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Research Article

Inhibitory Activity of α-Glucosidase of Bark of *Ceiba pentandra* Linn.

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ABSTRACT

Bark of Kapok (*Ceiba pentandra* (L.)) contains tannin, flavonoid, alkaloid, terpenoid, and saponin compounds. Flavonoid known to have inhibitory activity of α-glucosidase. The purpose of this study was to observe the n-hexane, ethyl acetate and water extract of bark of *Ceiba pentandra* (L.) to inhibitory activity of α-glucosidase. The method used for the activity measurement of the fractions was spectrophotometry UV-Vis. Water fraction analyzed by LCMS/MS to identify the chemical compounds. The pure compound suspected analyzed using molecular docking to predict the complementarity related to the model of isomaltase α-glucosidase using Autodock Vina, therefore the bond of the antidiabetic active compounds can be predicted. The results show the α-glucosidase inhibitory activity of the bark in n-hexane fraction is 4.60μg/mL, ethyl acetate fraction is 8.55μg/mL and water fraction is 5.61μg/mL. Water fraction analyzed by LCMS/MS eludated at retention time of 3.61min be fathomed as (+) catechin derivate and at retention time of 8.70min be fathomed as vavain derivate. The vavain derived compound, (+) catechin derived compound, acarbose and quercetin each shows docking score of -8.1; -8.8; -6.2 and -7.6 kkal/mol and have similarity to amino acid Glu276 bond which is a catalytic residue that plays role in hydrolysis process. The similarity of chemical bond of the active site be expected to have the same activity as α-glucosidase inhibitory agent.

Key words: α-glucosidase; AutoDock Vina; LCMS/MS; vavain derivative

INTRODUCTION

Diabetes mellitus (DM) is a chronic disease occurs either when the pancreas does not produce enough insulin or when the body cannot effectively use the insulin produced. World Health Organization (WHO) states that there are 422 million adults living with diabetes in 2014 in the world. The prevalence of people with diabetes in Indonesia has been increased from 5.7% in 2007 to 6,9% in 2013 and it becomes highest cause of death number 3 in Indonesia (World Health Organization, 2016).

DM treatment may be conducted in two ways, pharmacological and non-pharmacological method. Non-pharmacological therapy can be formed with diet control and exercises. Pharmacological therapy can be proceed with oral antidiabetic medication that is one of them α-glucosidase inhibitor acarbose, miglitol, voglibose, and deoxynojirimycin. These drugs work as competitive inhibitor of enzymatic breakage of maltase, isomaltase, sucrose and glyucoamylase inhibitor to delay the decomposition of sucrose and complex of carbohydrate in the intestine. The main effect is reducing glucose level in blood. (Wells *et al.,* 2009).

The side effect of acarbose as α-glucosidase inhibitory agent encourages many researches of other α-glucosidase inhibitor drugs from plants. Bark of *Ceiba pentandra* (L.) has potency as an antidiabetic. The α-glucosidase inhibitory activity in 80% ethanol extracted by reflux method shows IC_{50} of 5.16μg/mL (Mun'im *et al.,* 2013). According to another research, water extract of bark of *Ceiba pentandra (L.).* Shows hypoglycemic activity at dose of 250mg/kg in normal rats or diabetic rats induced by Streptozocin (Ladeji *et al.,* 2003).

Molecular docking is a computational method that is used to view the activity of any compound and predict the chemical bonds

among macromolecules and active compounds efficiently without any synthesis process at the first place, therefore it may reduce the cost and time of the research. This research is conducted to obtain the IC_{50} value of α-glucosidase using *in vitro* method from fractions of n-hexane, ethyl acetate and water of kapok bark. Water fraction identified by LCMS/MS to know the molecular weight of the bioactive compound, and in silico test performed afterward.

