INTERNATIONAL JOURNAL OF AGRICULTURE & BIOLOGY
ISSN Print: 1560–8530; ISSN Online: 1814–9596
23–0031/2023/29–4–307–314
DOI: 10.17957/IJAB/15.2034
http://www.fspublishers.org

Full Length Article

In Vitro and *In Silico* Analysis of *Chlorella* sp. CHS1 Extracellular Metabolites: An Antivibriosis Candidate for Sustainable Aquaculture

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Received 24 January 2023; Accepted 02 March 2023; Published 17 March 2023

Abstract

An isolate of *Chlorella* sp. CHS1 from a shrimp pond has been confirmed morphologically and molecularly. It is necessary to understand how a candidate for vibriosis biocontrol interacts with the *Vibrio* protein. Information on the interaction of functional groups can be used to modify the ligands and their derivatives to increase their effectiveness. The goal of this study was to evaluate the *in-vitro* effect of a *Chlorella* sp. CHS1 on *Vibrio harveyi* and the interaction of those with hemolysin protein of *V. harveyi* using a molecular docking study. The extracellular metabolite of *Chlorella* sp. CHS1 had an inhibitory effect on *V. harveyi*. *Chlorella* sp. extracellular metabolite increased zone inhibition as CHS1 levels increased. The GCMS analysis revealed eight putative compounds with greater than 80% similarity to the database. AAG25957.1 was hemolysin *V. harveyi* target protein used in this study. According to the Swiss model protein modelling, the accuracy of the protein sequence and model template reached up to 0.92. Through the evaluation of physicochemical properties, Lipinski's role compliance and molecular docking, three most promising anti-vibriosis compounds were identified, i.e., (a) 1,2,3-propane tricarboxylic acid, 2-hydroxy, triethyl ester (ID 17), (b) hexadecenoic acid, methyl ester (ID 28), and (c) tricosane (ID 22). Hydrophobic and hydrogen bond interactions formed the interaction between a potential ligan from the extracellular metabolite of *Chlorella* sp. CHS1 and the hemolysin protein.

Keywords: Microalgae; Shrimp; *V. harveyi*; Vibriosis; Molecular docking; Biocontrol

Introduction

Vibrio harveyi is a negative-gram bacteria that lives in aquatic environments, particularly in tropical and warm waters (Zhang *et al.* 2018; Firmino *et al.* 2019). Many warm-water fish and invertebrates have been linked to *V. harveyi*-related diseases, including grouper (Shen *et al.* 2017; Zhu *et al.* 2018), sea bream (Haldar *et al.* 2010), barramundi (Dong *et al.* 2017), tiger puffer (Mohi *et al.* 2010), seahorses (Raj *et al.* 2010; Qin *et al.* 2017), abalone (Wang *et al.* 2018), rock lobster (Diggles *et al.* 2000), shrimp (Manilal *et al.* 2010; Zhou *et al.* 2012; Muthukrishnan *et al.* 2019), and sea cucumber (Becker *et al.* 2004). This bacterium has been linked to a variety of fish symptoms, including opercula nodules, scaling, skin ulcers, tail rot, vasculitis, necrotizing enteritis, and gastroenteritis are all symptoms (Zhang *et al.* 2020). Muscular necrosis caused white or opaque lesions in the tails of the affected shrimp (Zhou *et al.* 2012). *V. harveyi* caused significant mortality in captivity seahorses and was identified by white spots on the surface and anorexia (Raj *et al.* 2010). The presence of white spot was also detected in abalone, which was followed by pustules and foot muscle atrophy (Wang *et al.* 2018). In immunosuppressed organisms, the severity of disease-related *V. harveyi* appears to be very high.

