umsurabaya
UNIVERSITAS MUHAMMADIYAH SURABAYA
Camellia
Clinical, Pharmaceutical, Analitical and Pharmacy Community Journal
http://journal.um-surabaya.ac.id/index.php/CAM

Physicochemical Properties and In silico Binding Interaction With Gyrase B Of Antibacterial Compounds From Aquous Extract of Hibiscus sabdariffa L.

Karakteristik Fisikokimia dan Interaksi Ikatan In silico dengan Gyrase B dari Senyawa Antibakteri dalam Ekstrak Air Hibiscus sabdariffa L.

Nuzul Wahyuning Diyah[1], Khairunnisa Andini[1], Isnaeni[1,2,*]

[1] Department of Pharmaceutical Science, Faculty of Pharmacy, Universitas Airlangga, Surabaya
[2] Pharmacy Study Program, Faculty of Health Science, Universitas Muhammadiyah Surabaya, Surabaya

**Corresponding author :* isnaeni@um-surabaya.ac.id

INFO ARTIKEL

Dikirim:
17 Maret 2024

Direvisi:
30 April 2024

Diterima:
31 Mei 2024

Terbit *Online*:
30 Juni 2024

ABSTRACT

Hibiscus sabdariffa L. (Hs) is recognized to have various pharmacological activities such as antibacterial, antiedema, anti-fungal, antihypertensive, anti-inflammatory, and antiviral. The aqueous extract of Hs' calyx which is rich in polyphenols and flavonoids displayed antibacterial activity against several bacteria so that the compounds are important to be developed further as antibacterial agents. This study aims to find out which compounds contained in the extract have the antibacterial potency based on in silico interaction with gyrase B. Physicochemical descriptors of ten compounds were evaluated before molecular docking study, and the docking simulation was executed with Autodocktools 1.5.6. The selected compounds based on Lipinski's Rules and their lower ΔG^0 were subjected to prediction of pharmacokinetic and toxicity (ADMET) using pKCSM online tool. The results gave five test compounds showed the higher binding affinity on gyrase B compared to quercetin which served as inhibitor of the enzyme. The glycosides displayed better scores due to the existence of more hydroxyl groups. The potential aglycones were myricetin and delphinidin. According to the ADMET parameters, the two compounds have the best profile where they were neither hepatotoxic nor carcinogenic and also safer due to the higher LD_{50}. In conclusion, the myricetin and delphinidin generated better binding interaction than the ligand inhibitor of gyrase B which indicated that they have higher potential as antibacterial agents.

Keywords: in silico, gyrase B, antibacterial, ADMET, Hibiscus sabdariffa

ABSTRAK

Hibiscus sabdariffa L. (Hs) memiliki berbagai aktivitas farmakologi seperti antibakteri, antiedema, antijamur, antihipertensi, antiinflamasi, dan antivirus. Ekstrak air kuntum bunga Hs yang kaya akan polifenol dan flavonoid menunjukkan aktivitas antibakteri terhadap beberapa bakteri sehingga senyawa-senyawa tersebut dapat dikembangkan lebih lanjut sebagai agen antibakteri. Penelitian ini bertujuan untuk mengetahui senyawa apa saja dalam ekstrak yang mempunyai potensi sebagai antibakteri berdasarkan interaksi pengikatannya dengan girase B secara in silico. Sepuluh senyawa dievaluasi sifat fisikokimia sebelum dilakukan docking molekul. Simulasi docking dilakukan dengan program Autodocktools 1.5.6. Kemudian senyawa terpilih, berdasarkan persyaratan hukum Lipinski dan energi bebas (ΔG^0) yang rendah, diprediksi sifat farmakokinetik dan toksisitasnya (ADMET) menggunakan pKCSM online tool. Dari hasil penelitian diketahui lima senyawa uji menunjukkan afinitas pengikatan yang lebih tinggi pada girase B dibandingkan dengan kuersetin yang merupakan inhibitor enzim girase B. Glikosida menunjukkan interaksi yang lebih baik karena mengandung lebih banyak gugus hidroksil. Aglikon yang potensial adalah mirisetin dan delfinidin. Berdasarkan parameter ADMET, kedua senyawa tersebut memiliki profil terbaik, tidak bersifat hepatotoksik dan karsinogenik serta lebih aman karena LD_{50} yang lebih tinggi. Sebagai kesimpulan, mirisetin dan delfinidin menghasilkan interaksi pengikatan yang lebih baik dibandingkan ligan inhibitor girase B yang mengindikasikan bahwa keduanya memiliki potensi lebih tinggi untuk dikembangkan sebagai obat antibakteri.

