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Original Article

Molecular Dynamic Simulation of Hydroxymethylglutaryl-CoA Reductase Inhibitors from *Gnetum gnemon* L. Seed Extract

Yuditya Artha, Arif Arrahman, Azminah, Arry Yanuar*

ABSTRACT

Objective: *Gnetum gnemon* L. (melinjo) seed extract contained trans-resveratrol which has been shown to inhibit hydroxymethylglutaryl-CoA (HMG-CoA) reductase. Therefore it has a potent activity for lowering blood cholesterol. This study was carried out to determine the molecular dynamics simulation of HMG-CoA reductase inhibitors from *Gnetum gnemon* L. seed extract. **Methods:** Molecular dynamics simulation using AMBER was used. The simulation was set at 300 K as default temperature and 310 K, average human body temperature. The main parameters of this study were ligand-residue interaction, binding affinity, root mean square deviation (RMSD), root mean square fluctuation (RMSF), hydrogen bonds analysis, molecular mechanics Poisson Boltzmann surface area (MMPBSA), and molecular mechanics generalized born surface area (MMGBSA). **Results:** In the simulation study, trans-resveratrol, trans-piceid, gnemonol M, gnemonoside B, viniferin and gnetin C had shown lower energy than HMG (PDB ID: MAH), the substrate of HMG-CoA Reductase. Free energy binding obtained from simulation was between 11.1 to -31.38 kcal/mol. **Conclusion:** The simulation at 310 K was preferable than 300 K as more interactions were performed and higher affinity was obtained.

Key words: *Gnetum gnemon* L, Hydroxymethylglutaryl-CoA reductase inhibitor, Molecular dynamics, Trans-resveratrol.

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INTRODUCTION

Hypercholesterolemia is elevation the level of blood plasma cholesterol and one of the risk factor for cardiovascular diseases (CVD) occurrence (e.g., stroke and heart attack). Stroke places as the first leading cause of death in Indonesia according to data from World Health Organization in 2015. Food diet gives significant effect on CVD that caused by blood cholesterol level.[1] Statin was invented in 1971 has a potential activity to inhibit cholesterol synthesis. Currently, statin and its analogs were chosen as primary prevention of CVD.[2]

From time to time, research on drugs, including statins was conducted to discover another alternative treatment of CVD. Development of drugs now focus on plants derived drugs to avoid unpredicted event during synthetic drugs research.[3] *Gnetum gnemon* (melinjo) contains resveratrol abundantly including resveratrol derivatives: gnetin C, gnemonoside A and gnemonoside D.[4] Resveratrol inhibits HMG CoA to mevalonate conversion up to 32,4% while simvastatin reaches up to 43,0%.[5] Another benefit of resveratrol is their intervention against dyslipidemia and obesity.[6] An experiment extracted melinjo seed to analyse several compounds composed within and its activity against hydroxymethylglutaryl-CoA reductase (HMGCR).[7] The experiment shows dichloromethane extract gives highest inhibitory activity against HMGCR based on *in vitro* study. The extract predicted contains a compound that has potential inhibitory activity toward HMGCR and is expected performs higher affinity compared to HMG, the original substrate of HMGCR also the precursor of mevalonate.

Molecular dynamics simulation provides the structure as a dynamic system with transformed continuously structural conformation.[8] The computational study, molecular dynamics (MD) were carried as a complementary study to *in vitro* experiment. The affinity each of the extracted compound is compared to statins and HMG by employing the computational method.

MATERIALS AND METHODS

Viniferin, piceid, gnemonol M, gnetol, isorhapontigenin, latifolol, resveratrol, gnemoside B, gnetin C, HMG, rosuvastatin, and pravastatin were selected for the ligands in this study. These ligands were parameterized by Sander adding AM1-BCC charge in Antechamber.[9]

Best energy conformation from docking study was chosen to be used as preparation material on MD. Several studies state their inhibitory activity towards HMGCR.[4-6,10] Thus, Piceid and Resveratrol were included in the simulation as well to rescore binding affinity. The interaction between residues and ligands was visualized by LigPlot.[11]

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The simulation was running in Amber using pmemd.cuda in GPU environment.[12] ADP as the cofactor was included in this simulation and prepared. The required files were downloaded at http://research.bmh.manchester.ac.uk/bryce/amber.[13] Simulation was set at 300 K and 310 K, water TIP3P octahedron was selected as the solvate with 12.0 Å size. The charges of each ligand were generated using antechamber. Na^+ as counter ion was added to create a system with neutralized pH. Dynamics of the system was observed and analyzed for 20 ns subsequently visualized by VMD.[14] Minimisation was carried to equilibrate the solvated complex (volume pressure npts nvts constant). Constant heat and density until 600 ps were preceded by 50 ps heat and density equilibration, time step was set at two femtoseconds. The residues from 1 – 786 were monitored during the equilibration. Hydrogen bond interactions were limited to less than 3 Å and 60° for the distance and bonding angle respectively.

