In Vitro and In Silico Analysis for Antibacterial Activities of Various Extracts of Gracilaria salicornia (Rhodophyta) from Selayar Islands, Indonesia

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In Vitro and In Silico Analysis for Antibacterial Activities of Various Extracts of *Gracilaria salicornia* (Rhodophyta) from Selayar Islands, Indonesia

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Abstract

This study aims to evaluate the toxicity, antibacterial activity, and chemical profiling of *Gracilaria salicornia* extract. The research stages included the extraction, bioactivity test, GC-MS, and docking analysis. The extraction of samples was carried out by gradient maceration. The toxicity of the extracts was analyzed using the BSLT method, and the antibacterial activity was analyzed by paper disc diffusion. The antibacterial activity showed better sensitivity against *Escherichia coli* than *Staphylococcus aureus*. The inhibition zone against *E. coli* ranged from 7.73-17.30 mm, while *S. aureus* ranged from 6.91-13.44 mm, and all of the extracts were toxic to *Artemia salina* Leach. The chloroform extract showed the highest activity with the clear zone diameter was 17.3±1.65 mm and 13.44 ± 0.04 mm to *E. coli* and *S. aureus* respectively. The presence of steroids, terpenoids, fatty acids, and alkaloids based on GC-MS and docking analysis were responsible for these activities. Molecular docking analysis showed that Cholest-5-en-3-ol and Cholesta-4,6-dien-3-ol showed better interactions than control with binding energy -9.99 and -9.67 kcal.mol^{-1} against 5BMM, -8.00 and -7.93 kcal.mol^{-1} against 3LPS respectively. Based on this data, it can be stated that *G. salicornia* extract is highly potential as the source of antibacterial compounds.

Keywords: Antibacterial activity; Binding energy; Docking; *Gracilaria salicornia*; GC-MS; and Toxicity

1. Introduction

Searching for antibiotics to fight infectious diseases have many obstacles and have reached a turning point [1]. However, microorganisms with different mechanisms may form a self-defence system to be resistant to certain antibiotics [2]. This condition can be a serious threat if it continues because it will become an obstacle for the therapy of various diseases [3]. The issue has become a trigger for researchers looking for new antibiotic candidates [4, 5].

Marine organisms such as macroalgae have massive potential for the discovery of secondary metabolites as the new medicinal candidate [6]. This was inseparable from the extreme environmental conditions and other factors, such as the presence of microorganisms. Research data have shown that macroalgae have secondary metabolites such as phlorotannins, fatty acids, polysaccharides, peptides, and terpenes that are pharmacologically active as antibacterials [7].

Gracilaria was one of the genera on the Indonesian coast with a fairly large population [8]. The macroalgae were high in secondary metabolites and had pharmacological impacts against cell oxidation processes and bacterial infections [9]. The pharmacological effects are derived from the class

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of compounds such as alkaloids, flavonoids, saponins [10], steroids, terpenoids, and fatty acids [11]. *Gracilaria salicornia* was one of several algae species that were not widely explored for its bioactive compound [12], especially on the Indonesian coast. This study was carried through an in vitro and in silico analysis of the antibacterial activity of *Gracilaria salicornia* extracts. This report was also the first of the Indonesian *Gracilaria salicornia* extract metabolite profiling based on GC-MS.

2. Experimental

a. Collection and Identification of Algal Material

The sample was obtained from the shore of Selayar Island (5°54'59.45" S and 120°26'43.98" E) at a snorkelling depth of 1.5 meters. The sample obtained was *G. salicornia* based on identification the Productivity and Water Quality Laboratory, Faculty of Marine Science and Fisheries Hasanuddin University and morphological analysis based on Guiry & Guiry, 2020 [13].

b. Extraction and Sample Preparation

Samples that have been taken from the growing site were immediately washed several times using seawater to remove epiphytes and other impurities. Then the salt attached to the samples was rinsed using distilled water. After drying, the sample was then cut into pieces and ground into powder. The sample was then extracted by graded maceration method with n-hexane, chloroform, ethyl acetate and methanol. The extract obtained was then concentrated using a rotary evaporator.

c. Toxicity Test

The toxicity of the various extracts of *G. salicornia* was tested using the *Brine Shrimp Lethality Test* (BSLT) method. The 48-hour-old larva was used as an object for this test. A total of 100 µL of seawater with 10-15 larvae was inserted into the microplate, then the extract solution was added so that the final concentrations of 7.81, 15.63, 31.25, 62.50, and 125 µg.mL^{-1} were obtained. The toxicity of the extract was expressed by LC_{50} (µg.mL^{-1}) obtained through linear regression equations from the log concentration against probit values. The probit value was the conversion of % of larval death after incubation for 24 hours.

