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Inhibition of HMG-CoA Reductase Activity from Date palm (Phoenix dactylifera L.) fruit's polyphenols: In-Silico Study

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Inhibition of HMG-CoA Reductase Activity from Date palm (*Phoenix dactylifera* L.) fruit's polyphenols: In-Silico Study

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Abstract

Hyperlipidemia is a term that encompasses various genetic and acquired disorders that describe elevated lipid levels within the body. It is a very common disorder, especially in Indonesia, but also throughout the world. Hyperlipidemia itself does not typically lead to critical symptoms itself, however, having this underlying pathology will often lead to serious illnesses that may ultimately lead to death. HMG-CoA Reductase (HMGCR) was the receptor responsible for antihyperlipidemic agent. Recent studies suggested that a diet based on date fruits presents various health benefits, as these fruits are naturally enriched in plant polyphenols. The aim of this study was to determine the inhibitory activity of polyphenols from date palm on HMG-CoA reductase in-silico through molecular anchoring. The various polyphenols from date palm (*Phoenix dactylifera* L.) fruit used in this study were Gallic Acid, Cathechin, Rutin, Quercetin, isoquercetin, Kaempferol, Ferulic Acid, Caffeic Acid, Cinnamic Acid, Syringic Acid, and Vanillic Acid. Biological activity of Date palm polyphenols could be predicted with molecular docking and use score of binding affinity as a parameter for the ability on HMG-CoA reductase enzyme inhibition. The step consist of preparation of ligand and target protein, molecular docking, and druglikeness test. The result shows that the Date palm polyphenols potential as antilipidemic, the compound who has the highest binding affinity is Cathechin of -8.5. Comparative compounds used is atorvastatin which is first-line drugs for the treatment of dysplipidemia.

Keywords: Date Palm, Polyphenol, HMG-CoA Reductase, antilipidemic, Molecular docking

1. Introduction

In humans, the mevalonate pathway is responsible for the endogenous synthesis of cholesterol. In cholesterol synthesis, the enzyme 3-hydroxy-3-methylglutaryl-coenzyme A reductase (HMGR) (enzyme nomenclature [EC] 1.1.1.34), which catalyses the reaction converting HMG-CoA to mevalonate, is the rate-limiting enzyme of the mevalonate pathway that produces cholesterol and other isoprenoids. The enzyme, HMGR, represents an important molecular target for several antihypercholesteremic drugs known as statins. Human HMGR enzyme contains 888 amino acids, with the first 339 residues as the membrane anchor domain located in the endoplasmic reticulum. A linker region is located between residues ranging from 340 to 449, while the catalytic domain, from residues ranging from 450 to 888, resides in the cytoplasm. The structure of the catalytic portion of human HMGR consists of a proteinic tetramer containing four actives sites formed by residues of two monomers. This active site is characterized by the so-called *cis*-loop which is involved in the reduction of the substrate HMG-CoA. Several of the polyphenolic compounds, such as tea catechins (mainly gallate ester derivatives), have been tested successfully both in vitro and in vivo as cholesterol-lowering agents, apart from their antioxidant activities. The evidence indicates that polyphenols exert concerted, multiple-level action involving the upregulation of the low-density lipoprotein receptor, the reduction of cholesterol absorption, and the modulation of both synthetic and metabolic pathways. Considering the complexity of action and the short half-life of HMGR, the direct effect on HMGR activity is very difficult to recognize and isolate. [1]

Phoenix dactylifera belongs to the Arecaceae family; its leaves, barks, pits, fruits and pollens have antioxidant, anticancer, hepatoprotective, neuroprotective, gastrointestinal protective, antidiabetic, antihyperlipidemic, sexual improvement, and antimicrobial potentials. The broad pharmacological effects of *p.dactylifera* may be attributed to the powerfull and beneficial ingredients including phenolics, flavonoids, carotenoids, vitamins, minerals, amino acid, fatty acids and organic acids.[2]

Plant polyphenols have been extensively studied and have recently gained increased interest among researchers and clinicians due to their biological properties

including antioxidant activity, cholesterollowering properties, and other potential health benefits such as chemoprevention of cancer, prevention of diabetes, and cardiovascular diseases. [3,4]

High total phenolic contents of P.Dactylifera, in the range of 10.47 to 22.11 mg/100 g FW. In details, Ajwa Al Madinah had the highest content (22.11 mg/100 g DW), followed by Nabt Saif (22 mg/100 g DW), while Khla Al Qassim had the lowest content (10.47 mg/100 g DW). Some polyphenol likes gallic, *p*-coumaric, and ferulic acid derivatives were the most dominant phenolic compounds, respectively. Moreover, different classes of flavonoids were identified in the tested varieties; quercetin, luteolin, apigenin, isoquercetrin, and rutin. [5]