Kata Kunci : *in silico, gyrase B, antibakteri, ADMET, Hibiscus sabdariffa*

INTRODUCTION

Hibiscus sabdariffa L. (Hs), known as roselle is an ideal plant for developing countries because it is relatively easy to grow and can be used as food and fiber. Roselle is known to have some pharmacological activities such as antibacterial, antiedema, antifungi, antihypertensive, antiinflammatory, and antiviral (Ross, 2003). One of the biological activity of roselle's calyx is antibacterial activity. The aquous extract of roselle's calyx is rich in polyphenol compounds, such as phenolic acid, and flavonoid compounds such as anthocyanins, flavonols, and flavanols (Da-Costa-Rocha, et al., 2014). The compounds contained in the extract which were suspected to provide antibacterial effect were polyphenols and flavonoid compounds. Poly-phenols and flavonoid compounds have phenolic hydroxyl groups which formed hydrogen bonds with protein in microorganisms and resulting damage in the bacteria protein structure then kill microorganisms (Palczar & Chan, 1988). The part of flavonoid structures that play a role in inhibiting the synthesis of bacterial nucleic acids are rings A and B which generated hydrogen bonds by stacking nucleic acid bases. This mechanism inhibited the formation of DNA and RNA.

The hydroxyl groups in position of 2',4' or 2',6'-dihydroxylation in ring B and 5,7-dihydroxylation in ring A played an important role in the antibacterial activity of flavonoids. Flavonoids damaged the permeability of bacterial cell walls, microsomes, and lysosomes as a result of its interactions with bacterial DNA (Cushnie & Lamb, 2005). Polyphenols compounds contained in roselle petal water extract which have the potential as antibacterial is protocatechuic acid (Isnaeni, et al., 2020), while flavonoid compounds contained in the extract that have characteristics of antibacterial pharmacophore were quersetin, gossypetin, methyl epigallocatechin, delphinidin-3-sambubioside, cyanidin-3-sambubioside, myricetin-3-arabinogalactoside, and quersetin-3-rutinoside.

Many antibacterial substances work by inhibiting gyrase B in bacteria at the process of

DNA replication (Campbell & Farrell, 2009). Research related to new antibacterial drugs is being explored to fight emerging bacterial resistance, such as DNA gyrase (Barancokova , et al., 2018). In this study, eleven compounds found in the aqueous extract of roselle's calyx were docked into gyrase B (PDB: 4PRV) using AutodockTools 1.5.6 software, and their ADMET properties were predicted. Identification of ADMET at an early stage will lead the compound towards the development of new drugs because undesirable properties will lead to failure in the next step of drug development.

MATERIALS AND METHODS

Materials and Tools

The test compounds in this study included protocatechuic acid, gossypetin, delphinidin, cyanidin, delphinidin-3-sambubioside, cyanidin-3-sambubioside, methyl epigallocatechin, myricetin, myricetin-3-arabinogalactoside, and quercetin-3-rutinoside, in two-dimensional (2D), three-dimensional (3D) structures, and SMILES code.

Acer Swift SF314-41 computer set with Windows 10 Home Single Language 64-bit operating system and AMD Ryzen 5 3500U processor with Radeon VGA Mobile Gfx (8 CPUs), ~2.1GHz equipped with programs ChemOffice 18.1, AutoDockTools-1.5.6, Discovery Studio Visualizer software were used to build the structure and performed docking simulation.

Preparation of structure of test compounds

The test compounds are drawn in 2 Dimensional (2D) structures using the ChemDraw 18.1 program then the physicochemical properties were obtained by choosing the Show Chemicals Properties Window tools. Then 3D structures were prepared in ChemDraw 18.1 and then subjected to energy minimization using MMFF94 method. The molecular structure in optimized geometry was save in mol2 format and also SDF format.

Receptor and Ligand Setup

The protein structure of Gyrase B (PDB. 4PRV) was downloaded from the Protein Data Bank through the https://www.rcsb.org site. This structure contained native ligand ADP. The protein used as receptor was processed in Autodock Tools 1.5.6 program, which included removing the original water molecules and its native ligands, correcting the missing atoms, and giving charge. The prepared receptor was saved in pdbqt format and ready to be used in docking simulation.

The test ligands and native ligand ADP were also prepared by using the Autodock Tools 1.5.6 program. Ligand molecules were charged and observed for the number of their rotatable bonds, then make sure that the number of active torsion was equal to the number of rotatable bonds. Ligands were saved in pdbqt format prior to docking.

Redocking native ligand

Redocking the native ligand ADP in pdbqt format into the prepared receptor Gyrase B was a validation procedure in molecular docking. The parameter used for validation is the RMSD (Root Mean Square Deviation) value; if RMSD≤ 2Å then the docking method was valid and can be used for docking simulation (Jain and Nicholls, 2008).

Molecular docking study

The AutodockTools 1.5.6 program was opened, then the prepared receptor ligand was selected. The coordinates of grid box were set so that the box can covered the surfaces of binding site and test ligand, then the grid parameters were store in the GPF file. Reopen AutodockTools 1.5.6 program and the prepared receptor ligand were selected again, then the docking parameters were set and save it in DPF file. Then, docking simulations were running by the AutoGrid program using command prompt. The docking process of each test ligands were performed in triplicates.

