

# Angiotensin-converting enzyme inhibitory activity of polyphenolic compounds from *Peperomia pellucida* (L) Kunth: An *in silico* molecular docking study

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## ABSTRACT

This study aimed to predict the potential activity and interaction conformation of polyphenolic compounds from *Peperomia pellucida* (L) Kunth (nine compounds) with angiotensin-converting enzyme (ACE) macromolecule by *in silico* molecular docking study. The crystal structure of ACE as a molecular target was obtained from the PDB database (PDB ID: 1UZF) with captopril as a native ligand. Molecular docking analysis was performed using AutoDockZn (100 docking runs) based on the active site of Zn<sup>2+</sup>, the central grid was placed on Zn<sup>2+</sup> with a box size of 40 Å × 40 Å × 40 Å and a center of 40.835 Å × 34.382 Å × 44.607 Å for selective inhibitors (MCO702) with a spacing of 0.375 Å. Based on the docking results demonstrated that the prediction of each polyphenol compounds from *P. pellucida* has the potential of active as ACE inhibitors, it was indicated that docking results of each compound has lower affinity compared to captopril (with binding affinity of -6.36 kcal/mol and the inhibition constant 21.81 μM), where the most moderate binding affinity (the most potential) was tetrahydrofuran lignin ((1R,2S,3S,5R)-3,5-bis(4-hydroxy-3,5-dimethoxyphenyl)cyclopentane-1,2-diyl)bis-(methylene) diacetate) of -8.66 kcal/mol and the highest binding affinity (the less potential) was dillapiole (6-allyl-4,5-dimethoxybenzo[d][1,3]dioxole) of -4.99 kcal/mol, although with different forms of interaction, bond, and constant inhibition. Based on the interaction of ACE binding site, 5,6,7-trimethoxy-4-(2,4,5-trimethoxyphenyl)-3,4-dihydronaphthalen-1(2H)-one showed the most similar interaction with the captopril ligand. These results are preliminary data for further research with predictions of target compound biological activity and interaction quickly, accurately, and inexpensively.

## INTRODUCTION

Hypertension or high blood pressure is one of the most top prevalent diseases in the world (Sorlie *et al.*, 2014). In 2010, it was estimated 31.1% of adults worldwide had hypertension

(Mills *et al.*, 2016) and is predicted to increase by about 60% by 2025 (Kearny *et al.*, 2005). Also, hypertension may increase risk factors for cardiovascular disease and has been associated with complications, such as stroke, heart attack, and kidney failure (Hartmann and Meisel, 2007).

One of the primary therapeutic agents for the treatment of hypertension is angiotensin-converting enzyme (ACE) inhibitors. ACE is one component of the renin-angiotensin-aldosterone system and plays a crucial role in the regulation of blood pressure (Skeggs *et al.*, 1953; 1957). Metalloprotein residue of Zn<sup>2+</sup> in ACE cleaves the His-Leu dipeptide from the inactivated C angiotensin I terminal (Ang I) and converting it into a potent vasoconstrictor in the form of angiotensin II (Unger, 2002). ACE has two isoforms,

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including: (1) glycoprotein form in somatic tissue with single large polypeptide chain of 1,277 amino acid and (2) in germinal cells that are synthesized in the form of lower molecular mass and play a role in sperm maturation and sperm binding of oviduct epithelium (Natesh *et al.*, 2003; 2004). Currently, the crystal structure data from ACE is available in the form of PDB files that can be used for *in silico* molecular docking study.

In recent years, modeling methods based on computer simulations have become a useful tool in solving many scientific and engineering problems (Forli *et al.*, 2016). In some conditions, computer-based modeling methods have become a bridge between applied or experimental science and theories of natural sciences, such as physics, chemistry, and biology quickly and precisely. When the simulation is compared with the experiment, it sometimes gives slightly different results (Ramachandran *et al.*, 2008). Molecular docking is a process that searches for possible bonds of two molecules interacting under topographical conditions or energy considerations, the goal being to match the two molecules to the conformation in which has the best interaction (Santos-Martins *et al.*, 2014; Seeliger and de Groot, 2010). One molecule (ligand) is docked to another molecular region (receptor) and from both molecules are calculated its interaction energy. In docking, its interaction energy is calculated by Van der Waals energy and Coulombic energy involved between all the atoms of both molecules (Ghosh and Gemma, 2014).