MATERIAL AND METHODS

Ceiba pentandra bark was obtained from BALITRO, Bogor, Indonesia. α-Glucosidase Type I: from yeast *Saccharomyces cerevisiae* (EC 3.2.1.20), bovine serum albumin and p-nitrophenyl-α-D-glucopyranoside (p-NPG) as synthetic substrate of α-glucosidase were purchased from Wako Pure Chemical Industries, Ltd (Osaka, Japan), Quercetin (Sigma), ethanol 80%, n-hexane (Merck), ethyl acetate (Merck), methanol (Merck), distilled water, sodium carbonate, buffer of pH 7, dimethyl sulfoxide, acetonitrile (Merck), formic acid 0,1% (Merck), receptor enzyme of isomaltase *Saccharomyces cerevisiae* in the Protein Data Bank (PDB ID: 3AJ7).

Reflux, spectrophotometer UV-vis (Shimazu UV-1800), UPLC-MS (Waters), Incubator (Mermet), AutoDock Tools, AutoDock Vina, Discovery Studio 2017R2, MacPymol (Schrodinger), Swiss-Model, iBabel and MarvinSketch (ChemAxon).

Extraction of the bark

The bark dried in the sun for 7 days, grinded to the powder form. The herbal powder of kapok bark was weighed 100g and was extracted by reflux method with 750mL of 80% ethanol for 30min. The extraction process is repeated four times. The extract obtained was filtrat and filtrate was evaporated with rotary evaporator to become viscous extract.

Fractionation

The ethanolic extract of kapok bark fractionated with liquid-liquid extraction method. Ten gram of viscous extract was dispersed with 100mL of distilled water until homogen suspension obtained. The suspension was fractionation with 100mL of n-hexane, shaken for 15min and awaited 15min for separation of polarity difference of the solvents. Non-polar fraction separated from polar fraction, and the polar fraction was again fractionated with ethyl acetate:water = 1:1 until there are 2 layer formed. Those 3 fractions collected and each evaporated using rotary evaporator to obtain viscous fractions.

Inhibitory activity enzyme α-glucosidase

α-glucosidase inhibitory activity test of kapok bark was based on the method of (Dewi *et al.,* 2007). Each mixture consists 5 µL extract, 495µL buffer phosphate pH 7, and 250µL p-nitrophenyl-α-D-glukopyranocide (pNPG) substrate with optimum consentration, mixture incubated for 5min at temperature of 37°C. The reaction stopped with addition of 1000µL sodium carbonate (Na_2CO_3) concentration of 200mM. The absorbance of final mixture measured by Spectrophotometer UV-Vis in wavelength of 400nm.

Preparation of instruments LCMS/MS

Sample of fraction dissolved in 100µL methanol before analyzed by LCMS as much as 5µL of the mixture. LC-MS/MS system used was (UPLC QTOF MS/MS: Waters), be equipped with UPLC BEH C_{18} (particle dimension of 1.7µm, 2.1mm x 50mm) and MS with XEVO-G2QTOF (Waters) in resolution method of ESI positive and Masslynk software (v.4.1). The eluent consisted of formic acid 0.1% in water (A), and formic acid 0.1% in acetonitrile. Total running time was 23min with flow rate of 0.2mL/minutes at temperature of 50°C. Component identity determined by fragmentation pattern of the spectra compared to KnapSack as literature.

Homology modelling

3D structure of α-glucosidase of *Saccharomyces cerevisiae* (maltase, EC 3.2.1.20) is not available. However, there is X-ray structure available of isozyme isomaltase (EC 3.2.1.10). Isomaltase with entry of (PDB ID: 3AJ7, Resolution of 1.30Aº) has high resolution X-ray structure and high sequence identity (73.51%) and score of sequence similarity (0.54) with α-glucosidase MAL32 (UniProt P38158) of *Saccharomyces cerevisiae.*

Table I. α-glucosidase inhibitory activity of Kapok (*Ceiba pentandra* L.) bark fractions.