The docking results were contained in a working folder as DLG file. To observe the ligand-receptor interaction, the Analyze menu was used and the pose of ligand-receptor complexes were stored in pdbqt format. The docking results included free energy of binding (ΔG^0) obtained for the ligands, inhibition constanta (Ki), hydrogen bonding and amino acid residues involved in the interactions (Praja, et al., 2018). The visualization of the ligand interactions with amino acid residues were carried out by using Discovery Studio Visualizer (DSV) software.

Tabel 1. Physicochemical properties of test compounds

Compound	MW	CLogP	HBA	HBD	pKa	tPSA	LogS	CMR
Gossypetin	318.24	0.84	8	6	5.7	147.68	-2.45	7.59
Delphinidin	303.25	1.91	6	6	-0.9	132.68	-0.53	7.47
Delphinidin-3-sambubioside	597.50	-1.97	15	11	-0.9	270.75	0.40	13.59
Cyanidin	287.25	1.76	5	5	-0.8	112.45	-0.62	7.32
Cyanidin-3-sambubioside	581.50	-1.31	14	10	-0.8	250.52	0.30	13.44
Myricetin	318.24	0.84	8	6	6.9	147.68	-2.47	7.59
Myricetin-3-arabinogalactoside	570.46	-2.16	16	12	6.6	287.51	-0.80	12.81
Quercetin-3-rutinoside	610.52	-1.36	16	10	6.6	265.52	-2.32	14.03
Methyl epigallocatechin	320.30	0.38	7	6	8.4	130.61	-1.50	7.95
Protocatechuic acid	154.12	1.06	3	3	3.9	77.76	-1.65	3.65

Notes: MW= Molecular weight (g/mol); ClogP= Calculated Logarithm of the Partition Cofficient; CMR= Calculated Molar Refractivity; tPSA = Topological Polar Surface Area (Å^2); E_{total}= molecular energy in optimized geometry; LogS = Logarithm of the Solubility; pKa= $-\log_{10}K_{a1}$ (acidity constant); HBA = Hydrogen Bond Acceptors; HBD = Hydrogen Bond Donors.

ADMET Prediction

ADMET prediction test was conducted by the pKCSM online tools. The structure of the compounds in the SDF format were converted to SMILES code by using SMILES online translator. The prediction of pharmacokinetic properties (absorption, distribution, metabolism, excretion) and toxicity of the compound were done by input the SMILES code of each compound then ADMET box were clicked.

RESULTS AND DISCUSSION

The physicochemical properties of the ten test compounds including molecular weight, CMR, CLogP, LogS, HBA, HBD, pKa, and tPSA, are listed in the Table 1.

MW and CMR are steric parameters used to describe the molecular size of a compound. The molecular weight of the value is compared to the CMR (Vargas, et al., 2009). Compounds with a molecular weight of less than 500 g/mol have good absorption and permeases (Lipinski, et al., 2001). Among the 10 test compounds, there are 6 compounds that meet the five Lipinski rules based on molecular weight parameters, namely protocatekuat acids, gossypetin, delphinidin, cyanidin, methylepigallocatechin, and myricetin. While the other 4 test compounds have an MW of more than 500 g/mol, namely delphinidin-3-sambubioside, cyanidin-3-sambubioside, myricetin-3-arabinogalaktoside, and quercetin-3-rutinoside, these compounds have a relatively large molecular weight due to glycoside groups that make the structure of the compound become larger.

CLog P is the result of calculating the coefficient of the solubility partition of fat in water. A logP value greater than 5 will cause drug compounds tend to have a high level of toxicity, as they will be held longer on lipid bilayer and distributed more widely in the body, so that the selectiveness of bonds to receptors becomes reduced. However, logP values that are too negative are also not good, because the molecules are too hydrophilic, so they cannot pass through the lipid bilayer. Therefore, a drug compound will have good absorption or permeation if the log value of P is less than 5 and greater than -0.4 (Lipinski, et al., 2001) (Syahputra, et al., 2014). Base on ClogP data in Table 1, there are 6 test compounds that have Clog P greater than -0.4 and smaller than 5, namely protocatechuic acid, gossypetin, delphinidin, cyanidin, methyl epigallocatechin, and myricetin. While the other 4 compounds have ClogP less than -0.4, namely delphinidin-3-sambubioside, cyanidin-3-sambubioside, myricetin-3-arabinogalaktoside, and quercetin-3-rutinoside. These compounds are glycosides of delphinidin, cyanidin, myricetin, and quercetin, which contain more hydroxyl groups in their sugar groups.

