Hiroshima J. Med. Sci
Vol. 67 Special Issue, May 2018

Sojagol from Mung Beans as A Potential Antagonist of Mineralocorticoid Receptor

Ratna KUSUMAWATI[1)], Heru SULASTOMO[2)], Arifin Nur SETYAWAN[3)], Ratih Dewi YUDHANI[4)], MUTHMAINAH[5)] and Dono INDARTO[1,6,*)]

1. *Department of Physiology, Faculty of Medicine, Universitas Sebelas Maret, Surakarta, Indonesia*
2. *Department of Cardiology and Vascular Medicine, Faculty of Medicine, Universitas Sebelas Maret, Surakarta, Indonesia*
3. *Faculty of Medicine, Universitas Sebelas Maret, Surakarta, Indonesia*
4. *Department of Pharmacology, Faculty of Medicine, Universitas Sebelas Maret, Surakarta, Indonesia*
5. *Department of Anatomy, Faculty of Medicine, Universitas Sebelas Maret, Surakarta, Indonesia*
6. *Biomedical Laboratory, Faculty of Medicine, Universitas Sebelas Maret, Surakarta, Indonesia*

ABSTRACT

This bio-computational study aimed to explore phytochemicals derived from Indonesian plants which inhibited the mineralocorticoid receptor (MR) for cardiovascular diseases treatment. A total of 516 phytochemicals was used in this study which was derived from the HerbalDB database and screened with Lipinski Rule of Five. Three dimensional structure of MR was obtained from a protein data bank (access code 3VHU) and the structure of aldosterone antagonists (spironolactone and eplerenone) as standard ligand was obtained from the ZINC database (ZINC03977913 and ZINC72187491) respectively. MR-standar ligand binding complexes were validated using AutoDock Vina 1.1.2 software three times. Interaction between MR and phytochemicals was molecularly dock with the same software and visualized using Chimera 1.9 software. Spironolactone had -9.5 kcal/mol docking score and MR binding site at Gln^{776}, Arg^{817}, and Cys^{942}. Whereas -9.7 kcal/mol docking score was observed in eplerenone and it had binding site at Arg^{817} and Thr^{945}. There were six phytochemicals with lower binding score againts MR than the standards but only Sojagol interacted with MR at Gln^{776} and Arg^{817} residues. More over, Sojagol had a lower molecular size (336.338 Da) compared with the standards and was commonly found in mung beans. In conclusion, Sojagol might become in silico antagonist of aldosterone.

Key words *: Mineralocorticoid receptor, Aldosterone antagonist, Spironolactone, Phytochemical*

In recent years, cardiovascular and circulatory diseases have become the world health burden and the leading causes of death worldwide. In 2013, there were up to 54 million deaths globally and cardiovascular diseases were attributable to 32% of these deaths (17 million).(1) Moreover, 80% of cardiovascular diseases mortality occurs in low-income and middle-income countries. It is estimated that cardiovascular diseases will give rise to 23.6 millions of death in 2030.(2)

Aldosterone has been known for many years as an endogenous hormone that plays an important role in the pathogenesis of heart disease.(3)(4) Growing evidence had indicated that aldosterone was involved in the progression of end-organ damage since it induced vascular smooth muscle hypertrophy, vascular matrix impairment (remodelling) and endothelial dysfunction.(5)(6) Inhibition of aldosterone and other mineralocorticoid steroids to the MR has been demonstrated to have beneficial therapy in various cardiovascular disease.(7) Hence, aldosterone antagonist has become a new therapeutic agent, based on its sodium retention properties in the management of cardiovascular diseases.(8)(9)

Spironolactone is firstly generated as MR antagonist. It is able to inhibit not only the MR but also other families of steroid receptor. Therefore, it is not surprised if long term administration of spironolactone has unwanted effects like progestational and antiandrogenic activities.(3)

Virtual screening which uses molecular docking methods is one of the most effective ways to explore new agents or phytochemicals as a novel candidate of aldosterone antagonist.(10) Molecular docking is a computer program that can predict the complex structure of two molecules in silico efficiently.(11) So, this study aimed to explore phytochemicals of Indonesian herbal plants which were able to interact with

Corresponding author : Dono INDARTO, M.D.
Department of Physiology, Faculty of Medicine, Universitas Sebelas Maret, Surakarta, Indonesia
Biomedical Laboratory, Faculty of Medicine, Universitas Sebelas Maret, Surakarta, Indonesia
Email : donoIND323@gmail.com

mineralocorticoid receptor (MR) and potentially developed as a new aldosterone antagonist.