RESULTS AND DISCUSSIONS

Molecular dynamic

PDB ID 1HW8 was selected as macromolecule target and was validated using redocking Mevastatin to the macromolecule using Autodock.[15,16]

Equilibration

The system was equilibrated in 300 K and 310 K until it reached temperature stability at 300 K and 310 K precisely, density close to water (1 g/ml) at atomic pressure (1 atm). The stability of the system was examined by observing their plot of density, total energy, and temperature visually.

RMSD AND RMSF

RMSD and RMSF measures system dynamic in which higher values signify poor stability. A system with 310 K tends to be more fluctuate than 300 K as shown on RMSD (root mean square deviation) graph Figure 1 and 2. RMSD = 0.00 indicate perfect overlapping between structure coordinate and reference coordinate, lower value signifies more likeness towards reference coordinate. RMSF (root mean square fluctuation) is a measurement of average primary chain mobility.[17] In contrast, a variation of temperature did not show the meaningful impact on RMSF Figure 3 and 4.

Hydrogen bond

A hydrogen bond is an interaction between X—H-------A, wherein H is positively charged, and A is partially or entirely negatively charged.[18] A hydrogen bond is classified based on the occupancy percentage: weak (25-50%), strong (50-75%) and very strong (75-100%).[19] System 310 K formed more hydrogen bonding and obtained higher occupancy implying higher temperature induces the system to be more active, consequently established more opportunity to initiate contacts with neighbouring atoms (Table 1, 2).

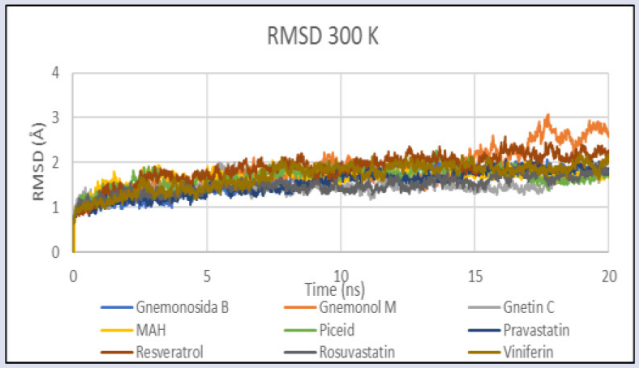

Figure 1: RMSD plot of residues on Microsoft Excel at 300 K during 20 ns.

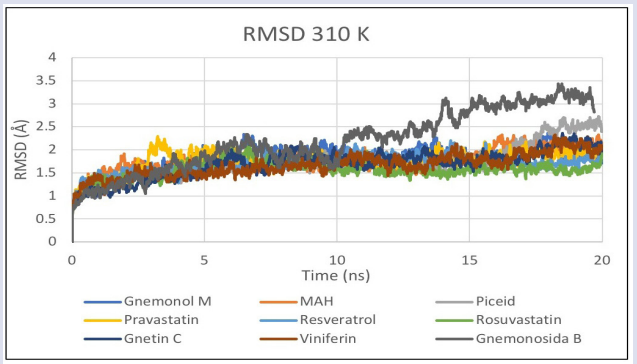

Figure 2: RMSD plot of residues on Microsoft Excel at 310 K during 20 ns.

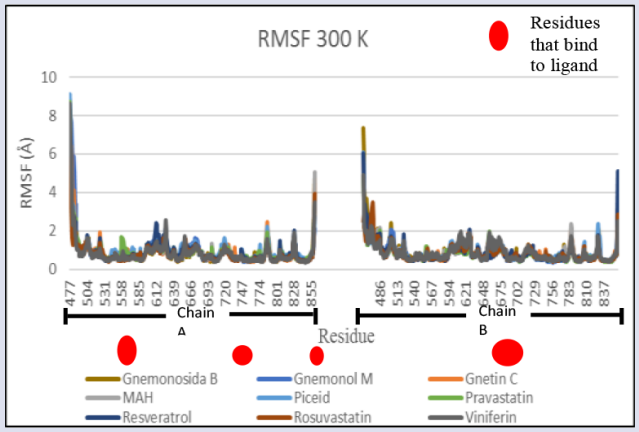

Figure 3: RMSF plot of residues on Microsoft Excel at 300 K during 20 ns.

