RASĀYAN *J. Chem.*
Vol. 16 | No. 3 |1849-1857| July - September | 2023
ISSN: 0974-1496 | e-ISSN: 0976-0083 | CODEN: RJCABP
http://www.rasayanjournal.com
http://www.rasayanjournal.co.in

AGENTS FROM THE LICHEN SUBSTANCE OF *Cladonia crispata* (Ach.) Flot. AND THEIR IN-SILICO APPROACHES

F. Ismed[1,2,✉], V. F. Sardi[2], P. P. Putra[2] and D.P. Putra[1,2]
[1]The Laboratory of Natural Resource of Sumatra (LBS) Universitas Andalas, Padang 26163, Indonesia
[2]Faculty of Pharmacy, Universitas Andalas, Padang 26163, Indonesia
[✉]Corresponding Author: friardi@phar.unand.ac.id

ABSTRACT

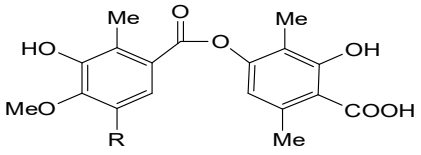
In the exploration of Sumatran lichen, phytochemical investigations were conducted on the lichen *Cladonia crispata* (Ach.) Flot. The working process started with maceration, non-polar, semi-polar, and polar solvents. For the purification process, column chromatography was used to obtain squamatic acid (1) and barbatic acid (2). These compounds were identified spectroscopically (UV-vis-IR-MS-NMR). Furthermore, antibacterial activity from the extract and isolated compounds against seven pathogenic bacteria were evaluated. The ethyl acetate extract significantly inhibited all tested bacteria in the concentration range of 250–2000 µg/disc. Regarding the isolated compound, barbatic acid had the potential as an antibacterial agent at a concentration of 12.5–50 µg/disc, followed by squamatic acid. In the in silico study, the absorption, distribution, metabolism, excretion, and toxicity (ADMET) prediction and molecular docking simulation were conducted for both compounds to understand the action mechanism of the two compounds as antibacterial agents.

Keywords: Antibacterial, Barbatic Acid, Depsides, Squamatic Acid, Lichen.

RASĀYAN *J. Chem.*, Vol. 16, No. 3, 2023

INTRODUCTION

Lichen is a symbiotic organism between mycobionts (fungi) and photobionts (algae or cyanobacteria) with unique chemical substances. Approximately 1050 compounds have been isolated from lichen,[1] collected in several groups such as monoaromatic, depsides, depsidones, depsones, dibenzofurans, terpenes/steroids, and miscellaneous. From a pharmacology aspect, the chemical substance activity of lichen has great potential as a medicinal candidate, specifically as an antimicrobial agent. Furthermore, lichen extracts and compounds often inhibit the growth of microbial pathogens and act at low concentrations.[2] This activity can aid in solving and innovating to overcome various antibiotic and bacterial resistance problems. To obtain medicinal candidates with optimal pharmacological potency, Molecular docking, or an *in silico* approach, is a method with a high success rate (60%–75%) and fast acceleration. In particular, this method is capable of predicting the orientation of a molecule to another for stable complex formation, specifically with biological molecules.[3] The advantage is that further pharmacological tests can be carried out. Molecular docking can also simulate medicine–receptor interactions, explain the medicinal mechanism, and improve accuracy, sensitivity, specificity, and predictability.[4] In continuing the exploration of Sumatran lichen and search for antimicrobial candidates, this study collected several species in the West Sumatra region. One of them is *Cladonia crispata* (Ach.) Flot. from genus *Cladonia* (family: Cladoniaceae, Ascomycotina: Lecanorales). Research on this species' phytochemical and pharmacological activities is still limited, but from our preliminary tests, this species has interesting potential for further research. The bioactive compound, barbatic acid, and squamatic acids were isolated from thallus *C. crispata* (Fig.1).



R= Methyl (CH_3-), Squamatic acid
R= COOH, Barbatic acid

Fig.-1: Structure of the Isolated Compound

http://doi.org/10.31788/RJC.2023.1638258

These two compounds belong to the depside group, which is a lichen genera marker compound group reported in the *Stereocaulon* genus[5,6] and sections of *Cladonia*.[7] Moreover, seven pathogenic bacteria have been selected and used to assess the antibacterial activity of the isolated compounds. *In silico* analysis of the isolated compounds was conducted on these pathogenic bacteria.

EXPERIMENTAL

Lichen Material

Lichen *C. crispata* was taken from rocks in the Harau area, Lima Puluh Kota Regency, West Sumatra (coordinates 0°06′49.5″ S 100°39′50.4″ E). This was confirmed by sending a specimen voucher to Dr. Harrie Sipman (BGBM, Berlin), stored at the Sumatran Biota Laboratory, Universitas Andalas, West Sumatra (Indonesia), with collection number FF1901.

