Potential of antibacterial compound from extract of the green algae Bornetella sp

by

IOP Conference Series: Earth and Environmental Science

Potential of antibacterial compound from extract of the green algae *Bornetella sp*

Potential of antibacterial compound from extract of the green algae *Bornetella sp.*

Bahrun, N H Soekamto*, Firdaus and A Akbar

Chemistry Departement, Faculty of Mathematics and Natural Sciences, Hasanuddin University Jln. PerintisKemerdekaan Km 10, KampusTamalanrea, Makassar-Indonesia

*E-mail: Noek_1512@yahoo.com

Abstract. Antibiotics resistance has become an obstacle in the treatment of various types of diseases caused by bacterial infections. The investigation of secondary metabolites from macroalgae as antibiotic reference compounds can be done as an effort to overcome this problem. Potential test for antibacterial compounds from green algae *Bornetellasp* has been carried out against *Escherecia coli* and *Staphylococcus aureus* bacteria. The secondary metabolite extraction process was carried out using a multilevel maceration method using n-hexane, chloroform, ethyl acetate and methanol as solvents. The extract obtained was then tested for phytochemicals and their activity against the tested bacteria by the paper disc diffusion method. The test results obtained showed that the chloroform extract had the highest activity with an inhibition zone diameter of 16.33 ±1.18 mm against *E. coli* and 14.26 ±0.69 mm against *S. aureus*. The presence of secondary metabolites of the alkaloid, steroid, phenolic and flavonoid groups based on the results of phytochemical screening is the cause of this activity.

1. Introduction

Infectious diseases are one of the main causes of worldwide death cases, this is exacerbated by the emergence of antibiotic resistance cases [1]. The case of antibiotic resistance shows the large global health crisis and it is one of the most serious threats facing the world community today [2]. Antibiotic resistance occurs when bacteria can neutralize antibiotics by pumping them out of cells or changing the structure of their cell walls to minimize drug interactions with bacterial cells [3]. The research for new bioactive compounds is one of the solutions to overcome this problem [4, 5]. Natural products are a source of secondary metabolites with varying structures and bioactivity. Another advantage of natural products is the high level of availability and contains secondary metabolites which have similar properties with medicinal compounds (drug-likeness property) and have pharmacological effects which play as substances that are effective against drug resistance [4, 6].

Macroalgae is one of the marine natural products with a very diverse ~~of~~ structure and bioactivity of secondary metabolite compounds, one of which is as an antibacterial agent [7][8]. The diversity of structure and potential of secondary metabolites from macroalgae was formed naturally as an effort to survive in extreme environmental conditions also to fight predators. In this case, macroalgae produce secondary metabolites that significantly different from plant extracts to survive. This inspires the researchers to explore the bioactive compound from macroalgae [7, 9].

Bornetella sp is a green alga belonging to the Dasycladaceae family.Several studies have shown the potential of Dasycladaceae as a potential antibacterial source. *Acetabularia acetabulum* showed antibacterial activity against *S. aureus, S. pneumonia* [10] and *E. coli* [11]. *Cymopoliabarbata* exhibits as a potential broad-spectrum antibacterial agent [12]. Dasycladaceae is known to contain terpenoid compounds such as brominated sesquiterpenes [13, 14], flavonoid compounds such as catechins and epicatechin [15], polyisoprenylatedbromohydroquinones compounds such as

7-Hydroxycymopochromanone (PBQ1) and 7-Hydroxycymopolone (PBQ2) [16], phenolic compounds such as sulfated phenolic acids [17] and also contain sulfated coumarins compound [18, 19]. However, the secondary metabolites from the *Bornetella sp* species have not been explored, this was proven by the lack of published articles. This study aims to explore the potential of *Bornetella sp* and it is expected to be able to contribute to fighting antibiotic resistance.

2.Materials and Method

2.1 Sample collection and preparation

Samples were obtained from the coastal waters of the Selayar Islands, South Sulawesi, at the coordinates of 5°53'56.52"SL and 120°27'3.49"EL (figure 1). Sampling was carried out in July 2020 using a snorkelling device at a depth of 1-1.5 meters.

The samples that have been collected were immediately washed several times using seawater to remove remaining soil and epiphytes. Washing was continued using distilled water to remove the salt. The cleaned samples were then left to dry for 5-7 days. Furthermore, the sample is milled using a grinding machine to maximize the solvent diffusion process during the extraction process.

