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In Silico Studies on Bidara (*Ziziphus mauritiana*) Leaves Ethanol Extract Bioactive Ligands compared to Acarbose toward α-glucosidase Enzyme

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ABSTRACT

Introduction : *Diabetes mellitus has been widely concerned in human health. Diabetes mellitus has a huge amount of sufferers. Carbohydrate diet, which is needed for energy supply, contributes to uncontrolled postprandial blood glucose which worsens diabetes mellitus. α-glucosidase is an enzyme that had been targeted to modify oligosaccharides, to decrease postprandial blood glucose. This study aims to predict the ability of the active compound contained in Z. mauritiana leaves to inhibit α-glucosidase protein through an in silico study.*

Methods : *Identified local Z. mauritiana leaves simplicial were extracted with 96% p.a ethanol solvent. The extract then analyzed with LCMS to confirm 6 active compound in the extract. Confirmed active compounds then undergo docking procedure, by downloading active compounds and acarbose molecular structure via PubChem, then converted to be 3D structure in pdb format by Pymol. Protein target α-glucosidase molecular structure were obtained from Uniprot, and 3D structure built by Swissmodel. Docking process held by Pyrex by AutoDock Vina. Protein interaction ligands were visualized by Biovia Discovery Studio.*

Results : *LCMS analysis revealed that there are apigenin, quercetin, routine, kaempferol, isovitexin and quercetin-3-rhamnoside in the extract. Molecular docking analysis explained that rutin has higher binding affinity than acarbose as the control. Rutin shared the same hydrogen bonding with acarbose in Arg 275 and Val 544.*

Conclusion : *In conclusion, Z. mauritiana have potential as α-glucosidase inhibitor.*

KEYWORDS : *Ziziphus mauritiana ethanol extract Active Compound, Acarbose, in silico, α-glucosidase*

INTRODUCTION

In the world, diabetes patients have risen from 108 million in 1980 to 422 million in 2014. As a chronic disease, this number will increase in the future. Prevalence acceleration of this disease is much faster in middle- and low-income countries. A healthy diet is one key factor that can prevent or delay progression of the disease (1). On one side, carbohydrate is very important in the human diet. It will be split by enzymes to be glucose and undergo catabolism process to attain energy which immensely needed by organisms to live (2). However, high carbohydrate diet found to be related to diabetes risk. It was proved by meta-analysis report by Hu, et al, 2012, that low carbohydrate diet can help improve metabolic profile, including blood glucose (3).

Starch, one kind of carbohydrate, is split by α-amylase to become maltose and maltotriose. After that, maltase-glucoamylase (MGAM) alias α-glucosidase is an enzyme that split maltase and maltotriose to become two glucose molecules. α-glucosidase is a strong enzyme, a hundredfold more active than sucrose-isomaltase, in the same reaction. α-glucosidase is found on the surface of the intestine enterosit cellular membrane

(4). Thereby, this enzyme is very important for blood glucose control.

α-glucosidase inhibitor has been found effective to treat diabetes mellitus. Acarbose is classified into this group. This agent can decrease postprandial blood glucose by inhibiting intestinal α-glucosidase through reversible inhibition. Inhibition of this enzyme can decrease postprandial blood glucose, thus prevent triglyceride deposition in adipose, hepatic tissue and blood serum (5).

A review state that flavonoids have a great potential as the α-glucosidase inhibitor. 103 flavonoids had been reported in this study. These compounds consist of xanthones, flavanones, flavans, anthocyanins, chalcones, and others structural motives (6). α-glucosidase also can be inhibited by quercetin, vitexin, apigenin (7), kaemferol (8), and rutin (9)by enzymatic *in vitro* study.

Ziziphus mauritiana, or called Bidara in Indonesia, is a spiny shrub or small tree which can grow to 12 m (10). *Z. mauritiana* whole plant ethanol extract contains carbohydrate, flavonoid, alkaloid, and saponin. It was reported that the extract can decrease postprandial blood glucose in vivo, even though not greatly significant (11). Meanwhile, *Z. mauritiana* leaves methanol extract can decrease blood glucose on oral glucose tolerance test in Swiss albino mice (12).

Based on the facts above, this study aims to predict the ability of the active compound contained in *Z. mauritiana* leaves to inhibit α-glucosidase protein compared to acarbose through an in silico study.