d. Antibacterial Test

Antibacterial activity by paper disc diffusion method was obtained based on the clear zone around the disc paper. Inoculum of tested bacteria (*S. aureus* and *E. coli*) were suspended by sterile axle with OD 0.08-0.1 (107 CFU.mL^{-1}) (Martins *et al.*, 2011). Then the suspension was applied on the surface of the Nutrien Agar (NA) media using sterile swabs. Sterile disc paper (diameter 6 mm) was soaked with extract solution and control, then placed on the surface of the NA media that had been applied to the tested bacteria. The clear zone diameter around the disc paper was measured after being incubated for 24 hours at 37°C.

e. GC-MS Analysis

The *G. salicornia* extract was analyzed using GC-MS (Shimadzu GC-MS 2010 Mass Spectrometry plus) SH-Rxi-5Sil MS (30 m x 0.25 mm) column with flame ionization detector (FID) (operated in EI mode at 70 eV) was used in this instrument. Ion source temperature and interface were 200°C and 280°C. The solvent cut time was 3 minutes, 400-700 m/z. Samples were injected at 250°C injector temperature with splitless mode, the pressure of 76.9 kPa and a flow rate of 14 mL.min^{-1} with a 1:10 ratio. The sample analysis was done with the initial temperature of column 70°C. The hold time was 2 minutes and the temperature was raised to 200°C at 10°C.min^{-1} rate. The final temperature of the column was 280°C, lasting 9 minutes at a 5°C.min^{-1} rate. The abundance of each compound was expressed in terms of relative area (%) and identification was done by comparing the retention time and mass spectrum of the data library (NIST and WILEY 9).

f. Docking Analysis

The interaction of compounds with target proteins was carried out through in silico studies using the molecular docking method. Compounds identified by GC-MS were used as ligands. Molecular docking analysis was carried out on

5BMM *(E. coli)* [14] and 3LPS *(S. aureus)* [15] using the Autodock program. The crystal structure of the target protein was obtained from the RCSB Protein Data Bank (http://www.rcsb.org/-pdb) and separated from its natural ligands and other unnecessary molecules before use. Then, the structure added Kollman charges and polar hydrogen atoms. The search for the ligand conformation area was carried out with the coordinates of the grid box at position -51.895, 24.563, -24.979 for 5BMM and -5.988, 26.925, 62.406 for 3LPS. The results of the molecular docking analysis were visualized using the Discovery Studio Visualizer 2016 program.

3. Results and Discussion

a. Toxicity Test

The BSLT test was the preliminary test used commonly for extract or compound toxicity. The toxicity was determined from the extract's ability to inhibit *Artemia salina's* growth. The toxicity of the extract was expressed by LC_{50} ($\mu g.mL^{-1}$). The toxicity of *G. salicornia* extract can be seen in Table 1. The value was obtained through the linear regression equation of log concentration against the probit value. The graph can be seen in Figure 1. The toxicity of the whole extracts is classified as toxic. The LC_{50} values range from 83.44-662.23 $\mu g.mL^{-1}$ and chloroform extract was the most active extract with LC_{50} 83.44 $\mu g.mL^{-1}$.

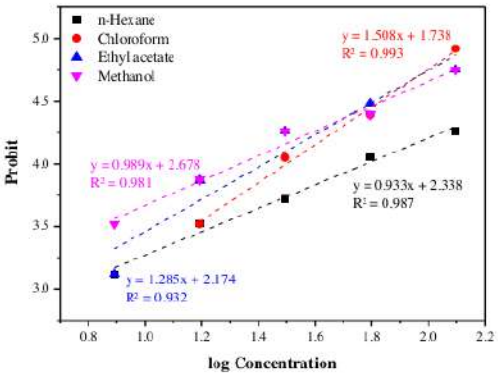


Fig. 1. The diagram of the correlation between log concentration of the extract and probit value

Similar findings were shown with this report from previous studies. Ethyl acetate extract of *G. salicornia* collected from the Persian Gulf belongs to the toxic category with LC_{50} 3 $\mu g.mL^{-1}$. However, methanol extract was not toxic with LC_{50} 1,349 $\mu g.mL^{-1}$ [16]. Several studies related to the toxicity test of *Gracilaria* extract showed different results from the research data obtained. Methanol extract *G. changii* (from Morib Beach, Selangor, Malaysia) [17] and ethanol extract *G. corticata* (J. Agardh) (from Indian coast) [18] were not toxic against *A. salina*. The LC_{50} values of each extract were 3.13 $mg.mL^{-1}$ and 1.081 $mg.mL^{-1}$ respectively.