Natural products (mainly from plants) are the primary source for new drugs discovery. One of the herbs empirically used as a hypertension drug by the community in some countries is *Peperomia pellucida* (L) Kunth herb. According to the study reported by Saputri *et al.* (2015), the methanol extract of *P. pellucida* has ACE inhibitory activity with IC<sub>50</sub> of 7.17 µg/ml and (Kurniawan *et al.*, 2016) has reported that fraction and isolated quercetin of *P. pellucida* herb has IC<sub>50</sub> of 3.44 and 7.22 µg/ml, respectively. Some polyphenols isolated from this herb includes secolignan (peperomins) and lignin (Xu *et al.*, 2006), pellucidin A (Bayma *et al.*, 2000), patuloside A (Khan *et al.*, 2010), dillapiole (Rojas-Martínez *et al.*, 2013), chromenes (Susilawati *et al.*, 2015), and quercetin (Kurniawan *et al.*, 2016). However, a study on ACE inhibitory activity (except quercetin) and the prediction of interaction conformation with ACE has not been reported.

In the present study, prediction of activity potency and interaction conformation of isolates from *P. pellucida* herbs with ACE have been conducted, which thus may expedite the development of further studies for new drugs discovery as ACE inhibitors.

## MATERIALS AND METHODS

### Materials

For *in silico* molecular docking study, a computer ASUS® (Taipei, Taiwan) with following specifications was used: processor Intel® Core™ i5-5200U CPU @2.20 GHz 2.19 GHz, 4.00 GB memory (RAM) DDR3, 64-bit Operating System, x64-based processor, Windows Education 10.1, and NVIDIA GEFORCE 820 M VGA. Software installed includes ChemOffice Pro v15.00 PerkinElmer, Chimera 1.10.2., Ligplot+ v.1.4.5, AutoDockZn (<http://autodock.scripps.edu/resources/autodockzn-forcefield>), AutoDock v4.2.6 and AutoDockTools (<http://autodock.scripps.edu/>), Amber14

(operated via putty to connect with the server), Pymol, OpenBabel GUI, and PMV 1.9.2 (*Phyton Molecular Viewer*).

### Receptor and native ligand structure preparation

The crystal structure of ACE (*angiotensin-converting enzyme*) in complex with captopril was downloaded from Protein Data Bank ([rcsb.org/pdb/](http://rcsb.org/pdb/)) with PDB id: 1UZF with 2.0 Å resolution. Ligand-protein docking was performed using *AutodockZn* program. AutoDockTools and OpenBabel programs were used to prepare and convert the type of the macromolecule complex and ligand file (O'Boyle *et al.*, 2011). Furthermore, water molecules were removed from the macromolecule protein 1UZF; then the complete structure was converted, and hydrogen atom addition was done using Leap module of AMBER 14. The ligand file was subjected to the calculation of AM1-BCC partial atomic charges using antechamber module of AMBER 14 and minimization was done using Amber force field ff99SB. Then, the output (.crd) was converted to PDB by an ambpdb module of AMBER 14.

### Ligand sample preparation

Structure of Ligand samples was acquired from some literature (as can be seen in Table 1) (Bayma *et al.*, 2000; Khan *et al.*, 2010; Kurniawan *et al.*, 2016; Rojas-Martínez *et al.*, 2013; Susilawati *et al.*, 2015; Xu *et al.*, 2006). 2D structure of ligand samples was built using ChemDraw® Pro 15 and was converted to the 3D structure using Chem3D® Pro 15. The MMFF94 atom types and charges of ligand samples were calculated, then minimization was performed using the MMFF94 force field minimization of Chem3D® Pro 15 (Wang *et al.*, 2015). The minimized structure was converted to PDB format.