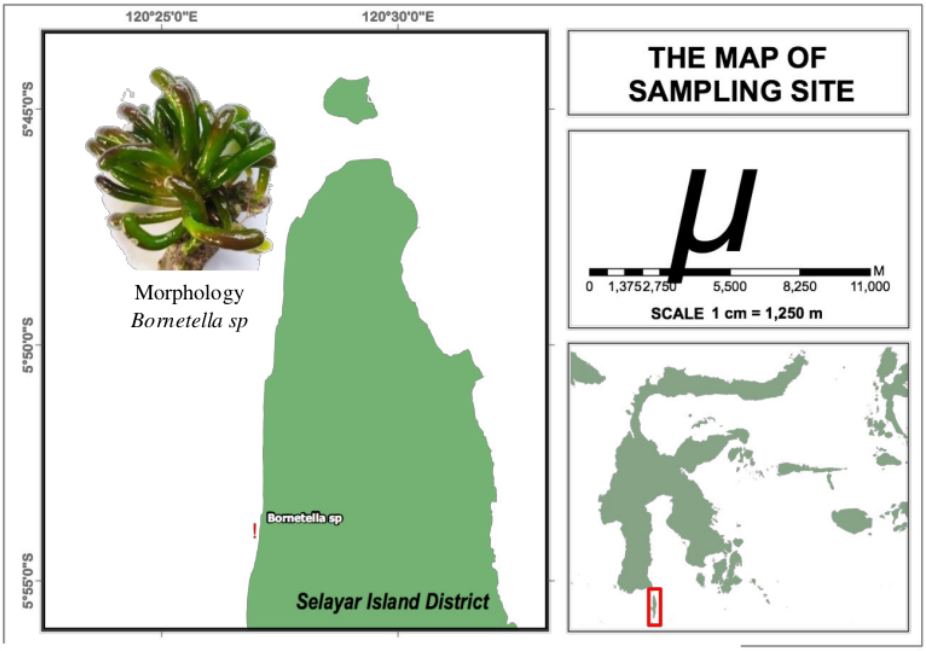

Figure 1. Map of sampling site with the figure of the samples

2.2. Sample extraction

A total of 0.5 kg of sample was macerated gradually in n-hexane, chloroform, ethyl acetate and methanol. Then filtered using Whatman filter paper no. 41. The extract then concentrated using a rotary evaporator.

2.3. Phytochemical screening

The phytochemical screening was carried out to determine the class of secondary metabolite compounds in the extract based on the protocol conducted by Harborne, 1987 [20]

2.4. Antibacterial activity of seaweeds extracts using disc diffusion method

The bacterial strains were prepared from 24 hours of old cultures in nutrient broth. Nutrient agar plates were prepared and the inocula then wiped using a sterile wipe on the surface of the Agar Nutrient media. Paper disc of 6 mm diameter was sterilized and saturated with the extracts and control then air-dried before placing on the seeded agar plate. After 24 hours of incubation at 37°C, the inhibition zone from the edge of the disc to the inner margin of the surrounding bacterial growth was measured in mm by using graduated scale and recorded [21].

3. Result and Discussion

3.1 Phytochemical profile of extracts

Phytochemical components of *Bornetellasp* were extracted continuously using solvents with different levels of polarity. The extraction process begins with a nonpolar solvent, namely n-hexane, then continues with chloroform, ethyl acetate and ends with a polar solvent, namely methanol. This method aims to obtain extracts containing groups of secondary metabolite compounds with varying polarity. The phytochemical profile of the extract can be seen in table 1.

Table 1. Preliminary phytochemical screening of various extracts of *Bornetellasp.*

Solvent	Phytochemical					
	Alkaloid	**Terpenoid**	**Steroid**	**Phenolic**	**Flavonoid**	**Saponin**
n-Hexane	++	-	+	+	+	-
Chloroform	++	-	++	+	+	-
Ethyl Acetate	+	-	+	+	+	-
Methanol	-	-	-	+	++	++

Phytochemical test results showed a positive reaction with the reagent used mostly except for the terpenoid test. The extracts of n-hexane, chloroform and ethyl acetate showed similar phytochemical profiles consisting of alkaloid, steroid, phenolic and tannin compounds. Meanwhile, the methanol extract consists of phenolic compounds, tannins and saponins. Phenolic and tannin compounds were detected in all extracts, but saponins were only detected in methanol extract. Each of these compounds has been reported to have antibacterial activity. A similar phytochemical profile has been reported to have potential antibacterial activity [22]. Other studies have also reported that the alkaloids [23], steroids [24] and flavonoids [25] from macroalgae show potential as antibacterial agents.

