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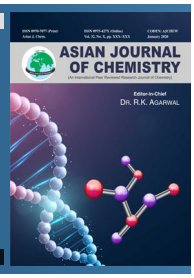

Virtual Screening of Flavonoid Compounds against Angiotensin II Type I Receptor Using Docking Method

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The aim of this study was to determine the affinities and interactions of five flavonoids, namely quercetin, epicatechin, genistein, luteolin and hesperetin, against AT_1R. Using the docking method, *in silico* studies were conducted, and AutoDock Vina and ChemOffice programs were used to analyse permeability and stability of atoms in the ligand. Discovery Studio was used for interaction visualization. Using ANOVA, ligand affinity was statistically analysed with 95% confidence level. The five flavonoids, namely quercetin, epicatechin, genistein, luteolin and hesperetin, and two positive controls, namely valsartan and losartan, had H donors < 5, H acceptors < 10 and molecular weights 302.24, 290.27, 270.24, 286.24, 302.28, 435.53 and 422.92 g/mol, respectively. Furthermore, the affinity of these ligands against AT_1R were -8.3, -7.8, -8.3, -8.3, -7.6, -8.7 and -9.2 kcal/mol, respectively. Few amino acid residues showed interactions similar to the control, such as van der Waals, hydrogen bond and pi-interactions. All ligands in the normality and homogeneity tests showed *p*-values > 0.05 and equal to 0.059, respectively. The five flavonoids had *p*-value < 0.05 against the controls. All the five flavonoids have good permeability and their statistical affinity was significantly different from the controls. Nevertheless, active site cavities and amino acid residues similar to the controls enabled the flavonoids to interact with AT_1R. The affinity of quercetin is statistically similar to that of genistein and luteolin, whereas that of epicatechin is similar to hesperetin.

Keywords: Angiotensin receptor II type 1 (AT_1R), Docking, Epicathecin, Genistein, Hespertein, Luteolin, Quercetin.

INTRODUCTION

Cardiovascular disease is one of the most fatal diseases [1]. An uncontrolled lifestyle is one of the causes of cardiovascular disease. Furthermore, hypertension is an important factor [2]. In Indonesia, hypertension prevalence in the year 2013 was approximately 9.4% [3]. By year 2025, approximately 80% increase in hypertension cases is expected worldwide, especially in developing countries. From 639 million cases in year 2000, the number is expected to increase to an estimated 1.15 billion by year 2025 [4].

Hypertension is a circulatory disorder characterized by increased blood pressure, causing various complications [5]. Adoption of a healthy lifestyle and appropriate medication can control hypertension. Medicinal plants are being studied to develop new drugs for hypertension because herbal drugs are safer than synthetic drugs [4]. Flavonoid, a secondary plant metabolite, has various biological activities [6,7]. Flavonoid has many known health benefits, such as blood pressure reduction [8]. Hypertension leads to oxidative stress, which causes vasoconstriction [9,10]. Flavonoid can dilate blood vessels through increasing nitric oxide (NO) activity in endothelial cells [11]. Quercetin genistein, epicatechin, hesperetin, and luteolin have shown to lower blood pressure of spontaneously hypertensive rats through increased NO production [2,12-18]. These flavonoids may inhibit AT_1R, but their exact affinity and interaction with AT_1R are unknown. AT_1R is present in the heart, brain, adrenal glands, kidneys, and liver [19,20]. AT_1R has 359 amino acids and molecular weight 4 kDa [21]. Amino acid residues of Arg167 an Tyr35 play a role in the interaction of ARB drugs with AT_1R [22].

Docking studies can be used to determine the affinity and interactions between natural compounds and receptors [23]. Docking is a preliminary study that is used to improve research accuracy, thus saving time, energy and cost [24]. Although preclinical studies have been used for this purpose, the high cost,

intraspecies extrapolation in drug development and a lack of structural information are challenging factors [22,25]. For developing a new drug, docking study with structure modification is beneficial to determine the activity and adverse effect of drugs. Therefore, this research analyzed quercetin, genistein, epicatechin, hesperetin and luteolin against AT_1R in terms of affinity and interactions in docking.

EXPERIMENTAL

Tools: The tools that were used in this research were laptop, Asus X455L Intel Core i3-inside™), Protein Data Bank (http://www.rcsb.org/pdb/) to collect protein, Discovery Studio 2016 Client (DS) program to observe interactions and active site of receptor, AutoDock Vina (Version 4.2, updated for version 4.2.6) for docking process, ChemOffice 2D (Version 15.0) to draw two dimensional (2D) structure and determine physicochemical properties, ChemOffice 3D (Version 15.0) to draw three dimensional (3D) structure.

