Sys Rev Pharm 2020;11(10):290-296
A multifaceted review journal in the field of pharmacy

In Silico Study on Antibacterial Activity and Brazilein ADME of Sappan Wood (Caesalpinia Sappan L.) Against *Escherichia coli* (Strain K12)

Dwi Krihariyani[1,2], Eddy Bagus Wasito[3*], Isnaeni Isnaeni[4], Siswandono Siswodihardjo[4], Wiwik Misaco Yuniarti[5], Entuy Kurniawan[6]

[1]Doctoral Program, Faculty of Medicine, Universitas Airlangga, Indonesia; [2]Health Polytechnics of Surabaya, Ministry of Health, Indonesia; [3]Faculty of Medicine, Universitas Airlangga, Indonesia; [4]Faculty of Pharmacy, Universitas Airlangga, Indonesia
[5]Faculty of Veterinary Medicine, Universitas Airlangga, Indonesia; [6]Health Polytechnics of Bandung, Ministry of Health, Indonesia

***Corresponding Authors**
Eddy Bagus Wasito, **E-mail**: eddy-b-w@fk.unair.ac.id ;
Dwi Krihariyani, **E-mail :** dwi.krihariyani-2018@fk.unair.ac.id

ABSTRACT
This study aims to perform in silico analysis for revealing the antibacterial activity of brazilein compounds contained in sappan wood against the ESBL enzyme derived from *Escherichia coli* multiresistant isolates of UTI patients together with the antibacterial activity of meropenem as the comparative compound. The in silico test was conducted to predict antibacterial activity by docking using the Molegro Virtual Docker computer program. The receptor used was beta-lactamase AmpC, PDB code: 1LL5, together with an imipenem ligand (IM2-370). In addition to predicting the antibacterial activity, this study also aims to predict physicochemical and pharmacokinetic properties (ADME) as well as toxicity of brazilein and meropenem with the pkCSM online tool program. Next, the data obtained were analyzed by comparing the docking bond energy between brazilein, imipenem ligand, and meropenem with the target receptor. The lower the ligand bond energy than the target receptor is, the more stable the bond is formed. Hence, this can be used to predict the biological activity of compounds. In silico test results showed that the bond energy of brazilein was -77.4202 kcal/mol, -98.2425 kcal/mol for meropenem, and -85.8187 kcal/mol for imipenem. Brazilein has potential as an antimicrobial even though it is lower than meropenem and imipenem. Based on the results of in silico test using the pkCSM online tool program, the brazilein compound also has good pharmacokinetic properties causing relatively low toxicity.

Keywords: ADME, AmpC beta-lactamase, brazilein, meropenem, in silico test

Correspondence:
Eddy Bagus Wasito
Faculty of Medicine, Universitas Airlangga, Indonesia
E-mail: eddy-b-w@fk.unair.ac.id
Dwi Krihariyani
Doctoral Program, Faculty of Medicine, Universitas Airlangga, Indonesia; Health Polytechnics of Surabaya, Ministry of Health, Indonesia
E-mail: dwi.krihariyani-2018@fk.unair.ac.id