The virulence of a *V. harveyi* strain has been shown to be highly dependent on the host species (Vera *et al.* 1992), dose, duration of exposure, host species age (Jun and Huai-Shu 1998), and pathogenic factors of the bacterial strain (Gomez-Gil *et al.* 1998; Esteve and Herrera 2000). Extracellular proteases, hemolysin, outer membrane protein, phospholipase, and the secretion system, all played important roles in *V. harveyi* pathogenesis (Austin and Zhang 2006; Natrah *et al.* 2011). Hemolysin is a toxin that contributes significantly to the virulence of *V. harveyi* (Chattopadhyay and Banerjee 2003; Qiao *et al.* 2012; Zhao *et al.* 2021). Protein hemolysin has a molecular weight of 47.3 kDa and 419 amino acids (Zhong *et al.* 2006; Zhao *et al.* 2021).

Some microalgal allelopathic chemicals have piqued the interest of researchers due to their roles in algal community succession and potential as biocontrol agents. Tohmola *et al.* (2011) reported that microalgae produce and secrete metabolites into the medium during growth.

To cite this paper: Yuniarti A, M Dailami, NB Arifin, R Yuwanita, AM Hariati (2023). *In Vitro* and *In Silico* analysis of *Chlorella* sp. CHS1 extracellular metabolites: An antivibriosis candidate for sustainable aquaculture. *Intl J Agric Biol* 29:307–314

Carbohydrates are the most common constituent of microalgal exudates, followed by nitrogenous compounds and vitamins (Watanabe *et al.* 2008). Extensive research will be required to determine the extracellular products of microalgae's chemical composition and mode of action. Several microalgae have been reported to have antimicrobial activity. *Chlorella* sp. produced the extracellular compound cysteine protease (ECPI-2) to protect cells from threats such as viruses and herbivorous animals (Ishihara *et al.* 2006). *Chlorella* sp. extracellular fluid can also be used against *Pseudokirchneriella subcapitata* (DellaGreca *et al.* 2010). Natrah *et al.* (2011) discovered that extracts from *Chlorella vulgaris* CCAP211/12 and *C. saccharophila* CCAP211/48 inhibited QS-regulated violacein production in CV026 indicating the presence of N-hexanoyl homoserine lactone.

Chlorella sp. CHS1 has been identified morphologically and molecularly as a species of microalgae indigenous to shrimp ponds. The potential biocontrol of vibriosis caused by *V. harveyi* by an extracellular metabolite of *Chlorella* sp. CHS1 was investigated. Knowing how a candidate for vibriosis biocontrol interacts with the virulence protein is important when considering a candidate for vibriosis biocontrol. The goal of this study was to evaluate the in-vitro effect of a *Chlorella* sp. CHS1 extracellular metabolite on *V. harveyi* and the interaction of those with the hemolysin protein from *V. harveyi* using a molecular docking study.

Materials and Methods

Culture of microalgae

Chlorella sp. CHS1 from ponds in the Situbondo area was used in this study for microalgae culture. Cultures of *Chlorella* sp. CHS1 have been identified morphologically and molecularly. *Chlorella* sp. CHS 1 was grown in Walne medium (Jayasankar and Valsala 2008) at 25°C, pH 7, 25 ppt salinity, and a light intensity of 4,500 lux.

Extraction of extracellular metabolites of *Chlorella* sp. CHS1

The method of Natrah *et al.* (2011) for preparing of algal supernatant extract for microalgae was used. During the late stationary period, algae were harvested. The culture was centrifuged for 5 min at 5000 rpm, and the supernatant was collected at -20°C. A 10 mL of the *Chlorella* sp. supernatant was thoroughly mixed with 10 mL of ethyl acetate. The solution was centrifuged at 3000 rpm for 10 min, and the ethyl acetate fraction was collected. This extraction was carried out twice. The sample was then evaporated at 30°C dissolved in 100 µL of 100% acetonitrile, and finally diluted with 300 µL of ddH_2O. All samples should be stored at -20°C in glass sample vials until use.