Instrumentation

The instrumentation used to characterize the isolated compounds included UV-VIS Spectrophotometer (Shimadzu Pharmaspec 1700®), FT-IR Spectrophotometer Frontier Spectrometer (PerkinElmer), Fisher-Johns Melting Point Apparatus, LC-MS/MS (LC: ACQUITY UPLC and MS: Xevo G2-S QTof) and NMR Spectroscopy (^{1}H and ^{13}C-NMR spectra (500 and 125 MHz), on a JEOL-NMR Spectrometer).

Reagents and materials

Some chemicals used were Silica gel 60, silica gel plate 60 F254, dimethyl sulfoxide (DMSO), and analytical grade solvents obtained from Merck. The materials in the antimicrobial activity testing were nutrient agar (NA), Mueller–Hinton agar (Merck®), and human blood type O from the Indonesian Red Cross.

Isolation marker compounds

The air-dried whole thallus lichen *C. crispata* (1904 g) was sequentially extracted by non-polar to polar solvents (*n*-hexane, ethyl acetate, and methanol). The chemical substances were monitored using the thin layer chromatography method by developing developer B (*n*-hexane-diethyl ether-formic acid = 120:30:5) and C (toluene-ethyl acetate-formic acid = 75:20:5). The visualization was conducted using a UV lamp (λ 254 and 356 nm) and Anisaldehyde-H_2SO_4 reagent.[8] Ethyl acetate dry extract of 50 g was separated using the vacuum column chromatography method and then was preadsorbed with silica gel 60 in a 1:1 ratio. This was followed by vacuum column chromatography (silica gel 60, 500 g), eluted with a step gradient polarity pattern (*n*-hexane-EtOAc-MeOH v/v, 100:0 → 0:100) as the mobile phase. TLC monitored the column results to obtain nine subfractions. The second and third subfractions formed a precipitate in the form of crude crystals. The crystallization process purified the residue, and compound 1 (1805 mg) was obtained. Furthermore, the filtrate from the subfraction (18 g) was purified using the column chromatography method, and compound 2 (163 mg) was obtained.

Antibacterial Assay

Antibacterial testing was carried out by diffusion method against seven bacterial pathogens, i.e., *Escherichia coli* ATCC 25922 (EC), *Staphylococcus aureus* ATCC 25923 (SA), *Enterococcus faecalis* ATCC 12228 (EF), *Pseudomonas aeruginosa* ATCC 27853 (PA), *Klebsiella pneumoniae* ATCC 1706 (KP), *Moraxella catarrhalis* ATCC 25240 (MC) and *Propionibacterium acnes* ATCC 11827 (PAC). The growth of bacteria EC, SA, EF, PA, and KP was on MHA media. Meanwhile, MC and PAC have grown on 5% MHA+blood media. The evaluated extracts are *n*-hexane, ethyl acetate, and methanol extracts with concentrations of 2000, 1000, 500, and 250 µg/disc and isolated compounds (compounds 1 and 2) with concentrations of 5, 2.5, 1.25, and 0.625 µg/disc, all diluted in DMSO.[9,10] Furthermore, 10 µL of the test solution was pipetted onto the paper disc. Chloramphenicol and Usnic acid were used as the positive control and DMSO as the negative control, and the test was repeated three times.

Molecular Docking Studies

Physico-Chemical Properties Analysis

Two compounds are coded as compounds 1 and 2 with IUPAC names 2-hydroxy-4-(3-hydroxy-4-methoxy-2,5-dimethyl benzoyloxy)-3,6-dimethyl benzoic acid and 5-[(4- carboxy-3-hydroxy-2,5-dimethyl phenoxy)

carbonyl]-3-hydroxy-2-methoxy-4-methyl benzoic acid. Furthermore, the rule of five was determined using the SWISS ADME Web Server (www.swissadme.ch).

ADMET prediction with pkCSM software

The ADMET profile of isolated chemical molecules can be predicted utilizing an open-source *in silico* application. The structure is distorted using the SMILES format and inputted to the pkCSM website https://biosig.lab.uq.edu.au/pkcsm/.