LogS is a physicochemical property that states the solubility of a compound in water (de Brito, 2011). Based on research of compounds

with LogS≥ 0 fall into the category of compounds that are very soluble in water, compounds with LogS between -2 and 0 belong to soluble category, LogS between -4 to -2 is slightly soluble, and <-4 is insoluble according to the classification (Sorkun , et al., 2019). Highly soluble test compounds based on these criteria are delphinidin-3-sambubioside and cyanidin-3-sambubioside, which belong to flavonoid compounds of the anthocyanin group. This can be revealed in the flavonoid nucleus there is a cation that is a positively charged oxygen atom. Based on the above results it can be known that compounds from rosela flowers such as anthocyanin group compounds solubility in water is high, this can explain that anthocyanins are compounds with the highest concentrations in rosela flower water extract compared to other compounds (Ramirez-Rodrigues, et al., 2011).

The hydrogen bond acceptors (HBA) and hydrogen bond donors (HBD) are associated with energy needed which is higher the bonding capacity of hydrogen, the energy needed for absorption processes to occur is higher (Syahputra, et al., 2014). Compounds with HBA ≤10 and HBD ≤5 have good permeability. Too much hydrogen bonding causes the compound to be more bound to the water phase so that the absorption of a compound will be disrupted (Lipinski, et al., 2001). Within 10 test compounds, there are 6 test compounds that have the amount of HBA ≤ 10 and there are 2 compounds that have HBD≤ 5. Delphinidin-3-sambubioside, cyanidin-3-sambubioside, myricetin-3-arabinogalactoside, and quercetin-3-rutinoside compounds have HBA> 10 and HBD> 5, this is influenced by the greater number of hydroxyl groups compared to flavonoid aglicon compounds, so in the absorption process will require higher energy because it will be more bound to the polar phase.

The pKa is an important factor, whether the drug administered per oral is absorbed more in the stomach than in the intestines. A drug compound has good absorption if its ionization degree is in between of 6-8 (Patrick, 2013). Based on the result in Table 1, there are 3 compounds that have pKa in the range of 6-8 which are myricetin, myricetin-3-arabinogalactoside, and quercetin-3-rutinoside. Three compounds are strong acids namely delphinidin-3-sambubioside, cyanidin-3-sambubioside, delphinidin, and cyanidin. Strong acid compounds are more soluble in water (hydrophilic), this may explain that delphinidin-3-sambubioside, cyanidin-3-sambubioside, delphinidin, and cyanidin compounds, which are the most numerous anthocyanin groups in the water extract of roselle's petal (Ramirez-Rodrigues, et al., 2011). In addition, the compounds delphinidin-3-sambubioside, cyanidin-3-sambubioside, delphinidin, and cyanidin, are the compounds responsible for the acid pH in roselle petal water extract. The pH of the roselle petal water extract is acidic, which is 2.5 (Higginbotham, et al., 2014).

The topological polar surface area (tPSA) is a molecular descriptor widely used for the study of ADMET and bioavailability of drug compounds (Li, et al., 2005). A compound has good bioavailability if the tPSA value≤ 140Å^2 (Veber, et al., 2002). In this study, protocatechuic acid had the smallest tPSA than the flavonoids due to the number of polar groups in protocatechuic acid was less than presented in flavonoids. The tPSA of aglycones, such as cyanidin, delphinidin, and myricetin compounds are smaller when compared with their glycosides. This is because the number of hydroxyl groups in the glycosides were higher than hydroxyl contained in the aglycone. From the result on physicochemical properties, it can be recognized two anthocyanin glycosides, delphinidin-3-sambubioside and cyanidin-3-sambubioside, were very soluble in water and possessed acidic properties. There were two water soluble and acidic compounds which have good tPSA values, namely cyanidin and delphinidin. Almost all the requirements of Lipinski's rules were not fulfilled by the test compounds of glycosides, except logP. However, their aglycones fulfill almost all the requirements, except for HBD which is more than 5. Among all test compounds, cyanidin met all requirement of Lipinski's rule.

Docking Result of ligands on Gyrase B

In this study ligands docked into binding site of Gyrase B including 10 test ligans, the native ligands ADP,and reference ligand. Quercetin was used as reference because it was the largest part of flavonoid compounds contained in the water extract of roselle's petal (Da-Costa-Rocha, et al., 2014), and it was reported as the potential antibacterial agent. It was known that quercetin could bound to gyrase B of *Escherichia coli* in experiment *in vitro* so that the DNA replication process was disrupted (Fang, et al., 2016) (Plaper, et al., 2003). The mechanism of inhibition of Gyrase B by quercetin was to inhibit the ATP