In vitro analysis

This study was conducted to evaluate antimicrobial growth against *V. harveyi*, which was cultured for 24 h in 10 mL of TSB containing 2% of NaCl at 30℃. The broth was washed and suspended with PBS after being centrifuged at 2000 rpm (pH 7, 2). This bacterial suspension (1 mL) was placed in a TSA plate containing 2% NaCl. TSA wells were formed, and 10 µL of *Chlorella* sp. extracellular metabolites was added. The plates were incubated at 30°C for 24 and 48 h, and the zones of inhibition around the wells were measured at both times.

GC-MS analysis

Gas chromatography-mass spectrometry (GC-MS) analysis was performed using an Agilent Technologies-7890A gas chromatograph with a mass-selective detector (5975C NICI-MS). A capillary column DB-5HT was used in the chromatography system (30 mm × 0.320 mm, film thickness 0.10 m). The injection was carried out automatically at a temperature of 250°C in the injector. The programmed temperature was applied with column starting at 180°C, and gradually increasing by 50°C per min until it reached 325°C and held there for 1 min. In split-less mode, helium gas (99.99% purity) is used as the carrier gas, with a constant flow rate of 3.5 $mL.min^{-1}$ (1 L).

In silico analysis

Homology modeling of hemolysin protein: The SWISS-MODEL template library was searched for template using BLAST (Camacho *et al.* 2009; Bienert *et al.* 2017). BLAST was used to search the target sequence of the *V. harveyi* (AAG25957.1) against the primary amino acid sequence in the database (Zhang *et al.* 2001). A total of eight templates were discovered. Models were created using the target-template alignment. The QMEANDisCo scoring function was used to evaluate the global and per-residue model (Studer *et al.* 2020).

Preparation of the ligand: In the current docking study, several identified compounds from *Chlorella* sp. CHS1 extracellular metabolite were used. PubChem (http://pubchem.ncbi.nlm.nih.gov) was used to download the 3D structures. The inhibitor molecule was given the Gasteiger and Kollman united atom charges. The 3D structures were minimized to obtain the most stable energy and then converted to *.pdb format with open babel oftware (O'Boyle *et al.* 2011) and compiled in Pyrx 0.8.

Molecular docking and ligan interaction: The Auto Dock Vina program (Trott and Olson 2010) compiled in Pyrx software was used for molecular docking. To evaluate the ligand binding energies across the conformational search space, the Lamarckian genetic algorithm was used. A polar hydrogen atom was added to the receptor protein. The active site of the protein accommodated in the docking was

defined by a grid box region of 25 × 25 × 25 with a 0.375 Å span and center coordinates of x (204.54), y (15.42), z (34.74). The interaction of Ligan protein was investigated using Discovery Studio tools. SwissADME web-based platform (http://www.swissadme.ch/) was used to analyze the physicochemical properties and fulfillment of Lipinski's (Daina *et al.* 2017).

Results

In vitro analysis

The antimicrobial properties of *Chlorella* sp. CHS1 extracellular metabolites were tested against *V. harveyi* using a well diffusion assay. Table 1 shows the results of the antagonism assays used in this study. The zone inhibition increased as the concentration of extracellular metabolites of *Chlorella* sp. CHS1 increased, according to the well diffusion assay. As a control, there was no zone of inhibition. After 48 h, the inhibitory zones in all concentrations were reduced by 5.86–29.76%.

GM-MS analysis

The GC-MS analysis of *Chlorella* sp. extracellular metabolites CHS1 was successful in detecting 41 different of active compounds. The active compounds were identified using a comparison of retention time and mass spectrum in the GC-MS library (Fig. 1). Only eight putative compounds shared more than 80% similarity with the C-MS library database (Table 2). Following that, the eight compounds were chosen for molecular docking analysis.

