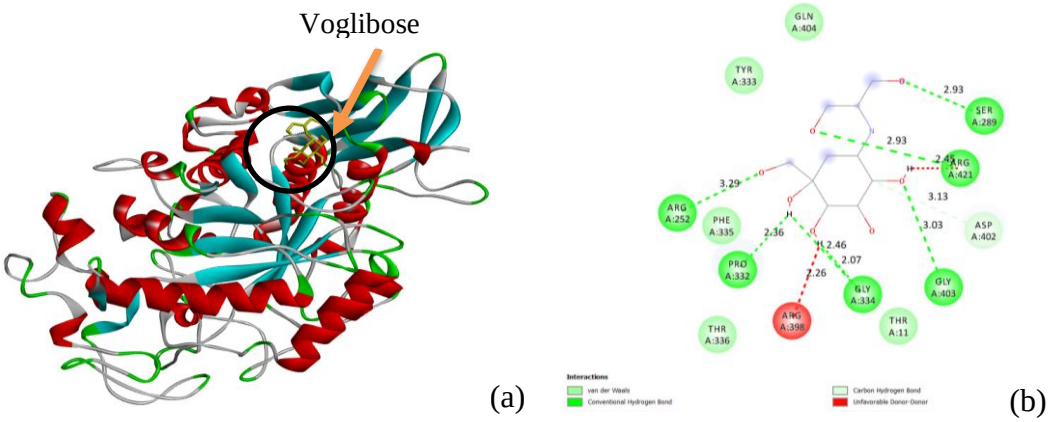

Figure 7. The 3D (a) and 2D (b) plot of α-amylase (PDB ID: 4GQR) interactions with voglibose

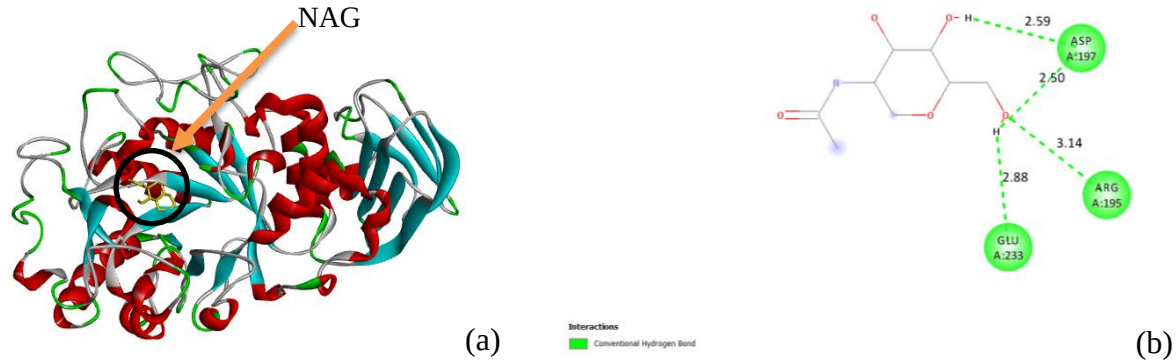

Figure 8. The 3D (a) and 2D (b) plot of α-amylase (PDB ID: 4GQR) interactions with NAG

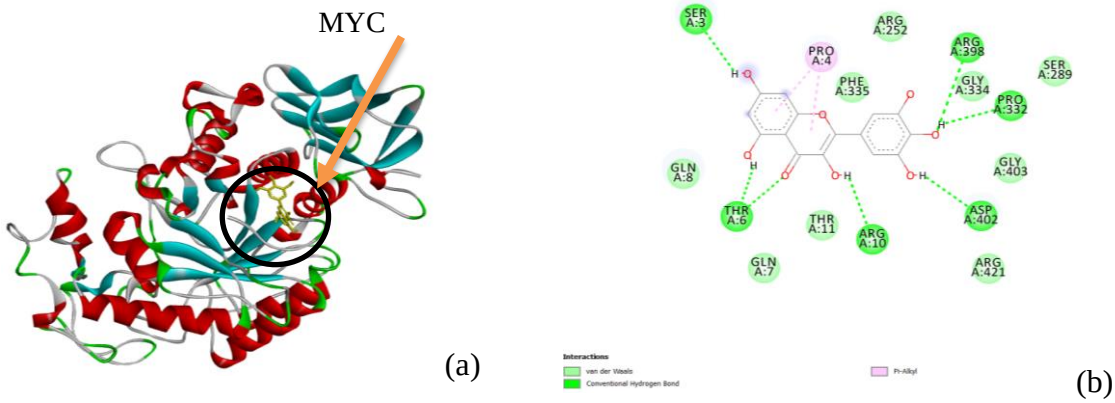

Figure 9. The 3D (a) and 2D (b) plot of α-amylase (PDB ID: 4GQR) interactions with MYC