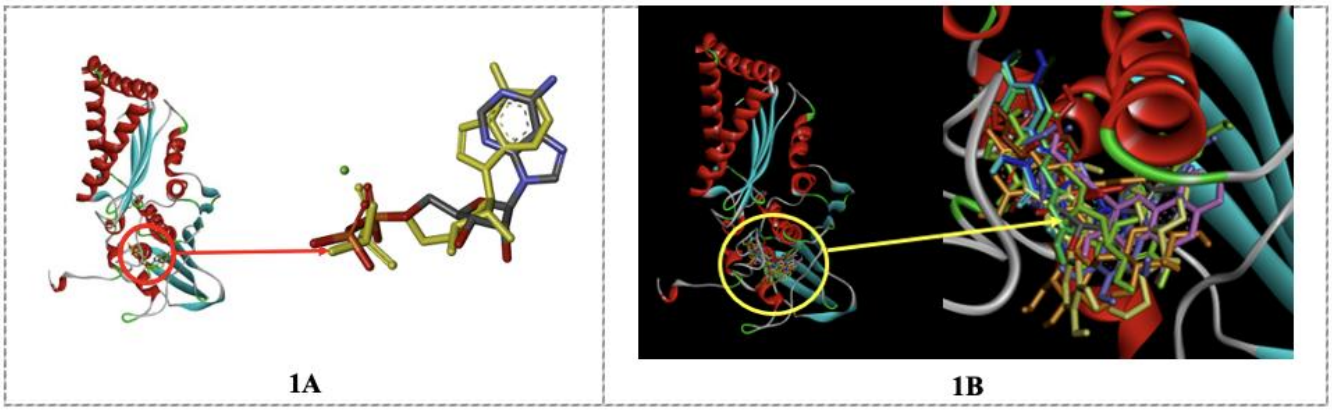

Figure 1. Overlap pose of native ligands before docking (blue color) and after docking (yellow color) in ATP binding site Gyrase B in the validation process (1A); Docked conformations of test ligands in the binding site of gyrase B

used for the activation of Gyrase B. When ATP was inhibited, Gyrase B would be inactive, and could not change the shape of bacterial DNA from circular to supercoil. Bacterial DNA which is not supercoil becomes easily degraded and there is no DNA replication (Campbell & Farrell, 2009). Research related to gyrase B as the new target for antibacterial drugs are being explored to fight the resistance of emerging bacteria (Barancokova , et al., 2018).

Docking validation resulted average RMSD value of 1.73±0.25Å, from three re-dockings of the original ligand in ATP binding site of gyrase B. A small RMSD value indicates that the conformation of the ligand complex with gyrase B is stable (Praja, et al., 2018). The docking method is declared valid if RMSD< 2Å (Jain and Nicholls, 2008). Visualization of the docking pose of native ligand after validation can be seen in Figure 1.

The docking results from AutodockTools 1.5.6 are ΔG^0, Ki, hydrogen bonding and amino acid residues (Praja, et al., 2018). The lower ΔG^0 indicates the higher stability of ligand-receptor interactions, and the lower Ki indicates more stable ligand-receptor complex (Dinata, et al., 2014). The docking results of test compounds can be seen in the table 2. Docking result showed the average ΔG^0 value of quercetin was -7.38 kcal/mol and the Ki was 4.22 μM. There are seven test compounds that have ΔG^0 smaller than quercetin, including cyandin, delphinidin, delphinidin-3-sambubioside, myricetin, cyandin-3-sambubioside, quercetin-3-rutinoside, and myricetin-3-arabinogalactoside.

Hydrogen bonding showed that test ligands had the same interaction as reference ligand. In addition to hydrogen bonds (H-bond), there were also hydrophobic interactions that showed predictions of test ligands occupying the binding site of the receptor as well as the reference ligand in gyrase B (Prasetiawati, et al., 2019). Based on docking results on Gyrase B the test ligands bound the same amino acid residues as in the quercetin, so it was concluded that the test compounds had the same mechanism of interaction. The majority of test compounds showed hydrogen bond with the same amino acid as in the quercetin, such as Gly102, except gossypetin and myricetin-3-arabinogalactoside. Most of test compounds interacted with the amino acid Lys103, except delphinidin, delphinidin-3-sambubioside, and cyanidin-3-sambubioside. For hydrophobic interactions, all test compounds interact with the same amino acid, Lys103 and Ile94, as in quercetin, except myricetin-3-arabinogalactoside and protocatechuic acids.

It was assumed that the binding affinity of delphinidin, cyanidin, and myricetin in their glycoside forms were higher than their aglycone. Myricetin showed higher binding affinity than delphinidin and cyanidin, and binding of delphinidin was higher than cyanidin for both form, glycosides and aglicones. This difference seemed to be related to the number of hydroxyl groups, and the conjugation of C4 carbonyl in myricetin compounds which lead to differences in the interaction with amino acids. The test compounds performed H-bonds by the OH group at position-4' except the glycoside of delphinidin, cyanidin, myricetin, and quercetin. This was influenced by the presence of sugar groups that can block the OH group at position-4' to interact with the amino acids. The H-bonds interaction in 2D was displayed in Figure 2.