Homology modeling of protein hemolysin *V. harveyi*

Hemolysin, which play an important role in the virulence of *V. harveyi*, has the ability to lyse red blood cells by creating pores in the cytoplasmic membrane. The amino acid sequence of the *V. harveyi* hemolysin protein, accession number of AAG25957.1, was obtained from the Gen Bank (Zhang *et al.* 2001). Three-dimensional (3D) structure modeling was performed using the Swiss model (https://swissmodel.expasy.org/) and a template from *V. vulnificus* protein hemolysin. There were eight protein structures from a protein database (pdb) with sequence identities ranging from 75.36 to 76.79% (data were not shown). Model 7 was chosen as the best model based on the highest QMEANDisCoGlobal value (0.90) among the other models. The alignment of the amino acid sequence of *V. harveyi* hemolysin protein and the template model (PDB ID 6jl2.2.A) is shown in Fig. 2. The Ramachandran plot was used to assess the structure quality of the modeled hemolysin protein (model 7). Fig. 3a–b show the 3D structure of the modeled hemolysin protein as well as the Ramachadran plot result. According to the Ramachandran plot analysis, 97.37% of the amino acids in that protein were in the favored region.

The molecular docking and ligand interaction

The docking analysis revealed that seven (7) compounds and the naturally occurring ligand (hexa-ethylene glycol) could bind to the same active site of the hemolysin protein. However, butylated hydroxytoluene (ID11) was unable to bind to the same active site. Fig. 4 depicts the binding positions of eight (8) putative compounds and the natural ligand for the hemolysin protein. Table 3 shows the binding affinity of eight putative compounds and natural ligands with hemolysin *V. harveyi*. There were five compounds with lower binding affinity values than natural ligands (hexa-ethylene glycol); (1) Hexadecanoic acid, methyl ester, (2) Heneicosane, (3) Docosane, (4) Tetracosane and (5) Tricosane. This suggested that those five compounds are potential inhibitors of *V. harveyi* protein hemolysin. The ADME (adsorption, distribution, metabolism and elimination) related physicochemical and pharmacokinetic properties were evaluated using SwissADME (Daina *et al.* 2017) (http://www.swissadme.ch/). Table 4 shows the psychochemical and pharmacokinetic properties of the eight putative compounds. The scoring was done based on the value of binding energy, Lipinski violation, bioavailability, and potential for chemical synthesis to select the compound with high potential (Table 5). The scoring results ranged from 24.84 to 26.97. (1) 1,2,3-propanetricarboxylic acid, 2-hydroxy-, triethyl ester, (2) hexadecanoic acid, (3) tricosane were the top three candidates for anti-vibriosis compounds. Various chemical interactions can cause a ligan to bind to the target protein. To discover this relationship, the discovery studio was used to analyze how ligands and receptors interact. Previous research suggested that the three chemicals might have anti-vibrio properties. Fig 5 depicts the interactions of these three compounds with the hemolysin protein of *V. harveyi*.

Discussion

The zone inhibition increased as the amount of *Chlorella* sp. extracellular metabolites increased. This decrease demonstrated that *Chlorella* sp. extracellular metabolites CHS1 is bacteriostactic (suppresses growth) rather than bacteriocidal (kills bacteria). High cell density microalgae cultures were discovered to excrete growth inhibitors into supernatant, as previously reported in *Nannochloropsis* sp (Richmond and Zou 1999) and *C. vulgaris* (Javanmardian and Palsson 1991). Diatom extracellular polyunsaturated aldehydes also influenced bacterial development (Ribalet *et al.* 2008). However, depending on the type of bacteria, these chemicals have varying effects on bacterial growth, either suppressing or stimulating. Natrah *et al.* (2011) discovered no evidence of inhibitory growth activity caused by the cell-free supernatant of microalgal cultures. The chemicals, on the other hand, inhibited acyl-homoserine lactone-regulated violacein production and bioluminescence in the aquaculture pathogen *V. harveyi*. Haloperoxidase enzyme

Table 1: Extracellular metabolites of *Chlorella* sp CHS1 were evaluated *in vitro*

