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COMPARATIVE DOCKING STUDY OF 'DUKUNG ANAK' PHYTONUTRIENT COMPOUNDS WITH ANTI-CHOLESTEROL PROPERTIES

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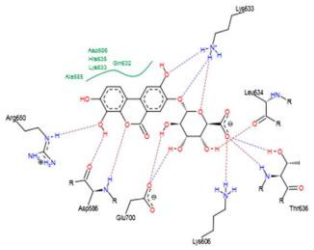
Graphical abstract



Abstract

Inhibition of 3-hydroxy-3-methyl-glutaryl-CoA reductase (HMGR) has been a useful strategy in the treatment of cardiovascular disease. Molecular docking study was carried out to study the effects of geraniin and its metabolites on 3-hydroxy-3-methyl-glutaryl-CoA reductase. 3-hydroxy-3-methyl-glutaryl-CoA reductase acts on melavonate pathway for cholesterol production in the liver but high level of cholesterol in the body may lead to cardiovascular disease where low density lipoprotein accumulates and forms atherosclerotic plaque. In clinical treatment, drug statin is used to block 3-hydroxy-3-methyl-glutaryl-CoA reductase function to reduce the risk of cardiovascular disease but there are unwanted effects where drug statin may cause muscle pain and liver damage. Naturally obtained phytonutrient compounds, geraniin and urolithin groups from dukung anak or scientifically known as *Phyllanthus sp*. were evaluated for 3-hydroxy-3-methyl-glutaryl-CoA reductase inhibitory activity using *in silico* docking studies. Most of these compounds were found to be potent inhibitors of 3-hydroxy-3-methyl-glutaryl-CoA reductase in comparison with known drugs of cardiovascular disease. The binding energies of urolithin A, urolithin B-glucoronide and urolithin D-glucoronide were compared with that of geraniin and it was found that these phytonutrient compounds may have more potent inhibition of 3-hydroxy-3-methyl-glutaryl-CoA reductase comparable with the current drug for cardiovascular disease.

Keywords: Geraniin, *Phyllanthus sp*., Docking, 3-hydroxy-3-methyl-glutaryl-CoA reductase, Cardiovascular disease

Abstrak

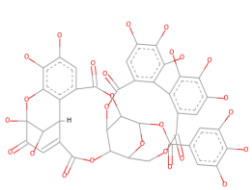
Perencatan fungsi 3-hydroxy-3-methyl-glutaryl-CoA reductase (HMGR) merupakan strategi dalam rawatan penyakit kardiovaskular. Kajian pengedokan molekular telah dijalankan bagi mengkaji kesan tindakan geraniin dan metabolitnya terhadap fungsi 3-hydroxy-3-methyl-glutaryl-CoA reductase. 3-hydroxy-3-methyl-glutaryl-CoA reductase bertindakbalas melalui tapak jalan melavonate bagi penghasilan kolesterol di dalam hati namun begitu kandungan kolesterol yang tinggi di dalam badan mendorong kepada penyakit kardiovascular dimana lipoprotin berketumpatan rendah berkumpul dan membentuk plak aterosklerotik. Dalam rawatan klinikal, ubat statin digunakan untuk merencat fungsi 3-hydroxy-3-methyl-glutaryl-CoA reductase bagi mengurangkan risiko penyakit kardiovaskular namun begitu terdapat kesan sampingan ubat statin dimana ia mengakibatkan kesakitan otot dan kerosakan hati. Fitonutrient kompoun semulajadi, geraniin dan kumpulan urolithin dari pokok dukung anak dengan nama saintifik *Phyllanthus sp.* telah digunakan bagi menilai aktiviti perencatan fungsi 3-hydroxy-3-methyl-glutaryl-CoA reductase melalui kajian pengedokan '*in silico*'. Kebanyakan kompoun ini berpotensi merencat fungsi 3-hydroxy-3-methyl-glutaryl-CoA reductase apabila dibandingkan dengan ubatan semasa dalam merawat penyakit kardiovaskular. Tenaga pengikatan bagi urolithin A, urolithin B-glucoronide dan urolithin D-glucoronide telah dibandingkan

dengan geraniin dan didapati bahawa kompoun fitonutrien tersebut adalah berpotensi tinggi dalam merencat 3-hydroxy-3-methyl-glutaryl-CoA reductase berbanding ubatan semasa yang digunakan dalam rawatan penyakit kardiovaskular.