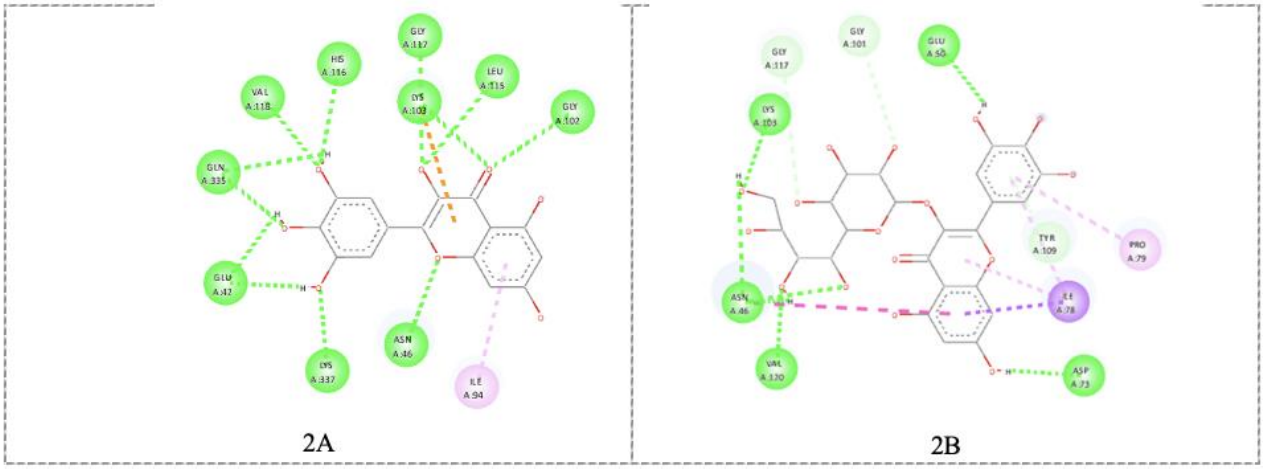

Figure 2. Comparison of H-bond interactions between myricetin, aglycone (2A) and its glycoside (2B)

Based on the docking results, myricetin-3-arabinogalactoside was the best binding ligand in gyrase B, its aglycone (myricetin) also showed higher binding than delphinidin and cyanidin. The percentage of potential compounds against gyrase B in the aqueous extract were quercetin-3-rutinoside 2.89%, cyanidin-3-sambubioside 14.25%, and delphinidin-3-sambubioside 29.90%, while the content of myricetin and its glycosides were not stated. This was may be due to their few amounts (Ramirez-Rodrigues, et al., 2011). The ratio of myricetin-3-arabinogalactoside with delphinidin-3-sambubioside was 1:47, and the myricetin content in the extract of roselle's calyx cannot be identified (Herranz-Lopez, et al., 2012).

When it was assumed based on the percentage of content in the extract, then the compounds that gave more contribution to the antibacterial activity of the extract was delphinidin and its glycoside. However, for the isolated compound, myricetin has the higher potential to be developed as antibacterial agent. Based on physicochemical properties and docking results, seven compounds were selected for ADMET study.

ADMET Profiles

The ADMET properties of selected compounds were displayed in Table 3. Based on absorption parameters, there are no compounds showed good caco2 permeability. However, three compounds have good absorption across intestinal membrane, two of them were aglycone.

Quercetin-3-rutinoside was the glycoside that was predicted to be absorbed via intestinal membrane. Cyanidin has the highest percentage in intestinal absorption due to its physicochemical properties that met the criteria of Lipinski's rule.

All compounds have a moderate VDss (volume of distribution), meaning that the compounds were distributed into body tissues but they did not show ability to penetrate the blood-brain barrier (BBB permeability less than -1). Distribution is the stage of pharmacokinetics where the drug reaches systemic circulation. Large volume of distribution cause low plasma concentrations due to wide distribution into body tissues (Aslam, et al., 2003).

Metabolism is the change of lipophilic drugs that are pharmacologically active into hydrophilic (water soluble) and pharmacologically inactive drugs (Ratnadi and Sujana, 2017). Enzymes that worked in the drug metabolism are Cytorochrome P450 (CYP450) which consists of several isforms. The drugs or xenobiotics could be a substrate or an inhibitor of the CYP450 that will interfere in metabolism of the other drugs. CYP450 is an important detoxification enzyme in the body that is mainly found in the liver. As a substrate, drug can induce the enzyme which lead to increase in certain drug metabolism so that the drug becomes less effective. In contrast, as an inhibitor, drug inhibit the enzyme activity resulted in the increased concentration of other drug increased in the blood. Delphinidin was predicted to be CYP1A2 inhibitor and cyanidin was predicted to inhibit CYP1A2, CYP2C19 and CYP2C9. Further research is needed to study their *in vivo* metabolism and the side effects. Three compounds that did not act as substrates or inhibitors of CYP450 were glycosides of delphinidin, cyanidin, and quercetin. This finding was interesting because the attached sugar tend