MATERIAL AND METHODS

Protein preparation

Preparation of MR protein was performed using AutoDockVina program 1.1.2 version.(12) Three dimensional structure of MR was downloaded from Protein Data Bank (http://www.rcsb.org/pdb/) with access code 3VHU. This MR protein was crystallized with spironolacton and had 2.11 Å diffraction pattern. In addition, spironolacton was interacted with MR at Gln^{776}, Arg^{817}, and Cys^{942} residues.(13) Before running the molecular docking, spironolactone and water were removed from the MR structure. Hydrogen was then added to MR in order to increase polarity in the binding pocket. After that, grid box was made by selection of some amino acid residues that surrounded the binding pocket. Hence, three binding site of spironolactone to the MR had to be located in the center of grid box.

Standard ligand preparation

Aldosterone was used as the standard ligand agonist of MR while spironolactone and eplerenone became the aldosterone standard antagonists. Their three dimensional structures were obtained from ZINC database (zinc.docking.org) with access code ZINC03830183, ZINC03977913 and ZINC72187491 respectively. They were saved in *mol2 and *sdf format. Validation of MR-standard ligand interaction was molecularly docked with AutoDock Vina 1.1.2 software three times to obtaine the binding energy (Table 1). Visualisation of MR-standard ligand interaction was done using Chimera software (version 1.9, The Resource for Biocomputing, Visualization, and Informatics, University of California) (Fig.1). Their binding sites were compared with the binding sites of Hasui's study.(13) The validation results were then used as the reference to explore candidates of aldosterone antagonist.

Phytochemical preparation

Phytochemicals of Indonesian herbal plants were obtained from HerbalDB data base which was Indonesian herbal data base and developed by Department of Pharmacy, Universitas Indonesia http://herbaldb.farmasi.ui.ac.id/. The phytochemical structures were downloaded from a public data base at https://pubchem.ncbi.nlm.nih.gov/. All phytochemicals of Indonesian herbal plants were then screened using Lipinski's rule of five criteria(14) and 517 phytochemicals were used as the research samples. All selected phytochemicals were modified and prepared using PyRx program. They finally saved in *.pdbqt format file.

Table 1. Validation results of MR interacted with aldosterone, spironolactone or eplerenone

	Validation 1	Validation 2	Validation 3	Average of binding energy (kcal/mol)	Binding site
Aldosterone (ZINC03830183)	-9.3	-9.3	-9.3	-9.3	Asn^{770}, Gln^{776}, Arg^{817}
Spironolactone (ZINC03977913)	-9.5	-9.6	-9.5	-9.5	Gln^{776}, Arg^{817}, Cys^{942*}
Eplerenone (ZINC72187491)	-9.7	-9.7	-9.7	-9.7	Arg^{817} and Thr^{945}

*The first two binding sites were the same as binding sites of Hasui's work

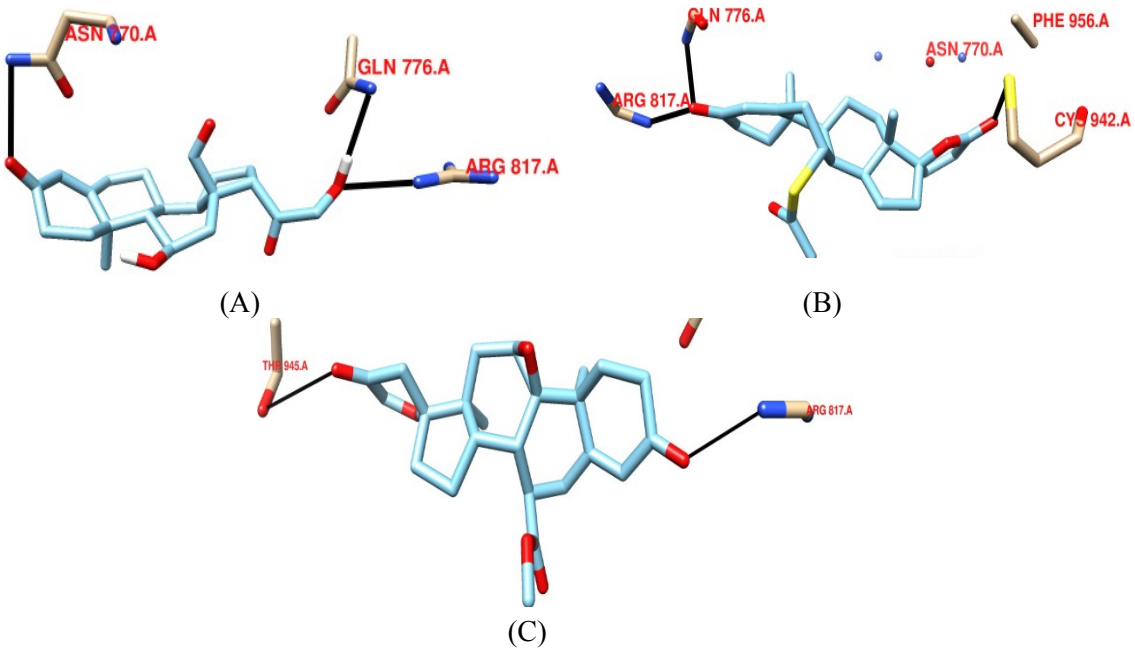


Fig 1. MR-standard ligand binding complexes were visualized using Chimera 1.9 software.