Kata kunci: Geraniin, *Phyllanthus sp*., Pengedokan, 3-hydroxy-3-methyl-glutaryl-CoA reductase, Penyakit kardiovaskular

1.0 INTRODUCTION

Atherosclerosis cardiovascular disease is a chronic inflammatory condition that leads to stroke or heart attack [1]. The main cause is due to the timely accumulation of fatty materials mainly low-density lipoprotein along the artery walls and formation of plaques that thickens and hardens over time causing the blockage of blood flow [2]. Naturally, low density lipoprotein is one of five lipoprotein groups that transports fat molecules such as cholesterol, phospholipids and triglycerides through the bloodstream but low density lipoprotein is prone to oxidation that contributes to plaque formation in the artery walls [3]. Human body requires certain level of cholesterol in the body to maintain membrane structural integrity and fluidity and it also plays important role as precursors in many biological functions [4-5], but elevated level of cholesterol may increase the number of low-density lipoprotein in the body [6]. One way to control the level of low density lipoprotein in the bloodstream is by using lipid-lowering agents, such as statin, where it acts on 3-hydroxy-3-methyl-glutaryl-CoA reductase, the first and key rate-limiting enzyme of the cholesterol biosynthetic pathway [7-8]. Inhibition of 3-hydroxy-3-methyl-glutaryl-CoA reductase slows the rate of mevalonate production, one of the main precursors in cholesterol production pathway in the liver [9]. Certain 3-hydroxy-3-methyl-glutaryl-CoA reductase binding molecules, such as statin group drugs are effective in inhibiting 3-hydroxy-3-methyl-glutaryl-CoA reductase actions but are prone to other side effects such as muscle pain, liver damage and also the causal onset of diabetes [10-13]. With the increasing number of cardiovascular disease cases worldwide [14], urgent needs for development of alternative drug for effective treatment strategy for cardiovascular disease patients are needed. Being a country rich in natural biodiversity, the exploitation of local herb known as dukung anak from *Phyllanthus sp.* reveals many of its health benefits due to the presence of its main compound, geraniin [15, 16]. Geraniin, which breaks down to urolithin metabolites, is well known for its high antioxidant activities and cytoprotective effects which protect cells from free radical attack [17-20]. Research on geraniin compound had proven its universal role in benefiting human health with its anti-inflammatory, anti-cancer, anti-obesity, anti-bacterial and anti-viral properties [21-25]. Furthermore, compounds from *Phyllanthus sp*. extracts were shown to inhibit atherosclerotic plaque formation in artery walls and decrease cholesterol synthesis by inducing the expression of low density lipoprotein receptor gene to degrade more low density lipoprotein [26, 27]. The intake of *Phyllanthus sp.* extracts were also proven to be safe based on toxicity studies on the consumption of *Phyllanthus sp.* extracts in rats which shows no adverse effects at very high dosage [28]. The aim of this study is to evaluate the effects of *Phyllanthus sp.* compounds, geraniin and its metabolites on 3-hydroxy-3-methyl-glutaryl-CoA reductase through *in silico* study and to further design these phytonutrient compounds as lead compound for anti-cholesterol drug development.