Table 3. ADMET profiles of test compounds

ADMET parameters		Compound No.						
		1	2	3	4	5	6	7
Absorption:								
Caco2 Permeability	($logP_{app}$,10^{-6} cm/s)	-0.011	-0.629	-0.472	-0.851	0.095	-0.229	-0.857
Intestinal Absorption	(% Absorbed)	78.14	26.15	71.024	16.126	65.93	77.207	32.633
Distribution:								
VDss (human)	(Log L/kg)	-0.033	0.291	-0.131	0.11	1.317	1.559	0.34
BBB Permeability	(log BB)	-1.407	-1.961	-1.766	-2.321	-1.493	-1.098	-1.756
Metabolism:								
substrate	CYP2D6	Yes	No	Yes	No	No	No	No
	CYP3A4	No	No	No	No	No	No	No
inhibitor	CYP1A2	Yes	No	Yes	No	Yes	Yes	No
	CYP2C19	Yes	No	No	No	No	No	No
	CYP2C9	Yes	No	No	No	No	No	No
	CYP2D6, CYP3A4	No	No	No	No	No	No	No
Excretion:								
Renal OCT2 substrate		No	No	No	No	No	No	No
Clearance total (Log ml/ min/kg)		0.684	-0.057	0.648	-0.196	0.422	0.407	-0.324
Toxicity:								
Rat LD_{50} (g/kg)		1616	1453	844	713	795	747	1509
AMES Toxicity		No	Yes	No	Yes	No	No	No
Hepatotoxicity		No	No	No	No	No	No	No

Notes: compound no. 1= Cyanidin, 2= Cyanidin-3-sambubioside, 3= Delphinidin, 4= Delphinidin-3-sambubioside, 5= Myricetin, 6= Quercetin, 7= Quercetin-3-rutinoside; BBB permeability= blood brain barrier permeability.

to decrease the absorption by passive diffusion, readily to be destroyed by gastric acid or enzyme, but on the other hand the survive glycoside could have contribution in maintain the metabolic stability of the compounds.

The parameter of excretion stage is clearance (Cl) which is a measure of the removal of a drug from plasma or blood volume containing the drug for a unit of time (Aslam, et al., 2003). Drug excretion is elimination of the drug from systemic circulation that generally conducted by kidneys along with urine. Cyanidin was the compound that has the highest total clearance, which related with its MW that smaller than 300 (MW= 285.25 g/mol), while the glycoside compounds have negative Cl value due to their large MW. The larger MW caused the clearance of the compound getting lower so the amount of compound removed from the body also smaller. In addition to clearance, the parameter of the excretion was the OCT2-substrate. Renal OCT2 is a transporter on the renal that has an important role for disposition and renal clearance of drug and endogenic compounds. This parameter can be used to know the potential contraindications that will arise (Pires, et al., 2015). All test compound were not acted as renal OCT2-substrate.

The importance to predict the toxicity of compounds to be developed as drugs are to figure out the safety of the compounds to the body. Rat LD_{50} is used as standard for determination of acute toxicity (Pires, et al., 2015). According to the Badan Pengawas Obat dan Makanan (BPOM), when $LD_{50}\geq$ 15g/kg, the compound falls into the category of relatively harmless (BPOM, 2014). All test compounds were predicted to be at 6^{th} level toxicity (LD_{50} greater than 15g/kg) which meant compounds relatively not acutely toxic. The parameter AMES toxicity is a broad method for assessing the occurrence of mutagens. When the test result is

positive (yes), then the compound could be a mutagen and can causes carcinogenicity (Pires, et al., 2015). Delphinidin-3-sambubioside and cyanidin-3-sambubioside were predicted to have AMES toxicity. The other important parameter of toxicity is hepatotoxicity. Hepatotoxic compounds have the capability to induced impaired function of liver (Pires, et al., 2015). Fortunately, all test compounds were not hepatotoxic. Based on ADMET profiles, the potential antibacterial compounds which showed good ADMET properties were delphinidin and myricetin.

CONCLUSION

Among ten compounds from aqueous extract of roselle's calyx, five compounds have higher inhibitory activity against gyrase B than quercetin as native ligand. Based on the high percentage of availability in the extract, the compounds that have high contribution to the antibacterial activity of the extract were glysosides of quercetin, cyanidin, and delphinidin.

As a single compound, the myricetin and delphinidin generated better binding interaction than the inhibitor of gyrase B which indicated that they have higher potential as antibacterial agents. Based on all evaluation of physicochemical properties, in silico binding on gyrase B, and ADMET profiles, myricetin and delphinidin were promoted to be developed further as antibacterial agents.

ACKNOWLEDGMENT

Thank to the Faculty of Pharmacy, Airlangga University for providing research facilities

REFERENCES

Barancokova , M., Kikelj, D. & Ilas, J., 2018. Recent progress in the discovery and development of DNA gyrase B inhibitors. *Future Medicinal Chemistry.*

Campbell, M. K. & Farrell, S. O., 2009. *Biochemistry.* 6 penyunt. Belmont: Thomson Higher Education.