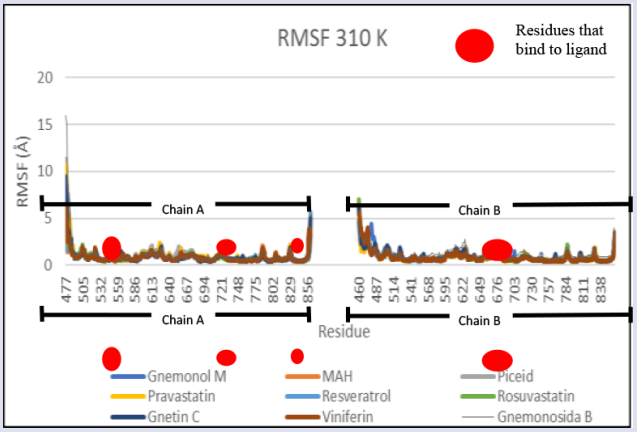

Figure 4: RMSF plot of residues on Microsoft Excel at 310 K during 20 ns.

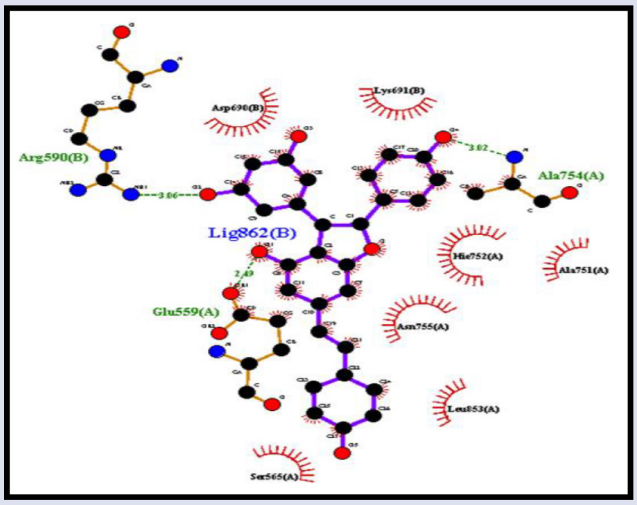

Figure 5: Interaction of Gnetin C during 20 ns simulation at 310 K.

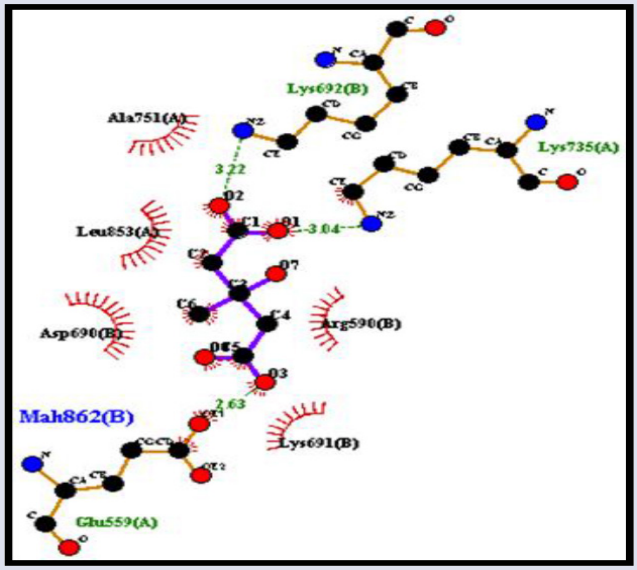

Figure 6: Interaction of HMG during 20 ns simulation at 310.

Gnetin C performed the highest number of total hydrogen bonds at 310 K, 5.361 bonds. Compared to the system at 300 K, gnetin C produced 2,397. The difference in total hydrogen bonding resulted in a significant gap of energy binding affinity between 300 K and 310 K during gnetin C simulation. Hence hydrogen bonds were a critical parameter to be highly considered on each ligand (Table 3, 4).

Although hydrogen bonding was essential in this study, hydrophobic interactions play a significant role as well. HMG as the substrate has less hydrophobic groups compared to other ligands resulting lower affinity as shown in Table 4.

Active binding sites 1HW8: Ser^{684}, Asp^{690}, Lys^{691}, Lys^{692}, Lys^{691}, Lys^{735} which essential for hydrogen bonding and Leu^{562} Val^{683}, Leu^{853}, Ala^{856}, Leu^{857} important residues for hydrophobic interaction.[20] In this study, ligands performed less contact with neighbouring hydrophobic amino acids compared to the statin.

