JNPDD
Journal of Natural Product for Degenerative Diseases
https://journal.univpancasila.ac.id/index.php/Natural

Molecular Structure Similarity Analysis using Tanimoto Coefficient and Its Correlation Analysis with Maltase-Glucoamylase Inhibitory Activity of *Nigella Sativa*'s Compounds

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Received: 31 August 2024 / Accepted: 29 September 2024

ABSTRACT: *Nigella sativa* is one of the medicinal plants that are efficacious for treating diabetes mellitus. Diabetes mellitus is a chronic metabolic disorder characterized by hyperglycemia due to damage of insulin action, insulin production, and/or both. In this study, the molecular structure similarity analysis of the compounds in *Nigella sativa* to acarbose and the correlation analysis of the similarity with its activity as antidiabetic with the mechanism of maltase glucoamylase (MGAM) inhibition was carried out. Similarity analysis has done used Tanimoto coefficient. The prediction of MGAM inhibitory activity has done using molecular docking with molegro virtual docker. The kaempferol 3-glucosyl-(1-2)-galactosyl-(1-2)-glucoside; (S)-2,3-epoxysqualene; quercetin 3-glucosyl-(1-2)-galactosyl-(1-2)-glucoside; oleic Acid has activity as an inhibitor of MGAM with rerank score -107.8770, -102.1760, -95.7338, -92.4246 respectively and these has Tanimoto score 0.426, 0.319, 0.413, 0.357 respectively. The correlation analysis obtained that there is a significant relationship between the Tanimoto Coefficient and rerank score with the opposite relationship because the correlation value is negative. Greater the degree of molecular structure similarity of *Nigella sativa*'s compounds to acarbose more likely has the similar biological activity as MGAM inhibitory.

KEYWORDS: *Nigella sativa*; MGAM; Tanimoto coefficient; Correlation

1. INTRODUCTION

Diabetes mellitus is a chronic metabolic disorder characterized by hyperglycemia due to damage to insulin action, insulin production, and/or both [1]. Most of the diabetes mellitus that occurs is type 2 diabetes mellitus, which is a condition in which blood sugar levels exceed normal values caused by the body not using the hormone insulin optimally [2]. Various drug developments for diabetes have been carried out, both synthetic drugs and herbal medicines. Acarbose is one of the famous drugs that can be used to treat diabetes mellitus type 2. Acarbose is able to inhibit the action of the alpha-glucosidase enzyme which is an important enzyme for the hydrolysis of carbohydrates into glucose. Inhibition of the alpha-glucosidase enzyme will have an effect on delaying glucose absorption [3]. Meanwhile, anti-diabetic drugs from natural ingredients that have been reported through in vitro and in vivo studies include *Nigella sativa* [3–5], garlic [6], ginger [7] , bitter gourd [7, 8], bangle rhizome [9], actinomycetes extract [10], cinnamon, bay leaf and brotowali [6, 7]. Giving black seed oil (*Nigella sativa*) to patients with type 2 diabetes mellitus showed that given black seed oil significantly reduced HbA1C levels in the control group compared to the standard group. Based on these study, black seed oil can be used as an additional therapy in patients with type 2 diabetes mellitus [3–5, 11–13]. The black seed oil has many bioactive compounds so it cannot be determined exactly what compounds act as Maltase-Glucoamylase inhibitors. In this study, an in-silico screening was carried out using the molecular docking method to predict compounds in *Nigella sativa* that have activity as a Maltase-Glucoamylase inhibitor accompanied by a similarity analysis of the bioactive compound *Nigella sativa* based on binary data and its correlation to activity. Analysis of the similarity of compound functional groups was carried out by the method of determining the Tanimoto coefficient to determine the closeness of the structure between the compounds

How to cite this article: Nurita A, Esti M. Molecular Structure Similarity Analysis using Tanimoto Coefficient And Its Correlation Analysis With Maltase-Glucoamylase Inhibitory Activity Of *Nigella sativa*'s Compounds. JNPDD. 2024; 2(1): 48-57.