Table 1 Phenolic classes of and identified compounds in date fruits [6]

Class	Identified compound
Benzoic acids and derivatives	Gallic acid, protocatechuic acid, p-hydroxybenzoic acid, vanillic acid, sinapic acid, and syringic acid
Cinnamic acid and derivative	Caffeic acid, hydrocaffeic acid, ferulic acid, p-coumaric acid, dactyliferic acid, 2 caffeoylshikimic acidhexosides, 3-caffeoylshikimic acid, 4-caffeoylshikimic acid,5-caffeoylshikimic acid, caffeoylsinapoyl hexoside, and dicaffeoylsinapoyl hexosid
Flavonoid glycosides and esters	Luteolin, quercetin and apigenin, quercetin rhamnosyl-hexoside sulfate, quercetin 3-O-rutinoside (rutin), quercetin hexoside sulfate, quercetinacetyl-hexoside, isorhamnetin-3-O-rutinoside, isorhamnetin hexoside, chrysoeriol rhamnosyl-hexoside, isorhamnetin acetyl-hexoside, quercetin 3-O-glucoside (isoquercitrin), chrysoeriol

	hexoside sulfate, andchrysoeriol hexosid
Flavan-3-ols	(+)-Catechin, and (−)-epicatechin

Catechins and their gallate esters are a class of polyphenolic compounds that has recently attracted a great deal of attention because of its beneficial effect on human health. They are functionally versatile and regulate a number of biological pathways to deal with various physiological or pathophysiological pathways. Catechins are mostly known for their potent antioxidants activity; however, depending on the doses, they may also show prooxidant effects. Catechins have been shown to suppress several key pathways linked to oncogenesis, including those involved in cell survival, proliferation, and invasion, along with angiogenesis. They are also helpful against disorders involving lipid and glucose metabolism such as type 2 diabetes and obesity and could also alleviate the risk of cardiovascular diseases.[7]

Kaempferol is a flavonoid compound that found in many fruits, vegetables and medicinal plants. it has anti-oxidant, anti-inflammatory, anti-cancer, and anti-obesity effects. Specially, the anti-adipogenic effects of kaempferol during adipocyte differentiation, Kaempferol inhibits lipid accumulation in adipocytes. [8.9]

Quercetin, one of the most widely distributed flavonoids in plants, has been demonstrated to reduce hyperlipidaemia and atherosclerotic lesion formation. Reverse cholesterol transport (RCT) plays a crucial role in exporting cholesterol from peripheral cells, which is one mechanism utilized in the prevention and treatment of atherosclerosis. [10] Quercetin and its glycone rutin, against high cholesterol diet (2%) induced hepatotoxicity and inflammation.[11]

Chlorogenic acid (CGA) is one of the most abundant polyphenols in the human diet and is suggested to be a potential anti atherosclerotic agent due to its proposed hypolipidemic, anti-inflammatory and antioxidative properties. Caffeic, ferulic and gallic acids may be the potential active compounds accounting for the in vivo effect of CGA. [12]

The phytochemical screening of Paspalum scrobiculatum L (Kodo millet) contains phenolic compounds such as quercetin, ferulic acid, vanillic acid and Syringic acid (SA) . Syringic acid is a phenolic compound of natural origin. SA is an excellent compound to be used as a therapeutic agent in various diseases (diabetes, CVDs,

cancer, cerebral ischemia, neuro and liver damage) and possess anti-oxidant, antimicrobial, anti-inflammatory and antiendotoxic activities. Comparison with standard hepatoprotective drug silymarin, SA exhibits potent hepatoprotective and antihyperlipidaemic activities in acetaminophen hepatotoxicity induced rats.[13]

The literature supporting a physiologically significant inhibition of the key rate-limiting enzyme, HMGR, in lipid metabolism by polyphenols is limited for mechanistic insights into their therapeutic use. With the variety of potential compounds contained in the *P.dactylifera* fruit, thus this study was conducted to prove the phytochemical compound potential from *P.dactylifera* fruit for as dyslipidemia treatment through molecular docking method, so it can be used as an alternative in the treatment of dyslipidemia.