Materials: The materials that were used in this research were 3D ligand structure of quercetin, epicatechin, genistein, luteolin, hesperetin and losartan and valsartan as control positive from ChemOffice (Version 15.0) in pdb format, 3D receptor structure of AT1R in pdb format.

Ligand preparation: Two dimensional (2D) quercetin, epicatechin, genitein, luteolin, hesperetin and control positive losartan and valsartan were drawn by ChemOffice 2D program (version 15.0). Their three dimensional (3D) were drawn by using ChemOffice 3D program (version 15.0), then minimized energy using MM2 minimize energy tools to find the most stable form compound conformation to bind to the receptor [26].

Physico-chemical properties analysis: Physico-chemial properties of quercetin, epicatechin, luteolin, hesperetin, genitein and control positive losartan and valsartan were analyzed by using ChemOffice 2D (Version 15.0) to predict their ability to penetrate biological membrane by looking the H donor, H acceptor, molecular weight and log P parameters [26,27].

Preparation of receptor: 3D structure of AT_1R (PDBID: 4YAY) was downloaded from RCSB Protein Data Bank (http://www.rcsb.org/pdb/). The downloading result was opened by Discovery Studio 2016 to remove water molecules, detach attached ligand and add hydrogen atoms in polar part. The result was stored in pdb format.

Molecular docking: Active site detection, bound ligand-receptor docking were performed using AutodockVina computer program (version 4.2, updated for version 4.2.6) by optimization using the losartan control positivevof the AT_1R [28]. The affinity and RMSD could then be viewed using Command Prompt.

Ligand receptor interactions analysis: Receptor and ligands of the .pdbqt format were inputted to the Discovery Studio 2016 Client program. Discovery Studio would show the 2D and 3D type of bond between the receptor and ligand. The final stage after obtaining the visualization results was to perform data analysis.

Data analysis: The chemical and physical properties (log P and BM) of quercetin, genistein, epicatechin, hesperetin, and luteolin were then analyzed by using Lipinski′s rule of five for predicting their absorption and permeability properties. Subsequently, amino acids involved in the receptor-drug interaction were identified and their docking scores were determined. Low affinity indicates a stable drug-receptor interaction and a high biological activity [29-31]. For the statistical analysis, ANOVA test was performed by using SPSS program version 23.0 with 95% confidence interval.

RESULTS AND DISCUSSION

Physiochemical properties: Physiochemical properties of the compounds were determined based on their structure (Fig. 1), molecular weight, log P and total steric energy (Tables 1 and 2). The structure indicates that H donors and H acceptors of valsartan are 2 and 5, losartan are 2 and 5, quercetin are 5 and 2, epicatechin are 5 and 1, genistein are 3 and 2, luteolin are