Geometry Optimization

The test compounds used are named compounds 1 and 2. The molecular structure was first drawn using Marvin Sketch, and the geometry was optimized using the extended tight-binding quantum chemistry method with xTB software.[11]

Macromolecular Preparation

First downloaded on the website www.rcsb.org, the target macromolecule was f and is a receptor on several microorganisms such as dihydropteroate synthetase from *S. aureus* with code PDB (1ad4), *P. aeruginosa* (3ix3), *E. faecalis* (6ep5), and *K. pneumoniae* (6pib). The target macromolecule crystal structure was downloaded from the Protein Data Bank website and then prepared and separated by the ligand and receptor. This target macromolecule preparation stage removes water molecules and natural ligands while adding hydrogen atoms. Molecular docking (docking) was conducted using a deep learning method, namely a convolutional neural network (CNN) from Gnina.[12]

Identification of Molecular Docking Simulation Results

The molecular docking simulation was selected from the best CNN pose score. The simulation analyzes each residue involved and the molecular interactions between the macromolecules and the test compound molecules. Amino acid residues formed were analyzed for bond type using BIOVIA Discovery Studio 2021.

RESULTS AND DISCUSSION

Compounds Isolated from Thallus *C. crispata*.

The isolated compound structure was determined by spectroscopy methods (UV, IR, MS, and NMR). Organoleptically, compound 1 is amorphous and has a brownish-white color. The UV-Vis spectrophotometer instrument identification showed λ_{max} (abs) 213.4 (0,571). The infrared spectrophotometer produces absorption peaks at several wave numbers (cm^{-1}), 1623 (C=O), 1179 and 1076 (OH), and 815 (aromatic). LC-MS/MS was performed using a negative mode ion with the spectrum values of 389.0867 *m/z* and 225.0398 *m/z* at a retention time (Rt) of 4.20 min. The *m/z* value refers to the squamatic acid compound based on the comparison with the study, namely 389.0862 *m/z* and 225.0405 *m/z*.[13] Compound 1 gives a chemical shift signal at δ: 2.04 (3H, s, methyl), 2.47 (3H, d, *J*= 2.65, methyl) 2.48 (3H, d, *J*= *2.65*, methyl), 3.87 (3H, s, methoxy), 6.65 (1H, d, *J*= 6.55), 6.66 (1H, d, *J*= 6.55) and 12.27 (2H, bs) for ^{1}H-NMR spectral (in solvent d_6-DMSO). In the data, ^{13}C-NMR (in a solvent of d_6-DMSO): 111.4 (C1), 159.9 (C2), 104.5 (C3), 160.8 (C4), 105.5 (C5), 143.8 (C6), 165.7 (C7), 170.1 (C8), 21.2 (C9), 56.3 (C4 -OMe), 111.9" (C1′), 161.3 (C2′), 115.9 (C3′), 152.0 (C4′), 115.9 (C5′), 139.0 (C6′), 173.2 (C7′), 9.1 (C8′), 22.9 (C9′). From several spectroscopic descriptions, it was concluded that compound 1 was squamatic acid—a secondary metabolite of the depside group found in Lichen. Squamatic acid is found in several species in the genus *Cladonia*.[14] A lack of studies and discussions concerns the pharmacological activities of squamatic acid. However, several reports have stated that the acetone extracts of *C. incrassata* and *F. cucullata* contained squamatic acid and had antibacterial and anticancer activity.[15] Compound 2 has a white crystalline form soluble in acetone and melts at 188.2ºC. The UV-Vis spectrophotometer instrument provides absorption at wavelengths (nm) (absorbance) of 213.5 (0.759) and 275 (0.342). From the IR spectrum data, the absorption peaks at several wave numbers (cm^{-1}) (functional group) are 1638 (C=O), 1506 (aromatic), 1118 (OH), and 724 (aromatic). Analysis of LC-MS/MS using negative mode ions obtained spectrum values at 359.2000 *m/z* and 181.3000 *m/z*, leading to barbatic acid compounds (359.11 *m/z* and 181.05 *m/z*).[16] From the ^{1}H-NMR spectrum, (the solvent d_6-DMSO) compound 2 gave a chemical shift of δ 1.95 (3H, d, *J*= 4.5, methyl), 1.96 (3H, d, *J*= 4.5, methyl), 2.44 (3H, s, methyl), 2.52 (3H,s, methyl), 3.82 (3H, s, methoxy), 6.56 (1H, s), 6.66 (1H,s), 10.69 (OH, s). The ^{13}C-NMR spectrum show

compound 2 gave a chemical shift at δ 116.6 (C1), 161.9 (C2), 110.5 (C3), 159.9 (C4), 106.8 (C5), 139.5 (C6), 169.2 (C7), 8.6 (C8), 23.4 (C9), 116.3 (C1′), 161.6 (C2′), 111.9 (C3′), 152.4 (C4′), 107.6 (C5′), 139.5 (C6′), 173.7 (C7’), 9.6 (C8′), 23.6 (C9′) and 56.3 (C4 -OMe). Comparison with the literature and spectroscopic observations (NMR, MS) showed that compound 2 was barbatic acid. As a depside group, barbatic acid combines two orsellinic units linked by an ester bridge. The acid is used as a marker compound to distinguish several sections and chemotypes, in particular, section Parviae in the genus *Cladonia*.[17]