INTRODUCTION
Urinary tract infection (UTI) is an infection caused by microorganisms in the urinary tract, which starts from an infection in the urinary tract, and then infects the genitalia and even the kidneys[1]. *Escherichia coli* is a gram-negative bacteria in the Enterobacteriaceae group that dominates the cause of UTI. *Escherichia coli* isolated from urine cultures are generally Multiple Drug Resistant (MDR), but are still sensitive to the antimicrobials of carbapenem and aminoglycosides. Carbapenem antimicrobials, nevertheless, are still considered as the last option in treatments of infection cases caused by *Escherichia coli* multiresistant[2].
MDR, moreover, usually occurs because of the resistant coding gene in the plasmid, so the bacteria can produce the enzyme Extended Spectrum Beta-Lactamase (ESBL). ESBL is an enzyme that has an ability to hydrolyze the antibiotics of penicillin, cephalosporins of generation I, II, and III, as well as aztreonam[3]. Besides, ESBL is most widely produced by Enterobacteriaceae, especially *Escherichia coli* and *Klebsiella pneumoniae*. ESBL is also derived from the mutated beta-lactamase enzyme, triggering an increase in the enzymatic activity of beta-lactamase so that this enzyme can hydrolyse generation III and IV cephalosporins and aztreonam[4]. Furthermore, the Canadian External Quality Assessment Advisory Group for Antibiotics states that genes controlling the production of beta-lactamase are located in the plasmid or chromosome. This facilitates the ability of the ESBL gene to move from one organism to another so that the spread of resistance is very easy between strains and even between species. Plasmids are also responsible for coding genes that carry resistance genes for other classes of drugs, such as aminoglycosides. This situation makes the choice of antibiotics against organisms that produce ESBL very limited[5].
Secang wood (Caesalpinia sappan L.) is one of the medicinal plants in Indonesia that can act as an antibacteria, antioxidant, and anti-inflammatory[6,7]. Secang wood contains five active compounds related to flavonoids. The five compounds are brazilin, brazilein, 3'-O-methylbrazilin, sappanin, chalcone, and sappancalchone. Brazilein is a major compound contained in sappan wood. Secang wood, according to some studies, reveal some benefits of sappan wood, such as inhibiting Mesenchymal Stem Cells Senescence[8], reducing liver damage level caused by excessive amounts of iron[9], increasing motility, percentage viability, and sperm concentration of male wistar rats[10], providing antigenotoxic effects on exposure to cyclophosphamide mutagen compounds based on micronucleus assays[11], increasing cytotoxicity and induction of apoptosis in T47D cells[12], inhibiting the activity of rheumatoid arthritis in rats induced by collagen arthritis type-II[13], and inhibiting catabolic processes in human osteoarthritic chondrocytes through inhibition of NFκB1 / p50[14].
Hence, to determine the activity of brazilein as an antibacterial MDR, it is necessary to perform an in silico

test with a computer program, both offline and online. In silico test is a test approach to predict physicochemical properties of molecules, pharmacokinetic properties (absorption, distribution, metabolism and excretion = ADME) of molecules, interaction of compounds with receptors, prediction mechanism of action, as well as selectivity and toxicity of compounds. This test also has several advantages, such as safe, free from chemical waste, easy, cost-effective, and fast.

In addition, as a comparison of the potential of brazilein against *Escherichia coli* MDR, a sensitive antibiotic, meropenem, was used. For ligands or molecules that have shown good biological activity and are able to bind the desired biological targets (receptors) (docking process) on protein data banks are imipenem (PDB ID: 1LL5)[15,16].

Brazilein secang wood actually can be developed in a drug design as an antibacterial drug. Drug design is often described as a systematic elaboration process to further develop existing drugs with the aims of obtaining new drugs with better activity and reducing or eliminating side effects through molecular manipulation. Molecular manipulation or structural modification is to synthesize a number of parent strains, identify structures, and test their biological activities. Changes in the structure of a compound can change the physicochemical properties of compounds, including lipophilic, electronic and steric properties, and also cause changes in the biological activity of compounds. To be more effective and efficient in modifying the structure, the physicochemical and pharmacokinetic properties (ADME) as well as toxicity of an synthesized compound should be predicted first to get the picture of drug interactions with receptors. As a result, this study aims to reveal the potential of brazilein as an antibacterial MDR using in silico method[17] .

MATERIALS AND METHOD

Materials

The 3-dimensional (PDB ID: 1LL5) X-ray crystal structure of AmpC WT beta-lactamase in complex with covalently bound imipenem was downloaded from http://www.rcsb.org/pdb/home.do. Next, the 3-dimensional structures of brazilein, meropenem, and imipenem were downloaded from https://pubchem.ncbi.nlm.nih.gov/compound/.

Tools

A set of computers with Windows 8 64 bit specifications and ChemDraw Professional 16.0, Chem3D 16.0, and Molegro Virtual Docker 5 programs.

Procedure

Activity prediction (molecular docking)