A). Aldosterone interacted with MR at Asn^{770} , Gln^{776} and Arg ^{817} residues. B). Spironolactone interacted with MR at at Gln^{776}, Arg^{817} and Cys^{942} residues while (C) eplerenone interacted with MR at Arg^{817} and Thr^{945}.

Docking and visualisation of herbal phytochemical

A total of 517 selected phytochemicals was molecularly docked with MR using AutoDockVina 1.1.2 program to analyze their binding energy. Molecular interaction of MR and phytochemicals was then visualized using Chimera 1.9 program. MR-phytochemical binding complexes which had lower binding energy than standard ligand antagonist and had similar binding sites were considered as a new candidate of MR antagonist.

RESULTS

Validation of standard ligands

Figure 1 showed that binding sites of MR agonist and antagonist. Aldosterone interacted with the MR at Asn770, Gln776 and Arg817 residues. Whist, MR antagonist (spironolactone) bound to MR at Gln776, Arg817 and Cys942 residues. These binding sites were different from the previous study which Asn770 was substituted with Cys942 residue. Arg817 and Thr945 residues were found in Eplerenone-MR interaction.

Molecular docking between MR and phytochemicals of Indonesian herbal plants

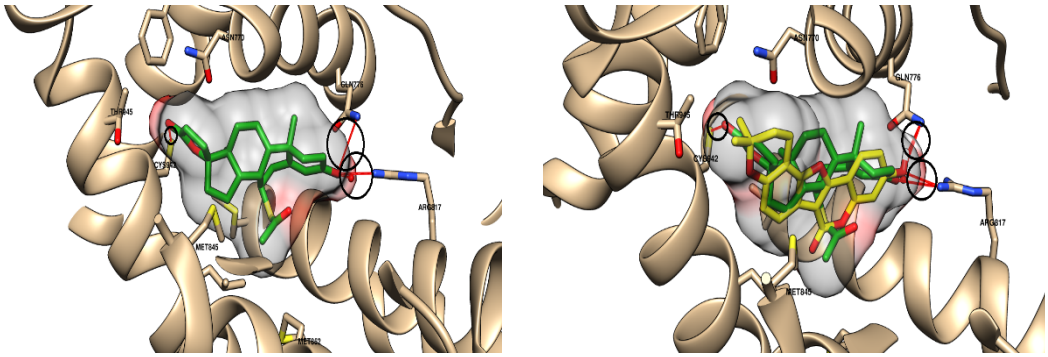
From 517 phytochemicals which full filled Lipinski's criteria, only 6 phytochemicals had lower binding energy than MR standard agonist and antagonists (Table 2). Progesterone had the lowest binding energy (-11.5 kcal/mol) whilst strychnine had the highest binding energy (-9.8 kcal/mol). Eurycomalactone and sojagol shared similar binding energy. In terms of binding site, there was only sojagol that had similar binding sites (Gln776 and Arg817) to aldosterone and spironolactone (Figure 1 and Table 1). Other phytochemicals interacted with MR only at one amino acid residue (Asn770, Leu810, Arg817, or Cys942). Additionally, molecular weight of all phytochemicals was lower than molecular weight of MR agonist and antagonists. The lowest molecular weight was observed in gentisin (258.226 Da) while the highest molecular weight was eurycomalactone (348.390 Da). Strychnine had molecular weight as similar as sojagol (approximately 335 Da).

Docking visualisation between MR and Sojagol

Figure 2 showed that sojagol occupied the MR binding pocket as similar as spironolactone except Cys^{942}. Sojagol bound to the MR at Gln^{776} and Arg^{817} residues and had similar conformation to spironolactone.