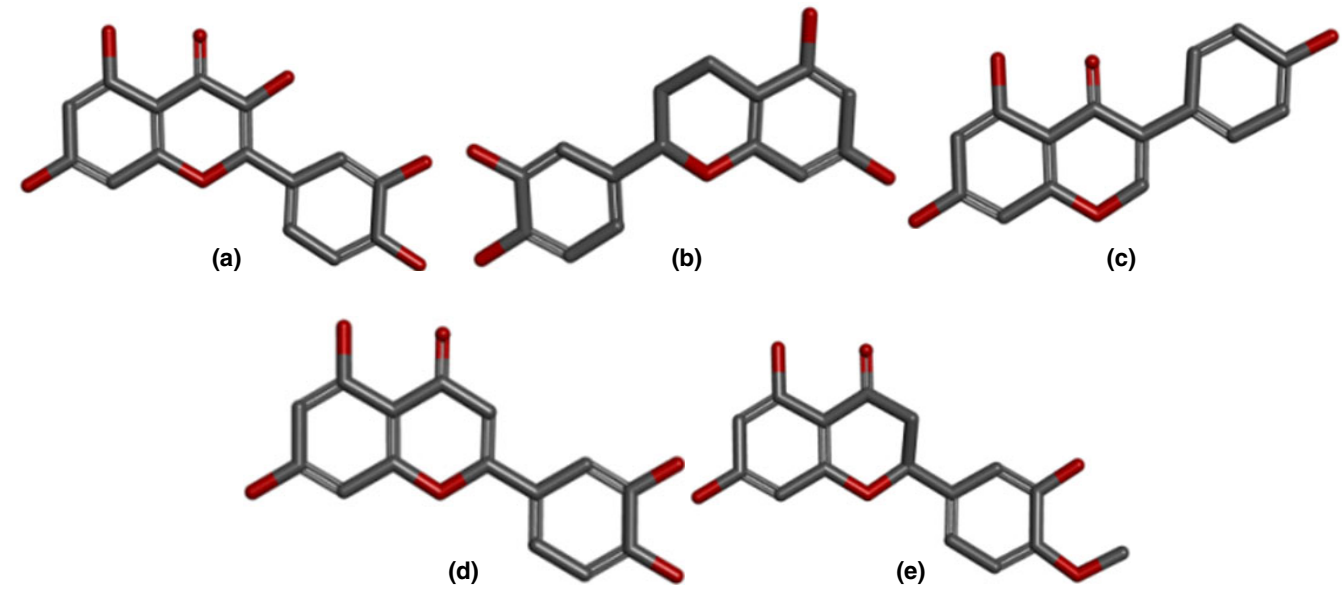

Fig. 1. Structure of flavonoid ligands (a) quercetin, (b) epicathecin, (c) genistein, (d) luteolin, (e) hesperetin

TABLE-1
DETERMINATION OF LIGANDS
PHYSCO-CHEMICAL PROPERTIES RESULTS

Molecule name	m.f.	log P	m.w. (g/mol)
Valsartan	$C_{24}H_{29}N_5O_3$	5.80	435.53
Losartan	$C_{22}H_{23}N_6OCl$	6.10	422.92
Quercetin	$C_{15}H_{10}O_7$	0.35	302.24
Epicatechin	$C_{15}H_{14}O_6$	1.50	290.27
Genistein	$C_{15}H_{10}O_5$	1.74	270.24
Luteolin	$C_{15}H_{10}O_6$	1.51	286.24
Hesperetin	$C_{16}H_{14}O_6$	1.50	302.28

TABLE-2
LIGAND STERIC ENERGY TOTAL ANALYSIS RESULTS

Molecule name	Steric energy before MM2 (kcal/mol)	Total energy (kcal/mol)
Valsartan	1860.586	41.7227
Losartan	1715.345	39.3801
Kuersetin	29.698	14.9431
Epikatekin	32.135	-4.3505
Genistein	80.643	29.6982
Luteolin	69.066	7.6356
Hesperetin	70.564	7.1458

4 and 2, and hesperetin are 3 and 3, respectively. The molecular weights of all the ligands were < 500 g/mol. The log P value of the five flavonoids were < 5, whereas those of the positive control were > 5.

After energy minimization by using MM2 tools, epicatechin was found to have the lowest total steric energy among the ligands.

Docking: Docking results obtained by using AutoDock Vina showed that the affinities of five flavonoids were higher than those of two positive controls (RMSD value = 0.000).

Interaction visualisation: Discovery Studio program can accurately determine the interaction between flavonoid compounds and AT_1R (Fig. 2). Fig. 3 shows five flavonoids occupying the active site cavity of AT_1R. Furthermore, both positive controls occupied the same cavity as the flavonoids.

Statistical analysis using SPSS: Statistical analysis was performed by using SPSS with 61 affinity data and 7 test groups, showing normal data distribution ($p > 0.05$) and homogeneity with p-value 0.059. The results of an ANOVA test with post hoc LSD revealed that the five flavonoid compounds were significantly different from the positive controls ($p < 0.05$) [32].

Regarding physiochemical properties, Lipinski′s rule showed that flavonoid compounds have < 5 H donors and > 10 H acceptors, molecular weight < 500 g/mol, and logP value <5. If the molecular weight of a compound is > 500 g/mol, penetrating the biological membrane becomes difficult. Furthermore, the log P value indicates the compound′s ability to dissolve in a biological membrane [33]. The log P value of the positive control valsartan is higher than required based on the Lipinski′s rule. The high log P value of valsartan is due to the high number of C atoms, increasing the partition coefficient, which results in low bioavailability. The high log P and low permeability of valsartan could be altered through an appropriate drug delivery system, such as proliposomes and self-nanoemulsifying drug delivery system (SNEDDS). Studies have suggested that orally administering valsartan by using proliposomes in a capsule and SNEDDS increased its bioavailability to 202.36 and 196.87%, respectively, *versus* valsartan suspension [34]. Moreover, losartan had a log P value of > 5 and low permeability. Studies have revealed that using self-microemulsifying drug delivery system increased the bioavailability of losartan 1.49 times that of losartan tablets [35]. Thus, flavonoid bonds meet all the requirements indicated by Lipinski′s rule, indicating that flavonoids are easily absorbed and have high permeability. When the atoms of a molecule are too close together, repulsion occurs due to the electron cloud on the atoms, resulting in a steric effect that alters molecular conformation, and the energy released during this process is called steric energy. During ligand preparation, before ligand docking, steric energy must be minimized (MM2 energy minimization). A molecular structure is stable if its intramolecular energy (total steric energy) is low. Hence, to form a stable 3D conformation, the repulsive force between the atoms must be minimal. Moreover, the number and position of hydroxyl groups in aromatic rings and unpaired electrons are involved in electron delocalisation [26,36-38]. Among the ligands, epicatechin had the most stable steric energy because the value of its total steric energy was small or negative. Hence, the repulsion between the atoms was small, making the molecule stable.