Concentration of *Chlorella* sp extracellular metabolites (µg.mL^{-1})	Inhibitory zone (mm) at	
	24 h	48 h
1	0.93±0.10	0.66±0.17
10	3.03±0.06	2.85±0.06
100	3.97±0.26	3.67±0.25
1000	5.89±0.21	5.41±0.22
DMSO	0.00±0.00	00.0±0.00

Mean ± standard deviation

Table 2: The putative compounds derived from *Chlorella* sp. CHS1extracellular metabolites

No	ID	RT	Area (%)	Library/ID	Quality	MF	MW (g.mol^{-1})
1	33	17.805	3.44	Heneicosane	96	$C_{21}H_{44}$	296.6
2	40	21.449	4.95	Docosane	98	$C_{22}H_{46}$	310.6
3	22	11.337	9.97	Tricosane	99	$C_{23}H_{48}$	324.6l
4	34	18.715	11.91	Tetracosane	97	$C_{24}H_{50}$	338.7
5	28	13.828	2.8	Hexadecanoic acid, methyl ester	90	$C_{17}H_{34}O_2$	270.5
6	11	9.454	4.22	Butylated Hydroxytoluene	98	$C_{15}H_{24}O$	220.35
7	26	13.093	20	1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester	86	$C_{16}H_{22}O_4$	278.34
8	17	10.639	6.38	1,2,3-Propanetricarboxylic acid, 2 -hydroxy-, triethyl ester	86	$C_{12}H_{20}O_7$	276.28

RT: retention time, MF: molecular formula, MW: molecular weight

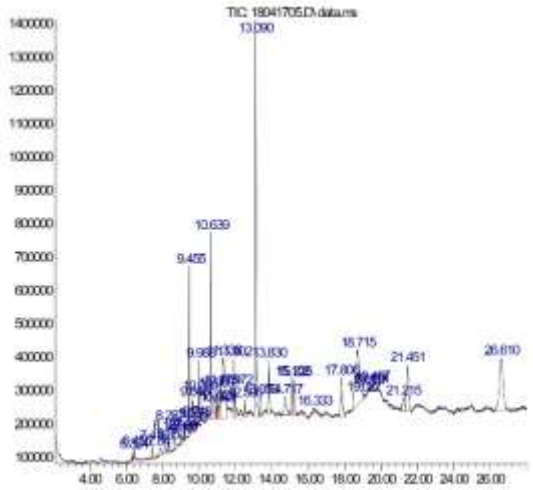


Fig. 1: *Chlorella* sp. CHS1 extracellular metabolites GC-MS analysis

Fig. 2: The amino acid sequences alignment of *V. harveyi* and template *V. vulnificus* hemolysins (6jl2.2.A)

from diatoms also interfered with quorum sensing by halogenating the acyl side chain and preventing AHL binding to the quorum sensing regulator (Amin *et al.* 2012).

Molecular docking, using either structure-based or ligand-based methods, has played an important role in the development of therapeutically important small molecules (Sliwoski *et al.* 2014). When the crystal structure of a protein cannot be determined empirically, homology modelling is used. Schmidt *et al.* (2014) demonstrated how modeling could result in precise target prediction in drug designing activity. Virtual screening of bioactive molecules is essential for weeding out potentially effective drugs in preclinical development.

According to Lipinski's rule, drug compounds that meet the criteria can penetrate cell membranes and be absorbed in the body: (1) a molecular weight (MW) <500

Table 3: The putative compounds derived from *Chlorella* sp. CHS1 extracellular metabolites

No	Compound ID	Molecular Formula	Affinity (kkal/mol^{-1})
1	28	Hexadecanoic acid, methyl ester	−7.1
2	33	Heneicosane	−6.5
3	40	Docosane	−6.4
4	34	Tetracosane	−6.3
5	22	Tricosane	−6.2
6	17	1,2,3-Propanetricarboxylic acid, 2 -hydroxy-, triethyl ester	−5.1
7	26	1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester	−5.1
8	11	Butylated Hydroxytoluene	−3.9
9	Natural ligand	Hexaethylene glycol	−5.4