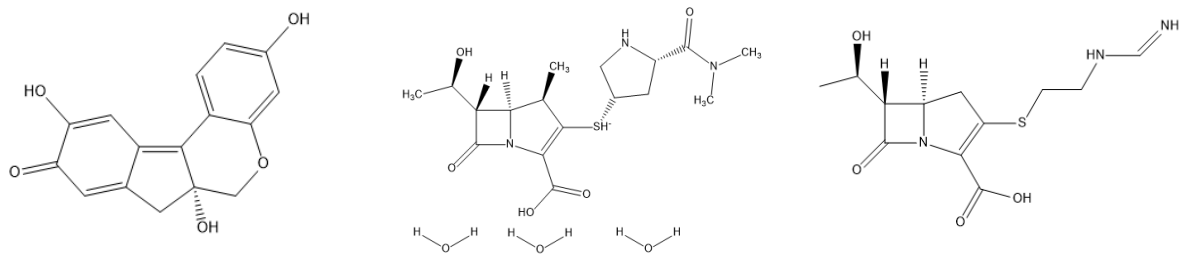
Compounds to be docked are brazilein, meropenem, and imipenem which 2-D structures were drawn using ChemDraw Professional 16.0., and then converted to the 3-D ones using Chem3D 16.0. determined as the most stable conformation. After measuring their minimum energy, they then were stored in the form of mol2 {SYBYL2 (*. Mol2)}. Meanwhile, the structure of the *Escherichia coli* ESBL protein (PDB ID: 1LL5) was obtained from the Protein Data Bank. The results obtained were in the form of Rerank Score (RS), namely energy required in the process of ligand-receptor interaction, and then based on these values the antibacterial activity of secang wood (Caesalpinia sappan L.) against *Escherichia coli* ESBL could be predicted.

Prediction of physicochemical and pharmacokinetic properties as well as toxicity of compounds (pkCSM)

Prediction of physicochemical properties, such as: Molecular Weight (BM), logarithm of octanol / water partition coefficient (Log P), number of bonds between rotating atoms (Torsion); Hydrogen Bond Acceptors (HBA), Hydrogen Bond Donors (HBD), and Polar Surface Activity (PSA), were performed using the pkCSM online tool. Similarly, prediction of pharmacokinetic properties (ADME: absorption, distribution, metabolism and excretion) as well as toxicity of brazilein, meropenem, and imipenem compounds were conducted using the pkCSM online tool. First, the 2-D molecular structures of brazilein, meropenem, and imipenem compounds were drawn with the ChemDraw Professional 16.0 program, and then copied in the Chem3D 16.0 program to make their 3-D structures before stored in *.sdf files. Second, the structures of brazilein, meropenem, and imipenem were translated into the SMILES format using the Online SMILES Translator (https://cactus.nci. Nih.gov/translate/). In the SMILES format, those compounds then were processed using the online pkCSM tool (http://biosig.unimelb.edu.au/pkcsm/prediction) to predict ADME and compound toxicity. Afterwards, to predict oral toxicity (LD50) in the globally harmonized system (GSH), Protox online tool was also used (http://tox.charite.de/tox/)[17,18].

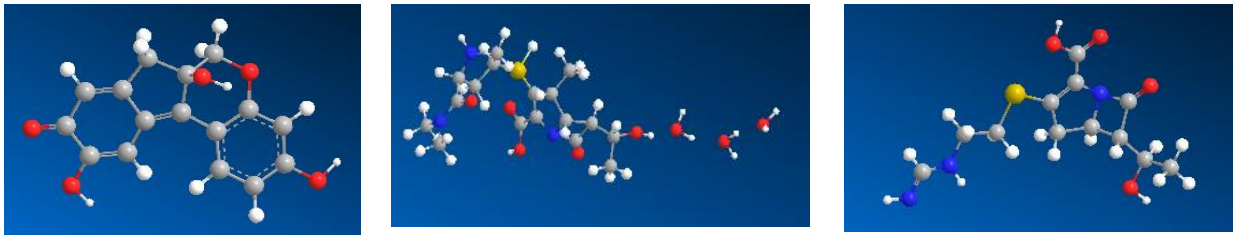
RESULTS AND DISCUSSION

The 2-D structures made with ChemDraw Professional 16.0 were shown in (Figure 1). Next, the 2D structures then were altered into the 3-D ones with Chem3D 16.0. The 3D structures then were used at all docking stages as illustrated in (Figure 2).



(a) (b) (c)

Figure 1. The 2D structures of (a) Brazilein; (b) Meropenem; (c) Imipenem compounds



(a) (b) (c)

Figure 2. The 3-D structures of (a) Brazilein; (b) Meropenem; (c) Imipenem compounds stored in SYBYL2

Activity prediction with docking and amino acid analysis

Protease receptors already downloaded in Protein Data Bank with code 1LL5 and then imported in the Molegro Virtual Docker program were depicted in (Figure 3). The detection results of the place where ligands and receptors (cavity) were interacting on the Protease 1LL5 receptor [A] were shown in (Figure 4). The Cavity used was Cavity 1 (volume 433,152) with active Ligand IM2_370 [A] since it has an area where the original ligand interacts with the protease enzyme.