https://doi.org/10.58511/jnpdd.v2i1.7385
ISSN: 3031-6847

and the proximity of the structure of the compounds in *Nigella sativa* to acarbose which has been used as an antidiabetic type 2 drug. Similarity measurement of the chemical structure of a compound refers to the similarity of chemical elements, molecules, or chemical compounds. It is assumed that similar compounds tend to have similar properties. From a statistical point of view, chemical structure similarity measurement is essentially a measurement of object similarity based on binary variables because the chemical structure of a compound is represented in the molecular fingerprint. Tanimoto coefficient is most often used in the measurement of similarity among chemical structure of compounds. The chemical structure is represented as binary data is a molecular fingerprint features such as either the presence or absence of chemical elements, a single bond, a ring, and etc [14][15–18]. MGAM (Maltose Glucoamylase) with pdb code 3TOP is a protein that plays a crucial role in the production of glucose in the human lumen and acts as an efficient drug target for type 2 diabetes and obesity so that these proteins can be targeted in the analysis of in silico in the activity of inhibiting the performance of MGAM in an effort to find active and potential compounds to be developed as drugs antidiabetic type 2 [19]. In principle, it is assumed that compounds with similar chemical structures have similar biological properties [18].

2. MATERIALS AND METHODS

2.1. Materials

Nigella sativa's metabolite that obtained from KnapSack Family database i.e m-thymol, carvacrol, alpha-thujene, (+)-alpha-pinene, beta-pinene, beta-myrcene, lauric acid, oleic acid, anisaldehyde, apiol, methyl chavicol, myristicin, (+)-R-citronellol, p-cymene, fenchone, alpha-phellandrene, gamma-terpinene, longifolene aldehyde, beta-amyrin, linolenic acid, (S)-2,3-epoxysqualene, thymoquinone, kaempferol 3-glucosyl-(1-2)-galactosyl-(1-2)-glucoside, quercetin 3-glucosyl-(1-2)-galactosyl-(1-2)-glucoside, quercetin 3-(6''''-feruloylglucosyl)-(1-2)-galactosyl-(1-2)-glucoside, salfredin B11, fuzitine, nigellicine, nigellidine, nigellimine, 4-terpineol, sabinene, nigeglanine, nonane, carvone, alpha-longipinene, dihydrocarvone, nigellidine 4-O-sulfite, the structure of MGAM protein crystals, the structure of acarbose as comparison compound.

Experimental Tools. A set of computer with 16 Gigabyte RAM, 62 bit, core i7 Processor operating system, Molegro Virtual Docker 7.0.0 Trial License software with oxygen password, R and SPSS applications.

2.2. Procedure

2.2.1. Data preparation

The data used in this study were the chemical structure of the compounds contained in *Nigella sativa* seeds. These compounds were obtained from the KNApSACK Core System database http://www.knapsackfamily.com/knapsack_core/top.php with the key word *Nigella sativa* [20]. Meanwhile, the structure of acarbose as comparison compounds were obtained from the drugbank database https://go.drugbank.com/ [21]. The structure of MGAM protein crystals were obtained from the database https://www.rcsb.org/ [22].

2.2.2. Molecular Structure Similarity Test

In the molecular structure similarity test, the first stage was to change the chemical structure of each compound in the form of SMILES into binary data using PubChem fingerprints with 881 features. In this step, use the R application with the RCDK package and fingerprint. After knowing the binary data of each fingerprint, the next step is to determine the features of each pair of compounds through a contingency table with a size of 2x2 as shown in Table 1.Each compound will have 2 types of code namely 0 and 1, 0 meaning that there is no corresponding fingerprint feature in compound, and 1 if there is a fingerprint feature on the compound.