MATERIALS AND METHODS

Materials

Acetonitrile HPLC grade and methanol were obtained from Merck. Water used for LC-MS/MS analysis was purified with a deionized water system from PT. Ikhapharmindo Putramas Pharmaceutical Industries. *Z. mauritiana* leaves obtained from Sampang, Madura island were identified by Balai Materia Medica, Batu, East Java, Indonesia.

Z. mauritiana Simplicia Preparation and Extraction

Z. mauritiana extraction was done in Faculty of Medicine, Malang Islamic University. *Z. mauritiana* leaves are sorted, washed and dried at 40˚C temperature. Subsequently, dry simplicial crushed until it becomes powder. 400 grams of leaves was extracted by kinetic maceration method using 3 L 96% p.a solvent, then stored at room temperature for 7 days. The obtained macerate was filtrated using Buchner until the liquid extract was obtained. The liquid extract was then collected and evaporated using a rotary evaporator at 70˚C until obtained a viscous extract (45,1 g).

Extract Sample Preparation

About 0.2 g the individual sample extract was weighed in centrifuge tubes with screw caps. 10 ml of the solvent methanol was added. Shaken vigorously for 1 minute in Branson Model 5510 ultrasonic bath for 5 minutes. The sample was centrifuged at 3500 rpm for 10 minutes, transfer supernatant to centrifuge tubes with screw caps. Supernatants filtered with a 0.2 μm syringe filter, and the solution was transferred to an autosampler vial and an aliquot was injected into the LC–MS/MS system for analysis.

Liquid Chromatography Mass Spectrometry

LCMS analysis was done in Malang State Polytechnic. UHPLC was performed using a Thermo Scientific (USA) Accela 1250 system. It was consisting of a

quaternary pump solvent management system, an online degasser, and an autosampler sample was maintained at 16°C. They were programmed by x-Calibur 2.1 software. Chromatographic separation was achieved using a Hypersil Gold column (50 mm × 2.1 mm,1.9 µm, from Thermo Scientific) was maintained at 35°C. A linear gradient method was compound separation in analytes. The eluent flow rate was set 300 µL/min and the elution solvents were 0.1% formic acid in water (A) and 0.1% in acetonitrile (B). The gradient elution was programmed as follows: 0.0-0.6 min, 10%B, 3.5-5.0 min 90%B, 5.2 min 10 %B and sample injection volume was 2 µL, and the run time was 7.5 min.

Mass spectrometric detection was performed using Triple Quadrupole MS (TSQ Quantum Access Max, Thermo Scientific, USA). It was equipped with an electrospray ionization source (ESI and the ESI source was set in negative ion mode. The HESI ionization source parameters were set as follows: Spray Voltage (3000 V); Vaporizer Temperature (250 °C); Capillary Temperature (300 °C); Sheath Gas (15 arbitrary units) and Auxiliary Gas (35 arbitrary units). The MS/MS instrument was operated in SIM (Selected Ion Monitoring) mode with the details listed in Table 1.

Table 1. SIM-MS Events and Parameters

$[M-H]^-$	Analyte
269	Apigenin
285	Kaemferol
301	Quercetin
431	Isovitexin
447	Quercitrin
609	Rutin

Molecular Docking with In Silico Method

The design of this study is a descriptive-analytic study(13). The active compounds obtained from the LCMS test then downloaded the 3D structure via http://pubchem.ncbi.nlm.nih.gov/ which is stored in sdf format. To convert sdf format to pdb format, we used Pymol programme. The target protein α-glucosidase downloaded via http://uniprot.org/. Homology α-glucosidase target protein was developed via http://swissmodel.expasy.org/, then the data was stored in pdb form. Docking process was held using pyrex programme with Autodock Vina. After the docking process is complete, protein interaction with ligands were visualized using Biovia Discovery Studio software.

RESULTS AND DISCUSSION

LCMS Analysis of Ziziphus mauritiana extract

LCMS analysis has been done to know the active compounds contained in *Z. mauritiana* leaves ethanol extract. The result had identified at least six active compounds of flavonoid group namely apigenin, quercetin, routine, kaempferol, isovitexin and quercetin-3-rhamnoside (table 2).