3.2 Antibacterial activity

The antibacterial activity of various extracts from *Bornetellasp* can be seen in table 2. The potential antibacterial activity tests were carried out on two types of bacteria representing gram-negative and gram-positive bacteria, namely *E. coli* and *S. aureus*. Antibacterial activity by the paper disc diffusion method was determined based on the diameter of the clear zone formed. The results obtained showed that the overall inhibition of the extract against *E. coli* was greater than

aureus bacteria. The diameter of the zone of inhibition against *E. coli* ranged from 8.55–16.33 mm, while the zone of inhibition against *S. aureus* ranged from 7.01–14.26 mm. This difference is caused by differences in the components of the cell wall structure in each of these bacterial groups [26]. The data obtained is in line with research conducted by Alghazeer et al [23].

Table 2. Antibacterial activity of various extract of *Bornetellasp.*

Extract	The diameter of Inhibition Zone (mm)*	
	E. coli	***S. aureus***
n-Hexane	13.25 ±2.45	7.01 ±0.09
Chloroform	**16.33 ±1.18**	14.26±0.69
Ethyl acetate	8.55 ±0.13	7.96±0.31
Methanol	12.19 ±4.31	7.53±0.23
Control Positive	**42.10 ±0.80**	**39.73±0.73**
Control Negative	0	0

*Values are mean ±standard deviation
Inhibition zones > 15 mm were classified as strong (bold), those from 8 to 15 mm were classified as moderate, and those between 1 and 8 mm were classified as weak [27]

The comparison of the diameter of the inhibition zone showed that the chloroform extract shows broad-spectrum activities with the highest inhibition zone. This is due to the relatively large permeability of the semipolar compounds against the bacterial cell wall, making it easier for them to penetrate the bacterial cell wall. The ability of chloroform extract extraction and sensitivity to inhibit bacterial growth have been reported by Sastry and Rao [28]. Based on the classification of antibacterial activity according to Kim (2007) [27] the inhibitory level of chloroform extract against *E. coli* is categorized as a strong antibacterial (inhibition zone> 15 mm). Meanwhile, the inhibition of the same extract against *S. aureus* bacteria was categorized as moderate antibacterial (Inhibition zones 8-15 mm). The antibacterial activity in the medium category was also shown by n-hexane, ethyl acetate and methanol extracts against *E. coli* bacteria. However, the same extract had a weak category of activity against *S. aureus* bacteria. Meanwhile, ciprofloxacin as positive control showed broad-spectrum activities with strong inhibition category so that it can be used as a standard reference to the antibacterial activity of the test extract.

4. Conclusion

The chloroform extract of *Bornetellasp* shows potential as a broad-spectrum antibacterial candidate so that it can be used as a source of antibacterial compounds in the future. The overall phytochemical profile of the extract showed the presence of alkaloid, steroid, phenolic, flavonoid and saponin compounds.

Acknowledgements

Author's thanks toHasanuddin University which has facilitated the further study of the author on the doctoral program. Also thanks to the Ministry of Research, Technology, and Higher Education of the Republic of Indonesia which has supported this research through the PMDSU program.