Sample	IC_{50} (µg / mL)	Strength Inhibition
N-hexane fraction	4.60 ± 0.14	Active
Ethyl acetate fraction	8.55 ± 0.03	Active
Water fraction	5.61 ± 0.12	Active
Quercetin	6.04 ± 0.14	Active

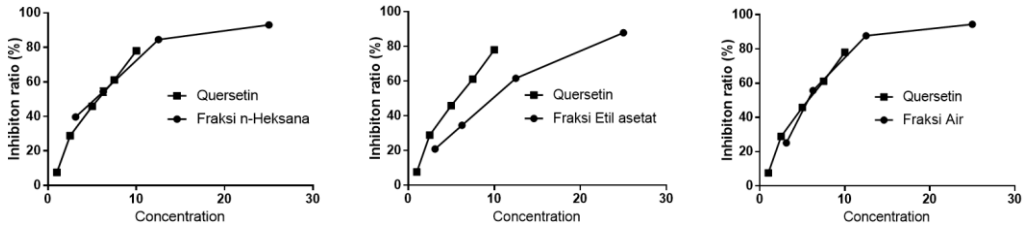

Figure 1. Graphic of α-glucosidase inhibitory activity of Kapok (*Ceiba pentandra* L*)* bark equation of logarithmic equations.

Homology modelling quality verified by PROCHECK (Sari *et al.,* 2016). Using MAL32 target sequence and structure with PDB ID 3AJ7 as template, homology modelling of α-glucosidase created by SWISS MODEL based on harmony of template of target using ProMod3. Model created has Global Model Quality Estimation (GMQE) of 0.93 and QMEAN of -0.23 (Proença *et al.,* 2017).

Molecular docking

3D structure of bioactive compounds created with MarvinSketch program and saved as .pdb format. Energy minimized using iBabel programme. Molecule attached by Autodock Vina 1.5.6. Optimized using Autodock Tools with addition of hydrogen polar and compute gasteiger and saved in .pdbqt format. Grid box quested of isomaltase α-glucosidase were center_x: 19.676; center_y: -7.243; and center_z: 21.469 with dimension of size_x: 15, size_y: 15, dan size_z: 15 and exhaustiveness: 20. The best score rated by Vina docking score selected and analyzed visually with MacPyMOL (Schrödinger®) software.

RESULT AND DISCUSSION

Dried bark obtain was 775g which was 40% herbal rendement of crude bark. Reflux method chosen because of the selectivity and effectivity of time. 400g powder of bark extracted by 3 liters of ethanol 80% for 30min, rendement obtained of the extract was 17.04%.

α-Glucosidase activity test was done using phosphate buffer solution pH 7 because the enzyme is working optimally at the condition. Quercetin used as control positive based on research that fenolic compound has α-glucosidase inhibitory activity from *Saccharomyces cerevisiae* is more powerful than acarbose (Tadera *et al.,* 2006). According to IC_{50} values, ≤100mg/ mL extract with active classified as antidiabetic (Lee *et al.,* 2001). α-glucosidase inhibitory activity of kapok bark fractions (Table I).

Phytochemical study of Ceiba pentandra revealed the presence of isoflavone, sesquiterpen (Ngounou *et al.,* 2000) and catechin (Noreen *et al.,* 1998). In this study to obtain high amounts of catechin compounds in plants dissolved in water (Demir *et al.,* 2016). Sample of water fraction injected into LCMS/MS to identify the secondary metabolites based on molecular weight. Water fraction selected because the fraction may dissolve in the eluent (acetonitrile and methanol). First peak selection based on abundance detected on the chromatogram of the sample. LCMS/MS has high sensitivity, small amount of sample could be detected and may detect almost all organic compounds. Chromatogram of water fraction sample using LCMS (Figure 2).

Eluent of LCMS/MS system used has been programed in such way with to diminish the polarity gradually until it reached its peak in the time of 18min and back to normal.