Antibacterial Assay of Extracts and Isolated Compounds

The test compares the activity of extracts and isolates compounds with broad-spectrum antibiotics to those in the study using chloramphenicol (positive control) against seven pathogenic bacteria. The extract concentration used four concentrations at 2000, 1000, 500, and 250 µg/disc. Meanwhile, the positive control employed a 30 µg/disc concentration and DMSO (negative control). Bacteria sensitivity showed a clear zone around the disc. The inhibition zone measurement from each sample can be seen in Fig.-2. Antimicrobial activity is grouped based on the inhibition zone diameter ≥12 mm, 9–12 mm, and 6 mm, showing very strong, moderate, and weak activities.[18] The ethyl acetate extract of *C. crispata* showed potent antibacterial activity against SA, PA, KP, EF, EC, MC, and PAC bacteria with inhibitory diameters in the range of 11–18 mm and a concentration of 2000 µg/disc. Meanwhile, a concentration of 250 µg/disc shows an inhibition diameter in the 7–16 mm range, which was considered moderate. The *n*-hexane and methanol extract was inhibited at a concentration of 2000 µg/disc with a diameter in the 8–16 mm range against all test bacteria and had an inhibitory power of 6–13 mm at 250 µg/disc. Therefore, the ethyl acetate extract was isolated and had better activity than others. Similar to the antibacterial assay towards the extracts, compounds 1 and 2 were evaluated using seven pathogenic bacteria and compared with chloramphenicol and usnic acid (concentrations of 30 and 50 µg/disc) and negative control (DMSO; Fig.-2). Based on test results, compound 1 with a concentration of 50 µg/disc, had weak activity against all bacteria. With the same concentration, compound 2 had potent activity against SA, PA, EF, and KP with an inhibition range of 12–17 mm. EC, MC, and PAC have a moderate inhibition category in the 9–12 mm range. For a concentration of 6.25 µg/disc, compound 2 had a weak inhibition effect on EF, EC, MC, and PAC, with a range of 7–9 mm. PA had moderate activity with an inhibition range of 9 mm. Meanwhile, SA and KP had a strong activity range of 13–14 mm.

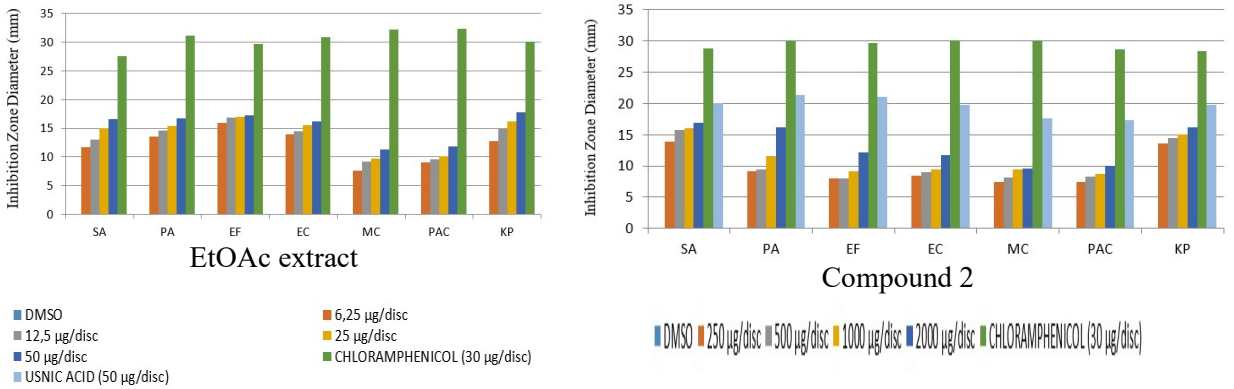


Fig.-2: Graph of Antibacterial Activity of EtOAc Extract and Isolated Compounds Against Seven Bacterial Pathogens: SA=*S. aureus*, PA=*P. aeruginosa*, EF=*E. faecalis*, EC=*E. coli*, MC=*M. catarrhalis,* PAC=*P. acnes*, and KP=*K. pneumoniae*.