### *In silico* molecular docking analysis

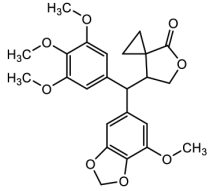
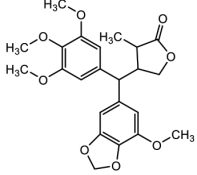
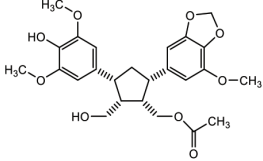
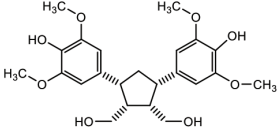
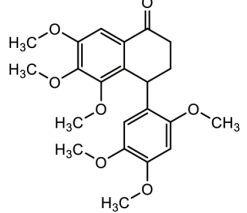
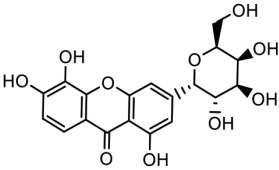
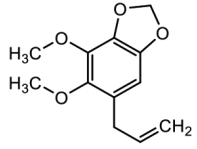
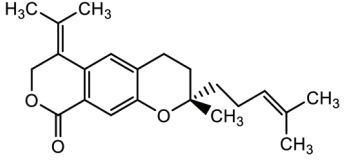
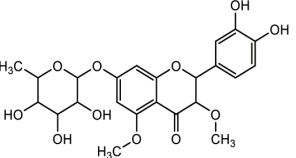
Docking of all the ligand samples was conducted using AutoDockZn (O'Boyle *et al.*, 2011). The program was begun with ligands in different conformations and found the best docking on binding-site proteins using a Lamarckian Genetic Algorithm (LGA) to make one possible formation. The docking simulation was performed for the isolated compound using a protein receptor (PDB id: 1UZF). The autogridZn program was used to calculate the position of the grid. Based on the active site of Zn<sup>2+</sup>, the central grid was placed on Zn<sup>2+</sup> with a box size of 40Å × 40Å × 40Å and a center of 40.835, 34.382, and 44.607 for selective inhibitors (MCO702) with a spacing of 0.375. The docking procedure was done using autodockZn program with 100 docking runs. Docking results were visualized using *LigPlot+* and *Poseview* (Laskowski and Swindells, 2011; Stierand and Rarey, 2010).

## RESULTS AND DISCUSSION

*In silico* molecular docking study aims to determine the potential of active compounds from natural products or synthetic. It is an approach for predicting potentially active compound based on the interaction and bonds types between the ligand and the active site of the receptor. This method is beneficial to predict the biological activity and interaction of ligand compounds quickly, accurately, and inexpensively.

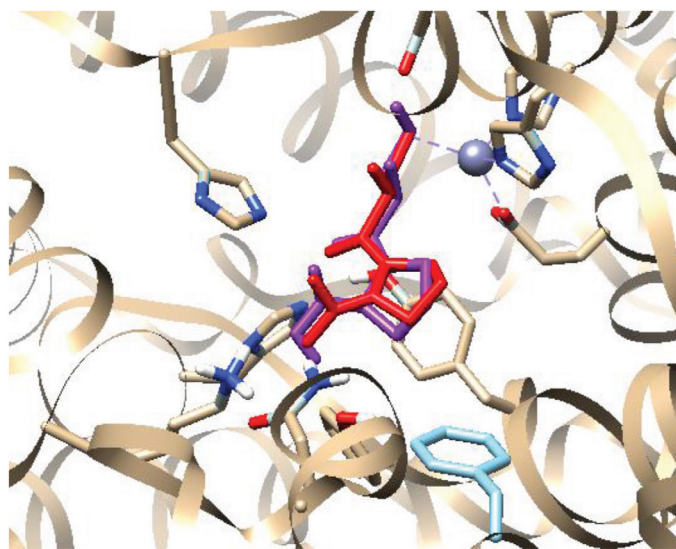
In present study, *in silico* molecular docking study was performed to predict the potency of activity and interaction between ligand molecules in the form of native ligand (captopril)