Table 1. Contingency table of compound pairs.

		compound-j		
		1	0	Total
Compound-i	1	a	b	$a+b$
	0	c	d	$c+d$
	Total	$a+c$	$b+d$	$n=a+b+c+d$

Note:

a : the frequency of the i-th and j-th compounds is 1
b and c : the frequency of one of the i-th or j-th compounds is 0 or 1
d : the frequency of the i-th and j-th compounds
n : total number of features

Calculating the similarity of compounds using the Tanimoto coefficient shown in equation 1.

$$T = a / (a + b + c) \quad \text{.....(1)}$$

2.2.3. Ligand – Protein Interaction Prediction of Test Compounds with MGAM Protein using Molecular Docking

The 3D structure of the compound was energy minimized using ChemDraw Chem3D software with the Molecular Mechanics + (MM+) minimization method. The target protein is downloaded via the rcsb.org webserver with the pdb code 3TOP which is a Maltase Glucoamylase complex with alpha acarbose native ligand. The docking process is carried out using rigid docking mode, MolDock Score, and MolDock SE scoring function on the Molegro Virtual Docker 7.0.0 program. The all of error structures on protein was repaired before docking.

2.2.4. Correlation Analysis of Molecular Structure Similarity with Compound Activity

The similarity test for the structure of the compound with the compound activity test using molecular docking will produce different values. However, both are used to measure the closeness between the target compound and other compounds. The relationship from the results of the structural similarity test which is expressed in the form of the Tanimoto similarity coefficient (x) and the molecular activity of the compound is expressed in the rerank score (y) using the correlation shown in equation 2.

$$\rho_{xy} = (\text{cov}(x, y)) / ((\sigma_x \sigma_y)) \quad \text{.....; (2)}$$

note:
ρ_{xy} : the correlation between the Tanimoto similarity coefficient and the docking ranking value
cov (x, y) : covariance between the Tanimoto similarity coefficient and the rerank score
σ_x : standard deviation of the Tanimoto similarity coefficient
σ_y : the standard deviation of the rerank score

3. RESULTS

3.1. Molecular Structure Similarity Test of *Nigella sativa*'s Compounds

Nigella sativa seed contains several very beneficial secondary metabolites. Extract of *Nigella sativa* seeds is reported to have biological activity as anti-diabetes type 2. In the analysis of the molecular structure similarity of compounds used acarbose as a control compound. Acarbose is a drug that has been approved by the FDA as a drug in type 2 diabetes therapy [21]. In this study, 38 compounds contained in *Nigella sativa* seed were then determined by the SMILES (Simplified Molecular - Input Line Entry System) of each compound.

The compounds that have been in the form of SMILES have been converted into several substructures based on the features of the molecular fingerprint (fingerprint). Determination of the molecular fingerprint feature using the R program with the RCDK package and fingerprint with the fingerprint development from PubChem. The number of molecular fingerprints developed by PubChem has 881 features (https://www.rdocumentation.org/packages/rcdk/versions/3.5.0/topics/get.fingerprint). In the molecular fingerprinting process of a compound, a compound that has the same substructure of 881 features is symbolized by 1 while other features are symbolized by 0. Thus, the data structure formed is a matrix with a size of 38 x 881 which contains a binary data set. The similarity of the chemical structure of a compound has been calculated using the Tanimoto coefficient with the resulting similarity range of 0 to 1. The compound pair has a high similarity value if the result of the Tanimoto coefficient is close to 1. In this study the acarbose compound was used as the main compound. Acarbose is a compound that has been used as type 2 antidiabetic

therapy so that by assessing the structure similarity of the compounds in *Nigella sativa* can be predicted the similarity of the activity of the compounds in *Nigella sativa* to acarbose activity as type 2 anti-diabetes drug. The Tanimoto coefficient value is shown in Table 2.

Table 2. Tanimoto Coeffisien of *Nigella sativa's* compound to Acarbose.