Several reports stated that squamatic acid’s antibacterial activity is weak[19] and has more potential as an antifungal.[20] Barbatic acid is reported to have the potential as an antibacterial and inhibits the growth of gram-positive and negative bacteria with four multi-resistant *S. aureus* strains.[21]

***In silico* Approach**

The structure of compounds 1 and 2 were analyzed for physical and chemical properties and molecular bonding, as shown in Fig.-1. The molecular weight and log P fulfilled the Lipinski rule of five in the prediction of physicochemical properties.[22] Meanwhile, the medicinal compound has a molecular weight of <500 g/mol and a log P < 5 and can be more easily absorbed orally.[23] Topology surface area (TPSA) is

a QSAR equation with a TPSA score greater than 140 angstroms, which it difficult to enter the cell membrane. In Table-1, Compound 2 having TPSA >140 is predicted to be challenging to penetrate the cell membrane. The prediction of Log S is a logarithm that expresses solubility in water, and the compounds are categorized as Moderately Soluble.

Table-1: Predicted Results of Physical and Chemical Properties

Compounds	Molecular weight (g/mol)	TPSA Å^2	Log P	Log S	Rule of Five*	Solubility
1	360.36	113.29	3.05	−4.69	Yes	Moderately soluble
2	390.34	150.59	2.42	−4.25	Yes	Moderately soluble

* Rule of Five: Number of violations of Lipinski's rule of five.

ADME Prediction

The pkCSM is an open-access tool developed by Pires *et al*.[24] The platform will predict the pharmacokinetic optimization and toxicity properties implemented in a web interface. This prediction result will balance potency, safety, and pharmacokinetic properties. Table-2 shows the ADMET predictions of compounds 1 and 2.

Table-2: ADMET (Metabolism, Distribution, Excretion,Absorption, And Toxicity)Attributes of Compounds 1 and 2

	Compound 1	Compound 2
Adsorption and Distribution		
Model Name	Predicted Value	Predicted Value
Water solubility	−3.787	−3.453
Caco2 permeability	0.788	−0.45
Intestinal absorption (human)	55.791	23.335
Skin Permeability	−2.736	−2.735
Substance recognized by P-glycoprotein	Yes	Yes
P-glycoprotein I inhibitor	No	No
P-glycoprotein II inhibitor	No	No
Volume of distribution at steady state in humans (VDss)	−0.445	−0.289
Fraction unbound (human)	0.199	0.216
Permeability across the blood-brain barrier (BBB)	−1.098	−1.542
Permeability across the central nervous system (CNS).	−2.986	−3.148
Metabolism		
(CYP3A4 and CYP2D6) substrate	No	No
(CYP1A2, CYP2C19, CYP2C9, CYP2D6 and CYP3A4) inhibitor	No	No
Excretion and Toxicity		
Total Clearance	0.802	0.834
Renal OCT2 transporter substrate	No	No
Toxicity assessment using the AMES test	No	No
Max. tolerated dose (human)	1.033	1.15
hERG I inhibitor	No	No
hERG II inhibitor	No	No
LD_{50} for acute toxicity in rats following oral administration	2.141	2.751
Lowest Observed Adverse Effect Level (LOAEL) using rat	2.144	2.658
Hepatotoxicity	No	No
Allergic reaction of the skin	No	No
Tetrahymena pyriformis toxicity	0.282	0.285
Minnow toxicity	0.418	1.5

Geometry

Before molecular docking, Geometry optimization is conducted to quickly and efficiently calculate electronic structures. An optimization in the electronic structure is calculated in a search for minimum energy and transition structures.[25] The optimization aims to obtain the ideal conformation of the compound 1 and 2 structure with the lowest energy. Geometry optimization results can be seen in Table-3.

Table-3: Energy Value of Geometry Optimization Results Using xTB

Compound	Electronic energy (Eh)	HOMO-LUMO Gap
1	−79.4 Eh	2.71 eV
2	−86.5 Eh	2.86 eV

Macromolecular Preparation

In this study, Gnina software was used for molecular docking to ensure that the method has an accurate geometric pose. The parameter uses a natural ligand redocking technique on each protein from the microorganisms. The parameter is declared to meet criteria similar to crystallographic results when the RMSD value of the natural ligand tethering to the receptor is 2.0.[26] The RMSD results from the docking method are shown in Table-4.

Table-4: Validation Method and Value of Bond-Free Energy from Docking Simulation

No	PDB Code	Organism	RMSD (Å)	Active Site	Binding Site	Test Compound Bond Free Energy (kcal/mol)	
						Compound 1	Compound 2
1	1ad4	*S. aureus*	1.68	–	Arg52, Asp84, Asn103, Asp167, Lys203	-5.06	-5.06
2	3ix3	*P. aeruginosa*	1.12	–	–	2.85	-1.60
3	6ep5	*E. faecalis*	0.29	–	Mg^{+2}	-9.20	-9.08
4	6pib	*K. pneumoniae*	0.30	–	Asp122, Ser160, Asn164, Lys167, His195	-9.47	-9.22