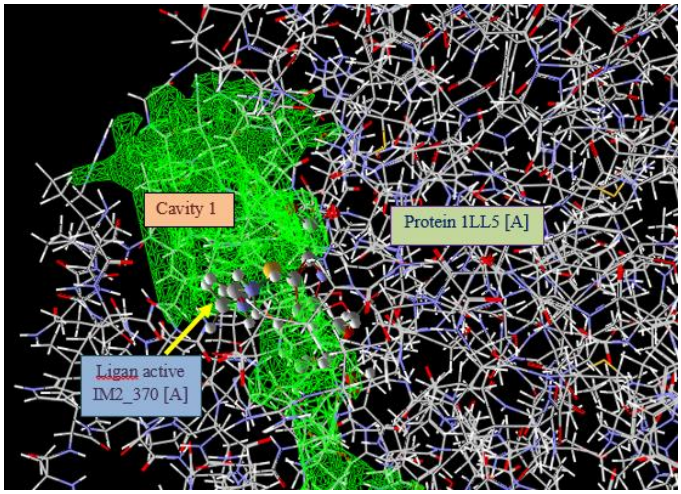
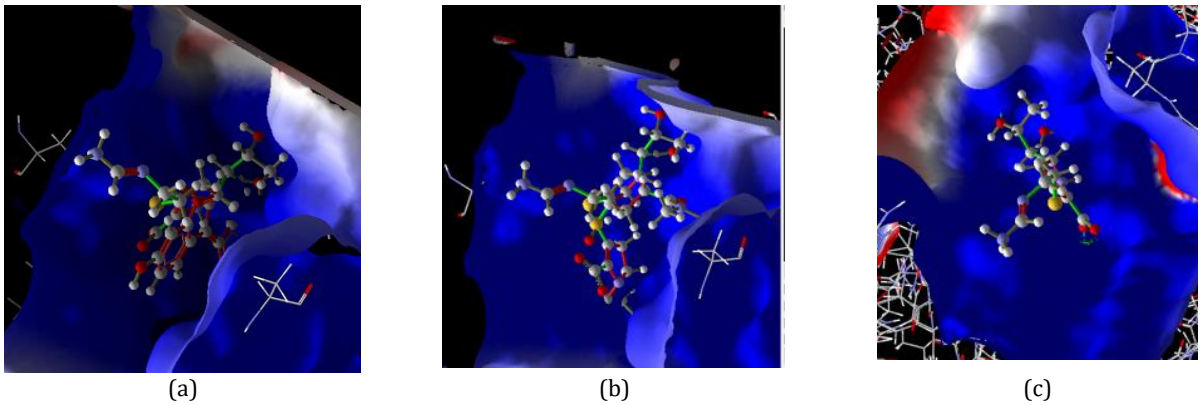


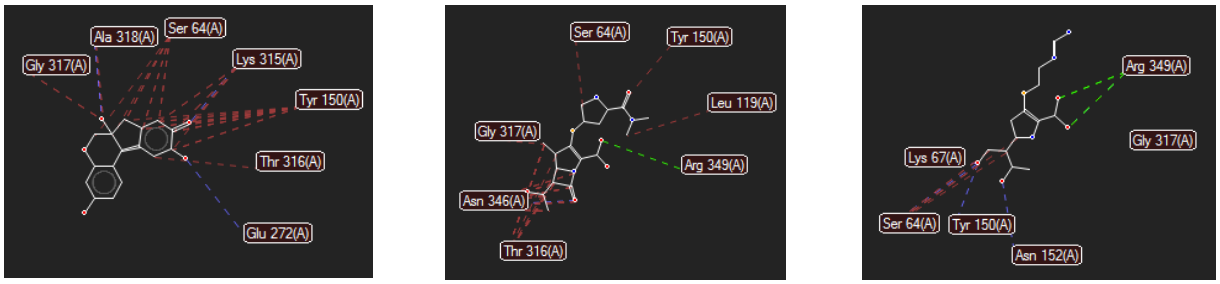
Figure 3. Protease Receptors (PDB code 1LL5) with cavity 1 (volume 433,152) and active Ligand IM2_370 [A]



(a) (b) (c)

Figure 4. Cavity 1 and Ligan (a) Brazilein; (b) Meropenem; (c) Imipenem

In the interaction of the ligand and the receptor, there is also an interaction of the ligand with some amino acid residues derived from Protease receptor 1LL5. Amino acids involved in the process of interaction of brazilein, meropenem and imipenem compounds with Protease receptors 1LL5 can be seen in Figure 5 and Table 1. The interactions of amino acid receptor residues with compounds occur through lipophilic / hydrophobic, electronic, and steric receptors. In (Figure 5) and Table 1, it can actually be seen the differences of interactions between each brazilein, meropenem and imipenem compounds with Protease receptor 1LL5 since there are differences in the spatial configuration of the structure of the three compounds.