No	Compounds	Tanimoto Coefficient
1	Kaempferol 3-glucosyl-(1-2)-galactosyl-(1-2)-glucoside	0.426
2	Quercetin 3-glucosyl-(1-2)-galactosyl-(1-2)-glucoside	0.413
3	Quercetin 3-(6-feruloylglucosyl)-(1-2)-galactosyl-(1-2)-glucoside	0.41
4	Fuzitine	0.368
5	Linolenic acid	0.357
6	Oleic acid	0.357
7	Salfredin B11	0.339
8	beta-Amyrin	0.323
9	(S)-2.3-Epoxysqualene	0.319
10	4-Terpineol	0.308
11	(+)-R-Citronellol	0.307
12	Anisaldehyde	0.302
13	Apiol	0.301
14	Carvone	0.297
15	Nigellicine	0.297
16	Myristicin	0.296
17	Nigellidine	0.295
18	Thymoquinone	0.282
19	Nigellimine	0.266
20	Dihydrocarvone	0.258
21	Lauric acid	0.254
22	Carvacrol	0.253
23	m-Thymol	0.253
24	Nigeglanine	0.244
25	Methyl chavicol	0.237
26	(+)-alpha-Pinene	0.217
27	gamma-Terpinene	0.212
28	alpha-Longipinene	0.211
29	alpha-Phellandrene	0.203
30	alpha-Thujene	0.197
31	beta-Myrcene	0.196
32	p-Cymene	0.176
33	beta-Pinene	0.171
34	Fenchone	0.169
35	Sabinene	0.161
36	Longifolene aldehyde	0.155
37	Nonane	0.099
38	Nigellidine 4-O-sulfite	0

The value of the Tanimoto coefficient is in the range of 0.099 to 0.426, this value describes the level of similarity structure of compounds in *Nigella sativa's* seeds with acarbose. Of the 38 compounds tested, there were three compounds that had a Tanimoto coefficient value above 0.4, namely kaempferol 3-glucosyl-(1-2)-galactosyl-(1-2)-glucoside, quercetin 3-glucosyl-(1-2)-galactosyl -(1-2)-glucoside, quercetin 3-(6-feruloylglucosyl)-(1-2)-galactosyl-(1-2)-glucoside. This means that the three compounds have the greatest degree of similarity with the structure of acarbose compared to other compounds.