The affinity of five flavonoids was higher than that of two positive controls. However, they could form bonds with AT1R because it has a negative affinity had good receptor stability, considering that positive affinity suggested no interaction of the ligand with the receptor [39]. The docking study generates two files. One file is in a log.txt format with a docked affinity value and RMSD data, whereas the other file is in an out_ligand_"mode".pdbqt format with tethered ligand conformation data. In molecular tethering, an RMSD value of < 2.00 Å was commonly used as a standard value. The RMSD value indicates calculation accuracy [40]. The highest negative affinity was shown by mode 1, with RMSD value of 0.000 and therefore, mode 1 exhibited the best interaction among all the modes [41]. On the basis of the docking results of the five flavonoids and two positive controls, mode 1 has an RMSD value of 0.000. Amino acid residues obtained through the docking of five flavonoids were not entirely similar to those of the positive controls (Table-3). Quercetin, genistein, epicatechin, hesperetin and luteolin bind to the AT1R position of the Tyr35 amino acid similar to the positive control losartan. Furthermore, the five flavonoids bind to the AT1R position of Arg167 amino acid similar to the positive control valsartan. The literature on various ARB drug classes showed that Tyr35 and Arg167 were present is all classes [25]. Thus, if the docking results are appropriate, five flavonoids might have effects similar to those of ARB drugs. However, the amino acid residues were not entirely same and the only visible interactions, namely hydrogen and hydrophobic effects.

The five flavonoids occupied the active site cavity of AT_1R (Fig. 3). Both positive controls share the active site cavity with flavonoids. The active site cavities of receptors and ligands have a lock and key characteristic, which calculated based on steric, geometric, bonding, affinity and electronic properties directly related to atoms or clusters on amino acid residues