References

[1] Hoffman P S 2020 Antibacterial discovery : 21st century challenges.*Antibiotics* **9**(213):1–10.
[2] Breijyeh Z, Jubeh B and Karaman R 2020 Resistance of Gram-Negative Bacteria to Current Antibacterial Agents and Approaches to Resolve It.M*olecules Review*, **25** (1340):1–23.
[3] Silhavy T J, Kahne D and Walker S 2010 The bacterial cell envelope.*Cold Spring HarbPerspectBiol*, **2**: 1–11.
[4] Yuan R 2017 Natural products to prevent drug resistance in cancer chemotherapy: A review.*Annals of the New York Academy of Sciences*, **1401** (1): 19–27.
[5] Liu, M., El-hossary, E.M., Oelschlaeger, T.A., Donia, M.S., Quinn, R.J. and Abdelmohsen, U.R., 2019, Review Potential of marine natural products against drug-resistant bacterial infections, *The Lancet Infectious Diseases*, **3099** (18): 1–9.
[6] Qin Y 2018*Health benefits of bioactive seaweed substances*(Qingdao, China: Elsevier Inc.)
[7] Gómez-Guzmán M, Rodríguez-Nogales A, Algieri F and Gálvez J 2018 Potential role of seaweed polyphenols in cardiovascular-associated disorders.*Marine Drugs*, **16** (8): 1–21.
[8] Singh J, Meshram V and Gupta M 2020*Bioactive Natural Products in Drug Discovery*. ed J Singh *et al* (Singapore: Springer Nature Singapore Pte Ltd)
[9] Durán A G, Rial C, Gutiérrez M T, Molinillo J M G and Macías F A 2020*Sesquiterpenes in Fresh Food*(Singapore: Springer Nature Singapore Pte Ltd)
[10] Sivaramakrishnan T 2017 Analysis of proximate composition and in-vitro antibacterial activity of selected green seweeds from South Andaman Coast of India.*International Journal of Current Microbiology and Applied Sciences*, **6** (12): 1739–49.
[11] Karthick P, Mohanraju R, Kada N M and Ramesh C H 2015Antibacterial activity of seaweeds collected from South Andaman, India.*Journal of Algal Biomass Utilization*, **6** (1): 33–6.
[12] Platas G, Basilio A, Gorrochategui J, Suay I., Portillo E and Jime M. 2001 Screening of antimicrobial activities in red, green and brown macroalgaefrom Gran Canaria (Canary Islands, Spain).*International Microbiology*, **4**: 35–40.
[13] Lumbang W A and Paul V J 1996 Chemical defenses of the tropical green seaweed NeomerisannulataDickie: Effects of multiple compounds on feeding by herbivores, *Journal of Experimental Marine Biology and Ecology*, **201**: 185–95.
[14] Paul V J, Cronan J M and Cardellina J H 1993 Isolation of new brominated sesquiterpene feeding deterrents from tropical green algae*Neomerisannulata* (Dasycladaceae : Chlorophyta), *Journal of Chemical Ecology*, **19** (9): 1847–1860.
[15] Yoshie Y, Wang W, Petillo D and Suzuki T 2000 Distribution of catechins in Japanese seaweeds, *Fisheries Science*, **66**: 998–1000.
[16] Badal S, Gallimore W, Huang G, Tzeng T J and Delgoda R 2012 Cytotoxic and potent CYP1 inhibitors from the marine algae *Cymopoliabarbata.Organic and Medicinal Chemistry Letters*, **2** (21): 1–8.
[17] Kurth C, Welling M and Pohnert G 2015 Sulfated phenolic acids from *Dasycladalessiphonous* green algae.*Phytochemistry*, **117**: 417–23.
[18] Hartmann A, Ganzera M, Karsten U, Skhirtladze A and Stuppner H 2018 Phytochemical and analytical characterization of novel sulfated coumarins in the marine green macroalga*Dasycladusvermicularis*(Scopoli) Krasser.*Molecules*, **23** (11).
[19] Kayser O and Kolodziej H 1999 Antibacterial activity of simple coumarins: Structural requirements for biological activity, *Zeitschrift fur Naturforschung - Section C Journal of Biosciences*, **54** (4): 169–174.
[20] To A G, Techniques M and Analysis O F P 1984*A Guide To Modern Techniques Of Plant Analysis*. (New York: Chapman And Hall)
[21] Radhika D, Veerabahu C and Priya R 2012 Antibacterial activity of some selected seaweeds from the Gulf of Mannar Coast, South India.*Asian Journal of Pharmaceutical and Clinical*

Research, **5** (4): 89–90.

[22] Alshalmani S K, Elshibani F A, Bengleil M S, Alamami A and Elmsllati S 2019 Investigation and antibacterial activity of libyanmacroalgae*Ulvafasciata*Salmin.*The Libyan Conference on Chemistry and Its Applications*, **1** (1): 48–51.