Table 2. Each chromatogram identification of *Z. mauritiana* leaves ethanol extract by LCMS qualitatively

Retention time (min)	Molecular Weight	Alleged compounds
2.37	268.50	Apigenin
2.81	608.50	Rutin
3.26	300.50	Quercetin
3.12	284.50	Kaempferol
2.33	446.50	isovitexin
2.96	446.50	Quercetin-3-Rhamnoside

At minute 2.37 with abundance 100 identified active compound with molecular weight 268.50-269.50. Based on the molecular weight, the active compound identified is a class of flavonoids, alleged apigenin compound

(figure 1). Apigenin is thought to have activity in the inhibition of α-glucosidase by an enzymatic in vitro study on *Quercus gilva* Blumes leaves methanol extract (7).

Figure 1. LCMS Chromatogram from Apigenin

At minute 3.26 identified molecular weight of 300.50-301.50 with abundance 100. Based on the molecular weight, the active compound identified is alleged quercetin compound (figure 2). Quercetin is reported having a strong activity of inhibition toward α-glucosidase, even compared with isoquercetin and rutin in an enzymatic study (14).

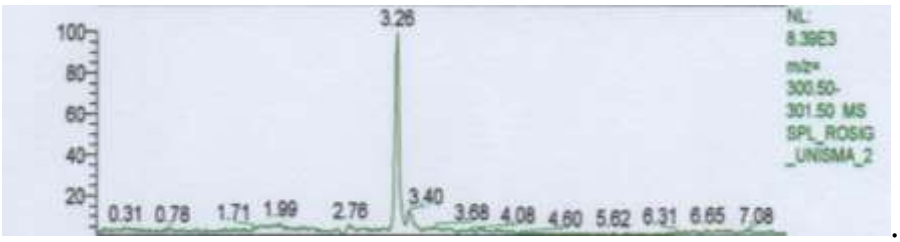


Figure 2. LCMS Chromatogram from Quercetin

At minute 2.81 identified molecular weight of 608.50 – 609.50 with abundance 100. Based on the molecular weight, the active compound identified is alleged rutin compound (figure 3). Rutin is reported of having a strong activity in inhibition of α-glucosidase, even not as much as quercetin in an enzymatic study (14).

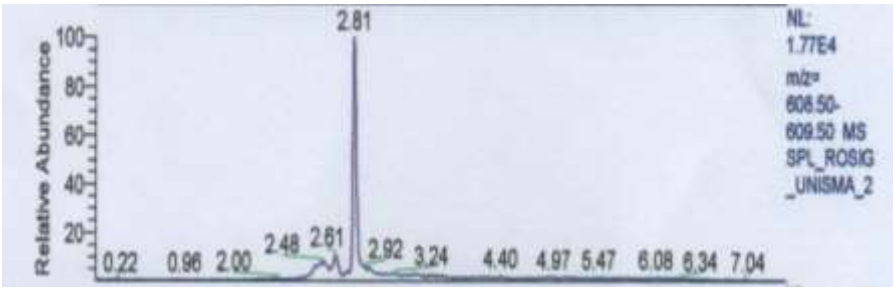


Figure 3. LCMS Chromatogram from Rutin

At minute 3.12 identified molecular weight of 264.50-265.50 with abundance 100. Based on the molecular weight, the active compound identified is alleged kaempferol compound (figure 4). Kaempferol is reported also having a strong activity in inhibition of α-glucosidase in an in vitro study (8).

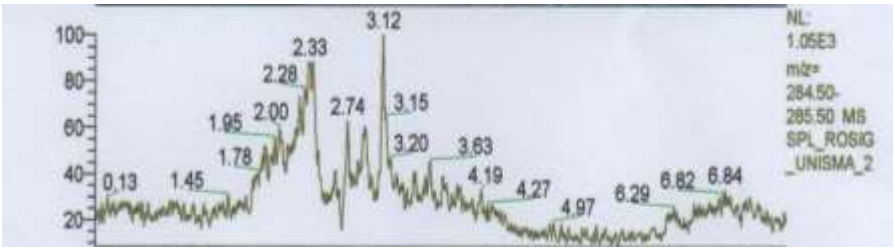


Figure 4. LCMS Chromatogram from Kaemferol

At minute 2.33 identified molecular weight of 430.50-431.50 with abundance 100. Based on the molecular weight, the active compound identified is alleged isovitexin compound (figure 5). Isovitexin is reported having activity in inhibition of α-glucosidase in an in vitro study of *Vigna angularis* beans ethanol extract with IC50 value is 4,8 mg/mL (15).