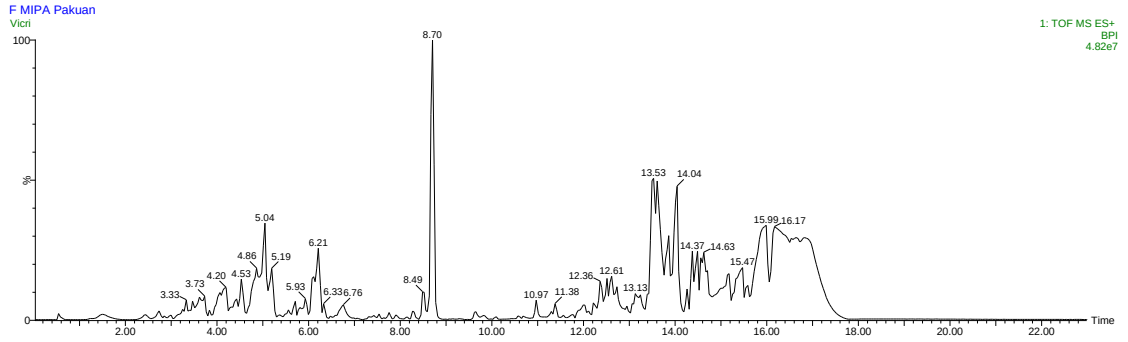

Figure 2. Chromatogram of water fraction sample using LCMS reached its peak in 18min.

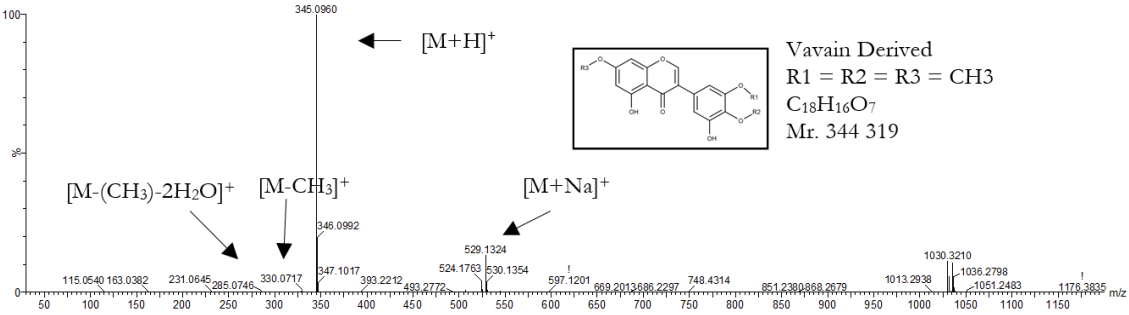

Figure 3. The mass spectrum of the compound at a retention time of 8.70min

The results show compounds appear in the chromatogram from polar to nonpolar ones. Nonpolar compounds bind strongly with C_{18} column which is nonpolar, therefore polar compounds were detected at the first place. Structure of compound eluted manually, because of limited database of LCMS/MS (Verpoorte *et al.,* 2008). The predicted result compared by molecular weight (MW) with Knapsack plant metabolites database.

Mass spectra results predicted the peak of 8.70min is base peak with abundance of vavain derived compounds compared by molecular weight (MW) contained in Ceiba pentandra. There are ion products on m/z 345.096 which indicates presence of $[M+H]^+$ from the component makes the molecule weight of 344. The presence of $[M-15]^+$ ion in MS indicates the loss of methyl group. On ion of 330 and 285m/z indicate the loss of 3 methyl groups and water (H_2O). Based on other allegations tied to the vavain derivative compounds, glycosides on ion 529m/z $[M+Na]^+$ name vavain 3'-O-β-D-glucoside (Noreen *et al.,* 1998) (Figure 3). Discovery of other cations on the mass spectra of ions such as Na + is indicated because of contamination so detected $[M + Na]^+$ (Sugimura *et al.,* 2015).

A research (Venter, Senekal, Amra-Jordaan, Bonnet, & Van Der Westhuizen, 2012) shows that (+) catechin derived has as ion fragment of 139 m/z which is fragmentation result of Retro Diels Alder (RDA). Results alleged on ion 257m/z $[M-H_2O-CH_2]^+$, 165 m/z $[M-C_6H_4O]+$, and 123m/z $[M-C_8H7O_4]+$. Other allegations obtained is ion 442 m/z is (-) epigallocatechin. The mass spectrum of the compound at a retention time of 3.61min (Figure 4).

