Identification of ACE1 Inhibitor Derived from Ashitaba's Chalcones: An *In Silico* Approach

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ABSTRACT

The angiotensin-converting enzyme, ACE1, is one of enzymes important to blood pressure modulation. The inhibition of protein responsible for blood pressure regulation, the angiotensin-converting enzyme, ACE1, is considered as a method to alleviate the hypertension condition Ashitaba plant might be potent for anti-hypertension because of its activities, such as anti-inflammatory, vasodilation, artherosclerosis, blood sugar regulation, potassium content, stress reduction, and hyperlipidemia. This research aimed to analyse and identify compounds of Ashitaba, spesifically the chalcones which might be potential as ACE inhibitor candidates. The structures of Ashitaba's chalcones were collected from PubChem, whilst the 3D structure of ACE protein was obtained from PDB database. Further analysis of compound including drug likeness and toxicity were conducted using SWISS ADME and Protox-II softwares, respectively. Result showed that dihydroxychalcone had strong interaction with ACE1 protein as compared to captopril, with score -7.1 kkal.mol^{-1}. The chalcone analyses outcomes obeys all Lipinski Rule of Five (RO5) at all parameters and showed no toxicity result. In conclusion, dihydroxychalcone could be developed for further analysis as ACE1 inhibitor.

Keywords: *ACE protein, hypertension, inhibitor, interaction.*

1. INTRODUCTION

Ashitaba, *Angelica keiskei*, is a plant belongs to celery or Umbelliferae family plants. It has been traditionally used in Japanese herbal medicine for its potential health benefits. While research on Ashitaba is ongoing, some studies and anecdotal evidence suggest that it may have certain properties that could be beneficial for managing hypertension and overall cardiovascular health [1,2]. Ashitaba is known to have activity related to anti hypertension activities, such as antioxidant properties [3], anti-inflammatory effects [4], vasodilation, artherosclerosis, blood sugar regulation, potassium content, and stress reduction, and anti-hyperlipidemia [1,3,5]. The activities might be related to its phytochemical compounds, such as chalcones, coumarins, and flavanones. However, it's important to note that further research is needed to establish its effectiveness and safety for this purpose.

Hypertension is a medical condition when the blood pressure is higher than normal (140/90 mmHg or higher) [6]. Several factors can cause the hypertension. Unhealthy living habits, ages, organ disorder, and genetic disorder of enzyme (protein) system. The angiotensin-converting enzymes, ACEs, are responsible and playing a role in blood pressure regulation by synthesizing vasoconstrictor hormone for increasing blood pressure [7]. ACE1, is an enzyme found in the human body that plays a crucial role in the renin-angiotensin-aldosterone system (RAAS). This system is involved in regulating blood pressure, fluid balance, and electrolyte homeostasis. ACE1 cleaves a dipeptide from angiotensin I, transforming it into angiotensin II. Angiotensin II is a potent vasoconstrictor, which means it narrows blood

J. Sukweenadhi and F. Setiawan (eds.), *Proceedings of the Conference on Natural Resources And Life Sciences 2022 (NRLS-BIO 2022)*, Advances in Biological Sciences Research 38,
https://doi.org/10.2991/978-94-6463-322-1_14

vessels, leading to an increase in blood pressure. It also stimulates the release of aldosterone from the adrenal glands, which promotes sodium and water retention, further increasing blood pressure. The actions of ACE1 in converting angiotensin I to angiotensin II make it a target for medications known as ACE inhibitors.

ACE inhibitors are commonly prescribed to lower blood pressure in conditions like hypertension and congestive heart failure. These inhibitors reduce the formation of angiotensin II, leading to vasodilation and a decrease in blood pressure [8]. (So far, the inhibitor that commonly used is captopril. Captopril has vasodilator activity. Although is used as ACE inhibitor agent, captopril might cause unwanted side effect such dry cough, hypotension, and renal dysfunction. Therefore it develops a concern in hypertension medication and treatment [9].