[23] Alghazeer R,Whida F, Abduelrhman E and Gammoudi F 2013 In vitro antibacterial activity of alkaloid extracts from green, red and brown macroalgae from western coast of Libya. *African Journal of Biotechnology*, **12** (51): 7086-7091.

[24] Azizi R A M M A, Nazemi M, Ruhani S and M A N 2020 Isolation and identification of antibacterial steroid compounds from Ulvafasciata in the Persian Gulf, **19** (5): 2384–2393.

[25] Xie Y, Yang W, Tang F, Chen X and Ren L 2015 Antibacterial activities of flavonoids : structure-activity relationship and mechanism.Current Medicinal Chemistry **86** (731): 132–49.

[26] Razarinah W, Ross R, Rahim N F A, Faridon B S and Radzun K A 2018 Antimicrobial activity of marine green algae extract against microbial pathogens.*Malaysian Journal of Biochemistry and Molecular Biology*, **21** (2): 43–47.

[27] Kim I H 2017 Antibacterial activity of *Ulvalactuca* against Methicillin-Resistant *Staphylococcucaureus* (MRSA).*Biotechnology and Bioprocess Engineering*, **12**: 579–82.

[28] Sastry V M V S and Rao G R K 1994 Antibacterial substances from marine algae: successive extraction using benzene, chloroform and methano *Botanica Marina*, **37**: 357–60.

Potential of antibacterial compound from extract of the green algae Bornetella sp

ORIGINALITY REPORT

15%	11%	11%	1%
SIMILARITY INDEX	INTERNET SOURCES	PUBLICATIONS	STUDENT PAPERS

PRIMARY SOURCES

1	darshanpublishers.com Internet Source	3%
2	www.stikes-bth.ac.id Internet Source	2%
3	In Hae Kim. "Antibacterial activity ofUlva lactuca against methicillin-resistantStaphylococcus aureus (MRSA)", Biotechnology and Bioprocess Engineering, 10/2007 Publication	2%
4	Fadli, Nasril Nasir, Abdi Dharma, Syafrizayanti, Nurainas, Rani Asmarayani, Mai Efdi. " Antifungal activity of three species against in rubber trees ", Archives of Phytopathology and Plant Protection, 2022 Publication	1%
5	Rajan, R., M. Selvaraj, S. Palraj, and G. Subramanian. "Studies on the anticorrosive & antifouling properties of the Gracilaria edulis extract incorporated epoxy paint in the Gulf	1%

of Mannar Coast, Mandapam, India", Progress in Organic Coatings, 2016.
Publication

6	www.rsc.org Internet Source	1 %
7	rjptonline.org Internet Source	1 %
8	www.sdiarticle1.org Internet Source	1 %
9	Ankit Pandeya, Isoiza Ojo, Olaniyi Alegun, Yinan Wei. "Periplasmic Targets for the Development of Effective Antimicrobials against Gram-Negative Bacteria", ACS Infectious Diseases, 2020 Publication	1 %
10	S. Suganya, R. Ishwarya, R. Jayakumar, M. Govindarajan, N.S. Alharbi, S. Kadaikunnan, J.M. Khaled, M.N. Al-anbr, B. Vaseeharan. "New insecticides and antimicrobials derived from Sargassum wightii and Halimeda gracillis seaweeds: Toxicity against mosquito vectors and antibiofilm activity against microbial pathogens", South African Journal of Botany, 2019 Publication	1 %
11	jurnal.unimed.ac.id Internet Source	1 %

12	pubs.rsc.org Internet Source	1 %
13	Palashpriya Das, Vijayshree S. Karankar. "New avenues of controlling microbial infections through anti-microbial and anti-biofilm potentials of green mono-and multi-metallic nanoparticles: A review", Journal of Microbiological Methods, 2019 Publication	<1 %
14	www.isca.in Internet Source	<1 %
15	"Recent Advances in Micro and Macroalgal Processing", Wiley, 2021 Publication	<1 %
16	ir.uitm.edu.my Internet Source	<1 %
17	jebas.org Internet Source	<1 %
18	journals.guilan.ac.ir Internet Source	<1 %
19	jssm.umt.edu.my Internet Source	<1 %
20	www.teses.usp.br Internet Source	<1 %

21	"Handbook of Marine Macroalgae", Wiley, 2011 Publication	<1%

Exclude quotes	On	Exclude matches	< 5 words
Exclude bibliography	On		