Enzyme modelling evaluation performed to know whether isomaltase α-glucosidase is good or not. By looking at the value of QMEAN, similarity of amino acid sequence of receptor, Plot Ramachandran and comparing the results before Homology Modelling (Figure 5). Score of 72.5% obtained of similarity on amino acid 3AJ7 sequence with target on homology models. With sequence similarity of 0.54, this model has resolution of 1.3 Å, with total number of 577 amino acid residue tied to oligo-1,6-glucosidase found in BLAST database.

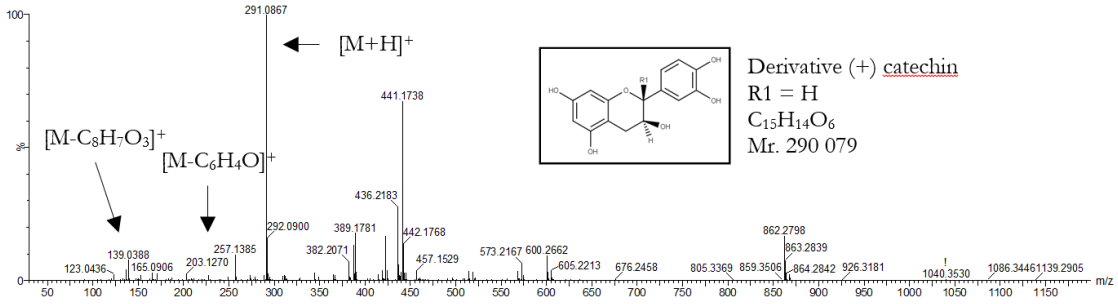

Figure 4. The mass spectrum of the compound at a retention time of 3.61min

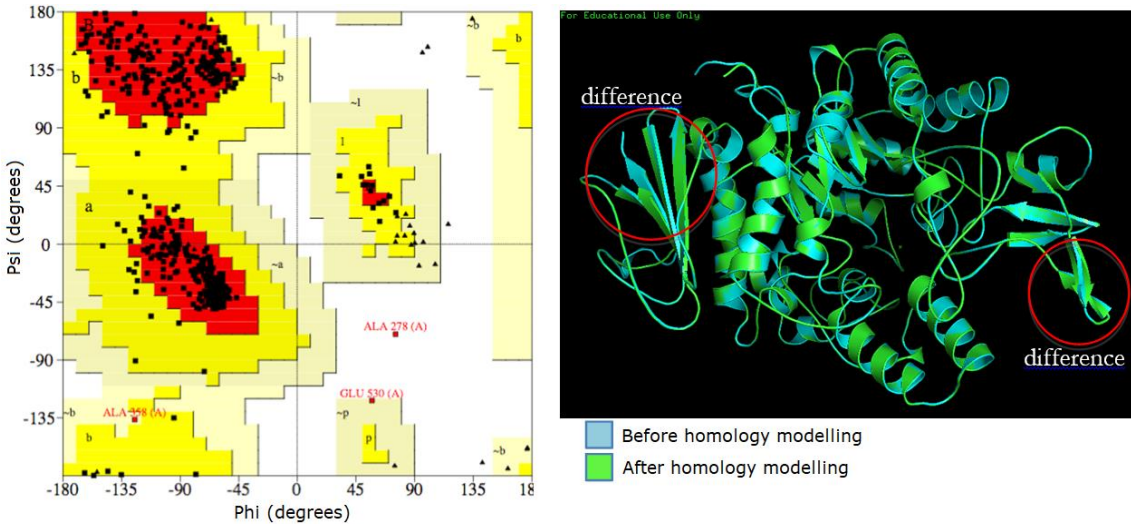

Figure 5. The results of model evaluation isomaltase enzyme α-glucosidase (PDB ID: 3AJ7) Plot Ramachandra has residual most favored regions amounted to 88.8%; additional allowed regions 10.6%; generously allowed regions 0.4% and 0.2% disallowed regions.