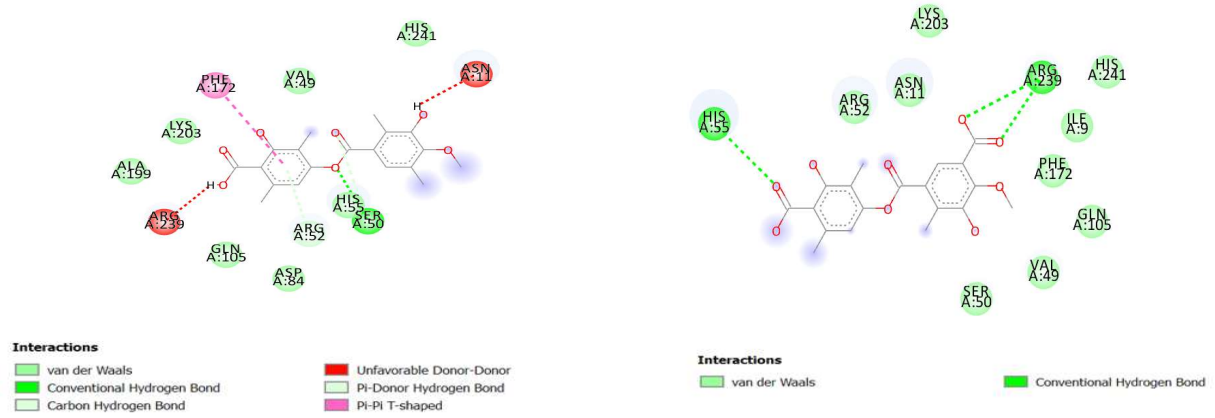
Identification of Molecular Docking Simulation Results

The docking of compounds 1 and 2 molecules will result in the energy prediction, and the interaction pose shape. The bond-free energy (ΔG) in the docking of molecules 1 and 2 to the microorganisms' protein is intended to predict the degree of binding affinity and the long-term stability of the formed complex (Table-4). Compounds 1 and 2 can inhibit the receptor by forming spontaneous bond energy, which is indicated by a minus Gibbs energy value. In the docking simulation on compound 1, *P. aeruginosa* and *M. catarrhalis* were positive, without spontaneous bonding. Figure-3A represents the molecular docking simulation result of the protein-coded 1ad4 *S. aureus*. Furthermore, 1ad4 is the gene encoding the dihydropteroate synthase.[27] Compound 1 obtained six hydrogen bonds and hydrophobic interactions on HIS51 and SER103, while compound 2 obtained two hydrogen bonds in the amino acids ARG239 and HIS55. Figure-3B displays the molecular docking simulation of the protein coded 3ix3 in *P. aeruginosa*, and 3ix3 can coordinate the target gene expression on virulence.[28] The interaction between compound 1 and 3ix3 obtained four hydrogen bonds on the amino acids ARG61, LEU110, PHE102, and SER106, and Van der Waals interactions on the amino acids LEU110, TYR 93, and TYR56. Compound 2 obtained two hydrogen bonds and hydrophobic interactions on ARG61 and GLY38. Table-4 also shows the molecular docking simulation result of the protein coded 6ep5 in *E. faecalis*. The 6ep5 protein regulates molecular regulation in bacteria by catalyzing post-translational modifications.[29]

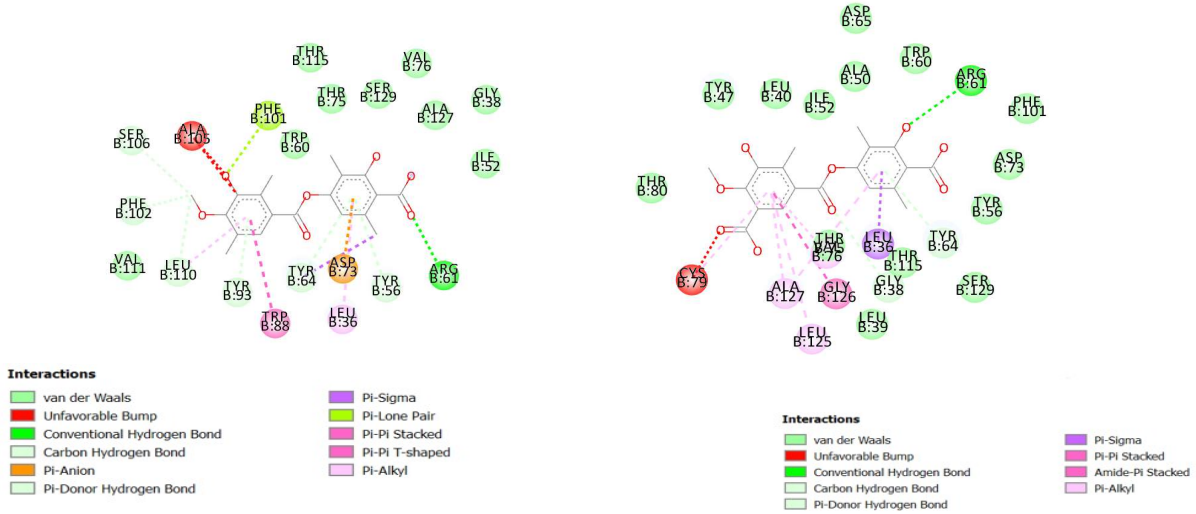
The interaction between compound 1 and protein obtained two hydrogen bonds in ASN 115 and GLU159. Compound 2 obtained three hydrogen bonds in PHE81, GLU159, and ASN115. The protein coded 6pib (UDP-2,3-diacylglucosamine pyrophosphate hydrolase) (Table-4) in *K. pneumoniae* plays a crucial role as a biosynthetic enzyme in gram-negative bacteria for the production of lipid A.[30]