**Table 1.** The IUPAC name and chemical structure of polyphenols compound from *P. pellucida*.

Compounds	IUPAC name	Structure formula	Ref.
1	7-((7-methoxybenzo[d][1,3]dioxol-5-yl) (3,4,5-trimethoxyphenyl)methyl)-5-oxaspiro[2.4]heptan-4-one (secolignan)		(Xu <i>et al.</i> , 2006)
2	4-((7-methoxybenzo[d][1,3]dioxol-5-yl) (3,4,5-trimethoxyphenyl)methyl)-3-methyldihydrofuran- 2(3H)-one (secolignan)		(Xu <i>et al.</i> , 2006)
3	((1S,2R,3R,5S)-3-(4-hydroxy-3,5-dimethoxyphenyl)-2- (hydroxymethyl)-5-(7-methoxybenzo[d][1,3]dioxol-5-yl) cyclopentyl)methyl acetate (tetrahydrofuran lignin)		(Xu <i>et al.</i> , 2006)
4	((1R,2S,3S,5R)-3,5-bis(4-hydroxy-3,5-dimethoxyphenyl) cyclopentane-1,2-diyl)bis(methylene) diacetate (tetrahydrofuran lignin)		(Xu <i>et al.</i> , 2006)
5	5,6,7-trimethoxy-4-(2,4,5-trimethoxyphenyl)- 3,4-dihydronaphthalen-1(2H)-one (methoxylated dihydronaphthalenone)		(Xu <i>et al.</i> , 2006)
6	1,5,6-trihydroxy-3-((2S,3S,4S,5S,6S)-3,4,5-trihydroxy-6- (hydroxymethyl)tetrahydro-2H-pyran-2-yl)-9H-xanthen-9-one (Patuloside A)		(Khan <i>et al.</i> , 2010)
7	6-allyl-4,5-dimethoxybenzo[d][1,3]dioxole (dillapiole)		(Rojas-Martínez <i>et al.</i> , 2013)
8	(S)-2-methyl-2-(4-methylpent-3-en-1-yl)-6-(propan-2- ylidene)-3,4,6,7-tetrahydropyrano[4,3-g]chromen-9(2H)-one (Chromene)		(Susilawati <i>et al.</i> , 2015)
9	2-(3,4-dihydroxyphenyl)-3,5-dimethoxy-7-((3,4,5-trihydroxy- 6-methyltetrahydro-2H-pyran-2-yl)oxy)chroman-4-one (quercetin)		(Kurniawan <i>et al.</i> , 2016)

and the isolated polyphenol compounds of *P. pellucida* (consisting of nine compounds as listed in Table 1) on the ACE macromolecule (PDB id: 1UZF).

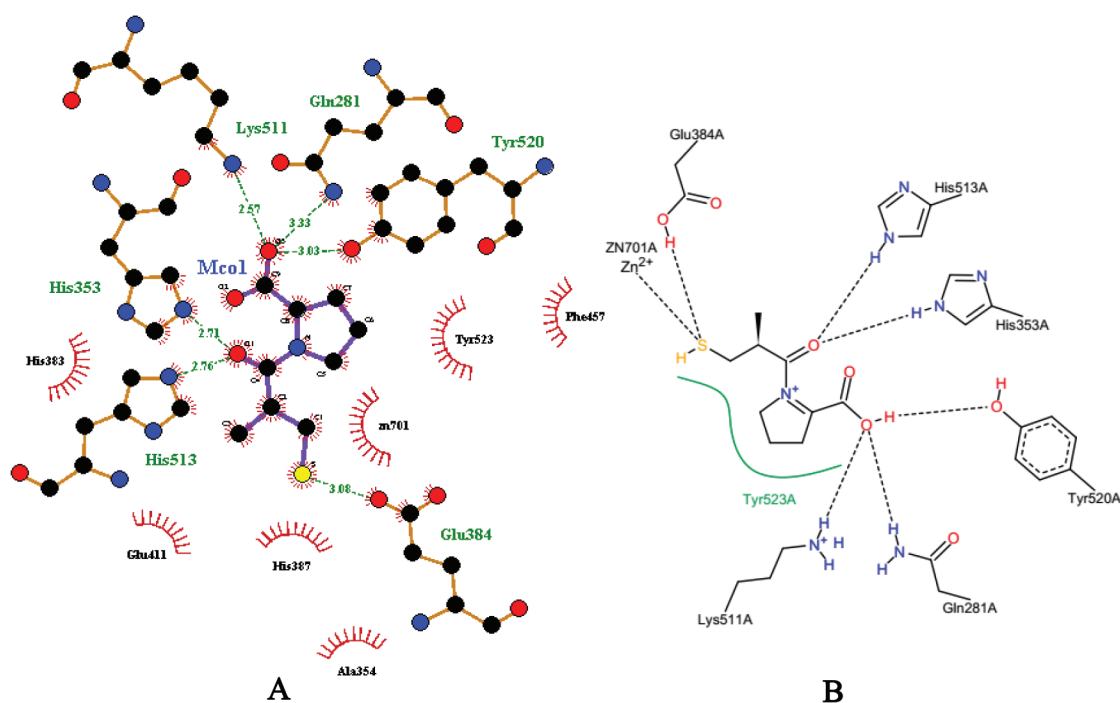
The docking of native ligand (captopril) gave Root Mean Square Deviation (RMSD) value of 0.96 Å (<2), which means the docking results were valid with a  $\Delta G$  value of -6.36 and clusters of 92% for the total of 100 times running. As can be seen in Figure 1, it shows the position between the native ligand and the docking results does not show significantly different locations



**Figure 1.** Visualization of comparison between original and docking results position of the native ligand. Where a red is original crystallography and purple is docking results.