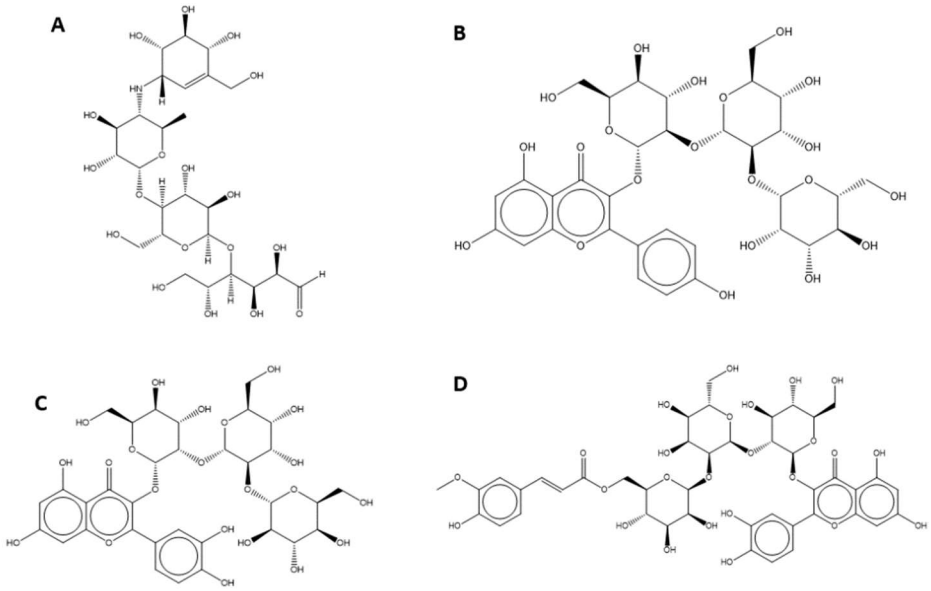

Figure 1. Structure of A) Acarbose B) Kaempferol 3-glucosyl-(1-2)-galactosyl-(1-2)-glucoside ; C) Quercetin 3-glucosyl-(1-2)-galactosyl-(1-2)-glucoside ; D) Quercetin 3-(6-feruloylglucosyl)-(1-2)-galactosyl-(1-2)-glucoside.

3.2. Molecular Docking

The biological activity test of type 2 antidiabetic was carried out through a molecular docking process with the mechanism of inhibition of the activity of the enzyme maltase glucoamylase (MGAM). The MGAM protein used is a crystal protein with the code pdb 3TOP (2.8 Å of resolution) which is Human maltase-glucoamylase (MGAM) hydrolyzes linear alpha-1,4-linked oligosaccharide substrates, playing a crucial role in the production of glucose in the human lumen and acting as an efficient drug target for type 2 diabetes. Inhibition of MGAM activity reduces blood glucose production so that it can reduce the effect of type 2 diabetes. Internal validation of docking protocol with Scoring function Moldock Score and MolDock SE algorithm in cavity 1 with binding site position at x = -45.572 y= 10.334 z=38.23 with radius constraints 15 shows the Root Mean Square Deviation (RMSD) value of 2.2 Å , superimposed native ligand (alpha-acarbose) with an alpha-acarbose redocking pose is shown in Figure 1.

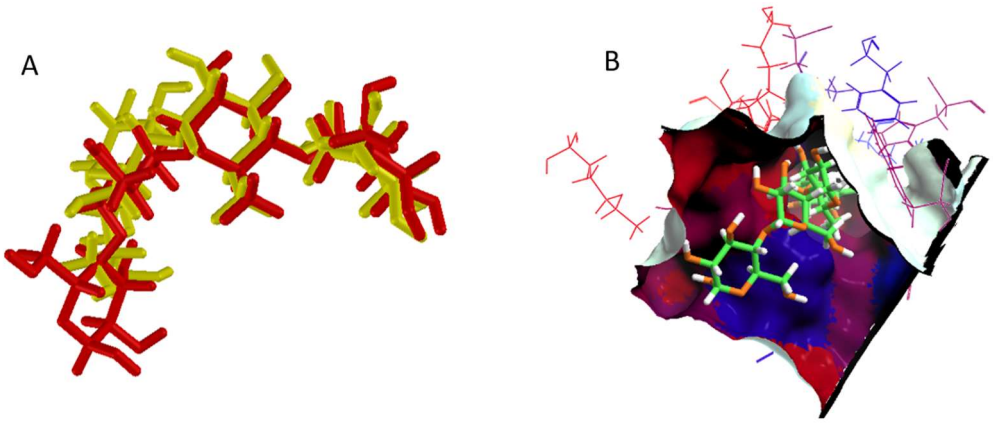

Figure 2. A) Superimpose of alpha acarbose (red) with pose redocking alpha acarbose (yellow) ; B) Alpha acarbose in the binding site of MGAM

The small RMSD value indicates that the shift between atoms after the docking process with the protocol that has been prepared for the docking process on the MGAM protein does not shift much from its previous position, so the protocol is reliable to be used for docking the test compounds [23]. RMSD is depending on the molecular weight of compounds. Small compounds can easily achieve low RMSDs even when placed

randomly and it can reach RMSD less than 2 Å, although in some cases with larger molecules the RMSD value of 2-3 Å is referred to as partial successes validation, and the total failed can be decide if RMSD of 8 Å [19, 23]. . The initial stage of docking was carried out on the comparison compound, the drugs officially used for the treatment of type 2 diabetes mellitus i.e alpha acarbose. The rerank score of acarbose againts 3TOP was -114.81.