The interaction between compound 1 and the 6pib protein obtained three hydrogen bonds. Moreover, hydrophobic interaction is seen on ARG80, ARG157, and GLU44. Meanwhile, compound 2 obtained three hydrogen bonds in ARG80, ARG157, and GLU44.

Compound 1 Compound 2



A. Interaction between *S. aureus* (PDB:1ad4) macromolecules and Compounds 1 and 2



B. Interaction between *P. aeruginosa* (PDB: 3ix3) macromolecules and Compounds 1 and 2

Fig.-3: Visualization of Interactions Between Macromolecules of Each Bacterial Pathogen and Isolated Compounds

CONCLUSION

Based on this study, barbatic acid (depside group) isolated from lichen *C. crispata* has significant potential as an antimicrobial agent. *In silico*, squamatic, and barbatic acid meet the requirements for good medicines to be absorbed orally according to the Lipinski rule of five.

ACKNOWLEDGMENTS

We are grateful to the DRTPM-DIKTI Grant 2023 – The Ministry of Education, Culture, Research, and Technology of the Republic of Indonesia to FI (contract No. 012/E5/PG.02.00.PL/2023). Dr. Harrie J. M. Sipman, BGBM Berlin-Dahlem, Freie Universitat Berlin, is acknowledged for identifying the lichen. We also thank the Institute of Tropical Disease (Lembaga Penyakit Tropis) team, Universitas Airlangga, for the NMR data.

CONFLICT OF INTERESTS

The authors declare that there is no conflict of interest.

AUTHORS CONTRIBUTION

All authors made significant contributions to this article. They started with designing, data analysis, reviewing, editing, and finally, publishing approval. The research profile of the authors can be verified from their ORCID identities, given below:

F. Ismed https://orcid.org/0000-0002-6200-7502
P. P. Putra https://orcid.org/0000-0001-9466-4569
D.P. Putra https://orcid.org/0000-0002-0930-3150s
V. F. Sardi https://orcid.org/0009-0007-3655-5963

