International Journal of Applied Pharmaceutics

ISSN- 0975-7058 Vol 14, Special Issue 2, 2022

Review Article

IN SILICO IDENTIFICATION TESTING OF TRITERPENE SAPONINES ON *CENTELLA ASIATICA* ON INHIBITOR RENIN ACTIVITY ANTIHYPERTENSIVE

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Received: 12 Nov 2021, Revised and Accepted: 15 Dec 2021

ABSTRACT

The renin-angiotensin-aldosterone system (RAAS) has an essential role in the occurrence of hypertension. Two drugs that act on SRAA are ACE Inhibitor and ARB. ACE inhibitor and ARB are two antihypertensive drugs that act on RAAS. However, both drugs are not fully effective because they produce incomplete suppression of RAAS. The therapeutic potential of the two drugs is limited, so it is necessary to find out other drugs that are more effective in inhibiting the RAAS system. Renin-inhibitors are evolving as options that can inhibit at the highest levels in RAAS. Discontinuation of renin inhibitor therapy does not cause rebound hypertension as in ACEIs and ARBs. The search for natural renin inhibitors began to be carried out as an alternative to aliskiren. Renin-inhibitors derived from natural ingredients generally come from the class of saponin compounds or polyphenol compounds. One of Indonesia's native plants that contain saponins and polyphenols is *Centella asiatica*. Thus, research on the antihypertensive potential of these plants was carried out. It is expected that the *Centella asiatica* plant has a renin inhibitor effect as a blood pressure-lowering drug derived from herbs but has the same effect as aliskiren. The research method was carried out *in silico* to test the activity of compounds that have potential as antihypertensives in the Centella asiatica plant. The method used is molecular docking, with the docking software used being Molegro Virtual Docker (MVD) 6.0. The results showed that madecasoside and asiaticoside from the *Centella asiatica* plant have intense renin inhibitor activity, with a mean re-rank score of-105.27 and-93.67, respectively. The conclusion is that madecasoside has similarities with aliskiren with a re-rank value of-105.27. The smallest re-rank value indicates the similarity and strong protein binding between the tested ligand compound and the comparison protein.

Keywords: Antihypertensive, Centtella asiatica, Triterpene saponines, Renin inhibitors, Aliskiren

DOI: https://dx.doi.org/10.22159/ijap.2022.v14s2.44737 Journal homepage: https://innovareacademics.in/journals/index.php/ijap

INTRODUCTION

Hypertension is a silent killer and can cause kidney damage, heart, stroke if not treated properly. In Indonesia the prevalence of the population with high blood pressure is 34.11%, with women 36.85% higher than men 31.34% [1]. Non-pharmacological therapy for hypertension is done by changing a healthier lifestyle, avoiding the stress of a low-salt diet, and exercising regularly from an early age. Pharmacological therapy of hypertension consists of a class of diuretic drugs, Angiotensin-converting enzyme (ACE-inhibitors), Angiotensin receptor blockers (ARBs), beta-blockers, Calcium channel blockers (CCBs), and renin inhibitors [2]. The renin-angiotensin-aldosterone system (RAAS) has an essential role in the occurrence of hypertension. Two drugs that act on RAAS are ACEIs and ARBs. Both have drawbacks in inhibiting RAAS and side effects. Renin is a crucial component of RAAS and has specificity for angiotensinogen. Renin-inhibitors can block RAAS at the highest levels. Indonesia has a variety of natural resources in the form of flora and fauna. However, there are still many plants that are either utilized or tested scientifically. This makes the authors interested in researching medicinal plants in Indonesia, which are thought to have activity as renin inhibitors used in the treatment of hypertension. Renin-inhibitors derived from natural ingredients generally come from the class of saponin compounds or polyphenol compounds. One of Indonesia's native plants that contain saponins and polyphenols is pegagan (*Centella asiatica*) [3]. Since ancient times, pegagan has been used empirically as a blood pressure-lowering drug, anti-bacterial, skin medicine and medicine for nervous disorders. Pegagan contains many active compounds and the most important compound is the triterpenoid saponin group. With the very high content of triterpenoid saponins in *Centella asitica* plants, the researchers wanted to see further the effect of asiatic acid, asiaticoside, madecasoside and centellasoside on the activity of renin inhibitors as antihypertensives. The secondary metabolite compound triterpenoid saponin has essential activity against renin inhibitor antihypertensive [4]. The triterpenoid saponin compounds in Centella asitica, which are very high in content, have not been investigated further on the activity of renin inhibitors as antihypertensives [5].