2. Materials and Method

2.1. Ligand Preparation

Chemical structure of Phoenyx Dactylifera fruit collected from literature study, 3D chemical structure, ligand SMILES and ID number is taken from PubChem (https://pubchem.ncbi.nlm.nih.gov/) (Table 1). The ligand then processed with Avogadro and saved with PDB format

Table 1. ID Number and Canonical Smiles of Phoenyx Dactylifera fruit compound

Compound	ID Number	Canonical SMILES
Gallic Acid	370	C1=C(C=C(C(=C1O)O)O)C(=O)O
Vanillic Acid	8468	COC1=C(C=CC(=C1)C(=O)O)O
Syringic Acid	10742	COC1=CC(=CC(=C1O)OC)C(=O)O
Caffeic Acid	689043	C1=CC(=C(C=C1/C=C/C(=O)O)O)O
Cinnamic Acid	444539	C1=CC=C(C=C1)C=CC(=O)O
Ferulic Acid	445858	COC1=C(C=CC(=C1)/C=C/C(=O)O)O
Cathechin	9064	C1[C@@H]([C@H](OC2=CC(=CC(=C21)O)O)C3=CC(=C(C=C3)O)O)O
Quercetin	5280343	C1=CC(=C(C=C1C2=C(C(=O)C3=C(C=C(C=C3

		O2)O) O)O)O)O
Kaempferol	5280863	C1=CC(=CC=C1C2=C(C(=O)C3=C(C=C(C=C3O 2)O) O)O)O
isoquercetin	5280804	C1=CC(=C(C=C1C2=C(C(=O)C3=C(C=C(C=C3 O2)O)O)O[C@H]4[C@@H]([C@H]([C@@H]([C@H](O4)CO)O)O)O)O)O
Rutin	5280805	CC1C(C(C(C(O1)OCC2C(C(C(C(O2)OC3=C(OC 4=CC (=CC(=C4C3=O)O)O)C5=CC(=C(C=C5)O)O)O) O)O)O)O)O

2.2. Target Selection Prediction of protein target performed with Pharmmapper (http://lilab.ecust.edu.cn), SuperPred (http://prediction.charite.de), and Swiss target prediction (www. swisstargetprediction.ch). the protein prediction then validated with UniProt (https://www.uniprot.org). The protein structure collected in Protein Data Bank (https://www.rcsb.org/) with PDB code 5HF5. The protein structure processed using PyMOL v1.7.4.5 to remove non-protein molecule. The target protein for this research is the HMG-KoA reductase enzyme.

2.3. Molecular Docking

Molecular docking performed with Vina Wizard feature integrated in PyRx 0.8. The ligand is were Gallic Acid, Cathechin, Rutin, Quercetin, isoquercetin, Kaempferol, Ferulic Acid, Caffeic Acid, Cinnamic Acid, Syringic Acid, and Vanillic Acid. The protein target is the HMG-KoA reductase enzyme. Atorvastatin is used as ligand control for the docking process.

2.4. Molecular Visualization and Small Molecule Interaction

Interaction between ligand, protein target, and control are visualized and analyzed with PyMOL v1.7.4.5. 2.5.

2.5. Drug-Likeness Test

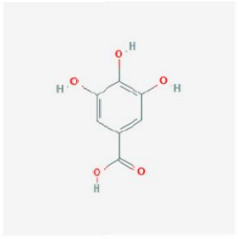
Drug-likeness test using physiochemical properties of ligand and matched with physiochemical of registered drugs. Drug likeness test using Lipinski rule

3. Results and Discussion

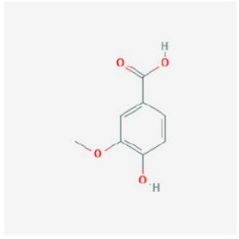
3.1.Ligand Preparation

The ligand is a active compound for being tested in target. Based on literature review, there are eleven ligand to use in this research, that is Gallic Acid, Cathechin, Rutin, Quercetin, isoquercetin, Kaempferol, Ferulic Acid, Caffeic Acid, Cinnamic Acid, Syringic Acid, and Vanillic Acid. Atorvastatin is used a a control for the research. After being download from PubChem website, the ligand format should be converted from SDF to PDB format to make the molecular docking process easier. The 2d structure of each ligand are shown in figure 1.

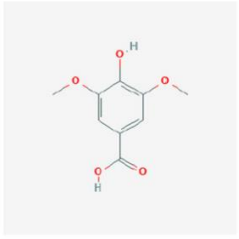
A.