REFERENCES

1. J. Elix, A Catalogue of standardized chromatographic data and biosynthetic relationships for lichen substances, 3rd ed. Canberra, 323(2014).
2. G. Shrestha and L. L. St. Clair, *Phytochemistry Reviews*, **12(1)**, 229(2013), https://doi.org/10.1007/s11101-013-9283-7
3. J. Cole, E. Davis, G. Jones, and C.R. Sage, Molecular Docking—A Solved Problem? In Comprehensive Medicinal Chemistry III, Elsevier, 297(2017), https://doi.org/10.1016/B978-0-12-409547-2.12352-2
4. G. Chen, A. J. Seukep, and M. Guo, *Marine Drugs*, **18**, 22(2020), https://doi.org/10.3390/md18110545
5. F. Ismed, F. Lohézic-Le Dévéhat, A. Guiller, N. Corlay, A. Bakhtiar, J. Boustie, *Phytochemistry Reviews*, **17(5)**, 1165(2018), https://doi.org/10.1007/s11101-018-9576-y
6. F. Ismed, H.E. Putra, N. Arifa, and D. P. Putra, *Jordan Journal of Pharmaceutical Sciences*, **14(2)**, 189(2021)
7. K. Huovinen, K. Ahti, and S. Stenroos, *Annales Botanici Fennici*, **26**, 297(1989)
8. S. Huneck and I.Yoshimura, Identification of lichen substances Springer Berlin Heidelberg, Berlin, Heidelberg, 125(1996)
9. D. Handayani, A. Rendowati, I. Aminah, N. P. Ariantari, P. Proksch, *Rasayan Journal Chemistry*, **13(4)**, 2633(2020), http://dx.doi.org/10.31788/RJC.2020.1345781
10. M.A Artasasta, T.M. Yanwirasti, A. Djamaan, D. Handayani, *Rasayan Journal Chemistry*, **12(3)**, 1463(2019), http://dx.doi.org/10.31788/RJC.2019.1235284
11. C. Bannwarth, E. Caldeweyher, S. Ehlert, A. Hansen, P. Pracht, J. Seibert, S. Spicher, and S. Grimme, *WIREs Computational Molecular Science*, **11(2)**, 49(2021), https://doi.org/10.1002/wcms.1493
12. A. T. McNutt, P. Francoeur, R. Aggarwal, T. Masuda, R. Meli, M. Ragoza, J. Sunseri, and D. R. Koes, *Journal of Cheminformatics*, **13(1)**, 20(2021), https://doi.org/10.1186/s13321-021-00522-2
13. Squamatic Acid [Internet]. 2020. Squamatic Acid Available from: https://mona.fiehnlab.ucdavis.edu/spectra/display/CCMSLIB00004751261
14. K. Huovinen, and T. Ahti, *Annales Botanici Fennici*, **23(3)**, 173(1986)
15. A. Dieu, M. Millot, Y. Champavier, L. Mambu, V. Chaleix, V. Sol, and V. Gloaguen, *Planta Medica*, **80(11)**, 931(2014), http://dx.doi.org/10.1055/s-0034-1382827
16. http://gnps.ucsd.edu/ProteoSAFe/gnpslibraryspectrum.jsp?SpectrumID=CCMSLIB00005719449
17. T. Ahti, S. Parnmen, and P. Mongkolsuk, *Sauteria*, **15**, 385(2008)
18. X. Pan, F. Chen, T. Wu, H. Tang, and Z. Zhao, *Food Control*, **20(6)**, 598(2009), https://doi.org/10.1016/j.foodcont.2008.08.019
19. E. Studzińska-Sroka, E. Hołderna-Kędzia, A. Galanty, W. Bylka, K. Kacprzak, and K. Ćwiklińska, *Natural Product Research*, **29**, (2015), https://doi.org/10.1080/14786419.2015.1005616
20. M. Millot, M. Girardot, L. Dutreix, L. Mambu, and C. Imbert, *Molecules*, **22**, 651(2017), https://doi.org/10.3390/molecules22040651
21. G. Selvaraj, A. Tinabaye, T. Ganesan, R.Ananthi, S. Senthil Kumar, and S. Arulmozhi, *International Journal of Scientific & Engineering Research*, **7(8),** 45(2016)
22. N. Lolok1, D. S. F. Ramadhan, S. A. Sumiwi, I. Sahidin, J. Levita, *Rasayan Journal Chemistry*, **15(1)**, 20 (2022), http://dx.doi.org/10.31788/RJC.2022.1516646
23. C.A. Lipinski, *Drug Discovery Today: Technologies*, **1(4)**, 337(2004) https://doi.org/10.1016/j.ddtec.2004.11.007

24. D.E.V. Pires, T.L. Blundell, and D.B. Ascher, *Journal of Medicinal Chemistry*, **58(9)**, 4066(2015), https://doi.org/10.1021/acs.jmedchem.5b00104
25. J. Zheng, and M.J. Frisch, *Journal of Chemical Theory and Computation*, **13(12)**, 6424(2017), https://doi.org/10.1021/acs.jctc.7b00719
26. E.W. Bell and Y. Zhang, *Journal of Cheminformatics*, **11(1)**, Article 40(2019), https://doi.org/10.1186/s13321-019-0362-7
27. I.C. Hampele, A. D'Arcy, G.E. Dale, D. Kostrewa, J. Nielsen, C. Oefner, M.G.P. Page, H.J. Schönfeld, D. Stüber, and R.L. *Journal of Molecular Biology*, **268(1)**, 21(1997), https://doi.org/10.1006/jmbi.1997.0944
28. P. Kiratisin, K.D. Tucker, and L. Passador, *Journal of Bacteriology*, **184(17)**, 4912(2002), https://doi.org/10.1128/JB.184.17.4912-4919.2002
29. S.Veyron, G. Oliva, M. Rolando, C. Buchrieser, G. Peyroche, and J. Cherfils, *Nature Communications*, **10(1)**, 10(2019), https://doi.org/10.1038/s41467-019-09023-1
30. J. Cho, M. Lee, C.S. Cochrane, C.G. Webster, B.A. Fenton, J. Zhao, J. Hong and P. Zhou, in Proceedings of the National Academy of Sciences of the United States of America, **117(8)**, 4109(2020), https://doi.org/10.1073/pnas.1912876117

[RJC- 8258/2023]