(a) (b) (c)

Figure 5. Interactions of Ligand and 1LL5 receptor [A] (H-Bond, electronic, steric) (a) Brazilein; (b) Meropenem; (c) Imipenem

Table 1. Interactions of ligands and 1LL5 receptors [a] (h-bond, electronic, steric)

Ligand	The Bonding of Hydrogen and Amino Acid Residues		Interactions of Electrostatics and Amino Acid Residues		Interactions of Steric and Amino Acid Residues	
Brazilein	3	Ala 318(A) Lys 315(A) Glu 272(A)	0	-	7	Ala 318(A) Gly 317(A) Ser 64(A) Tyr 150(A) Lys 315(A) Glu 272(A) Thr 316(A)
Meropenem	1	Asn 346(A)	1	Arg 349(A)	6	Gly 317(A) Thr 316(A) Asn 346(A) Ser 64(A) Tyr 150(A) Leu 119(A)
IM2_370[A]	5	Lys 67(A) Ser 64(A) Tyr 150(A) Asn 152(A) Arg 349(A)	1	Arg 349(A)	6	Lys 67(A) Ser 64(A) Tyr 150(A) Asn 152(A) Arg 349(A) Gly 317(A)

The re-docking results of brazilein, meropenem and imipenem compounds with 1LL5 Protease receptors can be seen in Table 2. The binding energy of brazilein with 1LL5 receptors [A] was higher than that of meropenem and imipenem. The results also showed that the rerank score of Brazilein was -77.4202 kcal / mol, -98.2425 for the rerank score of meropenem, and -85.8187 for the rerank score of imipenem. Based on those rerank scores, brazilein provided higher energy than meropenem and imipenem, so it was less stable in binding to receptors than meropenem and imipenem.

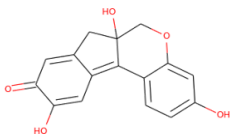
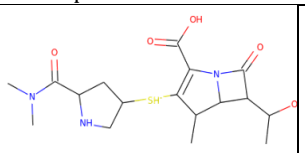
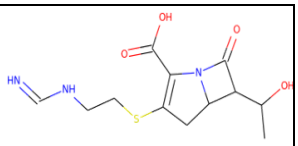
Table 2. Results of re-docking using the molegro virtual docker batchjob program

File name	Ligand	Rerank Scores
[02]Brazilein.mvdml	Brazilein	-77.4202
[03]Meropenem.mvdml	Meropenem	-98.2425
[00]IM2_370[A].mvdml	Imipenem	-85.8187

Prediction of physicochemical and pharmacokinetic properties as well as toxicity of compounds (pkCSM)

The in silico prediction results of the physicochemical properties of brazilein, meropenem, and imipenem compounds can be seen in Table 3. Based on a study conducted by Lipinski et al. (1997) on 2,245 drugs from the World Drugs Index baseline data argues that the compound would be difficult to absorb and its permeability would be low if the molecular weight is greater than 500, the log coefficient value of octanol / water (log P) is greater than +5, it has a donor H-bond (HBD), expressed in terms of O-H and N-H groups, greater than 5, or it has an H-acceptor (HBA) bond, expressed by the number of O and N atoms, greater than 10. The analysis above is known as the Lipinski's five law since all values are multiples of five[19]. Table 3 then indicates that brazilein, meropenem, and imipenem have molecular weights less than 500, logP values less than 5, as well as acceptor and donor values less than 10. Hence, it can be concluded that the three compounds are easily absorbed.

Table 3. In silico value predictions of the physicochemical properties of brazilein, meropenem, and imipenem compounds

SMILES Structures	 Brazilein	 Meropenem	 Imipenem
BM	284.267	384.478	299.352
LogP	1.624	-1.2263	-0.1760
Torsion	0	5	7
HBA	5	6	5
HBD	3	3	4
PSA (A^2)	119.652	156.137	120.082

Note: BM = Molecular Weight; Log P = logarithm of octanol / water partition coefficients; Torsion = a bond between atoms that can rotate; HBA = Hydrogen Bond Acceptors; HBD = Hydrogen Bond Donors; PSA = Polar Surface Activity.