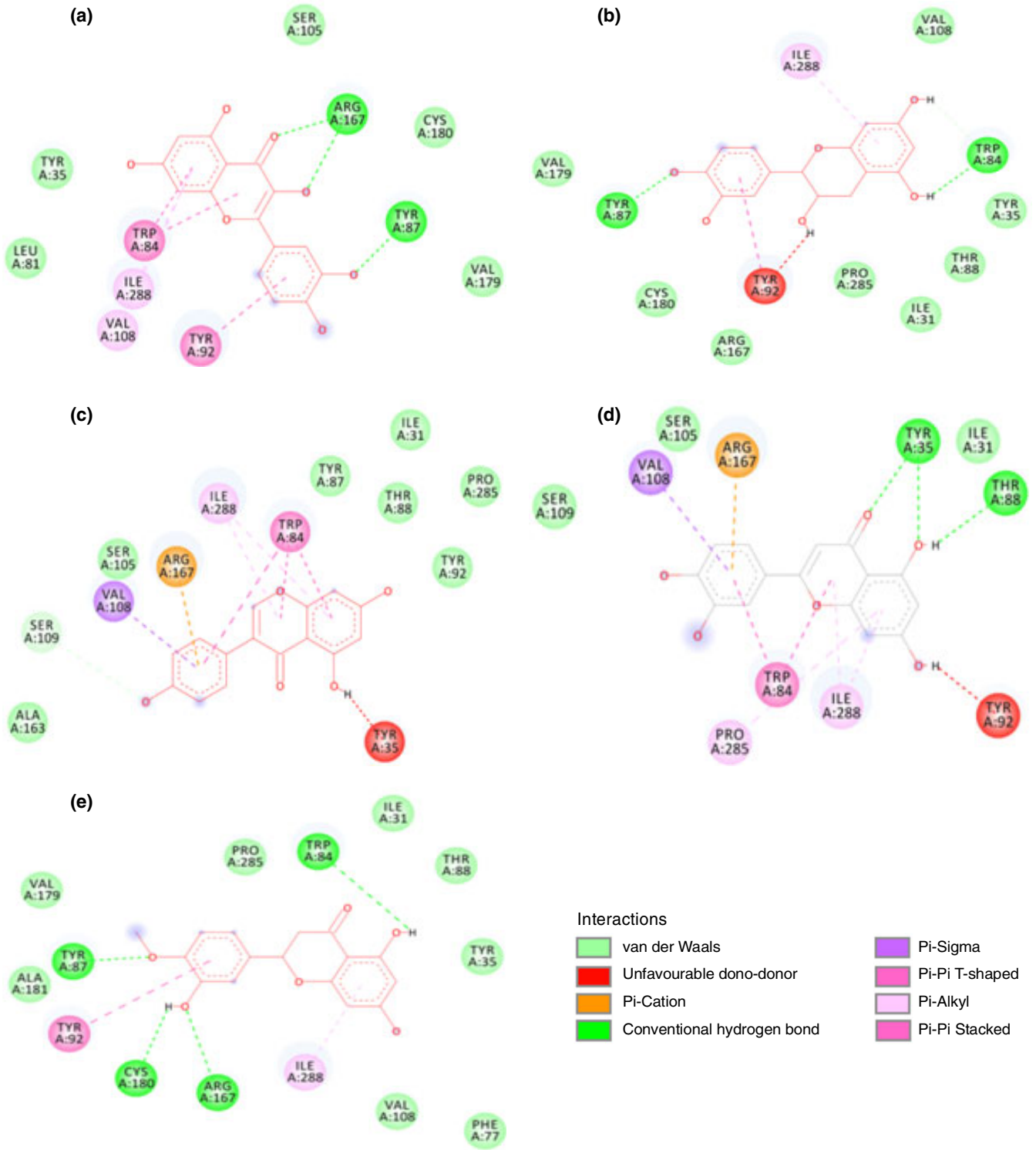

Fig. 2. Visualization results of the interaction types of flavonoid compounds (a) quercetin, (b) epicatechin, (c) genistein, (d) luteolin, (e) hesperetin using discovery studio

[29]. Statistical analysis of affinity data showed normal distribution and homogeneity but significant differences between the five compounds and two positive controls. However, according to the amino acid residues and the active site cavity (Figs. 2 and 3), all the five flavonoids contributed in lowering the blood pressure through involvement of AT_1R. The mechanism involves relaxing of the smooth muscle, thus promoting blood vessel vasodilation [21]. The docking study of flavonoids provided descriptive results, and therefore, this study should be used for *in vitro* studies.

Conclusion

According to Lipinski's rule, the amount of H donors and H acceptors, molecular weight and log P value of flavonoids

TABLE-3
SUMMARY OF LIGAND DOCKING ANALYSIS RESULTS AGAINST AT_1R

Molecule name	Affinity (kcal/mol)	Amino acid residue involved	Number of hydrogen bonds	RMSD
Quercetin	-8,3	Tyr35, Tyr92, Tyr87, Trp84, Arg167	3	0,000
Epicatechin	-7,8	Tyr35, Pro285, Tyr92, Thr88, Tyr87, Trp84, Ile288	1	0,000
Genistein	-8,3	Tyr35, Ile288, Thr88, Tyr92, Ser105, Arg167	0	0,000
Luteolin	-8,3	Tyr35, Pro285, Ile288, Tyr92, Trp84, Arg167, Ser105, Val108	1	0,000
Hesperetin	-7,6	Tyr35, Pro285, Thr88, Tyr87, Cys180, Trp84, Arg167, Ile288	1	0,000
Losartan	-8,7	Tyr35, Pro285, Tyr92, Tyr87, Cys180, Trp84, Val108, Ile288	1	0,000
Valsartan	-9,2	Ile288, Tyr292, Trp253, Trp84, Val108, Ser109, Ser105, Arg167	0	0,000