The receptor has a good model if the structure has a resolution of less than 2 Å, have an R-factor not greater than 20% and the most favoured more than 90% (Laskowski *et al.,* 1993). Based on target-template alignment, homology models generated have a Global Model Quality Estimation (GMQE) of 0.92 and QMEAN -0.23 (Waterhouse *et al.,* 2018). This study shows that α-glukosidase may recognize good result of model of structure, because the binding site of receptor after homology modelling with MAL32 as target is irreplaceable.

Molecular docking among isomaltase α-glucosidase with (+) catechin, vavain, quercetin and acarbose using Autodock Vina resulting the Gibbs Energy (ΔG). Optimization to parameters of receptors was using AutoDock Tools by removal of water, addition of polar hydrogen and gastegier docking with amount of gasteiger charge obtained -15.9969. The results show the best Gibbs energy (ΔG) is (+) catechin (-8.8kkal/mol), compared to vavain (-8.1 kkal/mol), acarbose (-6.2kkal/mol) and quercetin (-7.6 kkal/mol). The energy categories (kkal/mol) are: -15 to -40 is very strong, -4 to -15 is strong, and ≤ -4 is weak (Desiraju, 1999). Gibbs energy (ΔG) and "binding constant" (Ki) are related, therefore the more negative Gibbs energy and the lower Ki value result the stonger chemical bond. The results of molecular docking of isomaltase α-glucosidase using AutoDock Vina.