The docking of the test compounds was carried out on 38 compounds contained in *Nigella sativa* seeds. The results of the docking of the test compounds are shown in Table 3. The Rerank Score of 38 compounds in *Nigella sativa* was not more negative than acarbose. A more negative Rerank Score indicates that the affinity of the compound in protein binding sites is more stable and stronger, so that its role as an MGAM inhibitor is getting better. Kaempferol 3-glucosyl-(1-2)-galactosyl-(1-2)-glucoside, (S)-2,3-Epoxysqualene, Quercetin 3-glucosyl-(1-2)-galactosyl-(1 -2)-glucoside, Oleic Acid, and Linolenic acid were predicted the most have inhibitory activity of MGAM in producing glucose, with higher bond stability than others test compounds.

The compounds that have the best rerank score form several hydrogen bonds and hydrophobic interactions at the MGAM protein binding site with several amino acids. Acarbose form hydrogen bonds with amino acid residues ASP1279, MET1421, ARG1510, ASP1526, HIS1584. Kaempferol 3-glucosyl-(1-2)-galactosyl-(1-2)-glucoside form hydrogen bonds with amino acid residues TRP1369, GLN1372, LEU1367, TYR1251, ASP1281, MET1283, GLY1365, GLY1252. Quercetin 3-glucosyl-(1-2)-galactosyl-(1-2)-glucoside form hydrogen bonds wit amino acid residues TYR1251, ASP1281, ASP1377, GLN1372, THR1586, TRP1369. Oleic acid form hydrogen bond with ARG1250. Kaempferol 3-glucosyl-(1-2)-galactosyl-(1-2)-glucoside is the compound with the most negative rerank score, this shows a stronger affinity than other compounds, this is supported by the ability of the kaempferol structure to form 3 hydrophobic interactions and 14 hydrogen bonds.

3.3. Correlation of Molecular Structure Similarity with Compound Activity

In the correlation test, 37 compounds were selected which have a similar structure to Acarbose. The value of the similarity of the structure of the compound and the rank of the activity of the compound using docking have the same function in assessing the close relationship with the target compound (Acarbose), but the two values have different data intervals in interpreting the relationship between compounds. The relationship between the structural similarity values of compounds using the Tanimoto method with the rank score of compound activity using molecular docking is shown in the correlation value (ρ). The normality test of the data is carried out to determine the correlation test to be used as shown in Table 3.

Table 3. Data Normality Test.

Kolmogorov-Smirnov Test	Tanimoto Coefficient	Rerank Score
p-value	0.200 (>0.05)	0.000 (<0.05)

The normality test of the data is shown in Table 4, for the data of the Tanimoto coefficient, the data shows a normal distribution (p-value = 0.200) at a significance level of 0.05 and the Rerank score data shows that the data is not normally distributed (p-value = 0.000) at a significance level of 0, 05. Correlation test using the Spearman's Rho Test for the Tanimoto Coefficient and Rerank Score as shown in Table 3.

Table 4. Correlation Test of Rerank Score dan Tanimoto Coefficient.

Test Method	Correlation	p-value
Spearman's Rho	-0.714	0.00

The correlation test shows that the p-value of the Spearman's Rho test is 0.000 <0.05 (significant level). These results indicate that there is a significant relationship between the Tanimoto Coefficient and Rerank Score with the opposite relationship because the correlation value is negative, namely -0.714. Based on the correlation value between the Tanimoto coefficient and the rerank score of -0.714, it shows that the relationship between the type of assessment through the Tanimoto coefficient and the Rerank Score in measuring the proximity of 37 selected compounds with Acarbose is quite close in the opposite direction. The negative correlation value indicates that the greater the Rerank Score or the Rerank Score value goes to positive and Tanimoto Coefficient value smaller or goes to of 0 (zero). Meanwhile, if the value of the Rerank Score is getting smaller or the value of the Rerank Score is heading towards a more negative, then the value of the Tanimoto Coefficient will be getting bigger towards 1 (one). This is in accordance with the theory that the greater the degree of similarity in the structure of a compound, the more likely it is that the compound has the same biological activity.