The best interaction showed by between ligand and receptor along with the least binding affinity was considered as the highest inhibiting activity. As shown by Table 3, the least binding affinity of triterpenoids was lupenone (-9,3 kcal/mol), and the highest binding affinity was betulin. Therefore, the best interaction between ligand and α-amylase was the interaction of lupenone.

Analysis of potential as α-amylase inhibitors showed that lupenone (-9.3 kcal/mol), lupeol (-9.1 kcal/mol), betulin (-8.8 kcal/mol), betulinic acid (-8.9 kcal/mol) and betulonic acid (-9.2 kcal/mol) have an lower binding energy than the control acarbose (-8.5 kcal/mol), voglibose (-6.3 kcal/mol), or the ligan native NAG (-5,6 kcal/mol), MYC (-8 kcal/mol); which is predicted to have the potential as inhibitors of α-amylase.

The binding affinity value was validated by the RMSD of each ligand. The value was obtained from the optimization the best pose during molecular docking analysis of the selected ligands and receptor α-amylase. The lowest RMSD value performed the best ligand position approaching the conformation of ligan native, namely NAG and MYC.

In this study, two inhibitor references were used as control, namely acarbose and voglibose. Molecular docking between control and receptor performed four similar binding site amino acid residues such as GLY334, GLY403, ASP402 and PRO332. Two amino acid residues, ASP402 and PRO332, were also bound native ligand MYC (Figure 9). GLY334, GLY403, ASP402 and PRO332 are only linked to native ligan and control. It was indicated that triterpenoid identified from *C.decandra* leaves such as lupenone, lupeol, betulin, betulinic acid and betulonic acid inhibited α-amylase by non-competitive inhibition.

Several amino acid residues were bound at the binding site of α-amylase and triterpenoid compounds identified from *C.decandra* leaves namely ILE235, LEU162, ILE235, TRP58, TRP59, TYR62, and TYR151 by hydrophobic bond. Three amino acid residues such as ALA198, LEU165, and HIS201 were bound with betulin, and betulinic acid by hydrophobic bond. Among the five-triterpenoid compounds, only betulin and betulinic acid have conventional hydrogen bond with α-amylase (PDB ID: 4GQR). The compounds with more hydrogen bonding interactions was assumed that it has significant biological activities [24].

Human pancreatic α-amylase consists of three domain such as A, B and C. Active site of α-amylase was found at the amino acid residue ASP197, GLU233 and ASP300 [25]. In this study, betulin was bound to catalytic amino acid residue ASP197 by conventional hydrogen bond; therefore it was predicted that betulin inhibited α-amylase by competitive inhibition mode [26]. Betulin is a part of pectacyclic triterpenoid and derivate of lupane. Hydroxyl group (-OH) at C-28 of ligand or betulin was docked at ASP197 with the distance 2.39 Å by hydrogen bond (Figure 3). The result of this study related to the earlier result, hydroxyl group of γ-mangostin formed hydrogen bond with carboxyl group of amino acid residue ASP197 [27]. The binding energy of betulin (-8.8 kcal/mol) was lower than acarbose, voglibose and native ligan. Thus, it was predicted that betulin, one of triterpenoid compounds identified from *C.decandra* leaves potent as α-amylase inhibitors by competitive inhibition. Other triterpenoid compounds identified from *C.decandra* leaves were inhibited α-amylase activity by non-competitive inhibition.

4. CONCLUSION

Five triterpenoid compounds were identified from *C. decandra* leaves including lupenone, lupeol, betulin, betulinic acid and betulonic acid. Based on the molecular docking analysis, the binding affinity of triterpenoid compounds were lower than ligand native and control. Lupenone, lupeol, betulinic acid and betulonic acid was inhibited α-amylase activity by non-competitive inhibition. On the contrary, betulin inhibited α-amylase by competitive inhibition. According to the result of molecular docking, and druglikeness and toxicity analysis were concluded that betulin was potent as α-amylase inhibitors. Thus, it was suggested to carry out in vitro analysis to determine inhibitory concentration of *C. decandra* leaves extract as α-amylase inhibitors.

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