(with the RSMD value <2). Figure 2 demonstrates the interaction between groups of the native ligand with protein (amino acid) and  $Zn^{2+}$  metalloprotein groups of an ACE macromolecule using *LigPlus* (A), *PoseView* (B). The binding mode of captopril in ACE macromolecule complex was investigated from its crystal structure (ID 1UZF). It is shown that the COOH and C=O groups of captopril formed hydrogen bonds with Tyr520, Lys511, Gln281 (with COOH) and His353, His513 (with C=O), respectively. The S-H group formed interaction with metalloproteins  $Zn^{2+}$  (with an atomic distance of 2.3 Å) and formed hydrogens bonding with Glu384. The bond forms of ligands from the docking results involved the hydrogen and hydrophobic bonds on clusters of amino acids and its interactions in the  $Zn^{2+}$  metalloprotein according to studies has been reported by Chang and Alli (2012) and Sharifi *et al.* (2013). This interaction (with distances of < 3 Å) indicated that the active site of each ligand has interaction with ACE macromolecule. In Figure 3, the binding mode of each compound in macromolecule demonstrated that the groups of each compound (except Compound 8) formed interaction with  $Zn^{2+}$  and Glu384. Meanwhile, His353, His513, and Tyr523 had interactions (hydrogen and hydrophobic bond) with groups of each compound, and another hand, another amino acid group had different interactions.

Meanwhile, Table 2 demonstrates that the prediction of polyphenols compound from *P. pellucida* has potential to be developed as ACE inhibitors, it was indicated that docking results of each compound have lower affinity than captopril (with a binding affinity of -6.36 kcal/mol and the inhibition constant 21.81  $\mu M$ ) except Compound 7. Based on the results of calculation and docking analysis, similarity was exhibited between polyphenol compounds (except compound 7) with the native ligand. In the previous study, Guerrero *et al.* (2012) have predicted the interaction and bonds of luteolin (polyphenols