Computational biology and high bioinformatics potential in medical chemistry, no only speed up the drug discovery process but also changing the way drugs are discovered and designed.

One example of the method used is the docking of drug molecules with receptors also called molecular docking. Molecular docking is a process in which two molecules are matched via tethering in 3D space. Currently, molecular docking approaches have been widely used in modern drug design to help understand drug and receptor interactions [6]. The receptors here are the active site of drug action contributing to the pharmacological effect. It has been widely reported that computational techniques can support and assist the design of compounds for get a more potent inhibitor through the drug-receptor mechanism.

Based on the description above, a docking approach is carried out to estimate the chemical compounds of triterpene saponins from Centella asiatica, namely Asiatic acid, asiaticoside, madecasoside, and madecasid acid, in inhibiting renin activity. Molecular docking is also predictable orientation and affinity bonds of these compounds. This research was conducted using Molegro Virtual Docker (MVD) 6 for understanding the forms of interactions of compounds asiatic acid, asiaticoside, madecasoside, and madecasid acid n inhibiting the renin activity.

METHODS

This research is an *in silico* molecular docking study with a Post Test only Control design that uses triterpenoid saponin secondary metabolites from the *Centella asiatica* plant. The *in silico* test uses a docking molecule with the renin enzyme ligand. Docking software used is Molegro Virtual Docker (MVD) 6.0. Using compounds that will be seen for their activity against renin inhibition, namely triterpene saponins consisting of asiatic acid, asiaticoside, madecasoside, and madecasid acid. Then it can be seen the highest activity against renin inhibitors. The research was conducted for

three months at the Molecular Biology and Bioinformatics Laboratory of the BPPT (Research and Technology Center) Puspitek Serpong Indonesia.

The study was carried out by docking the ligands already present in the protein renin and then docking the molecular structure of the triterpene saponin compounds (asiaticoside, asiatic acid, madecasid acid, madecasoside) with aliskiren (Renin inhibitor). Then the comparison of the molecule docking between the triterpene saponin compounds and aliskiren was carried out.

Alikiren is a renin inhibitor that plays an important role in preventing cardiovascular complications and is renoprotective because it works by inhibiting the RAAS (Renin Angisotensin Aldosterone System). Renin-inhibitors are evolving as options that can inhibit at the highest levels in SRAA. Renin-inhibitors block angiotensin I and angiotensin II, resulting in no activation of the angiotensin type 1 receptor (AT-1). Aliskiren is the first renin inhibitor that can be administered orally and has progressed to phase III clinical trials. In addition, aliskiren is renoprotective and has minimal side effects [7].

A literature study was conducted to determine the content of Centella asiatica which has renin inhibitor activity. Namely from triterpene saponin compounds consisting of asiatic acid, asiaticoside, madecasid acid and madecasoside.

RESULTS

Results of docking molecules on compounds and ligands.

Table 1: Protein Re-rank value

Name	Ligand	MolDock score	Rerank score	H Bond
[00]PIV-HIS-PRO-PHE-HIS-LPL-TYR-TYR-SER [D]	PIV-HIS-PRO-PHE-HIS-LPL-TYR-TYR-SER [D]	-190.02	72.7901	-2.06608
[01]PIV-HIS-PRO-PHE-HIS-LPL-TYR-TYR-SER [D]	PIV-HIS-PRO-PHE-HIS-LPL-TYR-TYR-SER [D]	-150.091	143.011	-5.01333
[02]PIV-HIS-PRO-PHE-HIS-LPL-TYR-TYR-SER [D]	PIV-HIS-PRO-PHE-HIS-LPL-TYR-TYR-SER [D]	-150.016	17.3747	2.38394
[03]PIV-HIS-PRO-PHE-HIS-LPL-TYR-TYR-SER [D]	PIV-HIS-PRO-PHE-HIS-LPL-TYR-TYR-SER [D]	-113.311	3.68266	-1.90171
[04]PIV-HIS-PRO-PHE-HIS-LPL-TYR-TYR-SER [D]	PIV-HIS-PRO-PHE-HIS-LPL-TYR-TYR-SER [D]	-99.4847	337.197	-10.5637

Table 2: Re-rank value of asiatic acid

Name	Ligand	MolDock score	Rerank score	H Bond
[00]asiatic acid	asiatic acid	-122.286	-22.6932	-8.86246
[01]asiatic acid	asiatic acid	-117.703	-55.449	-4.77242
[02]asiatic acid	asiatic acid	-111.083	-94.3876	-6.01159
[03]asiatic acid	asiatic acid	-107.06	-65.5022	-1.05308
[04]asiatic acid	asiatic acid	-102.035	-63.073	-6.77448