Table 1
Toxicity of various extracts of *G. salicornia* base on BSLT method

Extract	LC_{50} ($\mu g.mL^{-1}$)
n-Hexane	662.23
Chloroform	83.44
Ethyl acetate	96.37
Methanol	276.69

b. Antibacterial Test

Paper disc diffusion was used to assess the antibacterial activity *G. salicornia* extract. The ability of the extract to inhibit the growth of tested bacteria is indicated by the clear zone diameter around the paper disc. The clear zone diameter data of various extracts of *G. salicornia* can be seen in Figure 2. The diameter of the clear zone against *S. aureus* varies from 6.91±0.14 mm to 13.44±0.04 mm. The value was less than the clear zone diameter against *E. coli*, which ranges from 7.73±0.15 mm to 17.3±1.65 mm. Extracts of n-hexane, chloroform, methanol, and positive control (ciprofloxacin) have greater clear zone against gram-negative bacteria *(E. coli)* than gram-positive bacteria *(S. aureus)*. This was in line with Arulkumar and colleagues' findings, which found that methanol extracts of *G. corticata* and *G. edulis* were more susceptible to *E. coli* than *S. aureus* [19]. The antibacterial activity of *G. dendroides* extract was also consistent with the findings of this report [20]. On the other hand, ethyl acetate extract showed greater clear zone against *S. aureus*. The difference in action was related to differences in the composition of the tested bacteria's cell walls as

well as the components of the compounds in each extract.

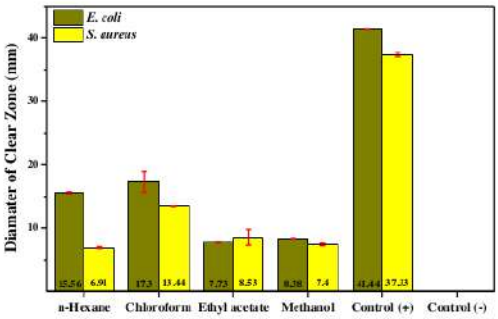


Fig. 2. Antibacterial activity of various extracts of *G. Salicornia*

As compared to the other extracts, the chloroform extract has the largest clear zone diameter for *S. aureus* and *E. coli* bacteria. The diameter was 13.44±0.04 mm against *S. aureus* and belongs to the moderate. However, it was strong inhibition against *E. coli* with the clear zone diameter of 17.3±1.65 mm. The ability of such clear zone can be juxtaposed with ciprofloxacin antibiotics of the same category, although ciprofloxacin has larger clear zone of 41.44±0.04 mm. The data shows that chloroform extract has the potential to be a broad-spectrum antibacterial candidate. Previous research has shown the ability of chloroform extract as potential antibacterial. Research conducted by Al-saif et al., 2014 showed that chloroform extract *G. dendroides* provide clear zones with diameters of 26.3 mm *(E. coli)* and 8 mm *(S. aureus)* [20]. The study also showed that chloroform extract has better antibacterial activity. Saeed et al., 2020, in their research has also proven that extract from chloroform solvent has better antibacterial activity than other solvents [21].

The inhibition of n-hexane extract also has fairly good potential with strong inhibition against *E. coli* (the clear zone diameter was 15.56 ± 0.16 mm). However, it has weak inhibition against *S. aureus*. Meanwhile, moderate inhibition was being observed in ethyl acetate extract against *S. aureus* and methanol extract against *E. coli*. However, the inhibition of ethyl acetate extract against *E. coli* and methanol extract against *S. aureus* belongs to weak inhibition.

c. GC-MS Analysis

Identification of compounds in various extracts of *G. salicornia* was carried out using GC-MS instruments. The results showed that n-hexane extract was dominated by steroid compound group (35.34%), fatty acids (27.38%), and terpenoids (11.09%). The chloroform extract was dominated by fatty acid compounds (66.31%), steroids (15.27%), and alkanes (6.28%). Ethyl acetate extract was dominated by ester compound (34.12 %), alkanes (25.07%), and fatty acids (16.05%). Meanwhile, methanol extract was dominated by steroid (44.21%), esters (19.88%), and triglycerides (5.49%). Also, there were minor compounds such as ketones, aldehydes, alkenes, and alkaloids. The compound was commonly found in this genera. The compound obtained bear similarities to the results of GC-MS *Gracilaria* species from the Suez Canal of Egypt [22]. Compounds such as fatty acids, terpenes, steroids, aromatic organic acids, alcohols, aldehydes, ketones, alkanes, and alkenes have been reported to have antibacterial activity [7].