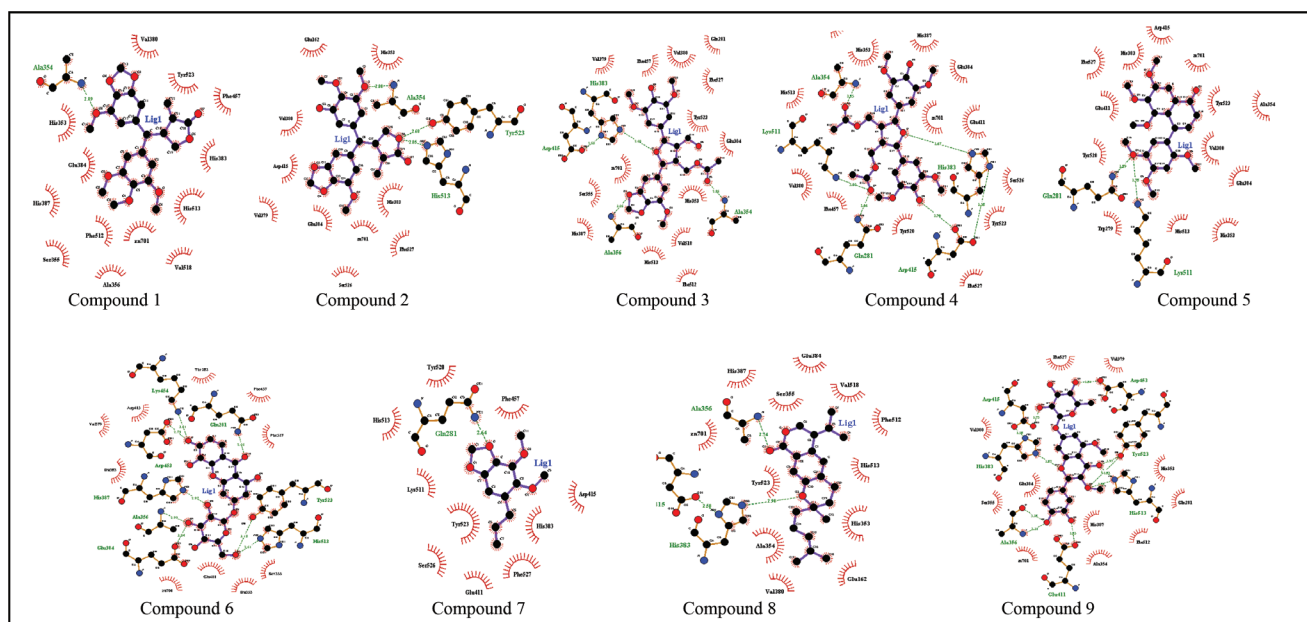


**Figure 2.** Visualization of docking results interaction between the native ligand and residue of ACE macromolecule (PDB: 1UZF) using (A) Ligplotplus and (B) dan Poseview.



group) using QSAR and molecular docking study of quercetin as a potential ACE inhibitor has been reported by [Muhammad and Fatima \(2015\)](#). Each amino acid residue can form seven different bonds or interactions with the ligand includes: (1) interaction of van der Waals bond, (2) interaction of aromatic face to face, (3) interaction of aromatic edge to face, (4) interaction of H-bond or protein as donor, (5) interaction of H-bond or protein as acceptor, (6) interaction of protein positively charged, and (7) interaction of protein negatively charged ([Radifar \*et al.\*, 2013](#)). In addition, [Table 2](#) also shows that some compounds have a potential active site with interaction distance between O atoms (ligand groups) and  $Zn^{2+}$  metalloprotein (protein receptor residue) at values less than 3Å, includes Compound 3, Compound 4, Compound 5, Compound 6, Compound 8, and Compound 9 with constant inhibition 752.52, 448.43, 10,740, 2,470, 517.42, and 831.04 nM, respectively. On other hands,  $Zn^{2+}$ , Glu384, His513, His353, Gln281, Tyr520,

Tyr523, and Lys511 are the binding site of ACE. The native ligand of captopril performs hydrogen bonding ( $Zn^{2+}$ , Glu384, His513, His353, Gln281, Tyr520, and Lys511) and hydrophobic interaction (Tyr523). Based on the results of docking analysis, compound 5 has the most similar interaction with the captopril ligand where the compound binds to the active side of ACE as can be seen in [Table 2](#), and these prediction results are in accordance with the molecular docking results of polyphenol compounds from *Phyllanthus niruri* herbs ([Ahmad \*et al.\*, 2018](#)). In addition, some study has reported that extracts and fractions (with rich in polyphenol content) of this herbs have activity as an ACE inhibitor using in vitro assay method ([Adhitia \*et al.\*, 2017](#); [Mun'im \*et al.\*, 2017](#); [Rinayanti \*et al.\*, 2013](#); [Saputri \*et al.\*, 2015](#)). Meanwhile, according to the previous study, [Kurniawan \*et al.\* \(2016\)](#) have done the assay of ACE inhibitory activity of Compound 9 (quercetin) with  $IC_{50}$  of 16.21 nM which has excellent correlation with the obtained docking



**Figure 3.** Visualization of docking results interaction of isolated polyphenols from *P. Pellucida* and ACE macromolecule receptor (PDB: 1UZF) by Ligplotplus software.

**Table 2.** The docking results of captopril and polyphenol compounds from *P. pellucida*.

No	Ligand	Binding energy (kcal/mol)	Inhibition constant (nM)	Atom distances Zn-S/O (Å)	Interaction of ACE binding site							
					$Zn^{2+}$	Glu384	His513	His353	Gln281	Tyr520	Tyr523	Lys511
1	Captopril (Native)	-	-	-	√	√	√	√	√	√	√	√
2	Captopril redocked	-6.36	21,810	2.3	√	√	-	√	√	√	√	√
3	Compound 1	-8.08	1,190	3.3	√	√	-	√	-	-	√	-
4	Compound 2	-8.14	1,080	4.7	√	√	-	√	-	-	√	-
5	Compound 3	-8.35	752.52	2.7	√	√	-	√	√	-	√	-
6	Compound 4	-8.66	448.43	2.2	√	√	-	√	√	-	√	-
7	Compound 5	-6.78	10,740	2.7	√	√	√	-	√	√	√	√
8	Compound 6	-7.65	2,470	2.1	√	√	√	√	√	-	√	-
9	Compound 7	-4.99	218,680	6.2	-	-	-	-	√	√	√	√
10	Compound 8	-8.58	517.42	2.9	√	√	-	√	-	-	√	-
11	Compound 9	-8.30	831.04	2.8	√	√	√	√	√	-	√	-

result. Therefore, it is essential to proceed to further study, mainly isolation and identification of active compounds as ACE inhibitors of this plant.