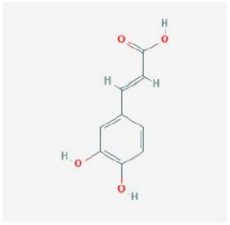
Gallic acid
(3,4,5-Trihydroxybenzoic Acid)



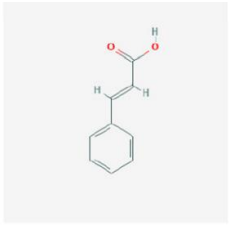
Vanillic acid
(4-HYDROXY-3-METHOXYBENZOIC ACID)



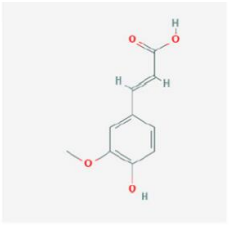
Syringic acid
(4-Hydroxy-3,5-Dimethoxybenzoic Acid)



Caffeic acid
(3,4-Dihydroxycinnamic Acid)

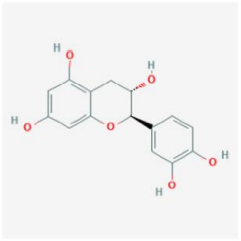


Cinnamic acid

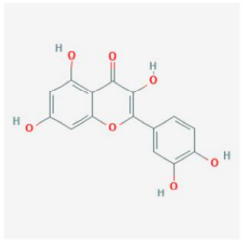


Ferulic acid
(4-Hydroxy-3-Methoxycinnamic Acid)

B.



Cathechin



Quercetin

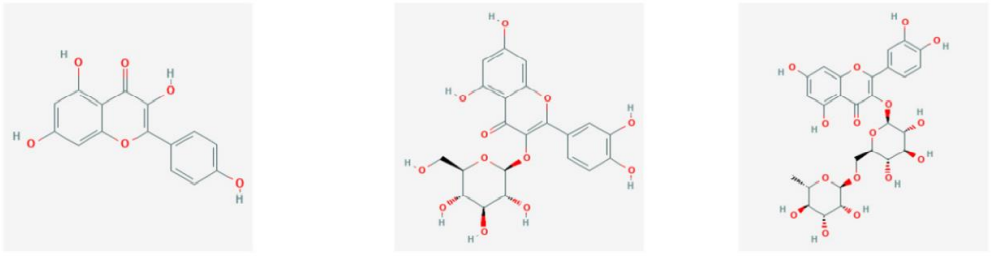


Fig. 1. Structures of polyphenols used in this study. A: benzoic acids and cinnamic acids derivatives. B: Flavonoids. Images taken from www.pubchem.ncbi.nlm.nih.gov/

3.2.Target selection

The target used in this research is HMG-KoA reductase enzyme with PDB. HMG-KoA reductase enzyme inhibition has proven to be achievable as therapeutic target because Conversion of 3-hydroxy-3-methyl glutaryl-CoA (HMG-CoA) to mevalonate by HMG-CoA reductase in the hepatocytes is the first and rate-limiting step in cholesterol biosynthesis. [15]. After taken the target from Protein Data Bank, the molecule being clean up from water molecule and other residues by using PyMol v1.7.4.5. The result is shown in Figure 2.

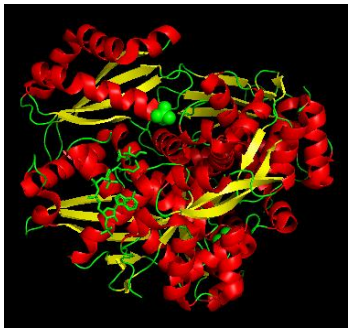


Figure 2.HMG coA enzyme after being processed with PyMol

3.3.Molecular Docking and Result

In silico is a method which uses database and software to do research. One of in silico technique is molecular docking. Molecular docking using computation method to predict potential activity from a compound before it is being tested. The advantage of this

method is to anticipate the failure of in vivo results by predict compound potential activity.

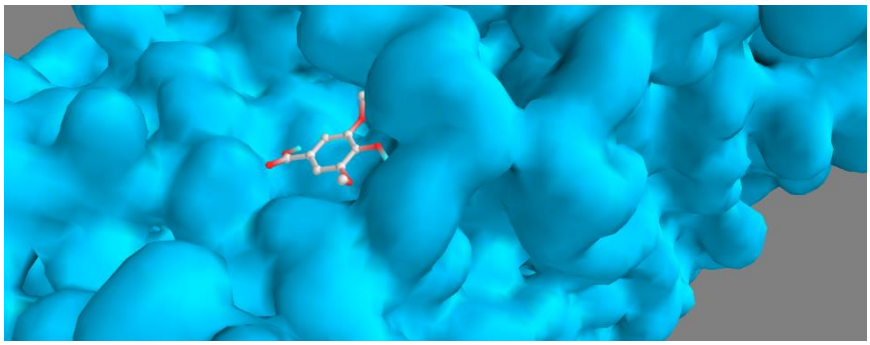


Figure 3. The Binding site of HMG co-A reductase (Blue) and Cathechin (grey) with PyMol v1.7.4.5

Binding affinity result from molecular docking of Phoenyx Dactylifera fruit compound are shown in Table 2. Binding affinity is a score to measure the compound ability to bind with the receptor. If the value is lower, then the affinity between receptor and ligand is higher, and vice versa , from all of the compound, cathechin has the lowest binding affinity value (Figure 3) and also the number is closest to Atorvastatin as control. It means that Cathechin in Phoenyx Dactylifera fruit has the highest potency to become drug compound for dyslipidemia treatment.