Table 4: The psychochemical properties of eight potential *Chlorella* sp. Compounds SwissADME-based CHS1

ID	Molecular Formula	MW	H-bond acceptors	H-bond donors	Consensus Log P	ESOL Log S	Violation of Lipinski	Bioavailability	Synthetic Accessibility
11	$C_{15}H_{30}O$	249.58	1	1	3.71	-4.32	0	0.55	4.19
17	$C_{12}H_{20}O_7$	295.43	7	1	0.80	-0.99	0	0.55	3.63
22	$C_{23}H_{48}$	324.63	0	0	9.00	-8.14	1	0.55	3.08
26	$C_{16}H_{28}O_4$	306.57	4	0	3.19	-3.85	0	0.55	4.51
28	$C_{17}H_{34}O_2$	304.72	2	0	5.42	-5.39	1	0.55	3.89
33	$C_{21}H_{44}$	340.92	0	0	8.08	-7.69	1	0.55	4.66
34	$C_{24}H_{50}$	338.65	0	0	9.35	-8.50	1	0.55	3.20
40	$C_{22}H_{46}$	310.60	0	0	8.64	-7.78	1	0.55	2.96

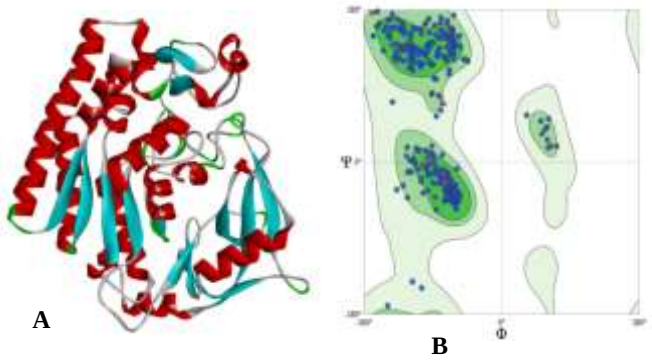


Fig. 3: (a) The 3D structure of *V. harveyi* hemolysin protein model (b). The Ramachandran plot's outcome

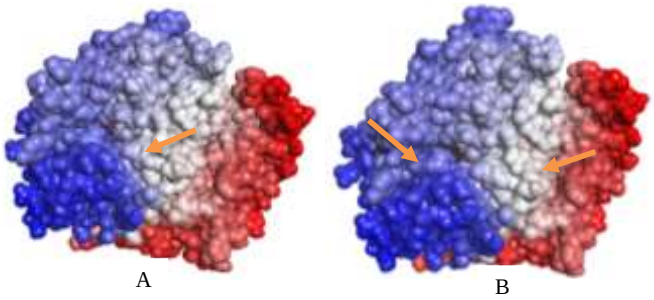
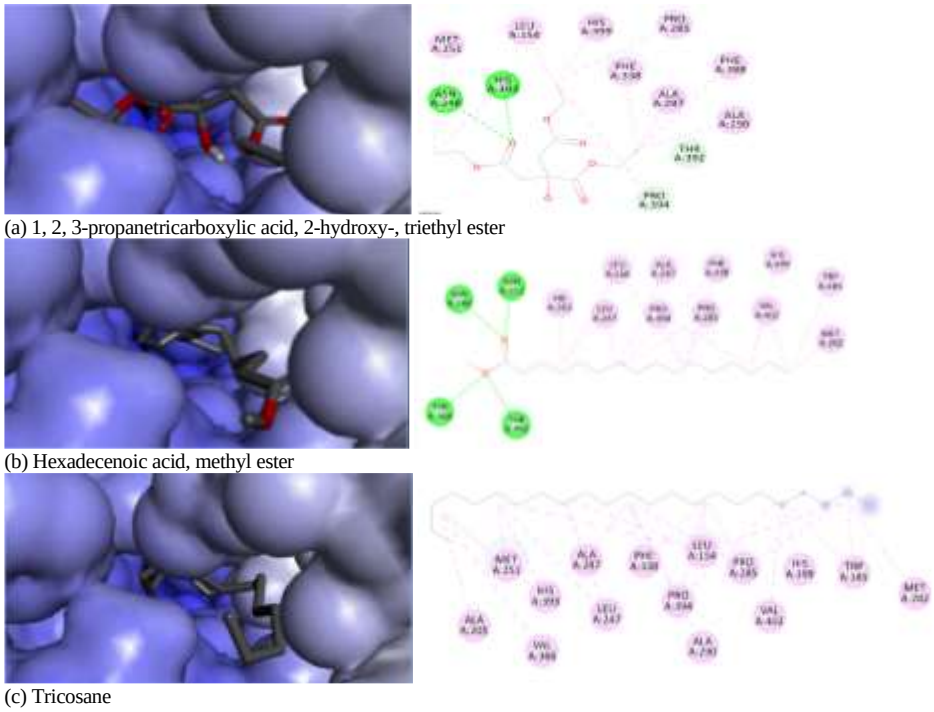