## CONCLUSION

In conclusion, we have conducted *in silico* molecular docking to study the potential of polyphenol compounds from *P. pellucida* as an ACE inhibitor. The crystal structure of ACE (PDB id: 1UZF) bound to captopril was used as a protein target. Based on the docking result showed that the native ligand (captopril) was obtained RMSD value of 0.96 Å (<2), which means the docking results were valid with a  $\Delta G$  value of -6.36 and clusters of 92% for the total of 100 times running. Based on the interaction of ACE binding site, Compound 5 shows the most similar interaction with the captopril ligand where the compound binds to the active side of ACE compared to other compounds. Therefore, these results are preliminary data for further research with predictions of biological activity and interaction quickly, accurately, and inexpensively from the target compound.

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## CONFLICT OF INTEREST

All author declared that there is no conflict of interest

## ABBREVIATIONS

ACE: Angiotensin-Converting Enzyme; DRPM: Direktorat Riset dan Pengabdian kepada Masyarakat; Gln: Glutamine; Glu: Glutamate; His: Histidine; IC<sub>50</sub>: half maximal inhibitory concentration; LGA: Lamarckian Genetic Algorithm; Lys: Lysine; MMFF94: Merck Molecular Force Field 94; *P. pellucida*: *Peperomia pellucida* (L) Kunth; PDB: Protein Data Bank; RMSD: Root Mean Square Deviation; Tyr: Tyrosine.

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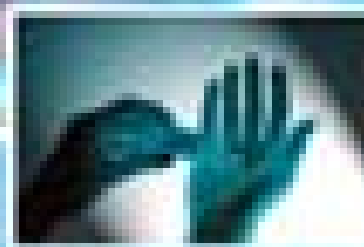
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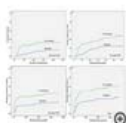


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Ravi Dutt Sharma, Jainendra Jain, Ratan Lal Khosa

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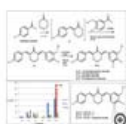


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Ajit Singh, Sheetal Chauhan, Ganesh Paramasivam, Tom Devasia, Yeshwanth Rao, Prasad Narayana Shetty, Navaneeth Amin, Deepak Uppunda, Hashir Kareem

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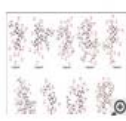


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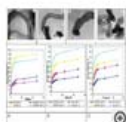


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Islamudin Ahmad, Azminah, Kamarza Mulia, Ary Yanuar, Abdul Munim

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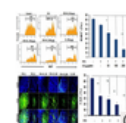


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Rokaya Aly Sobh, Hanaa El-Sayed Nasr, Wael Sabry Mohamed

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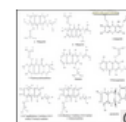


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Sofy Permana, Reny Purnama Hadi, Eviana Norahmawati, Agustina Tri Endharti

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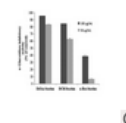


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Abdul Rohman, Mohamad Rafi, Gemini Alam, Muchtaridi Muchtaridi, Anjar Windarsih

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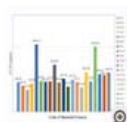
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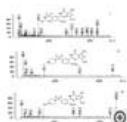


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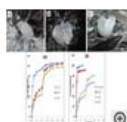


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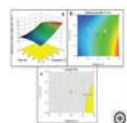


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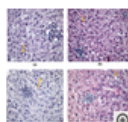


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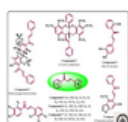


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Irmanida Batubara, Yanico Hadi Prayogo, Irma Herawati Suparto, Berry Juliandi, Susumu Uchiyama

DOI: [10.7324/JAPS.2019.90815](https://doi.org/10.7324/JAPS.2019.90815) Pages: 111-116

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Bayu Ardiansah

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