Table 2. Binding affinity of P.Dactylifera fruit polyphenols compound

Compound	**Binding Affinity**
Atorvastatin (control)	-8.2
Cathechin	-8.5
Kaempeferol	-8.0
Quercetin	-7.7
Rutin	-7.6
Isoquercetin	-7.6
Caffeic Acid	-6.9
Gallic Acid	-6.1
Ferulic Acid	-5.7

Cinnamic Acid	-5.6
Syringic Acid	-5.6
Vanillic Acid	-5.2

Drug-likeness Test After termolecular docking process, the next step is the drug-likeness test. Drug-likeness is a term to explain how physiohemical properties of a compound affect molecular properties in vivo. The majority rule for drug-likeness test using physiochemical properties of molecular structure and match with the registered drug. On of those rule is Lipinski rule, which is the molecular weight is ≤500 kDa, LogP is ≤5, Hydrogen bond donor is ≤5 and Hydrogen bond acceptor is ≤10. These criteria are similar with good drug oral bioavailability. The log value P states the solubility coefficient in fat/water which has a range of -0.4 - 5. The molecular weight of more than 500 Da cannot diffuse through the cell membrane. The high log P value indicates the more hydrophobic of the molecule. Molecules that are too hydrophobic tend to have high levels of toxicity because they will be retained longer in lipid bilayers and are more widely distributed in the body so that the selectivity of bonds to the target enzyme is reduced. The negative log P value is also not good because the molecule cannot pass through the lipid bilayer membrane. The number of donors and hydrogen bond acceptors describes the higher the hydrogen bond capacity, the higher the energy needed for the absorption process to occur. In general Lipinski's rules describe the solubility of certain compounds to penetrate cell membranes by passive diffusion. The result of drug-likeness test for each compound are shown in Table 3. Based on the Lipinski rule, all of the compound qualify criteria, except Rutin, with molecular weight is more than 500 kDa so it is difficult to absorbed by the human body. Furthermore, hydrogen bond donor and acceptor criteria is not eligible. Commonly, Lipinski rule describes a solubility of a compound to absorbed by cell membrane through passive diffuse. Thus, is it suspected that Rutin could be absorbed by human body but not through passive diffuse/

Table 3. Drug-likeness result of P.Dactylifera fruit polyphenols compound

Compound	Molecular weight	Hydrogen bond donor	Hydrogen bond receptor	(LogP)

Atorvastatin	558.6398032	4	7	6.38
Gallic Acid	170.12 g/mol	4	5	0.16
Vanillic Acid	168.15 g/ml	2	4	0.74
Syringic Acid	198.17 g/mol	2	5	0.77
Caffeic Acid	180.16 g/mol	3	4	0.75
Cinnamic Acid	148.16 g/mol	1	2	1.90
Ferulic Acid	194.18 g/mol	2	4	1.26
Cathechin	290.27 g/mol	5	6	0.98
Quercetin	302.24 g/mol	5	7	-0.56
Kaempferol	286.24 g/mol	4	6	0.03
isoquercetin	464.38 g/mol	8	12	-0.59
Rutin	610.52 g/mol	10	16	-3.89

In general, statins work by slowing the production of cholesterol and increases the liver's ability to remove cholesterol from the blood. Mechanism HMG-CoA reductase acts as an intermediary for the initial steps of sterol biosynthesis. Statins Inhibits HMG-CoA reductase by forming a type of open ring mevalonic acid. The presence of this inhibition causes the synthesis of cholesterol to be inhibited, so increase expression of LDL receptor and decrease LDL receptor degradation.[14] As result of this study, All Phoenyx Dactylifera fruit polyphenol compounds except Rutin have the potential to be used as a candidate material for a new drug to lower blood cholesterol levels.

Conclusion

Based on molecular docking result, Cathechin on Phoenyx Dactylifera fruit has the lowest binding affinity value compare to the other molecule, thus it has the highest potential to use for dyslipidemia treatment. From drug-likeness test result, all of the compound qualify the criteria except Rutin. It is suspected that Rutin could be absorbed by human body but not through passive diffusion.

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