Table 2. Result of docking between phytochemical of Indonesian herbal plant with MR

Phytochemical name	Average of binding energy (kcal/mol)	Binding site	Molecular weight (Da)
Aldosterone	-9.3	Asn^{770}, Gln^{776}, Arg^{817}	360.45
Spironolactone	-9.5	Gln^{776}, Arg^{817}, Cys^{942}	416.583
Eplerenone	-9.7	Arg^{817}, Thr^{945}	414.498
Progesterone	-11.5	Arg^{817}	314.461
Strychnine	-9.8	Asn^{770}	334.412
Eurycomalactone	-10.2	Cys^{942}	348.390
Strigol	-10.6	Cys^{942}	346.374
Gentisin	-9.9	Leu^{810}	258.226
Sojagol	-10.1	Gln^{776}, Arg^{817}	336.338



A B

Fig 2. A. MR-Spironolactone binding complexes were visualized using Chimera 1.9. B. Overlay of MR-spironolacton/Sojagol binding complexes. Green colour was spironolactone and Sojagol was yellow. Black circles were designated binding sites.

DISCUSSION

In this study, we found some differences of binding sites between aldosterone /spironolactone and MR using different docking methods. Aldosterone is the endogenous steroid hormone that occupies the ligand binding domain (LBD) of MR at Asn^{770}, Gln^{776} and Arg^{817} residues. Our results differ from other studies that reported aldosterone interacted with MR at Asn^{770}, Ser^{767}, Cys^{942}, Thr^{945} and Glu^{955} residues.(15) Asn^{770} residue is importantly required for stable binding to activate MR by which recruits some coactivators.(8) The two remaining residues (Gln^{776} and Arg^{817}) were reported to play an important role in stabilization of hydrogen bound with the MR.(9) Whereas, Ser^{767}, Cys^{942}, Thr^{945} and Glu^{955} residues are also needed to make hydrogen bond with activation function-2 of MR in helix 3 and 10.(15)

Spironolactone is passive MR antagonist because this compound has labile interaction with the LBD of MR and prevent recruitment of coactivators.(16) Spironolactone occupies Gln^{776} and Arg^{817} residues of MR binding pocket to block aldosterone-MR interaction. In our study, spironolactone also binds to the MR at Cys^{942} residue to make a lipophilic bond and to restrict the volume of binding space.(8) In contrast to our results, Hasui and co-workers reported that spironolactone has Asn^{770} instead of Cys^{942} residue to interact with MR. Asn^{770} is useful for binding stability and partial MR agonist or antagonist.(15) Further investigation is required to solve these different results.

From this bio-computaional study, we have demonstrated that sojagol was a new candidate of *in silico* MR antagonist regarding to binding energy, binding sites and molecular weight. A lower binding energy observed in sojagol will has a higher affinity to interact with the MR

compared with the standard MR antagonists. In addition to binding affinity, the lower energy also stabilizes sojagol-MR binding complexes. In general, binding score in molecular docking programs is calculated by summing up all molecular interactions like hydrophobic, hydrogen, van de Walls, electrostatic and solvation effect.(17)(18) Therefore, stronger molecular interaction will has lower binding energy and more stable ligand binding complex.

In our study, sojagol has two residues (Gln^{776} and Arg^{817}) which are very important for interaction with the LBD of MR. Although sojagol does not have Asn^{770} binding site, it will give a beneficial effect which lead to inactivation of MR.(8) This natural compound is different from some synthetic compounds that created by Hasui's lab center. The synthetic compounds bind to the MR at Asn^{770}, Gln^{776} and Arg^{817} residues that they probably have side effects as same as spironolactone for long term use although their selectivity is higher than spironolactone.

The next advantage of sojagol properties is its molecular weight. Sojagol has lower molecular weight compared with the existing MR antagonists. Lower molecular size of drug molecules will increase their absorption and bioavaibility in blood circulation.(19)

Sojagol is a secondary metabolite which is found in *Phaseolus radiates* plant (mung bean). One study has reported that administration of mung bean extracts lowers blood pressure in mice and exerts anti-inflammatory and antioxidant effects.(20) Another study indicated that mung bean extracts suppressed inflammation-induced by lipopolysaccharide in macrophage cell line.(21) Moreover, cardioprotective effect appears in rat model with cardiac damage which was given mung bean extracts. In the end of treatment. It significantly improved the integrity of heart tissues.(22) Overall, sojagol may potentially to be developed as a new MR antagonist. In conclusion, six phytochemicals have lower binding energy and molecular size than spironolactone and eplerenone but only sojagol has similar binding sites to the MR antagonist. Sojagol might become in silico MR antagonist. Further investigation should be performed to investigate whether or not sojagol has MR antagonist activity.

ACKNOWLEDGEMENTS

We would like to thank Mr. Yoga Mulia Pratama, MD, Biomedical Laboratory, Faculty of Medicine, Universitas Sebelas Maret Surakarta for his technical assistance of molecular docking.