Table 3: Re-rank value of asiaticoside

Name	Ligand	MolDock score	Rerank score	H Bond
[00]asiaticoside	asiaticoside	-188.059	-114.804	-16.5143
[03]asiaticoside	asiaticoside	-185.767	-125.03	-17.4695
[02]asiaticoside	asiaticoside	-183.213	-132.722	-11.8529
[04]asiaticoside	asiaticoside	-182.895	6.75091	-18.4229
[01]asiaticoside	asiaticoside	-182.574	-102.554	-12.4716

Table 4: Re-rank value of madecassid acid

Name	Ligand	MolDock score	Rerank score	H Bond
[03]madecassic acid	madecassic acid	-125.428	-20.9451	-3.99327
[01]madecassic acid	madecassic acid	-124.025	-83.1775	-7.46009
[00]madecassic acid	madecassic acid	-123.279	-26.9221	-9.28091
[04]madecassic acid	madecassic acid	-113.533	-51.5403	-2.88051
[02]madecassic acid	madecassic acid	-113.401	-103.812	-7.46469

Table 5: Re-rank value of madecassoside

Name	Ligand	MolDock score	Rerank score	H Bond
[00]madecassoside	madecassoside	-194.373	**-155.93**	-16.9351
[01]madecassoside	madecassoside	-179.026	-114.503	-20.0988
[02]madecassoside	madecassoside	-173.966	-47.7801	-11.9333
[04]madecassoside	madecassoside	-169.528	-79.5627	-11.697
[03]madecassoside	madecassoside	-167.134	-128.566	-12.3252

Table 6: Re-rank scores on renin inhibitors and triterpene saponins

Re-rank score	Renin inhibitor	Asiatic acid	Asiaticoside	Madecasid acid	Madecasoside
	-72.79	-22.69	-114.80	-20.95	-155.93
	-143.01	-55.45	-125.03	-83.18	-114.50
	-17.37	94.38	-132.72	-26.92	-47.78
	-3.68	-65.50	-6.75	-51.54	-79.56
	337.19	-63.07	-102.55	-103.81	-128.57
Average	-114.81	-22.46	-96.37	-57.28	-105.27

Table 7: Molecular value of docking score on renin inhibitors and triterpene saponins

Molecular value score	Renin inhibitor	Asiatic acid	Asiaticoside	Madecasid acid	Madecasoside
	-190.02	-122.29	-188.06	-125.43	-194.37
	-150.09	-117.70	-185.77	-124.03	-179.03
	-150.02	-111.08	-183.21	-123.28	-173.97
	-113.31	-107.06	-182.89	-113.53	-169.53
	-99.48	-102.04	-182.57	-113.53	-167.13
Average	-140.58	-112.03	-184.50	-113.40	-176.81

DISCUSSION

Hypertension is a disease that has a high prevalence in the world. Currently, there are 5 classes of antihypertensive drugs that we know (JNC 8). Those are diuretics, ACE-inhibitors, Angiotensin receptor blockers (ARBs), Beta blockers, CCBs (Chanel Calcium Blockers) [8]. Ace-inhibitors and ARBs have the highest mechanism of action activity, namely renin inhibitors. Where currently the existing renin inhibitor class of drugs is aliskiren. Aliskiren is currently the only chemically synthetic oral drug of the renin inhibitor class [9].

Currently, medical science in the fields of pharmacology and pharmacy is expected not only to provide synthetic (chemical) drug therapy but also to be supported by compounds from natural ingredients. Where these natural compounds are more natural free from chemicals so they can improve the quality of human life in the future by minimizing chemical contamination in the body [10]. Considering that the world is currently experiencing global warming which is related to external factors (free radicals) that can cause hypertension and other diseases. In addition to the field of medical pharmacology, other fields of science also support the development of natural material compounds, such as the fields of molecular biology, bioinformatics, biochemistry, and biomedicine, etc.

ACEi and ARB can increase plasma renin activity through negative feedback inhibition. This can increase target organ damage such as renal dysfunction and left ventricular hypertrophy. With the presence of renin inhibitors that can suppress renin activity, either with monotherapy or in combination with ACEIs or ARBs, it is expected to prevent target organ damage in hypertension [11].

Based on research conducted by the Akita Research Institute of Food and Brewing team in Japan in 2010 stated that the saponins contained in soybean seeds (Glucoronid saponins) have an essential renin inhibitor activity namely at the 3β-hydroxyl sugar chain position.