Fig. 4: (a) The natural ligand's pose and binding position (hexa-ethylene glycol), (b) the seven putative compounds from *Chlorella* sp. extracellular metabolites of CHS1

grams.mol^{-1}, (2) a hydrogen bond proton donor group <5, (3) a hydrogen bond proton acceptor group <10, and (4) the logarithm value of the partition coefficient in water and 1-octanol <5 (Lipinski *et al.* 2012). Table 4 shows that the chemicals with the ID numbers 11, 17 and 26 did not violate Lipinski's role. Meanwhile, the other chemicals violated Lipinski's role (ID 22, 28, 33, 34 and 40). The ability of a drug compound to absorb and circulate in the body is referred to as bioavailability (Daina *et al.* 2017). The SwissADME predictions revealed that all compounds identified in this study have a bioavailability value of 0.55, indicating a high possibility of absorption in the body. Synthetic accessibility (SA) score is based on the assumption that the frequency of molecular fragments in

Table 5: Scoring of eight putative extracellular metabolites of *Chlorella* sp. CHS1

ID	Molecular Formula	Affinity (kkal.mol^{-1})		Lipinski Role Violation		Bioavailability		Synthetic Accessibility		Total score	Rank
		Value	Score	Value	Score	Value	Score	Value	Score		
11	$C_{15}H_{30}O$	-3.9	3.9	0	10	0.55	5.5	4.19	5.81	25.21	7
17	$C_{12}H_{20}O_7$	-5.1	5.1	0	10	0.55	5.5	3.63	6.37	26.97	1
22	$C_{23}H_{48}$	-6.2	6.2	1	7.5	0.55	5.5	3.08	6.92	26.12	3
26	$C_{16}H_{28}O_4$	-5.1	5.1	0	10	0.55	5.5	4.51	5.49	26.09	5
28	$C_{17}H_{34}O_2$	-7.1	7.1	1	7.5	0.55	5.5	3.89	6.11	26.21	2
33	$C_{21}H_{44}$	-6.5	6.5	1	7.5	0.55	5.5	4.66	5.34	24.84	8
34	$C_{24}H_{50}$	-6.3	6.3	1	7.5	0.55	5.5	3.20	6.80	26.10	4
40	$C_{22}H_{46}$	-6.4	6.4	1	7.5	0.55	5.5	2.96	7.04	26.08	6



(a) 1, 2, 3-propanetricarboxylic acid, 2-hydroxy-, triethyl ester

(b) Hexadecenoic acid, methyl ester

(c) Tricosane

Fig. 5: The best pose and interaction of extracellular metabolites (a) 1,2,3-propanetricarboxylic acid, 2-hydroxy-, triethyl ester, (b) Hexadecenoic acid, methyl ester, and (c) Tricosane

"truly" attainable compounds correlates with the ease of synthesis (Daina *et al.* 2017). Furthermore, the SA score should be between 1 to 10. The higher the value, the more difficult it is for compound to be chemically synthesized. The SA values of the eight putative *Chlorella* sp. compounds CHS1 ranged between 2.96 to 4.66. Docosane (ID 40) was the simplest to synthesize, while Heneicosane was the most difficult (ID 33).