Table 5. Rerank Score of Compounds in *Nigella sativa* (cal/mol).

No	Ligand	Rerank Score
1	Kaempferol 3-glucosyl-(1-2)-galactosyl-(1-2)-glucoside	-107.8770
2	(S)-2,3-Epoxysqualene	-102.1760
3	Quercetin 3-glucosyl-(1-2)-galactosyl-(1-2)-glucoside	-95.7338
4	Oleic Acid	-92.4246
5	Linolenic acid	-86.0396
6	Nigellidine	-82.2884
7	Apiol	-77.4786
8	Quercetin 3-(6'-feruloylglucosyl)-(1-2)-galactosyl-(1-2)-glucoside	-76.4027
9	Lauric Acid	-75.5374
10	Myristicin	-74.7801
11	Nigellidine 4-O-sulfite	-72.9925
12	Salfredin B11	-72.2492
13	(+)-R-Citronellol	-69.6673
14	Fuzitine	-67.7267
15	Nigeglanine	-67.2640
16	Dihydrocarvone	-66.2078
17	Sabinene	-65.8284
18	Beta Myrcene	-65.5974
19	Calvacrol	-65.2293
20	Nigellimine	-64.6439
21	Carvone	-63.5489
22	Nigellicine	-63.3816
23	Beta-Amyrin	-62.8163
24	Alpha Tujene	-62.7140
25	Gamma-Terpinene	-61.0559
26	Thymoquinone	-60.1780
27	4-Terpineol	-59.4488
28	Anisaldehid	-58.9859
29	Nonane	-58.9793
30	p-Cymene	-58.5201
31	m-Thymol	-58.3036
32	Methyl Chavicol	-58.2637
33	alpha-Phellandrene	-57.9589
34	Fenchone	-57.4119
35	Beta Pinene	-52.4448
36	Alpha pinene	-50.1492
37	Longifolene aldehyde	-26.0960
38	alpha-Longipinene	55.1726