An in-depth breakdown of GC-MS data on chloroform extract was done to identify the complexity of the compounds involved in the high antibacterial activity of the extract. Compounds identified of chloroform extract can be seen in Table 2. The compound with the highest abundance was n-hexadecanoic acid with a percentage was 63.2%. n-hexadecanoic acid and other fatty acid compounds such as oleic acid, octadecanoic acid and pentadecanoic acid have been reported to have antibacterial activity [19, 23, 24]. Fatty acids were considered effectively to prevent infections caused by bacteria. It has been proven that fatty acids with long-chain (more than 10 carbon atoms) can cause lysis in bacterial protoplasms [25].

In addition, the steroid compounds such as Cholest-5-en-3-ol(3.beta.) (14.19%) has been reported to have inhibition against *E. coli, Salmonella,* and *S. aureus* [26]. Other compounds that have also been known to have antibacterial activity were phytol (2.41%), alkane hydrocarbon and 1,2-benzenedicarboxylic acid [19, 27]. Compound of 1,2-benzenedicarboxylic acid has also been reported to have an inhibitory effect on *Vibrio fluvialis* [28].

Table 2
Components identified in the chloroform extract of *G. Salicornia*

No	R. Time	Area%	Name	MW	MF	Compound Class
1	21.58	63.2	n-hexadecanoic acid	256	$C_{16}H_{32}O_2$	Fatty acid
2	39.6	14.19	Cholest-5-en-3-ol	386	$C_{27}H_{46}O$	Steroid
3	23.51	2.41	Phytol	296	$C_{20}H_{40}O$	Terpenoid
4	35.25	2.2	Hexatriacontane	506	$C_{36}H_{74}$	Alkanes
5	21.8	1.95	Ethyl hexadecanoate	284	$C_{18}H_{36}O_2$	Ester
6	19.78	1.67	2,6,10-trimethyl,14-ethylene-14-pentadecne	278	$C_{20}H_{38}$	Terpenoid
7	21.9	1.53	Heneicosane	296	$C_{21}H_{44}$	Alkanes
8	18.82	1.41	Tetradecanoic acid	228	$C_{14}H_{28}O_2$	Fatty acid
9	29.38	1.11	Pentacosane	352	$C_{25}H_{52}$	Alkanes
10	35	1.08	Cholesta-4,6-dien-3-ol	384	$C_{27}H_{44}O$	Steroid
11	18.04	1.06	Heptadecane	240	$C_{17}H_{36}$	Alkanes
12	29.67	0.99	9-octadecenal	266	$C_{18}H_{34}O$	Aldehyde
13	19.79	0.92	2-pentadecanone, 6,10,14-trimethyl-	268	$C_{18}H_{36}O$	Terpenoid
14	21.17	0.87	2-hydroxycyclopentadecanone	240	$C_{15}H_{28}O_2$	Ketones
15	28.36	0.8	Octadecanal	268	$C_{18}H_{36}O$	Aldehyde
16	20.07	0.77	Pentadecanoic acid	242	$C_{15}H_{30}O_2$	Fatty acid
17	23.97	0.7	Palmitaldehyde	338	$C_{22}H_{42}O_2$	Aldehyde
18	24.04	0.6	Oleic acid	282	$C_{18}H_{34}O_2$	Fatty acid
19	23.34	0.38	Tetratetracontane	618	$C_{44}H_{90}$	Alkanes
20	24.32	0.33	Octadecanoic acid	284	$C_{18}H_{36}O_2$	Fatty acid
21	33.96	0.3	2,6,10,14,18,22-tetracosahexaene, 2,6,10,15,19,23-hexamethyl-	410	$C_{30}H_{50}$	Terpenoid
22	29.89	0.29	1,2-benzenedicarboxylic acid	530	$C_{38}H_{58}O_4$	Ester
23	18.28	0.24	Pentadecanal	226	$C_{15}H_{30}O$	Aldehyde
24	20.87	0.23	Methyl hexadecanoate	270	$C_{17}H_{34}O_2$	Ester