Afterwards, the in silico prediction results of the pharmacokinetic properties (ADME) and toxicity of Brazilein, Meropenem, and Imipenem compounds can be seen in Table 4.

Table 4. The in silico predictions of the pharmacokinetic properties (ADME) and toxicity of Brazilein, Meropenem, and Imipenem compounds

ADMET	Brazilein	Meropenem	Imipenem
Intestinal absorption (human) (%)	92.111	33.227	37.266
Skin Permeability (log Kp)	-3.443	-2.735	-2.735
VDss (human) (log L/kg)	0.154	-0.951	-1.283
BBB permeability (log BB)	-0.709	-0.739	-1.139
CYP2D6 substrate (Yes/No)	No	Yes	Yes
CYP2D6 inhibitior (Yes/No)	No	No	No
Total Clearance (log ml/min/kg)	0.264	0.629	0.72
Renal OCT2 substrate (Yes/No)	No	No	No
AMES toxicity (Yes/No)	Yes	No	No
LD50 (mol/kg)	2.024	1.984	1.785

Note: VDSS: Steady State of Volume Distribution, BBB: Blood Brain Barrier, CYP2D6: Cytochrome P2D6, Renal OCT2: Renal Organic Cation Transporter 2

According to Chander et al. (2017), a compound can be considered to have a good absorption if its absorption value is > 80%; meanwhile its absorption is considered to be bad if it is <30%. Besides, intestine is considered as the main organ absorbing drugs given orally[20]. Based on Table 4 it can be seen that the intestinal absorption (human) value of brazilein, meropenem, imipenem compounds is more than 80% and not less than 30%. It indicates that the three compounds have good absorption. Moreover, according to Pires et al. (2015), a compound is considered to have relatively low skin permeability if it has a log value of Kp> -2.5. Based on Table 4, the values of Skin Permeability (log Kp) of brazilein, meropenem, and imipenem compounds ranged from -2.7 to -3.4, not less than -2.5. It means that all three of these compounds have good skin permeability[18].

Furthermore, volume distribution (VDSS) is a theoretical volume indicating the total dose of a drug distributed evenly to give the same concentration as in blood plasma. The higher the VD value is, the more drugs are distributed in the tissue rather than plasma. According to Pires et al. (2015), a compound is considered to have a low Volume Distribution when the Log VD value was <-0.15, and considered to have a high one if the Log VD value was > 0.45. Based on Table 4, the VDss (Steady State of Volume Distribution) value of brazilein compounds was 0.154, -0.951 for meropenem, and -1.283 for imipenem. Therefore, it can be said that all the derivatives of these compounds can be distributed evenly to give the same concentration as in blood plasma.

The ability of drugs to penetrate the blood brain barrier can actually be considered as an important parameter in reducing side effects and toxicity or in increasing the efficacy of drugs which pharmacological activity is in the brain. Brain-blood permeability measured in vivo in animal models then can be considered as logBB, which is the ratio of logarithmic concentrations in the brain to plasma.

In addition, according to Pires et al. (2015), a compound is said to be able to penetrate the blood brain barrier well if it has a Log BB value of > 0.3; meanwhile, it cannot be able to penetrate the blood brain barrier well if the BB log value is <-1. Based on Table 5, the BB log value of the brazilein compound was -0.709, -0.739 for meropenem, and -1.139 for imipenem. It indicates that all the derivatives of these compounds are able to penetrate the blood brain barrier in a moderate manner since the BB log value of each compound was greater than -1.

Besides, it is generally known that most metabolic reactions will involve oxidation processes. Cytochrome P450 is an important detoxification enzyme in the body, and mainly found in the liver. Cytochrome P450 can

oxidize foreign organic compounds, including drugs, as well as facilitate the excretion of these compounds. On the other hand, enzyme inhibitors, such as grapefruit juice, can affect the metabolism of the drug, contraindicating against the cytochrome P450 enzyme. Consequently, it is important to assess the ability of compounds that can inhibit cytochrome P450, represented by cytochrome CYP3A4 isoforms in this study. Besides, based on Table 4, brazilein, meropenem, and imipenem compounds are known not to be able to affect or inhibit the CYP3A4 enzyme. As a result, it can be predicted that these compounds tend to be metabolized by the P450 enzyme.