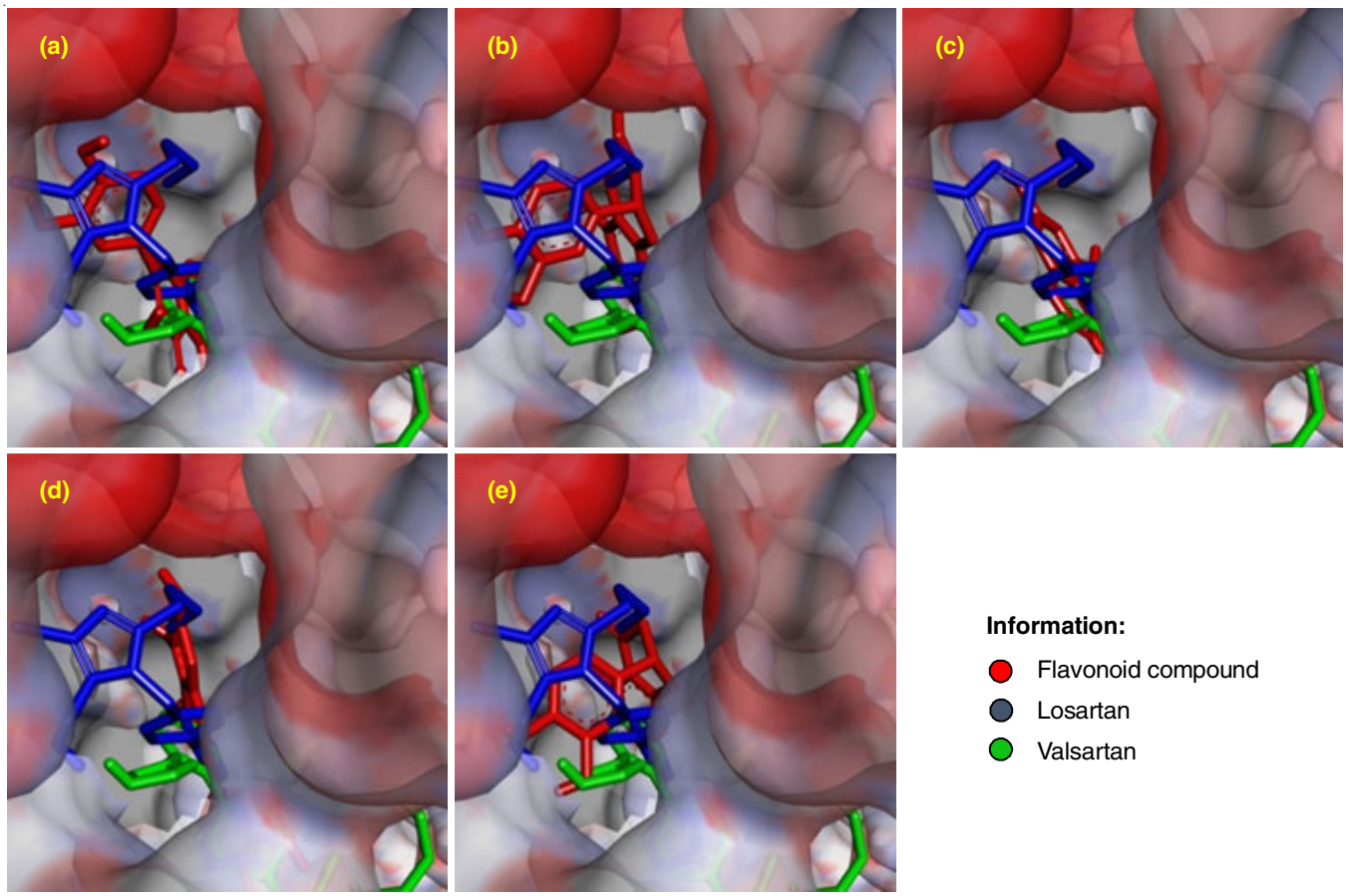

Fig. 3. Active site cavity of control positives-flavonoid (a) quercetin, (b) epicatechin, (c) genistein, (d) luteolin, (e) hesperetin

fulfil the criteria for permeability. Thus, quercetin, genistein, epicatechin, hesperetin and luteolin can effectively penetrate membranes. Energy minimization through the docking process ensures that ligands formed have a stable conformation for receptor binding. The docking results showed that the five flavonoids had a negative affinity value, indicating that flavonoids could bind to AT_1R. Regarding the interaction type, comparing flavonoids with positive controls, several amino acids positions were similar, particularly Arg167 and Tyr35. Regarding the active site cavity, the two positive controls and five flavonoids appeared to occupy the same active sites. ANOVA test results involving affinity data showed that the five flavonoids were significantly different from the two positive controls, but their amino acid residues and active site cavities were similar to those of the positive controls. Thus, quercetin, genistein, epicatechin, hesperetin and luteolin interacted with AT_1R and exhibited anti-hypertensive properties although they were not as effective as the positive controls.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

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