Note, MW: Molecular Weight; MF: Molecular Formula

d. Docking Simulation

Molecular docking analysis was performed to determine the components of secondary metabolite compounds in chloroform extract that contributed to antibacterial activity. Docking analysis was performed on compounds 1-13 and Ciprofloxacin as positive controls. Meanwhile, the redocking of native ligands with their respective proteins shows that the parameters used were valid. It was concluded based on the RMSD value of less than 2 Å, namely 0.65 Å for 3LPS and 0.85 Å for 5BMM, the structure can be seen in Figure 3. The figure shows that the conformation of the native ligand (grey) was not much different from the redoking ligand (green).

The summary of the docking analysis in Table 3 shows that generally, the tested ligands have better interaction with the target protein 5BMM *(E. coli)* than 3LPS *(S. aureus)*. This assessment was based on the binding energy and Ki values obtained, the lower the value, the better the interaction formed. This was consistent with the data from the results of antibacterial activity of chloroform extract which showed higher sensitivity to *E. coli* than *S. aureus*.

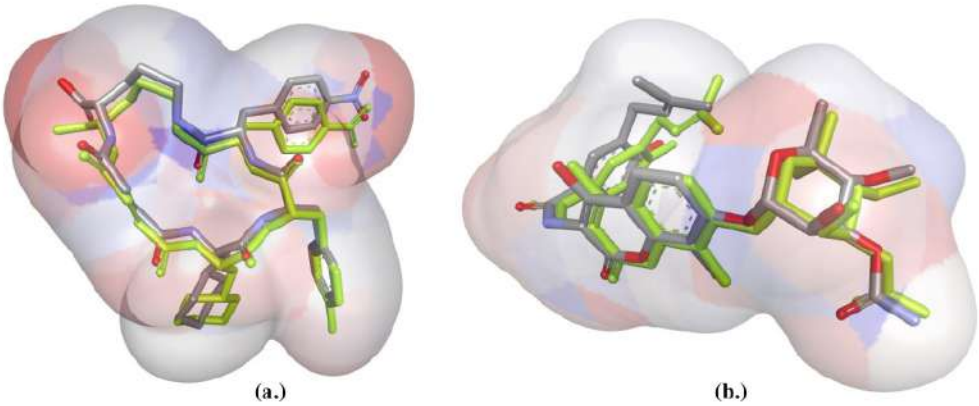
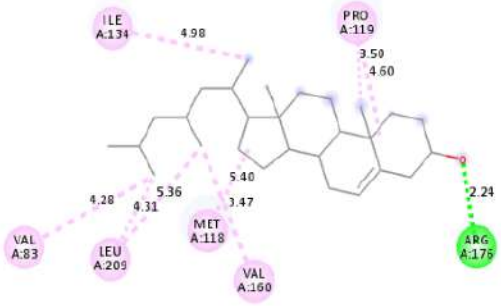


Fig. 3. Structure conformation comparison of the native (grey) and redocking ligands (green), **(a.)** Ligand for 5BMM, **(b.)** Ligand for 3LPS