The biological activity was thought to be caused by both H-bond and hydrophobic interactions between the ligands/inhibitors and the active regions of the receptor (Madeswaran *et al.* 2012). The interactions that occurred between the ligand and the hemolysin protein in this study were hydrogen bond (H-bond) and hydrophobic. According to Fig. 5, the compound 1,2,3-Propanetricarboxylic acid, 2-hydroxy, triethyl ester, and protein hemolysin were able to form five hydrogen bonds and ten hydrophobic interactions. ASN248, HIS393, THR392 and PRO394 amino acids were used to form hydrogen bonds. The methyl ester of hexadecenoic acid could form five hydrogen bonds and seven hydrophobic interactions. ASN248, ASN 252, TYR368, THR392 and ASN252 were the active sites of the residues involved. Furthermore, 28 hydrophobic interactions were found for tricosane, indicating that ligan can bind to the hemolysin protein. Small compounds bound to the target protein pocket via H-bonds and hydrophobic interactions may be able to prevent the conformational changes that result in fusion (Sivakumar *et al.* 2021). Although larger conformational changes degrade performance, smaller ligand-induced protein movements appear to have little effect on rigid docking performance (Verdonk *et al.* 2008). It was discovered that a flavonoid (found in papaya, apple and lemon) with anti-dengue activity could halt the dengue virus fusion process by impeding the movement of the hinge area and obstructing the conformational rearrangement in envelope protein (Mir *et al.* 2016).

The drug for neutralizing a target protein of *Vibrio* spp. was predicted using a molecular docking study against vibriosis. Sivakumar *et al.* (2021) used a compound from *Ulva fasciata* extract in a molecular docking study against the hemolysin protein *V. harveyi*. They discovered that methyl dehydroabietate had the highest binding affinity on the active pocket of hemolysin protein. Arunkumar *et al.* (2017) investigated several natural substances for their ability to suppress *Vibrio* hemolysin. Cyanidin and Bergapten have been identified as potential compounds for the development of novel and potentially effective drugs to treat vibriosis. Boronic acid derivatives bind to the binding site of the LuxP protein, according to *V. harveyi*, Rajamanikandan and Jeyakanthan (2017). The chemical inhibitor of *V. harveyi* biofilm development was discovered to be [2.2.1] hept-5-ene-2,3-dicarboxylic acid-2,6-dimethylpyridine 1-oxide.

Conclusion

An *in vitro* study revealed that the extracellular metabolites of *Chlorella* sp. CHS1 is bacteriostactic (growth suppressing) in a dose dependent manner. According to the virtual screening analysis using molecular docking, physiochemistry criteria, and Lipinski's drug similarity rule, three potential anti-vibrio compounds exist; 1,2,3-Propanetricarboxylic acid, 2 -hydroxy-, triethyl ester), Hexadecenoic acid, methyl ester), and Tricosane. The potent compounds interact with the hemolysin protein *V. harveyi* via hydrogen bond and hydrophobic interactions.

Acknowledgements

University of Brawijaya funded this study with grant number: 90/UN1/DITLIT/DIT-LIT/LT/2017.

Author Contributions

ATY designed the experiment and wrote the paper. MD carried out the in-silico analysis. The microalgae culture, extracellular metabolites, GC-MS analysis, and in-vitro study were prepared by NBA and RY. AMH reviewed the manuscript and analyzed the data.

Conflicts of Interest

This research has no potential conflict of interest.

Data Availability

The data described in this work will be made available upon reasonable request to the corresponding author.

Ethics Approval

Not applicable in this study.

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