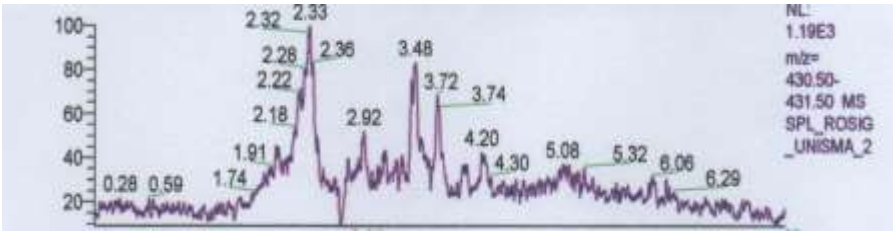


Figure 5. LCMS Chromatogram from isovitexin

At minute 2.96 identified molecular weight of 446.50-447.50 with abundance 100. Based on the molecular weight, the active compound identified is alleged Quercetin-3-rhamnoside compound (figure 5). Quercetin-3-rhamnoside still never being reported having activity in inhibition of α-glucosidase.

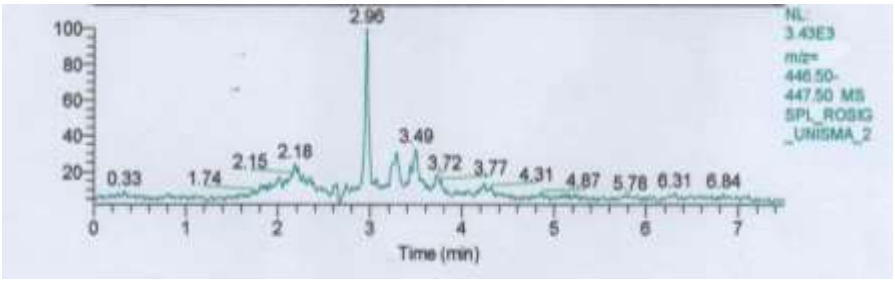


Figure 6. LCMS Chromatogram from Quercetin-3-rhamnoside

Molecular Docking of *Z. mauritiana* active compounds

The compounds like Apigenin, Kaemferol, Isovitexin, Rutin, Quercetin and Quercetin-3-rhamnoside have been isolated from the ethanol extract of the leaves. The results from in silico analysis, among the 6 compounds of *Z. mauritiana*, it is known that rutin shows higher binding affinity than control (acarbose) (table 3). Its ability to inhibit target protein (α-glucosidase) better than acarbose, suspected from the value of binding affinity -9.1 kcal/mol. This result expected that rutin has bigger activity than acarbose. Even though, other active compounds have binding affinity that not different too far with acarbose.

Hydrogen bonds on rutin residual protein Gln 121, Trp 126, Arg 275, Val 544. Rutin

shared the same hydrogen bonding with acarbose in Arg 275 and Val 544 (table 4). Even though, another amino acid which bound to Rutin, Gln 121, Trp 126, suspected to be involved in Rutin binding affinity that can surpass acarbose binding affinity value (picture 1).

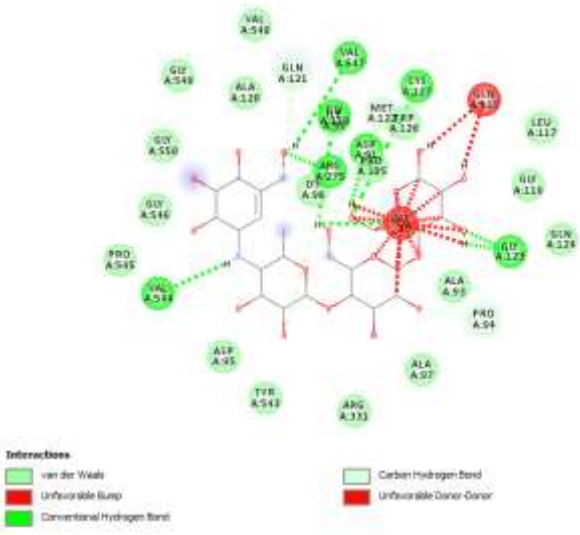
This result is consistent with another study that reports rutin has higher binding affinity value than quercetin and myricetin. However, enzymatic study showed quercetin have lower IC50 value than rutin. They have different hydrogen binding residues reference with our result, that are Glu322, Lys324, and Asp325 for rutin, Asp325 and Asp521 for quercetin (16).