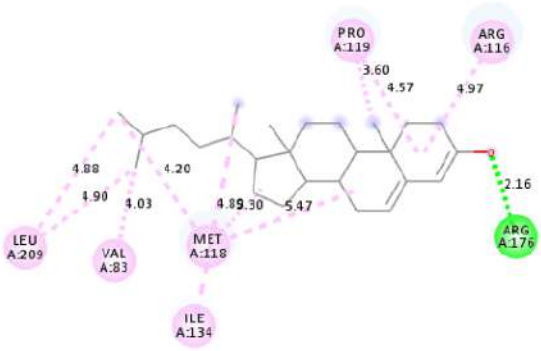
Table 3.
The result of docking analysis

Compound	5BMM		3LPS	
	Binding Energy (kcal.mol^{-1})	Ki (mM)	Binding Energy (kcal.mol^{-1})	Ki (mM)
Native Ligan	-13.60	10.821 x 10^{-6}	-5.70	6.583 x 10^{-2}
1	-4.65	38.898 x 10^{-2}	-4.56	45.144 x 10^{-2}
2	-9.99	4.759 x 10^{-5}	-8.00	0.137 x 10^{-2}
3	-6.00	3.994 x 10^{-2}	-4.89	25.939 x 10^{-2}
4	-4.14	91.73 x 10^{-2}	-2.60	12.34
5	-5.03	20.524 x 10^{-2}	-4.77	31.795 x 10^{-2}
6	-5.85	5.176 x 10^{-2}	-5.18	15.992 x 10^{-2}
7	-4.79	30.608 x 10^{-2}	-4.05	1.07
8	-4.40	59.388 x 10^{-2}	-4.85	27.971 x 10^{-2}
9	-4.86	27.383 x 10^{-2}	-2.87	7.89
10	-9.67	8.095 x 10^{-5}	-7.93	0.153 x 10^{-2}
11	-4.81	29.840 x 10^{-2}	-4.44	56.027 x 10^{-2}
12	-5.02	20.804 x 10^{-2}	-4.75	32.818 x 10^{-2}
13	-5.58	8.121 x 10^{-2}	-5.11	17.821 x 10^{-2}
Ciprofloxacin	-7.50	0.315 x 10^{-2}	-7.32	0.430 x 10^{-2}

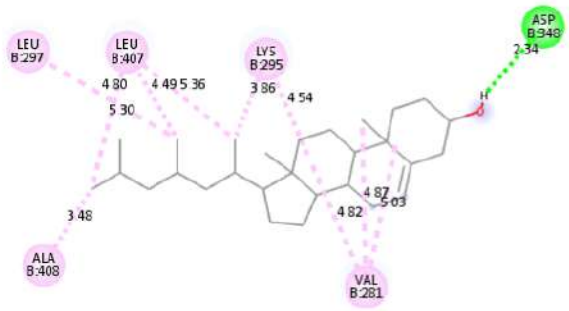
Structure orientation with binding energy lower than -5.0 kcal.mol^{-1} can be considered as the optimum interaction [29]. Based on these criteria, it can be judged that most of the tested ligands show good interactions with the 5BMM target protein. On the other hand, for the 3LPS target protein, most of the tested ligands have binding energy that was higher than this parameter. Another parameter that also needs to be considered was the inhibition constant (Ki). Based on research conducted by Arulanandam [30], the lower Ki values and binding energy indicate a good interaction and also correlate with in vitro assay. The binding energy data and inhibition constants obtained show a positive correlation, low binding energy values give low inhibition constants.



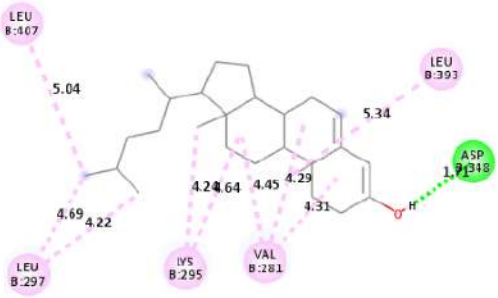
(a) Cholest-5-en-3-ol on active site of 3LPS



(b) Cholesta-4,6-dien-3-ol on active site of 3LPS



(c) Cholest-5-en-3-ol on active site of 5BMM



(d) Cholesta-4,6-dien-3-ol on active site of 5BMM

Fig. 4. Interaction of the tested ligand with amino acids on the active site of the target protein, green line was conventional hydrogen bond and pink line was alkyl interacton

Comparison of binding energy and Ki values for each of the tested and control ligands shows that compounds 2 and 10 which were steroid class of compounds have better interaction when compared to Ciprofloxacin which was commercial antibiotic compounds. The stability of the ligand interaction which was described by the energy binding value and Ki was determined by the type and number of ligand interactions against the amino acids on the active site of the target protein. The interaction can be seen in Figure 4.

forms hydrogen bond (green line) on the amino acid Asp348 residue with 2.34 Å of distance. The compound also showed Alkyl bonds (pink line) on the amino acid residues Val281 (4.82, 4.87 and 5.03 Å), Lys295 (3.86 and 4.54 Å), Leu297 (4.80 Å), Leu407 (4.49, 5.30 and 5.36 Å) and Ala408 (3.48 Å). The interaction of compound 10 involves amino acid residues which were approximately the same as the interactions in compound 2. Meanwhile, the interaction of compound 2 with the target protein 3LPS involves hydrogen bonding

interactions on the amino acid residue Arg176 with 2.24 Å length of the bond. Interactions in the form of Alkyl bonds were also formed on the amino acid residues Val83 (4.28 Å), Met118 (5.4 0 Å), Pro119 (3.50 and 4.60 Å), Ile134 (4.98 Å), Val160 (3.47 Å) and Leu209 (4.31 and 5.36 Å). The interaction of compound 10 with the target protein 3LPS involves almost the same amino acid residues as the interaction in compound 2. The presence of this interaction contributes to the stability of the interaction of the compound with the target protein. This also indicates that compound 2 and compound 10 have potential as antibacterial agents.