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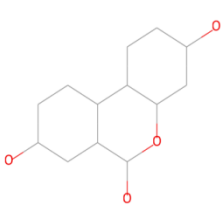

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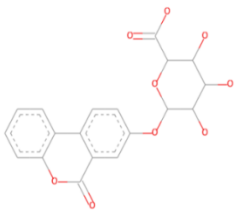

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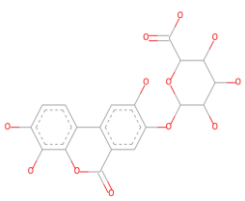

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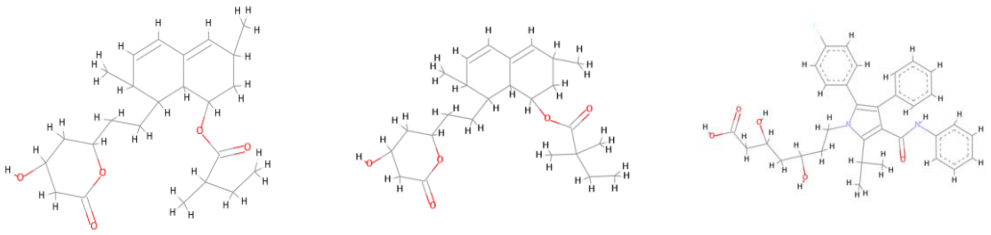

(5) (6) (7)

Figure 1 Ligands involved in molecular docking interactions with 3-hydroxy-3-methyl-glutaryl-CoA reductase protein

2.0 EXPERIMENTAL

2.1 Ligand Preparation

2D structure of geraniin [1] and statin groups; lovastatin [5], simvastatin [6], atorvastatin [7] were obtained from PubChem database of NCBI in mol2 format (Figure 1). ChemOffice Ultra 2004 software was used to draw the 2D structures of urolithin A [2], urolithin B-glucoronide [3] and urolithin D-glucoronide [4] and their SMILES notation was obtained from Discovery Studio application. The seven ligands were analysed for drug-relevant properties based on "Lipinski's rule of five" using Mol soft: Drug-Likeness and molecular property explorer (http://www.molsoft.com/mprop/). The molecular weight of the lead molecules range fixed between 35 to 350 and the value of xlogP fixed in range of 4 to 3.5. On the basis of binding affinity and drug like properties, one ligand that passed all of the screening tests was taken for further ADMET analysis. The adverse properties such as absorption, distribution, metabolism, excretion and the toxicity of geraniin and its metabolites were calculated using the ADMET SAR database.

2.2 Receptor Preparation

The 3D structure of 3-hydroxy-3-methyl-glutaryl-CoA reductase were obtained from RCSB Protein Data Bank (PDB ID: 3CCT). The overall stereochemical property of the protein was assessed by Ramachandran plot analysis [29]. The evaluation of structure model obtained from tool was performed by using Rampage Ramachandran plot analysis (http://mordred.bioc.cam.ac.uk). The active site of modelled structure of 3-hydroxy-3-methyl-glutaryl-CoA reductase protein was predicted by using DogSiteScorer Server (http://dogsite.zbh.uni-hamburg.de/) where it scans the protein surfaces for pockets and also interior of proteins for voids. It is a powerful tool to detect pockets and assess the protein druggability [30]. This is followed by energy minimization steps using YASARA to mimic the *in-vivo* environment. It is one of the important steps in optimizing the molecule towards stable state and removing bad contacts in the protein since stability is inversely proportional to its energy.

2.3 Molecular Docking

The ligands and the target 3-hydroxy-3-methyl-glutaryl-CoA reductase protein were docked using LeadIT software where it uses the FlexX package. Prior to the docking process, both ligands and protein were prepared and were assigned bonds, bond orders, explicit hydrogens, charges and flexible torsions. Using buildup algorithm, the ligands were flexibly located into the protein active site. This is being done through the superposing interaction points of the selected base fragment and the protein active site. The base fragment is then incrementally built up to the complete compound by modeling the ligand flexibility with a torsion library for the added components. Up to 200 poses are generated for each compound using FlexX package. The potent inhibitor for 3-hydroxy-3-methyl-glutaryl-CoA reductase inhibition was selected based on the best pose wise and the compound with the best docking score.