The chalcone compounds were chosen in this study for prediction as the ACE inhibitor agent because of their unique structure [10] and their pharmacological activity [5]. Chalcone could bind to ACE2, however no information about chalcone as ACE1 inhibitor. The purpose of this study was to investigate the possibility of Ashitaba's chalcones as ACE1 inhibitor candidates using in silico approach. This study offered some benefits, which increasing the functional properties of Ashitaba as herbal medicine for treating hypertension

2. MATERIALS AND METHODS

2.1. Protein target and compound preparation

The 3D structure of ACE protein (PDB ID 1UZF) was collected from PDB database and the 3D structure of Ashitaba chalcones were obtained from PubChem database. The Ashitaba chalchones consist of Isobavachalcone (PubChem ID 5281255), Deoxyxanthoangelol (PubChem ID 44566285), Dihydroxychalcone (PubChem ID 67766433), Jejuchalcone (PubChem ID 102429834), Deoxydihydroxanthoangelol (PubChem ID 11588137), Dorsmanin (PubChem ID 5472480), Xanthokeistal (PubChem ID 56666829), Xanthokeismin (PubChem ID 24970764), xanthoangelol (PubChem ID 643007), and Artocarmitin (PubChem ID 71451579). While the captopril (PubChem ID 44093) was used as a control.

2.2. Molecular Docking Simulation

The purposes of the analyses were to find out the possibility of Ashitaba compounds to interact with ACE1, based on RMSD score. Simulation of interaction between ACE protein and derivates of Ashitaba's chalcone was conducted by using MCULE webserver. The interaction strength was measured based on negativity score.

2.3 Druglikeness Analysis of Ashitaba's Chalcones

The purposes of the analyses were to find out ashitaba chalcones safety as drug candidate based on their obedience to Lipinski five and their toxicity state.

The drug likeness of compound derived from Ashitaba's chalcones were analysed based on Lipinski rule of five. The rule consists of several parameters, such as molecular weight, lipophilicity, number of hydrogen bond donor, number of hydrogen bond acceptor, and molar refractivity. Analysis of ADME parameters, pharmacokinetic properties, druglike nature was conducted by using SWISS ADME [11].

2.4. Toxicity Analysis of Ashitaba's Chalcones

Derivates of Ashitaba's chalcones were addressed for toxicity analysis. Several properties such as mutagenic activity, carcinogenic activity, immunotoxicity, hepato-toxicity, and cytotoxicity were identified by using Scfbio.

3. RESULTS

3.1. Collection of Ashitaba's chalcones and ACE1 protein structures

Various chalcone compounds of Ashitaba and human ACE1 protein were collected for the analysis (Table 1). In brief, the SMILE notation of Ashitaba's chalcones were collected from PubChem. Meanwhile, the 3D structure of ACE protein was collected from PDB database with ID 1UZF. The captopril compound is selected as a control for comparation.

3.2. Screening for inhibitor candidate derived from Ashitaba's chalcones.

Various chalcone compounds of Ashitaba were collected, such as Deoxyxanthoangelol, Dorsmanin, Xanthoangelol, Deoxydihydroxanthoangelol, Isobavachalcone, Jejuchalcone, Xanthokeismin, Xanthokeistal, Artocarmitin, and Dihydroxychalcone (Table 1). Subsequently, the interaction between those compounds and ACE1 protein were analyzed by using molecular docking simulation. Results showed that all Ashitaba's chalcones demonstrate lower binding affinity score than that of captopril (Table 2). It suggests that all Ashitaba's chalcones have stronger interaction with target protein than captopril. Deoxy-xanthoangelol demonstrated the strongest interaction with ACE1 protein.