Cushnie, T. T. & Lamb, A. J., 2005. Antimicrobial activity of flavonoids. *International Journal of Antimicrobial Agents,* 26(5), pp. 343-356.

Da-Costa-Rocha, I. et al., 2014. Hibiscus sabdariffa L. – A phytochemical and pharmacological review. *Food Chemistry,* Volume 165, pp. 424-443.

de Brito, M. A., 2011. Pharmacokinetic Study with Computational Tools in the Medicinal Chemistry Course. *Brazilian Journal of Pharmaceutical Sciencer,* 47(4), pp. 797-805.

Dinata, D. I., Rendrika, R. & Kustida, Y. N., 2014. Pengaruh Aktivitas Senyawa Flavonoid Yang Terdapat Dalam Madu Terhadap Reseptor H4R Sebagai Antiinflamasi. *Jurnal Farmasi Galenika ,* 01(02), pp. 61-70.

Fang, Y. et al., 2016. 3D-QSAR and docking studies of flavonoids as potent Escherichia coli inhibitors. *Scientific Reports,* Volume 6, pp. 1-13.

Herranz-Lopez, M. et al., 2012. Synergism of plant-derived polyphenols in adipogenesis: Perspectives and implications. *Phytomedicine,* Volume 19, pp. 253-261.

Higginbotham, K. L. et al., 2014. Aqueous Extracts of Hibiscus sabdariffa Calyces as an Antimicrobial Rinse on Hot Dogs Against Listeria Monocytogenes and Methicillin-resistant Staphylococcus aureus. *Journal of Food Control ,* Volume 40, pp. 274-277.

Isnaeni, I., Hendradi, E. & Zettira, N. Z., 2020. Inhibitory Effect of Roselle Aqueous Extracts-HPMC 6000 Gel on the Growth of Staphylococcus Aureus ATCC 25923. *Turk J. Pharm Science,* 17(2), pp. 190-196.

Lipinski, C. A., Lombardo, F., Dominy, B. W. & Feener, P. J., 2001. Experimental and Computational Approaches to Estimate Solubility and Permeability in Drug Discovery and Development Settings. *Advanced Drug Delivery Reviews,* Volume 46, pp. 3-26.

Li, S. et al., 2005. IV-IVC Considerations in the Development of Immediate-Release Oral Dosage Form. *Journal of Pharmaceutical Sciences,* 94(7), pp. 1396-1417.

Palczar, J. M. & Chan, E., 1988. *Dasar-dasar Mikrobiologi 2.* Jakarta: Penerbit UI Press..

Pires, D. E. V., Blundell, T. L. & Ascher, D. B., 2015. pKCSM: Predicting Small-Molecule Pharmacokinetic ann Toxicity Properties Using Graph-Based Signatures. *Journal of Medicinal Chemistry ,* Volume 58, pp. 4066-4072.

Plaper, A. et al., 2003. Characterization of quercetin binding site on DNA gyrase. *Biochemical and Biophysical Research Communications,* Volume 306, pp. 530-536.

Praja, R. A., Pratiwi, D. & N., 2018. Studi Penambatan Molekuler Senyawa Scopoletin dari Buah Mengkudu (Morinda

Citrifolia L.) Pada Enzim ACE Sebagai Antihipetensi. *Farmagazine,* V(1), pp. 40-47.

Ramirez-Rodrigues, M. M. et al., 2011. Physicochemical and Phytochemical Properties of Cold and Hot Water Extraction from Hibiscus sabdariffa. *Journal of Food Science,* 76(3), pp. 428-435.

Ratnadi , P. C. & Sujana, I. B. G., 2017. *Prinsip Dasar Farmakologi.* Denpasar : Rumah Sakit Umum Pusat (RSUP) Sanglah Denpasar.

Ross, I. A., 2003. *Medical Plants of The World.* 1 penyunt. Totowa, New Jersey: Humana Press Inc.

Sorkun , M. C., Khetan, A. & Er, S., 2019. AqSolDB, a Curated Reference Set of Aqueous Solubility and 2D Descriptors for a Diverse Set of Compounds. *Scientific Data,* 6(143).

Syahputra, G., Ambarsari, L. & Sumaryada , T., 2014. Simulasi Docking Kurkumin Enol, Bismetoksikurkumin dan Analognya Sebagai Inhibtor Enzim 12-Lipoksigenase. *Jurnal Biofisika ,* 10(1), pp. 55-67.

Vargas, F. M. et al., 2009. Development of a General Method for Modeling Asphaltene Stability. *Energy and Fuels ,* Volume 23, pp. 1147-1154.

Veber, D. F. et al., 2002. Molecular Properties That Influence the Oral Bioavailability of Drug Candidates. *Journal of Medicinal Chemistry ,* 45(12), pp. 2615-2623.