Conclusions

The chloroform extract of *G. salicornia* has the potential as antibacterial candidates. The inhibition of chloroform extract against *E. coli* was strong, the clear zone diameter was 17.3±1.65 mm, but the inhibition against *S. aureus* was moderate with a clear zone diameter of 13.44 ± 0.04 mm. This was confirmed by molecular docking analysis, which showed that the tested ligand has a greater affinity for the target protein 5BMM *(E. coli)* than 3LPS *(S. aureus)*. The GC-MS profile shows the presence of fatty acids, steroids, esters, terpenoids, and alkanes in the extract, which were thought to be responsible for the activity. Steroid compounds such as Cholest-5-en-3-ol and Cholesta-4,6-dien-3-ol that have been identified in chloroform extract can be used as antibacterial candidates. This was based on the better binding energy value compared to commercial antibiotic compound (Ciprofloxacin) based on molecular docking analysis.

Conflicts of interest

There is no conflict of interest.

Acknowledgment

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References

[1] B. Jubeh, Z. Breijyeh, R. Karaman, Resistance of gram-positive bacteria to current antibacterial agents and overcoming approaches, Molecules 25(12) (2020) 2888.

[2] T.J. Silhavy, D. Kahne, S. Walker, The bacterial cell envelope, Cold Spring Harb Perspect Biol 2(5) (2010) a000414.

[3] Y. Jia, X. Wen, Y. Gong, X. Wang, Current scenario of indole derivatives with potential anti-drug-resistant cancer activity, European Journal of Medicinal Chemistry 200 (2020) 112359.

[4] Z. Breijyeh, B. Jubeh, R. Karaman, Resistance of Gram-negative bacteria to current antibacterial agents and approaches to resolve it, Molecules 25(6) (2020) 1340.

[5] F. Agbaje-Daniels, A. Adeleye, A. Bola, S. Francis, D. Beukes, D. Nwankwo, Antibacterial activities of selected green seaweeds from West African Coast, EC Pharmacol Toxicol 4(8) (2020) 84-92.

[6] M. Liu, E.M. El-Hossary, T.A. Oelschlaeger, M.S. Donia, R.J. Quinn, U.R. Abdelmohsen, Potential of marine natural products against drug-resistant bacterial infections, The Lancet Infectious Diseases 19(7) (2019) e237-e245.

[7] E. Shannon, N. Abu-Ghannam, Antibacterial Derivatives of Marine Algae: An Overview of Pharmacological Mechanisms and Applications, Mar Drugs 14(4) (2016) 81.

[8] S. Rejeki, R.W. Ariyati, L.L. Widowati, R.H. Bosma, The effect of three cultivation methods and two seedling types on growth, agar content and gel strength of Gracilaria verrucosa, The Egyptian Journal of Aquatic Research 44(1) (2018) 65-70.

[9] S. Assaw, N.L. Rosli, N. Azmi, N.W. Mazlan, N. Ismail, Antioxidant and antibacterial activities of polysaccharides and methanolic crude extracts of local edible red seaweed Gracilaria sp. Malays. Appl. Biol 47(4) (2018) 135-144.

[10] S. Dayuti, Antibacterial activity of red algae (Gracilaria verrucosa) extract against Escherichia coli and Salmonella typhimurium, IOP conference series: earth and

environmental science, IOP Publishing, 2018, p. 012074.

[11] W.H. Gerwick, M.W. Bernart, Eicosanoids and related compounds from marine algae, Pharmaceutical and bioactive natural products, Springer1993, pp. 101-152.

[12] K. Chakraborty, T. Antony, M. Joy, Prospective natural anti-inflammatory drimanes attenuating pro-inflammatory 5-lipoxygenase from marine macroalga Gracilaria salicornia, Algal Research 40 (2019) 1-11.

[13] M. Guiry, AlgaeBase. World-wide electronic publication, National university of Ireland, Galway, 2020.

[14] T. Aycan, F. Öztürk, S. Demir, N. Özdemir, H. Paşaoğlu, Cobalt (III) complex of substituted nalidixic acid: Synthesis, characterization (IR, UV, EPR), single crystal X-ray, antimicrobial activity, Hirshfeld surface analysis and molecular docking, Journal of Molecular Structure 1225 (2021) 129043.