Table 1 Chalcone compounds of Ashitaba

Chalcone compound (PubChem ID)	SMILES	Structure

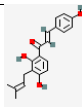
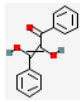
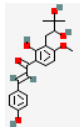
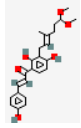
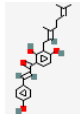
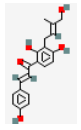
Isobavachalcone (5281255)	CC(=CCC1=C(C=CC(=C1O)C(=O)/C=C/C2=CC=C(C=C2)O)O)C	
Deoxyxanthoangelol (44566285)	CC1(CCC2=C(C=CC(=C2O1)C(=O)/C=C/C3=CC=C(C=C3)O)OC)C	
Dihydroxychalcone (67766433)	C1=CC=C(C=C1)C(=C(C(=O)C2=CC=CC=C2)O)O	
Jejuchalcone (102429834)	CC(C)(C(CC1=C(C=CC(=C1O)C(=O)/C=C/C2=CC=C(C=C2)O)OC)O)O	
Deoxydihydroxanthoangelol (PubChem ID 11588137)	CC1(CCC2=C(C=CC (=C2O1)C(=O)CCC3=CC=C(C=C3)O)OC)C	
Dorsmanin (PubChem ID 5472480)	CC1(CCC2=C(O1)C=CC(=C2O)C(=O)/C=C/C3=CC=C(C=C3)O)C	
Xanthokeistal (PubChem ID 56666829)	C/C(=C\CC1=C(C=CC(=C1O)C(=O)/C=C/C2=CC=C(C=C2)O)O)/CCC(OC)OC	
xanthoangelol (PubChem ID 643007)	CC(=CCC/C(=C/CC1=C(C=CC(=C1O)C(=O)/C=C/C2=CC=C(C =C2)O)O)/C)C	
Artocarmitin (PubChem ID 71451579)	C/C(=C\CC1=C(C=CC(=C1O)C(=O)/C=C/C2 =CC=C(C=C2)O)O)/CO	
captopril (PubChem ID 44093)	C[C@H](CS)C(=O)N 1CCC[C@H]1C(=O)O	

Table 2. Score of molecular docking simulation of Ashitaba's chalcones toward ACE1 protein

Compounds	Binding affinity score (kcal.mol^{-1})
Deoxyxanthoangelol	-8.5
Dorsmanin	-8.4
Xanthoangelol	-8.0
Isobavachalcone	-8.0
Deoxydihydroxanthoangelol	-7.9
Jejuchalcone	-7.9
Xanthokeismin	-7.8
Xanthokeistal	-7.6
Artocarmitin	-7.6
Dihydroxychalcone	-7.1
Captopril (control)	-6.5 -6.5

3.3. Lipinski Rule of Five (RO5) Analysis

Table 3. Druglikeness Analysis of Ashitaba's Chalcones

Compounds	Lipinski Parameters				
	Molecular weight (< 500Da)	Hydrogen Bond Donor (< 5)	Hydrogen Bond Acceptor (< 10)	Lipophilicity (< 5)	Molar Refractivity (40-130)
Artocarmitin	340	4	5	2.51	89.91
Deoxydihydroxanthoangelol	340	1	4	3.71	95.85
Deoxyxanthoangelol	338	1	4	3.51	93.51
Dihydroxychalcone	240	1	3	2.35	59.77
Dorsmanin	324	2	4	2.98	88.90
Isobavachalcone	324	3	4	2.81	89.04
Jeju-Chalcone	372	3	6	3.12	98.04
Xanthoangelol	392	3	4	4.14	114.04
Xanthokeismin	440	3	7	3.68	114.74
Xanthokeistal	412	3	6	3.81	112.22

According to Table 3, all Ashitaba's chalcones obey the Lipinski Rule of Five (RO5) at all parametres consisting of molar mass, hydrogen bond donor, hydrogen bond acceptor, lipophilicity, and molar refractivity.

3.4. Toxicity analyisis of Ashitaba's chalcones

Table 4. Toxicity analysis of Ashitaba's chalcones as ACE1 inhibitor candidates

Compounds	Toxicity Parameters				
	Hepato-toxic	Carsino genic	Immuno toxic	Mutag enic	Cyto-toxic
Arto-carmitin	No	No	**Yes**	No	No
Deoxydihydro-xanthoangelol	No	No	**Yes**	No	No
Deoxyxantho-angelol	**Yes**	No	**Yes**	No	No
Dihydroxy-chalcone	No	No	No	No	No
Dorsmanin	No	No	**Yes**	**Yes**	No
Isobavachalcon e	No	No	**Yes**	No	No
JejuChalcone	No	No	**Yes**	No	No
Xanthoangelol	No	No	**Yes**	No	No
Xanthokeismin	No	No	**Yes**	No	No
Xanthokeistal	No	No	**Yes**	No	No

According to Table 4, there is only dihydrochalcone that shows no toxicity. Furthermore, based on Table 5, the compound has toxicity category of 4 meaning that this compound is practically non-toxic and non-irritant.