3.0 RESULTS AND DISCUSSION

Nowadays, natural plant compounds had been widely used due to its therapeutic values in treatment of human diseases. Plants are the source for variety of beneficial bioactive chemicals with minimal side-effects compared to synthetic drugs and this increases the interest among researchers to identify any potential natural bioactive compounds in plants [31-34]. This type of studies have been widely grown in biomedical research and the discovery of natural bioactive compounds with anti-

cholesterol activity can act as an anti-cardiovascular disease drug by inhibiting the function of 3-hydroxy-3-methyl-glutaryl-CoA reductase in cholesterol production. In understanding drug-receptor interaction, the mostly used approach is by *in-silico* molecular docking analysis. These methods have been proven to give strong support in designing novel or more potent inhibitors involved in drug-receptor based on natural or synthetic compounds [35]. This computer oriented approach helps in identifying small molecules by orienting and scoring them in the active binding site a protein. The world drug index uses Lipinski's rule of five to filter the drug for its capability of drug for human use and it was proven that geraniin and its metabolites met the requirements and are expected to be active in humans after oral administration.

In this study, geraniin, urolithin A, urolithin B-glucoronide, urolithin D-glucoronide, lovastatin, simvastatin and artovastatin were treated as ligands for molecular docking interactions with 3-hydroxy-3-methyl-glutaryl-CoA reductase protein. Preparation of the ligands for docking process involves undergoing Lipinski rule of five analysis and ADMET analysis. The Lipinski rule of five for the ligands requirements where the cut off values includes, molecular mass less than 500Da, less than 5 hydrogen bond donors, less than 10 hydrogen bond acceptors, and high lipophilicity (expressed as Log *P* less than 5) were all being met [36]. The result shows that the compounds can be strongly recommended as a drug. As for the receptor protein, 3-hydroxy-3-methyl-glutaryl-CoA reductase undergoes Ramachandran plot analysis where Ramachandran plot revealed that the protein structure is suitable for docking studies. Through active site prediction analysis, we simulated the theoretical binding of the ligands to the active site of 3-hydroxy-3-methyl-glutaryl-CoA reductase protein and it these ligands correlates to one single active site of 3-hydroxy-3-methyl-glutaryl-CoA reductase protein that is proposed to be the main active site that has ability to inhibit 3-hydroxy-3-methyl-glutaryl-CoA reductase function. As for the energy minimization steps, it is essential to dynamically relax the conformation and remove steric overlap that produces bad contacts. In the molecular docking studies using LeadIT the results of interaction between 3-hydroxy-3-methyl-glutaryl-CoA reductase protein with the ligands; (A) geraniin (B) urolithin A (C) urolithin B-glucoronide (D) urolithin D-glucoronide (E) lovastatin (F) simvastatin and (G) atorvastatin were shown in Figure 2.

Bioactivity of the ligands was predicted through the scoring function generated by molecular docking interactions with 3-hydroxy-3-methyl-glutaryl-CoA reductase using LeadIT and FlexX. Molecular docking scores closely relates to the interacting amino acids at the site of interactions. Studies showed that 3-hydroxy-3-methyl-glutaryl-CoA reductase consists of 888 amino acids with three functional portions; residues 1-339 span the membrane of the endoplasmid renticulum eight times; residue 340-459 connect the membrane portion to the catalytic portion and residues 460-888 resides in the cytoplasm [37].