Table 1: **Total amount of hydrogen bonds during 20 ns.**

Ligand	Total Hydrogen Bonds	
	300 K	310 K
Gnemonol M	1,845	2,195
Gnetin C	2,397	5,361
HMG	2,088	2,731
Piceid	8,857	6,972
Pravastatin	5,771	7,001
Resveratrol	2,054	1,992
Rosuvastatin	7,917	8,856
Viniferin	4,073	4,380
Gnemonoside B	3,265	1,733

Table 2: **Hydrogen bonds occupancy at 300 K during 20 ns.**

No	Ligand	Donor	Acceptor	Occupancy (%)
1	Gnemonol M	LIG788-Side-O3	HIE861-Side-OXT	64.95%
2	Gnemonoside B	UNK788-Side-O4	GLU665-Side-OE1	50.25%
		UNK788-Side-O4	GLU665-Side-OE2	32.85%
3	Gnetin C	LIG788-Side-O4	ALA751-Main-O	28.5%
4	HMG	LYS735-Side-NZ	MAH788-Side-O1	38.75%
		LYS735-Side-NZ	MAH788-Side-O2	37.20%
5	Piceid	LIG788-Side-O1	GLU559-Side-OE1	54%
		LIG788-Side-O1	GLU559-Side-OE2	52.5%
		LIG788-Side-O6	ASP690-Side-OD1	48.3%
		LIG788-Side-O6	ASP690-Side-OD2	45.1%
		LIG788-Side-O2	GLU559-Side-OE1	42.45%
		LIG788-Side-O7	HIE861-Main-O	38.8%
6	Pravastatin	LIG1-Side-O5	ASP690-Side-OD1	91.10%
		LYS735-Side-NZ	LIG1-Side-O6	49.35%
		LYS735-Side-NZ	LIG1-Side-O5	44.70%
7	Resveratrol	LIG788-Main-O	ASN658-Side-OD1	14.25%
8	Rosuvastatin	LYS735-Side-NZ	LIG788-Side-O5	74.90%
		LIG788-Main-O	GLU559-Side-OE2	60.50%
		LIG788-Main-O	GLU559-Side-OE1	56.60%
		LIG788-Side-O4	ASP690-Side-OD1	39.15%
		LIG788-Side-O4	ASP690-Side-OD2	36.90%
		LYS691-Side-NZ	LIG788-Main-O	30.85%
		LIG788-Side-O1	ASP690-Side-OD2	25.40%
9	Viniferin	LIG788-Side-O5	ASP690-Side-OD1	63.85%
		LIG788-Side-O2	GLY560-Main-O	53.3%

MMPBSA AND MMGBSA

Some ligands were interacted with few of these residues carrying out hydrogen bonding and creating hydrophobic interaction. Rosuvastatin has sulphonyl functional group thus create hydrophilic properties and bind stronger compared to other statins.[21] Ligands displayed susceptible affinity compared to statins, however, enacted better affinity than HMG. These ligands have slightly inhibitory activity to HMGCR although not as strong as statins are. At 310 K, free binding energy tends to be lower as

Table 3: Hydrogen bonds occupancy at 310 K during 20 ns.

No	Ligand	Donor	Acceptor	Occupancy (%)
1	Gnemonol M	LIG788-Side-O2	GLU559-Side-OE1	42.20%
2	HMG	LYS735-Side-NZ	MAH788-Side-O1	48.85%
		MAH788-Side-O3	GLU559-Side-OE1	40.80%
3	Gnetin C	LIG788-Side-O1	GLU559-Side-OE1	67.95%
		LIG788-Side-O4	LYS691-Main-O	36.40%
		LIG788-Side-O3	ASP690-Side-OD1	27.55%
		LIG788-Side-O1	GLU559-Side-OE1	26.80%
4	Gnemonoside B	UNK788-Side-O4	HIE385-Side-OXT	38.5%
5	Piceid	LIG788-Side-O6	ASP690-Side-OD2	91.65%
		LIG788-Side-O1	GLU559-Side-OE1	58.30%
		LIG788-Side-O2	GLU559-Side-OE1	46.55%
		LIG788-Side-O1	GLU559-Side-OE2	35.80%
		LIG788-Side-O2	GLU559-Side-OE2	29.65%
6	Pravastatin	LIG1-Side-O4	ASP690-Side-OD2	94.85%
		LIG1-Side-O5	ASP690-Main-O	77.80%
		ASN755-Side-ND2	LIG1-Side-O2	50.50%
		LYS735-Side-NZ	LIG1-Side-O6	48.40%
		LIG1-Side-O2	GLU559-Side-OE1	30.25%
7	Resveratrol	LIG788-Main-O	ASP690-Side-OD2	24.40%
8	Rosuvastatin	LYS735-Side-NZ	LIG788-Side-O5	81.20%
		LIG788-Side-O4	ASP690-Side-OD2	64.90%
		LIG788-Side-O1	ASP690-Side-OD1	62.10%
		LIG788-Main-O	GLU559-Side-OE1	53.00%
		LIG788-Main-O	GLU559-Side-OE2	42.50%
9	Viniferin	LIG788-Side-O1	GLU559-Side-OE1	32.60%
		LIG788-Side-O2	CYS561-Main-O	27.95%