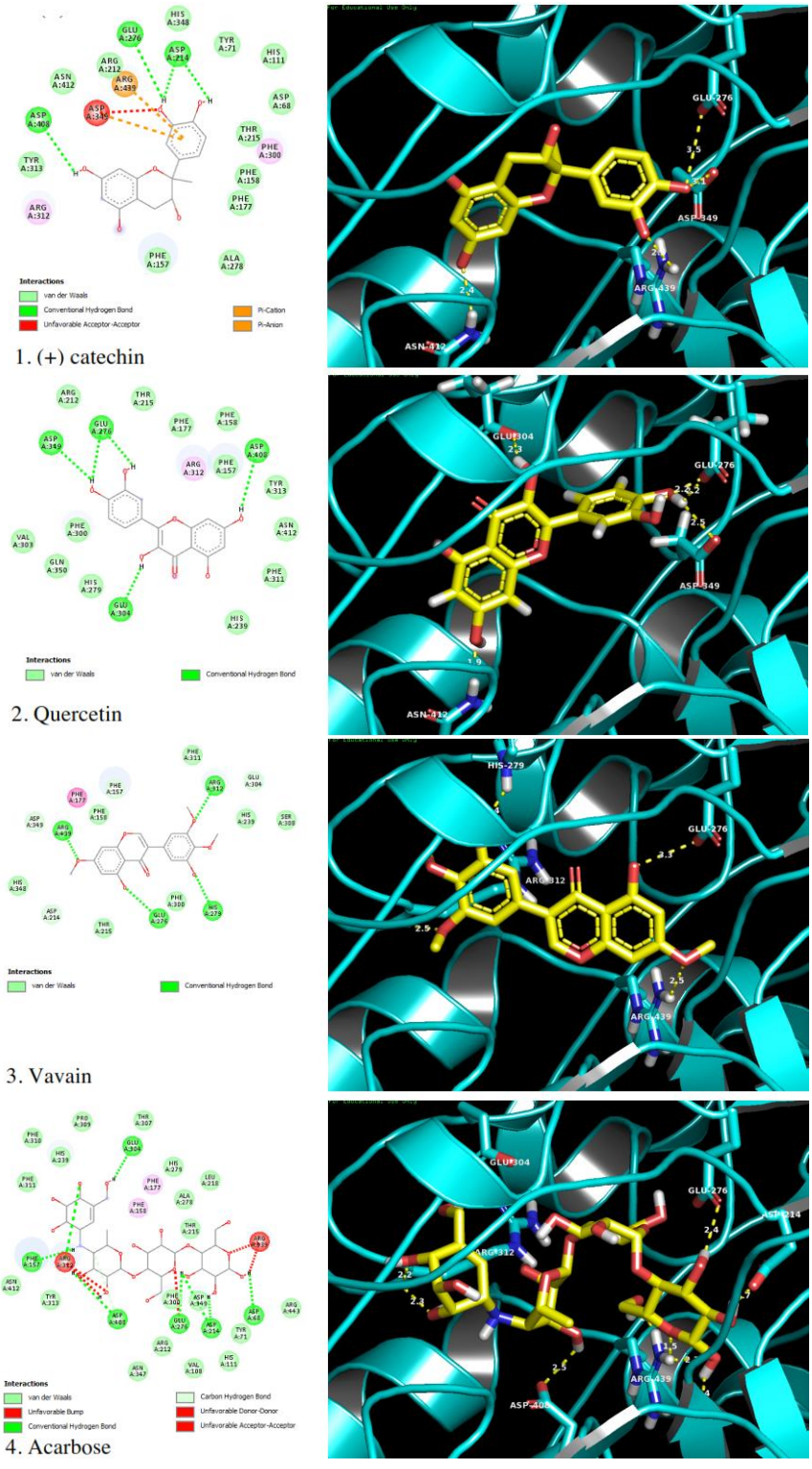

Figure 6. Visualization of results molecular docking isomaltase enzyme α-glucosidase (1) (+) Catechin, (2) Quercetin, (3) Vavain and (4) Acarbose. Amino acid residue Glu276 and Asp214 participate in the hydrolysis reaction and catalytic residue Asp349 an enzyme

Molecular interaction among the amino acids model of isomaltase α-glucosidase has obtained, interaction include the hydrogen bonds with chemical compound of quercetin, acarbose and compounds which be fathomed exist in the bark of kapok those are vavain and (+) catechin. Active site of isomaltase α-glucosidase formed hydrogen bond with quercetin and on amino acid Asn412, Glu304, Glu276 dan Asp349 residue which are catalytic residues. Acarbose formed hydrogen bond with amino acids Glu276, Glu304, Asp214, Asp68, Asp408, Arg439 dan Arg312 residues. (+) catechin formed hydrogen bond with amino acids Glu276, Asp349, Arg439, and Asn412 residues. Epicathecin isolated from another plant has regenation stimulating activity, insulin enhancement or insuline-like activity (Marles and Farnsworth, 1995). Furthermore, it is proven that phenolic compounds have α-glucosidase of *S. cereviceae* more powerful inhibition compared to acarbose, that vavain performed a test of hydrogen bond to amino acid Glu276, His279, Arg312 dan Arg439 residues. Isoflavone compounds show selectivity of certain COX-1 activities (Noreen *et al.,* 1998). From the result obtained, there was common hydrogen bond to the compound tested by acarbose and quercetin to the amino acid Glu276 residue. Amino acid Glu 276 and Asp 214 residues participated in hydrolysis process and Asp349 is catalytic enzyme residue (Yamamoto *et al.,* 2010). With the result that there are many common hydrogen bonds with quercetin and acarbose indicates potency of similar pharmacological activity. H-bond category by donor-acceptor distance were 1,2-1,5 Å (very strong) and 1,5-3,0 Å (weak or normal) (Jeffrey, 2013). Visualization molecular docking results of isomaltase α-glucosidase (Figure 6).

CONCLUSION

The α-glucosidase inhibitory activity of bark of Ceiba pentandra in n-hexane fraction is 4,60 mg/mL, ethyl acetate fraction is 8.55 mg/mL and water fraction is 5.61 mg/mL. Analysis of compound in water fraction with LCMS/MS eluted at retention time of 3,61 minutes be fathomed as (+) catechin derivate and at retention time of 8,70 minutes be fathomed as vavain derivate. The vavain derived compound, (+) catechin derived compound, quercetin and acarbose, each has docking score of -8,1; -8,8; -6,2 and -7,6 kcal/mol and they have a similar bond at amino acid Glu276 residue which has catalytic role in the hydrolysis process.

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