Table 3. Analysis of inhibiting potency of *Z. Mauritiana* leaves active compound against α-glucosidase receptor against α-glucosidase receptor with in silico method.

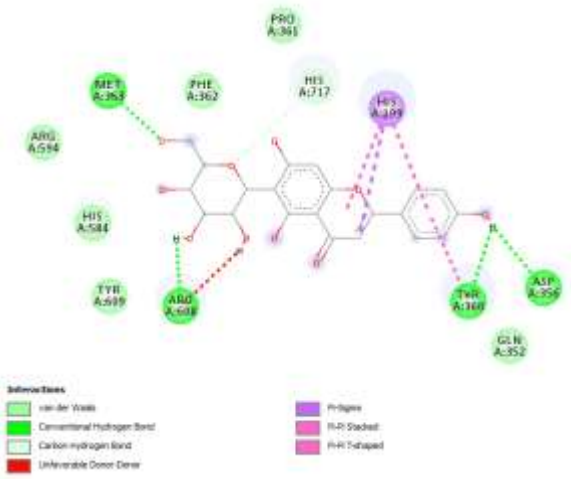
Compound	**CID**	**Receptor**	**Binding Affinity (kcal/mol)**
Rutin	5280805	A- glucosidase	-9.1
Acarbose (control)	**41774**	**A- glucosidase**	**-8.8**
Isovitexin	162350	A- glucosidase	-8.6
Quercetin-3-rhamnoside	5280459	A- glucosidase	-8.4
Apigenin	5280443	A- glucosidase	-7.9
Kaempferol	5280863	A- glucosidase	-7.8
Quercetin	5280343	A- glucosidase	-7.6

Table 4. Analysis of ligand interaction between active compound of Z. Mauritiana leaves active compound against α- glucosidase receptor by in silico method. Bold and underline letter indicates that the complex (α- glucosidase-ligand) has the same interaction with the α-glucosidase-inhibitor reference (acarbose)

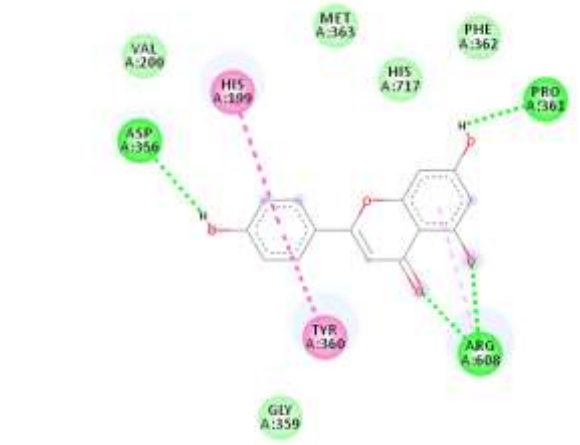
Ligand ID	Interaction
Apigenin	Hydrogen bond: Asp 356, Pro 361, Arg 608
Kaempferol	Hydrogen bond: Arg 608
Isovitexin	Hydrogen bond: Asp 356, Tyr 360, Met 363, Arg 608
Quercetin_3_ramnoside	Hydrogen bond: His 717
Quercetin	Hydrogen bond: Tyr 360, Arg 608
Rutin	Hydrogen bond: Gln 121, Trp 126, **Arg 275**, **Val 544**
Inhibitor reference/acarbose	Hydrogen bond: Asp 91, Gly 119, Gly 123, Cys 127, **Arg 275**, **Val 544,** Val 547


Interactions
van der Waals
Unfavorable Bump
Conventional Hydrogen Bond
Carbon Hydrogen Bond
Unfavorable Donor-Donor

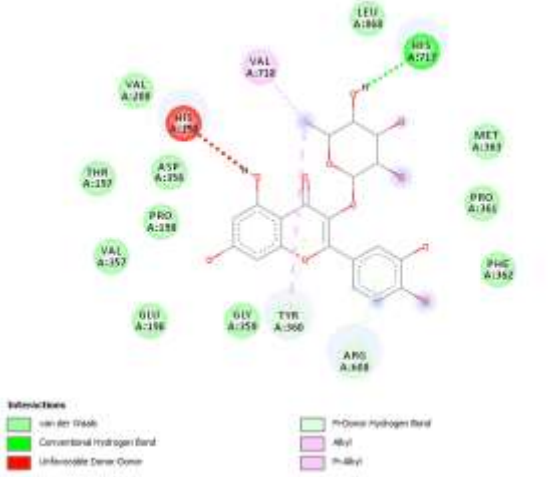
(a)