The present docking study is carried out for seven compounds against target protein within the active site 64-100 amino acid. The parameter used for identifying the best ligand binding position was the root-mean square distance (RMSD) value [38]. The docking interactions of geraniin and its derivatives molecules with 3-hydroxy-3-methyl-glutaryl-CoA reductase protein indicates that geraniin has the highest binding energy of -0.5003 kJ mol^{-1} followed by urolithin A; -18.3665 kJ mol^{-1}, urolithin B-glucoronide; -19.7511 kJ mol^{-1} while urolithin D-glucoronide exhibited lowest binding energy of -21.0387 kJ mol^{-1}. As for comparison with drug control, lovastatin, simvastatin and artovastatin exhibit binding energy of -16.3707 kJ mol^{-1}, -15.9768 kJ mol^{-1} and -24.5976 kJ mol^{-1} respectively as shown in Table 1. Identical amino acids that interacted with both geraniin and atorvastatin were found to be Ala585, Asp586, Glu632 and Lys633 as shown in Table 2. These findings suggested that the amino acids alanine, aspartic Acid, glutamic acid and lysine in the active site of 3-hydroxy-3-methyl-glutaryl-CoA reductase template were conserved and favouring the interactions with the ligands. The interaction of these compounds with target protein was found to be strong in docking models. Based on the docking score urolithin D-glucoronide have the lowest ranking compare to other compounds. These results revealed that urolithin D-glucoronide has the ability to bind towards the active site of 3-hydroxy-3-methyl-glutaryl-CoA reductase and inhibits the function of 3-hydroxy-3-methyl-glutaryl-CoA reductase in cholesterol production. Hence, this geraniin metabolite can be further developed as a potential drug for treatment of cardiovascular disease.