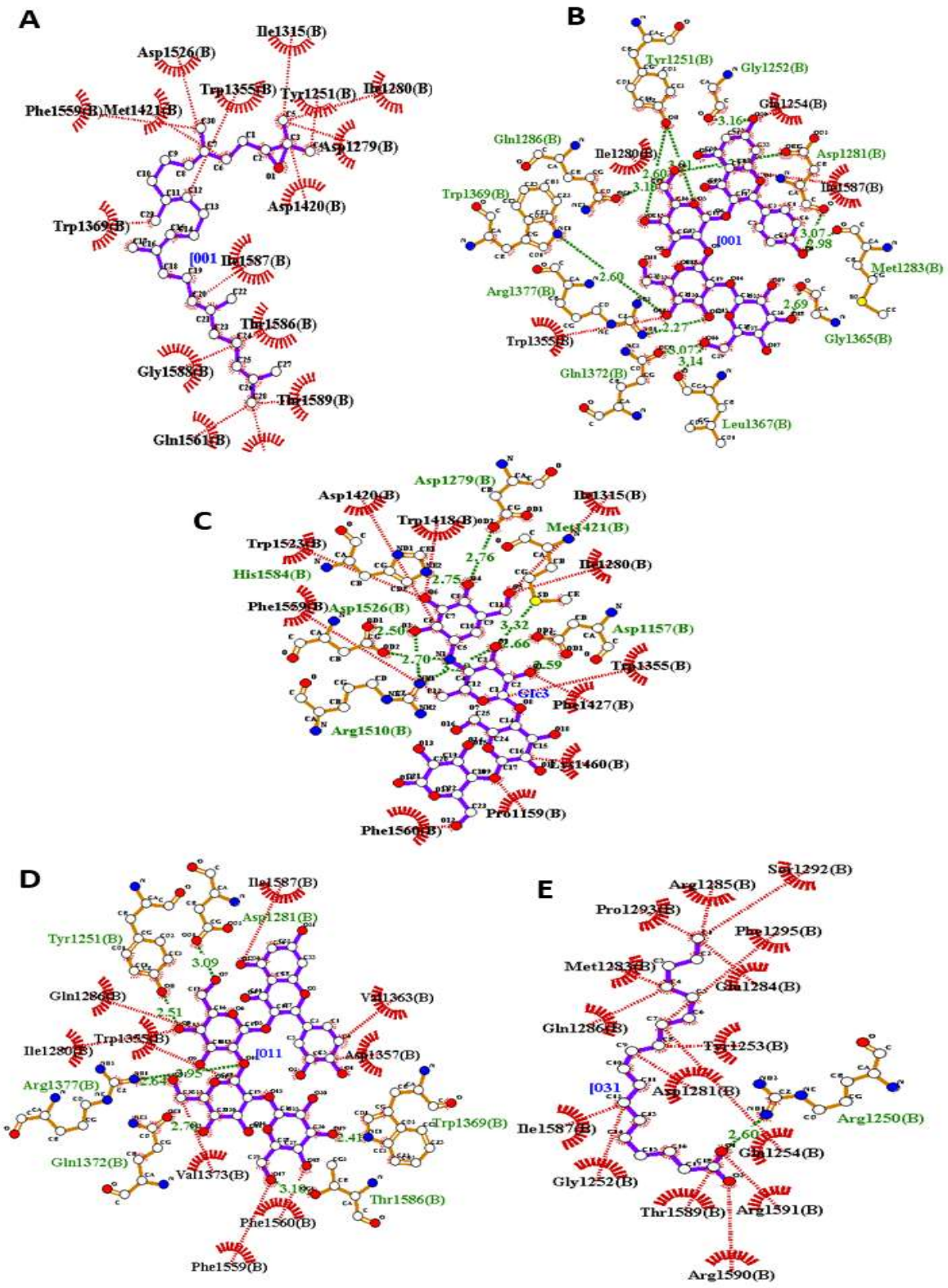

Figure 3. Interaction of Compounds Pose in the Binding Site of MGAM (hydrogen bond (green dot line) ; Hydrophobic interaction (red line)) : A) (S)-2,3-Epoxysqualene ; B) Kaempferol 3-glucosyl-(1-2)-galactosyl-(1-2)-glucoside; C) Alpha Acarbose ; D) Quercetin 3-glucosyl-(1-2)-galactosyl-(1-2)-glucoside ; E) Oleic Acid.

https://doi.org/10.58511/jnpdd.v2i1.7385
JNPDD 2024; 2(1): 46-55

4. CONCLUSION

Greater the degree of molecular structure similarity of *Nigella sativa*'s compounds to acarbose structure more likely has the same biological activity as MGAM inhibitory. The compounds of *Nigella sativa* seeds that predicted potential has biological activity as MGAM inhibitory i.e. Kaempferol 3-glucosyl-(1-2)-galactosyl-(1-2)-glucoside,Quercetin 3-glucosyl-(1-2)-galactosyl-(1-2)-glucoside, (S)-2,3-Epoxysqualene, and Oleic Acid but further analysis should be done regarding ADMET and Lipinsky rule of 5 for development as a drug candidate.

Acknowledgements: The authors are grateful for a research grant from Faculty of Pharmacy, Universitas Pancasila with the Internal Research Grant Program 2020.

Author contributions: Concept – E.M.; Design – N.A.; Materials, Data Collection and/or Processing, Analysis and/or Interpretation – E.M; N.A;

Conflict of interest statement: The authors have no conflict of interest.

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