To predict the compound excretion process, furthermore, the Total Clearance (CLTOT) and Renal Organic Cation Transporter 2 (OCT2) substrate were measured. CLTOT is a combination of hepatic clearance (metabolism in the liver and bile) and renal clearance (excretion through the kidneys). This is related to bioavailability, which is important to determine the dose level in achieving steady-state concentration. Based on Table 4, the CLTOT value of brazilein was 0.264, 0.629 for meropenem, and 0.72 for imipenem. Based on these values, the speed of excretion of compounds then can be predicted.

Organic Cation Transporter 2 is a transporter in the kidney which plays an important role in the disposition and clearance of drugs and endogenous compounds. OCT2 substrate also has the potential to cause side interactions if given together with OCT2 inhibitors. Based on Table 4, it then can be seen that the three compounds did not affect the OCT2 substrate. Hence, it can be predicted that the derivative is not an OCT2 substrate.

Subsequently, to determine the toxicity of a compound, Ames Toxicity test was carried out. The Ames Toxicity Test is a widely used method for assessing the mutagenic potential of compounds using bacteria. Positive test results indicate that the compound is mutagenic and can therefore act as a carcinogen. Based on Table 4, it then can be said that brazilein is assumed to cause mutagenic effects. Meanwhile, meropenem and imipenem are assumed not to cause mutagenic effects.

In addition, the results showed that brazilein bond energy is higher than meropenem and target receptors. It means that brazilein has potential as an antimicrobial. But, the in silico molecular docking value of brazilein was lower than that of meropenem and target receptors. Thus, it can be said that the physicochemical and pharmacokinetic properties as well as toxicity of brazilein compounds is assumed to be very well absorbed in the intestine since it can have good skin permeability, can be distributed evenly to provide the same concentration as in blood plasma, can be able to penetrate the blood-brain barrier moderately, can be metabolized by enzyme P450, can have a relatively low toxicity, and can have the greatest cytotoxic activity using the pkCSM online tool.

CONCLUSION

Finally, it can be concluded that Brazilein bond energy is higher than meropenem and target receptors. Based on the binding energy values, moreover, brazilein has potential as an antimicrobial, but its in silico molecular docking value is lower than meropenem and target receptors. It means that the physicochemical and pharmacokinetic properties as well as toxicity of brazilein compounds are assumed to be very well absorbed in the intestine, has good skin permeability, be distributed evenly to provide the same concentration as in blood plasma, be able to penetrate the blood-brain barrier moderately, be metabolized by enzyme P450, has a relatively low toxicity, and have the greatest cytotoxic activity using the pkCSM online tool.

ACKNOWLEDGMENT

We would like to extend our sincere gratitude and appreciation to Prof. Dr. Siswandono, Apt., M.S. from Faculty of Pharmacy, Universitas Airlangga who has a Molegro Virtual Docker program license.

FUNDING SOURCES

This research funded by recognition doctoral program from Health Human Resources Development and Empowerment (Badan Pengembangan dan Pemberdayaan SDM Kesehatan/PPSDM Kesehatan) Ministry of Health of the Republic Indonesia.

AUTHORS CONTRIBUTIONS

Dwi Krihariyani is currently pursuing doctoral education,
Eddy Bagus Wasito as promoter,
Isnaeni Isnaeni as co-promoter,
Siswandono Siswodihardjo, Wiwik Misaco Yuniarti, Entuy Kurniawan as revising it critically for important intellectual content; and final approval of the version to be published.