Indonesia has a variety of natural resources in the form of flora and fauna. However, there are still many plants that are either utilized or tested scientifically. This makes the authors interested in researching medicinal plants in Indonesia, which are thought to have activity as renin inhibitors used in the treatment of hypertension [12]. Renin-inhibitors derived from natural ingredients generally come from the class of saponin compounds or polyphenol compounds.

One of Indonesia's native plants that contain saponins and polyphenols is pegagan (Centella asiatica). This plant is a wild plant that grows in plantations, fields, roadsides and rice fields. This plant comes from tropical Asia, spread in Southeast Asia, including Indonesia, India, China, Japan and Australia. Since ancient times, gotu kola has been used empirically as a blood pressure-lowering drug, anti-bacterial, skin medicine and medicine for nervous disorders [13].

Centella asiatica contains many active compounds and the most important compound is the triterpenoid saponin group. The secondary metabolite content of triterpenoid saponins is very strong (4+), tannins, alkaloids and flavonoids are strongly positive (3+). Which includes triterpenoid saponins include asiatic acid, asiaticoside, madecassoside, centelloside [14]. In addition, pegagan also contains unidentified terpenes such as madecassic acid, thankuniside, brahmoside, brajmic acid, brahminoside, madasiatic acid, meso-inositol, carotenoids, hydrocotylin, vellarine [15].

Due to the very high content of triterpenoid saponins in Centella asitica plants, the researchers wanted to investigate further the effect of asiatic acid, asiaticoside, madecasoside and madecasid acid on renin inhibitors as antihypertensives, which was carried out with an *in silico* pre-clinical test [16].

Indonesia is a country that has natural wealth that is rich in medicinal plants, one of which is the Centella asiatica plant. Centella asiatica plant contains many secondary metabolites, including triterpenoid saponins. Among the triterpene saponins, there are pure compounds, namely Asiatic acid, asiaticoside, madecasid acid, and madecasoside [17].

The results of the *in silico* test on *Centella asiatica* containing triterpenoid saponins tested: Asiatic acid, asiaticoside, madecasid acid, madecasoside. Viewed from Protein in Humans 214Q with ligand compounds: asiatic acid, asiaticoside, madeca acid, madecasoside. The results obtained docking with RMSD: 0.644019, Radius: 15 armstrong.

The validation of the docking method was carried out by calculating the RMSD (Root Mean Square Deviation) value of the target protein and its original ligand. RMSD is a parameter used to see the similarity between the docking ligands and the crystallographic results. Validation can be accepted if the RMSD value obtained is less than 1.5. The smaller the RMSD value, the more similar the docking ligand position to the crystallographic ligand.

The result of docking is a grid score (re-rank). The grid score is the energy required by the ligand to bind to the protein. This energy is Gibbs energy, which is energy with a value if the more negative it means the bond between the ligand and protein is getting bigger.

The more<RMSD (Root Mean Square deviation) is the better ligand position, because it is closer to the original conformation. The smaller the re-rank score, the better and more potential to have renin inhibitor activity because the interaction is the strongest. Stable conformers have the least energy. From the results of the study, which has similarities with the function of aliskiren (renin inhibitor) is madecasoside, with a re-rank value of-105.27 (Homo sapiens). The smallest re-rank value indicates the similarity and strong protein binding between the tested ligand compound and the comparison protein [18].

To prove the first test was carried out by the *in silico* method. *In silico* test using docking software: molegro virtual docker (MVD) 6.0 obtained pure compounds that have the highest renin inhibitor activity in asiaticoside and madecasoside compounds (19). The average re-rank score for madecasoside is-105.27 and asiaticoside is-93.67 (table 6). And the average value of docking molecules madecasoside-176.81 and asiaticoside-184.50 (table 7).

CONCLUSION

Secondary Metabolite Compounds Tritrepenoid saponins in *Centella asiatica* plants have renin inhibitor activity which was tested *in silico*. Triterpenoid saponin compounds that have the best renin inhibitor activity are madecasoside compounds. The mechanism of the active compound in *Centella asiatica* is similar to that of aliskiren, namely madecasoside. Re-rank score: -105.27 (Homo sapiens).

ACKNOWLEDGMENT

Thank you to all those who have provided thoughts, suggestions and attention in guiding and directing the writing of this journal.

FUNDING

Nil

AUTHORS CONTRIBUTIONS

All the authors have contributed equally.

CONFLICTS OF INTERESTS

The authors declare that there is no conflict of interest in this reserach

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