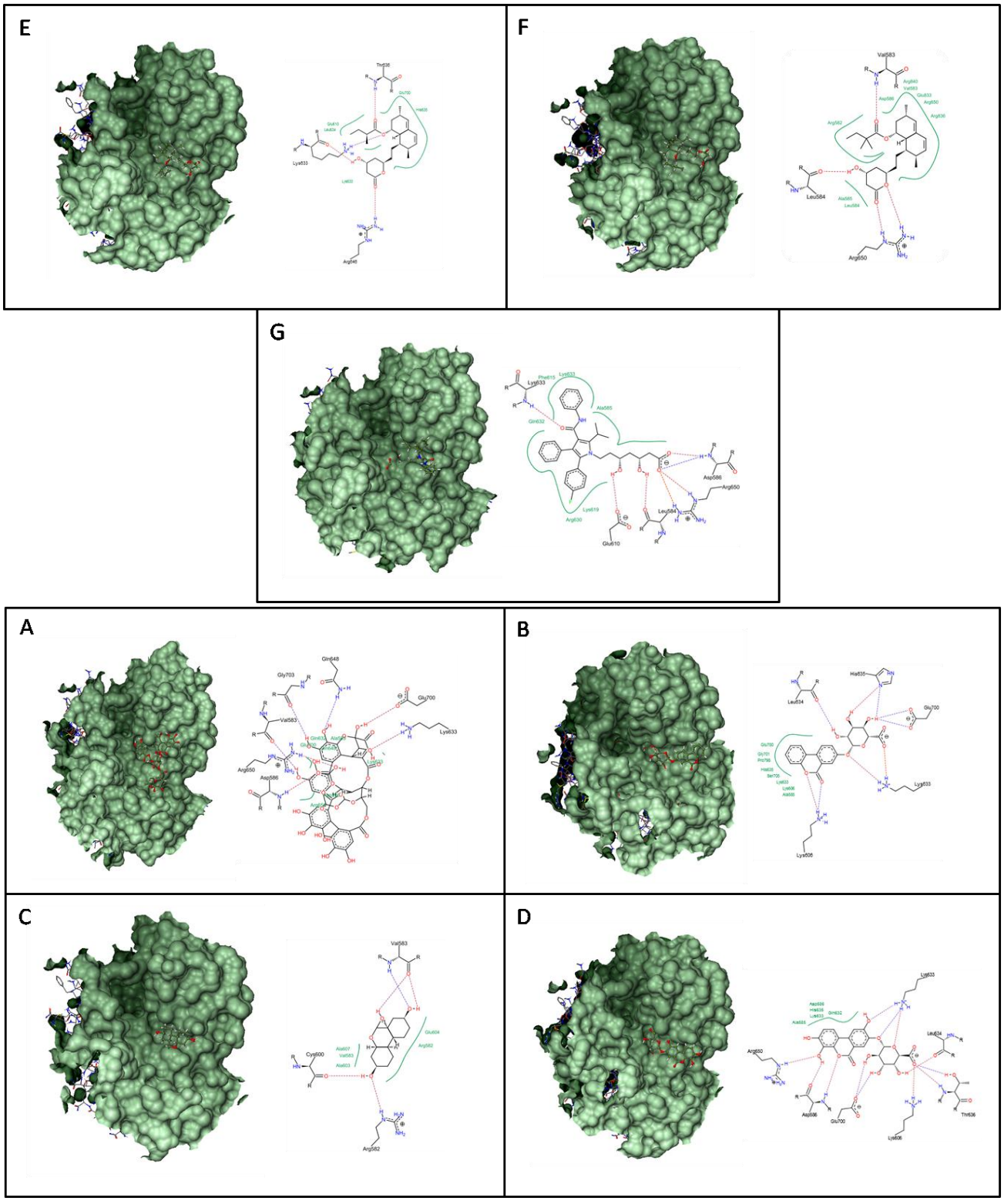

Figure 2 Molecular docking interactions of ligands with 3-hydroxy-3-methyl-glutaryl-CoA reductase protein using LeadIT. Each figure represents 3-hydroxy-3-methyl-glutaryl-CoA reductase 3D protein structure in green and accompanied with ligand 2D structure interacting amino acid

Table 1 Binding energy (docking scores) for molecular docking interactions between ligands and 3-hydroxy-3-methyl-glutaryl-CoA reductase protein

Name	Geraniin	Urolithin A	Urolithin B-glucoronide	Urolithin D-glucoronide	Lovastatin	Simvastatin	Atorvastatin
Total Poses	44	529	693	519	490	359	4
Docking Score	-6.5638	-15.5801	-24.9857	-31.6375	-17.6966	-12.8745	-24.337
Match	-18.1501	-16.8729	-26.7491	-39.8337	-19.6687	-18.2955	-34.2965
Lipo	-6.5909	-4.0734	-6.572	-6.3283	-13.2774	-10.4799	-9.068
Ambig	-13.4228	-5.2022	-10.5738	-9.5802	-12.161	-10.144	-6.7561
Clash	5.2	0.9685	5.1092	6.1048	10.8105	9.445	6.3836
Rot	21	4.2	8.4	12.6	11.2	11.2	14
#Match	7	5	14	14	6	4	13

Table 2 Amino acids involved in the interaction of molecular docking between ligands and 3-hydroxy-3-methyl-glutaryl-CoAreductase receptor

Ligands	Amino acids involved
Geraniin	Val583, Asp536, Ala585, Asp586, Glu632, Lys633, Gln648, Arg650, Glu700, Gly703
Urolithin A	Arg582, Val583, Cys600, Ala603, Glu604, Ala607
Urolithin B-glucoronide	Ala585, Lys606, Lys623, Lys633, Leu634, His635, Glu700, Gly701, Ser705, Pro798
Urolithin D-glucoronide	Ala585, Asp586, Lys606, Gln632, Lys633, Leu634, His635, Thr636 Arg650, Glu700
Lovastatin	Lys633, Leu634, His635, Thr636, Arg646, Glu610, Glu700
Simvastatin	Arg582, Val583, Leu584, Ala585, Asp586, Arg650, Glu833, Arg836, Arg840
Atorvastatin	Leu584, Ala585, Asp586, Glu610, Phe615, Lys619, Arg630, Gln632, Lys633, Arg650

4.0 CONCLUSION

Geraniin and urolithin groups from *Phyllanthus sp.* may be potent inhibitors of 3-hydroxy-3-methyl-glutaryl-CoA reductase comparable with current drug for cardiovascular disease treatment. Being herbal in nature, these compounds can be used as lead molecules to design potent inhibitors for 3-hydroxy-3-methyl-glutaryl-CoA reductase.

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