CONFLICT OF INTERESTS

The authors confirm that this article content has no conflicts of interest

REFERENCES

1. Pitt SJ. Clinical Microbiology for Diagnostic Laboratory Scientists. 2018. 298 p.
2. Sutandhio S, Alimsardjono L, Lusida MI. Distribusi dan Pola Kepekaan Enterobacteriaceae dari spesimen Urin di RSUD DR. Soetomo Surabaya Periode Januari - Juni 2015. 2015;2–9.
3. Mediavilla JR, Patrawalla A, Chen L, Chavda KD, Mathema B, Vinnard C, et al. Colistin- and Carbapenem-Resistant Escherichia coli Harboring mcr-1 and bla NDM-5 , Causing a Complicated Urinary Tract Infection in a Patient from the United States. MBio. 2016;7(4):1–4.
4. Godaly G, Ambite I, Svanborg C. Innate immunity and genetic determinants of urinary tract infection susceptibility. Curr Opin Infect Dis. 2015;28(1):88–96.
5. Haghighatpanah M, Mozaffari Nejad AS, Mojtahedi A, Amirmozafari N, Zeighami H. Detection of extended-spectrum β-lactamase (ESBL) and plasmid-borne blaCTX-M and blaTEM genes among clinical strains of Escherichia coli isolated from patients in the north of Iran. J Glob Antimicrob Resist. 2016;7:110–3.
6. Febriyenti, Suharti N, Lucida H, Husni E, Sedona O. Karakterisasi dan Studi Aktivitas Antioksidan dari Ekstrak Etanol Secang (Caesalpinia sappan L.). J Sains Farm Klin. 2018;5(1):23–7.
7. Uyo N, Tamat SR, Kosasih K. Granul Ekstrak Kayu Secang (Caesalpinia sappan L.) dan Rimpang Temu Mangga (Curcuma mangga Val & Zijp.) sebagai Antibakteri. J Biol Papua. 2018;10(1):11–6.
8. Putri AM, Putri NB, Rachmady R, Dilalah I, Murwanti R, Meiyanto E. Secang Heartwood Ethanolic Extract (Caesalpinia sappan L .) Inhibits Mesenchymal Stem Cells Senescence. 2017;(October):119–26.

9. Safitri R, Ratningsih N, Maskoen AM. The Effects of Caesalpinia sappan L . Extract Granule to Antioxidant Activity In Blood Serum of Wistar Rat (Rattus norvegicus) With Excessive Iron Condition. 2016;9(11):38–46.
10. Nadiyah, Rezano A, Sudigdoadi S. Effect of Sappan Wood Ethanol Extracts (Caesalpinia Sappan . L) To the Sperm Motility , Viability and Concentration of Male Wistar Rats. Althea Med Journal [Internet]. 2017;4(2):228–33.
11. Sugiyanto RN, Putri SR, Damanik FS, Sasmita GMA. Aplikasi Kayu Secang (Caesalpinia sappan L.) dalam Upaya Prevensi Kerusakan DNA Akibat Paparan Zat Potensial Karsinogenik melalui MNPCE ASSAY. Fak Farm Univ Gadjah Mada. 2013;(1):6.
12. Utomo RY, Novarina A, Tirtanirmala P, Kastian RF, Jenie RI. Enhancement of Cytotoxicity and Apoptosis Induction of Doxorubicin by Brazilein Containing Fraction of Secang (Caesalpinia sappan L .) on T47D Cells. 2018;(February):32–40.
13. Jung EG, Han K Il, Hwang SG, Kwon HJ, Patnaik BB, Kim YH, et al. Brazilin isolated from caesalpinia sappan L. inhibits rheumatoid arthritis activity in a type-II collagen induced arthritis mouse model. BMC Complement Altern Med [Internet]. 2015;15(1):1–11.
14. Weinmann D, Mueller M, Walzer SM, Hobusch GM, Lass R, Gahleitner C, et al. Brazilin blocks catabolic processes in human osteoarthritic chondrocytes via inhibition of NFKB1/p50. J Orthop Res. 2018;36(9):2431–8.
15. Siswandono. Rancangan Obat Rasional dan Pemodelan Molekul. 2015;(November).
16. Nugraha W, Suwartawan W, Prayoga A, Laksmiani L, Putra P, Ani S. Potensi Brazilein Potensi Brazilein dari Kayu Secang (Caesalpinia sappan L.) Sebagai Agen Depigmentasi Kulit Secara In Silico. J Farm Udayana. 2018;7(1):1.
17. Hardjono S. Prediksi Sifat Farmakokinetik , Toksisitas dan Aktivitas sebagai Calon Obat Antikanker melalui Pemodelan Molekul. 2017;14(2):246–55.
18. Pires DEV, Blundell TL, Ascher DB. pkCSM: Predicting small-molecule pharmacokinetic and toxicity properties using graph-based signatures. J Med Chem. 2015;58(9):4066–72.
19. Lipinski CA, Lombardo F, Dominy BW, Feeney PJ. Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. 1997;23.
20. Chander S, Tang CR, Al-Maqtari HM, Jamalis J, Penta A, Hadda T Ben, et al. Synthesis and study of anti-HIV-1 RT activity of 5-benzoyl-4-methyl-1,3,4,5-tetrahydro-2H-1,5-benzodiazepin-2-one derivatives. Bioorg Chem [Internet]. 2017;72:74–9.