Table 4: Recapitulation of MD affinity represented in kcal/mol.

No	Ligand	Molecular Dynamic			
		Binding Affinity (kcal/mol)	Temperature (K)	MMPBSA (kcal/mol)	MMGBSA (kcal/mol)
1	Pravastatin	-10.08	300	-27.2524	-23.9170
			310	-29.6295	-35.0508
2	Rosuvastatin	-9.40	300	-28.9507	29.8129
			310	-31.0991	-30.8993
3	Gnemonol M	-7.53	300	-13.3906	-11.0495
			310	-17.3838	-12.6914
4	HMG	-7.07	300	-7.9018	-10.6594
			310	-8.2101	-14.1883
5	Piceid	-7.07	300	-19.0170	-20.68882
			310	-15.7661	-21.5739
6	Resveratrol	-5.97	300	-13.4773	-14.7247
			310	-11.9587	-15.7353
7	Gnetin C	-7.33	300	-13.2123	-12.4022
			310	-19.3643	-31.3852
8	Viniferin	-7.39	300	-14.3328	-20.1497
			310	-17.0250	-14.4980
9	Gnemonoside B (aglycone)	-8.63	300	-16.4978	-13.4793
			310	-11.4466	-11.7893

shown in Gnetin C (Figure 5, 6). The increasing dynamic of the system develops more interactions, for instance, hydrogen bond contacts which bears stronger bond between ligands and residues.

CONCLUSION

On this simulation study, Gnetin C scored the best affinity.

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ABBREVIATIONS

HMG-CoA: Hydroxymethylglutaryl-CoA; RMSD: Reductase; root mean square deviation; RMSF: Root mean square fluctuation; MMPBSA: hydrogen bonds analysis; MMGBSA: molecular mechanics Poisson Boltzmann surface area; molecular mechanics, generalized born surface area; CVD: Cardiovascular diseases.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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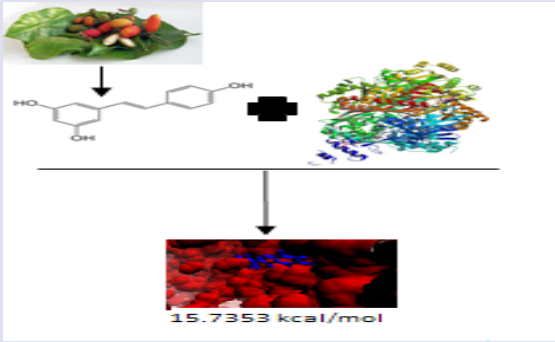
GRAPHICAL ABSTRACT



SUMMARY

- Trans-resveratrol inhibits hydroxymethylglutaryl-CoA (HMG-CoA) reductase, indicating it has a potent activity for lowering blood cholesterol. We performed molecular dynamics simulation of HMG-CoA reductase inhibitors from *Gnetum gnemon* L. seed extract to grasp better understanding of the ligands inhibitory properties. The simulation was set at 300 K as default temperature and 310 K, average human body temperature. The main parameters of this study were ligand-residue interaction, binding affinity, (RMSD), (RMSF), (MMPBSA), and (MMGBSA). We discovered that trans-resveratrol, trans-piceid, gnemonol M, gnemonoside B, viniferin and gnetin C had shown lower energy than HMG, the substrate of HMGCR. We concluded that Gnetin C was the most potent inhibitor according this study. The simulation at 310 K was preferable than 300 K as more interactions were performed and higher affinity was obtained.

ABOUT AUTHORS

Dr. Arry Yanuar, M.Si.Farm., Apt. Achieved Bachelor degree from University of Indonesia in Faculty of Math and Science and continued the Apothecary program. He graduated from Magister program at University of Gadjah Mada majoring Pharmacy. He conducted research at National Institute of Health (NIH), Bethesda, USA (2000). He completed his doctoral program at Nara Institute of Science and Technology (NAIST). He is working at Faculty of Pharmacy since 1990 until now

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