REFERENCES

1. **(GBD) GB of D**. Mortality and Causes of Death Collaborators 2015. Lancet. 2013;385:117–171.
2. **Mendis S, Puska P, Norrving B.** Global Atlas on Cardiovascular Disease Prevention and Control. Geneva: WHO; 2011.
3. **Cohn J., Colucci W**. Cardiovascular Effect of Aldosterone and Post-Acute Myocardial Infarction Pathophysiology. Am J Cardiol. 2006;97:4F–12F.
4. **Gaddam K., Pimenta E, Husain S, Calhoun D**. Aldosterone and Cardiovascular Disease. CurrProlCardiol. 2009;34:51–84.
5. **Mishra T., Rath P**. The Role of Aldosterone and Selective Aldosterone Receptor Antagonist Eplerenone in Cardiovascular Diseases. JIACM. 2006;7:211–6.
6. **Takeda Y**. Pleiotropic Actions of Aldosterone and The Effects of Eplerenone, a Selective Mineralocorticoid Receptor Antagonist. Hypertens Res. 2004;27:781–9.
7. **Ferrario C., Schiffrin E**. Role of Mineralocorticoid Receptor Antagonists in Cardiovascular Disease. Circ Res. 2015;116:206–13.
8. **Fagart J, Hillisch A, Huyet J, Barfacker L, Fay M, Pleiss U, et al**. A New Mode of Mineralcorticoid Receptor Antagonism by A Potent and Selective Nonsteroidal Molecule. JBiol Chem. 2010;258:29932–40.
9. **Jennings DL, Kalus J., O'Dell K.** Aldosterone Receptor Antagonism in Heart Failure. Pharmacotherapy. 2005;8:1126–33.
10. **Bielska E, Lucas X, Czerwoniec A, Kasprzak J., Kaminska K., Bujnicki JM**. Virtual Screening Strategies in Drug Design-Methods and Applications. JBCBB. 2011;92:249–64.
11. **Ferreira L., Dos Santos R., Oliva G**, Andricopulo A. Molecular Docking and Structure-Based Drug Design Strategies. Molecules. 2015;20:13384–133421.
12. **Trott O, Olson A**. Autodock Vina:Improving The Speed and Accuracy of Docking with A New Scoring Function, Efficient Optimization, and Multithreading. J Comput Chem. 2010;31:455–61.
13. **Hasui T, Matsunaga N, Ora T, Ohyabu N, Nichigaki N, Imura Y, et al**. Identification of Benzoxazin-3-One Derivates As Novel, Potent, and Selective Nonsteroidal Mineralocorticoid Receptor Antagonist. J Med Chem. 2011;54:8616–31.
14. **Lipinski C., Lombardo F, Dominy B., Feeney P**. Experimental and Computational Approaches to Estimate Solubility and

Permeability in Drug Discovery and Development Settings. Adv Drug Deliv Rev. 2001;46:3–26.
15. **Huyet J, Pinon G., Fay MR, Rafestin-Oblin M., Fagart J**. Structural Determinants of Ligand Binding to The Mineralcorticoid Receptor. 2012;350:187–95.
16. **Kolkhof P, Borden S**. Molecular Pharmacology of The Mineralocorticoid Receptor: Prospects for Novel Therapeutics. Mol Cell Endocrinol. 2012;350:310–317.
17. **Merz K., Ringe D, Reynolds C.** Drug Design: Structure-and Ligand-Based Approaches. Cambridge University Press; 2010.
18. **Rarey M**. Protein Ligand Docking in Drug Design. In: Bioinformatics-From Genomes to Drugs. Germany: Wiley; 2001. p. 315–60.
19. **Pollastri M.** Overview on The Rule of Five. Curr Protoc Pharmacol. 2010;49:1–8.
20. **Tang D, Dong Y, Ren H, Li L, He C.** A Review of Phytochemistry, Metabolite Changes, and Medicinal Uses of The Common Food Mung Bean and Its Sprouts (Vigna radiata). Chem Cent J. 2014;8:1–9.
21. **Chao W., Chung Y., Shih I., Wang H., Chou S., Hsu C**. Red Bean Extract Inhibits Lipopolysaccharide-Induced Inflammation And H2O2-Induced Oxidative Stress in RAW 264.7 Macrophages. J Med Food. 2015;18:724–30.
22. **Cheng D, Wang R, Wang C, Hou L**. Mung Bean (Phaseolus radiatus L.) Polyphenol Extract Attenuates Alumunium-Induced Cardiotoxicity Through an ROS-Triggered Ca2+/JNK/NF-kB Signaling Pathway in Rats. Food Funct. 2017;8:851–9.