Interactions
van der Waals
Conventional Hydrogen Bond
Carbon Hydrogen Bond
Unfavorable Donor-Donor
Pi-Sigma
Pi-Pi Stacked
Pi-Pi T-shaped

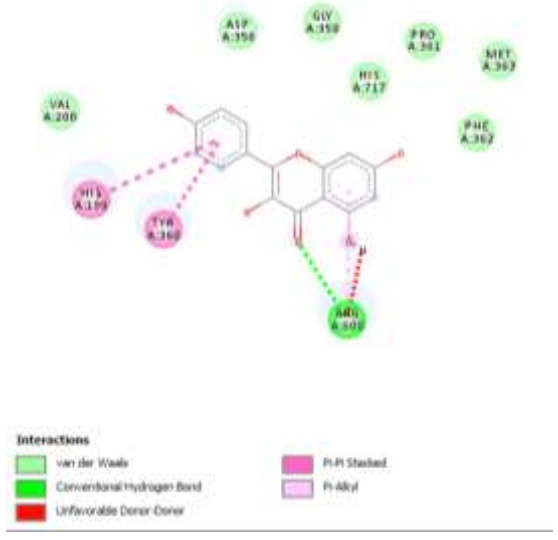
(d)


Interactions
van der Waals
Conventional Hydrogen Bond
Pi-Pi Stacked
Pi-Alkyl

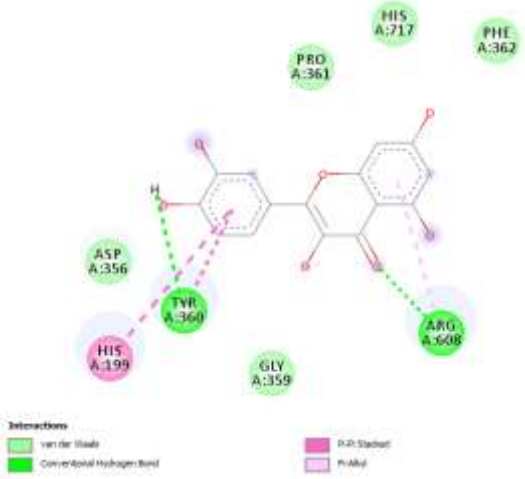
(b)


Interactions
van der Waals
Conventional Hydrogen Bond
Unfavorable Donor-Donor
Pi-Donor Hydrogen Bond
Alkyl
Pi-Alkyl

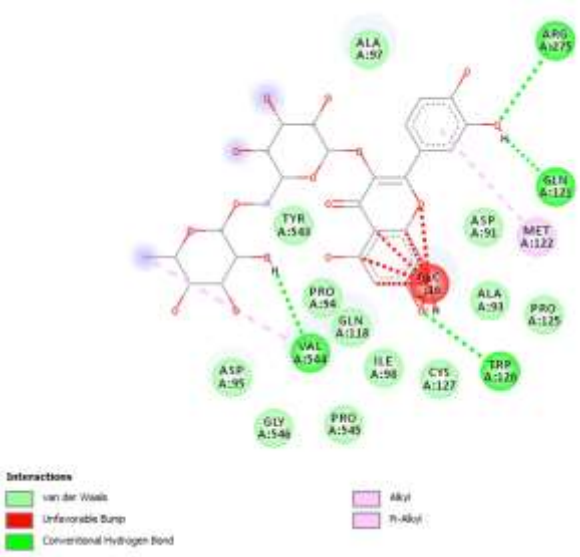
(e)


Interactions
van der Waals
Conventional Hydrogen Bond
Unfavorable Donor-Donor
Pi-Pi Stacked
Pi-Alkyl

(c)


Interactions
van der Waals
Conventional Hydrogen Bond
Pi-Pi Stacked
Pi-Alkyl

(f)



(g)

Figure 1. Interaction visualization between ligand and protein target α-glucosidase, (a) acarbose, (b) apigenin, (c) kaempferol, (d) isovitexin, (e) quercetin-3-rhamnoside, (f) quercetin, (g) rutin,

CONCLUSION

It can be concluded from research above that *Z. mauritiana* leaves extract active compounds have potential as α-glucosidase inhibitor.

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