Table 5. LD50 and Toxicity class of Ashitaba's chalcones as ACE1 inhibitor candidate

Compounds	LD50 ($mg.kg^{-1}$)	Toxicity class	Prediction accuracy (%)
Artocarmitin	1000	4	69.26
Deoxy-dihydroxantho-angelol	1060	4	69.26
Deoxyxanthoangelol	3800	5	68.07
Dihydroxychalcone	1190	4	100
Dorsmanin	3800	5	68.07
Isobavachalcone	1000	4	70.97
Jejuchalcone	3000	5	68.07
Xanthoangelol	1000	4	69.26
Xanthokeismin	25	2	67.38
Xanthokeistal	1000	4	68.07

4. DISCUSSION

Ashitaba extract was proven to have antihypertension antivity on human [2]. The active compounds was predicted as flavonoids and antioxidants contents. In this study, chalcones were used as ligands or inhibitors of ACE enzyme. Chalcone is an unsaturated form of α-β ketone. The result showed that all Ashitaba's chalcones gave negative docking score. When docking score of a compound is negative, it considers as a better bonding result between ligand and target. By this statement, it can be concluded that ashitaba's chalcones compound is potential as ACE1 inhibitor candidate based on their docking score. In Lipinski Rule of Five (RO5) analysis, all Ashitaba's chalcones obey all the rule: molar mass below 500 dalton, lipophilicity below 5, hydrogen bond donor group below 5, hydrogen bond acceptor group below 10, and molar refractivity at a range of 40-130. All chalcones compounds could be considered as drug molecules and could be candidates of ACE inhibitors.

From the molecular docking simulation, deoxyxanthoangelol had the lowest binding affinity score (which is -8.5 $kkal.mol^{-1}$) than that of the other compounds and captopril as control. However, it showed a positive result for hepatotoxicity parameter, while dorsmanin give a positive result for mutagenic parameter. Hence both compounds were not considered as ACE inhibitors and eliminated because of their unsafe property. In the analysis of immunotoxicity parameter, almost all chalcones give positive result, leaving dihydroxychalcone as the only chalcones giving no immunotoxicity. From carcinogenic and cytotoxicity parameters analysis, all chalcones give negative result, leaving dihydroxychalcone as the only chalcones with negative result for all parameters. From these results, it could be concluded that dihydroxychalcone is the only potential ACE inhibitor candidate.

Other studies reveal that chalcones of Ashitaba have many biological activities [5]. All these studies mentioned no toxicity effects occured during pre-clinical studies [5], and upon administration on rats [12]. However, based on the in silico analyses, there were a potensial toxicity of chalcones compounds other than dihydroxychalcone. It might be that in this in silico study, the analyzes was held on a pure compound, while in pre-clinical study the total effect was observed from the extracts. The mixed constituents might neutralize the side effects [13]. Further study is needed to prove the effect.

The chalcones might inhibit both ACEs. ACE1, based on the result of this in silico study, and ACE 2 based on other studies [14, 15]. ACE2 is also involved in modulating blood pressure [7]. Therefore, the chalcone of Ashitaba had strong activity as anti-hypertension. It is essential to emphasize that the use of Ashitaba's chalcones may have potential to cause hypotension as a side effect, it should not be used as a sole or primary treatment for hypertension. Lifestyle changes such as a balanced diet, regular exercise, and, if necessary, common medication is recommended to manage hypertension effectively.

5. CONCLUSION

Dihydroxychalcone is the only possible ACE inhibitor candidate among Ashitaba's chalcones which gives re-rank score -7.1, lower than captopril (the control). The chalcone analyses outcomes obeys all Lipinski Rule of Five (RO5) at all parameters and gives no positive result in toxicity analysis.

AUTHORS' CONTRIBUTIONS

TA: design, data collection, data processing, and analysis, manuscript preparation. YA: supervision, data interpretation, and writing manuscript. ADRD: conception, design, supervision, and funding. SWN: data analysis, data interpretation, critical review. PAK, LAW and HI: funding. MW: conception, design, supervision, data interpretation, writing manuscript, critical review.

ACKNOWLEDGMENTS

This study was funded by Grant Program Pengembangan Produk Unggulan Daerah (PPPUD), Kemenristekdikbud, contract number 004/SP2H/PPM/L7/2020.

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