[15] T.K. George, A. Tomy, M.S. Jisha, Molecular docking study of bioactive compounds of Withania somnifera extract against topoisomerase IV type B, Proceedings of the National Academy of Sciences, India Section B: Biological Sciences 90(2) (2020) 381-390.

[16] S. Saeidnia, P. Permeh, M. Nasiri, K. Mollazadeh, F. Farahani, Biological activity of two red algae, Gracilaria salicornia and Hypnea flagelliformis from Persian Gulf, Pharmacognosy Research 1(6) (2009).

[17] S. Sasidharan, I. Darah, K. Jain, In Vivo. and In Vitro. Toxicity Study of Gracilaria changii, Pharmaceutical biology 46(6) (2008) 413-417.

[18] K. Sreejamole, P. Greeshma, Antioxidant and brine shrimp cytotoxic activities of ethanolic extract of red alga Gracilaria corticata (J. Agardh) J. Agardh, Indian J Nat Prod Resour 4(3) (2013) 233-237.

[19] A. Arulkumar, T. Rosemary, S. Paramasivam, R.B. Rajendran, Phytochemical composition, in vitro antioxidant, antibacterial potential and GC-MS analysis of red seaweeds (Gracilaria corticata and Gracilaria edulis) from Palk Bay, India, Biocatalysis agricultural biotechnology 15 (2018) 63-71.

[20] S.S.A.-l. Al-Saif, N. Abdel-Raouf, H.A. El-Wazanani, I.A. Aref, Antibacterial substances from marine algae isolated from Jeddah coast of Red sea, Saudi Arabia, Saudi journal of biological sciences 21(1) (2014) 57-64.

[21] A. Saeed, S. Abotaleb, N. Alam, A. ELMehalawy, S. Gheda, In vitro assessment of antimicrobial, antioxidant and anticancer activities of some marine macroalgae, Egyptian Journal of Botany 60(1) (2020) 81-96.

[22] A. Nabil-Adam, M.A. Shreadah, N.M. Abd El-Moneam, S.A. El-Assar, Marine algae of the genus gracilaria as multi products source for different biotechnological and medical applications, Recent patents on biotechnology 14(3) (2020) 203-228.

[23] Z.-H. Pu, Y.-Q. Zhang, Z.-Q. Yin, X. Jiao, R.-Y. Jia, L. Yang, Y. Fan, Antibacterial activity of 9-octadecanoic acid-hexadecanoic acid-tetrahydrofuran-3, 4-diyl ester from neem oil, Agricultural Sciences in China 9(8) (2010) 1236-1240.

[24] A. Manilal, S. Sujith, B. Sabarathnam, G.S. Kiran, J. Selvin, C. Shakir, A.P. Lipton, Bioactivity of the red algae Asparagopsis taxiformis collected from the southwestern coast of India, Brazilian Journal of Oceanography 58 (2010) 93-100.

[25] K. Sivakumar, S. Kannappan, M. Dineshkumar, P.K. Patil, Evaluation of marine macro alga, Ulva fasciata against bio-luminescent causing Vibrio harveyi during Penaeus monodon larviculture, African J Microbiol Res 8(8) (2014) 803-813.

[26] M. Yusuf, U.A. Fitriani Nur, A. Rifai, In vitro antibacterial activity and potential applications in food of sea urchin (Diadema setosum) from Cape of Palette, South Sulawesi, Food Research 4(6) (2020) 2139-2146.

[27] A. Rahul, M. Gautham, T. Aswathy, S. Indu, A.S. Nair, Pharmacognostic and antibacterial activity evaluation of Acacia caesia (L.) Willd, J Pharmacogn Phytochem 9(3) (2020) 48-54.

[28] S.M.M. El-Din, A.M. El-Ahwany, Bioactivity and phytochemical constituents of marine red seaweeds (Jania rubens, Corallina mediterranea and Pterocladia capillacea), Journal of Taibah University for Science 10(4) (2016) 471-484.

[29] X.S. Yin, X.Q. Zhang, J.T. Yin, D.Z. Kong, D.Q. Li, Screening and identification of

potential tyrosinase inhibitors from Semen Oroxyli extract by ultrafiltration LC-MS and in silico molecular docking, J Chromatogr Sci 57(9) (2019) 838-846.

[30] C.D. Arulanandam, In silico approach on drug repurposing-Antimalarial drugs against HIV-1 protease, bioRxiv (2021).

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