JPBIO (Jurnal Pendidikan Biologi)
Vol. 9, No. 1, April 2024, 66 – 73 //e-ISSN 2540-802X
http://jurnal.stkippersada.ac.id/jurnal/index.php/JBIO/index

Antibacterial activity in vitro investigation of eucheuma cottonii extract from aru islands against pathogenic bacteria

Check for updates

Melda Yunita[1*], Juen Carla Warella[1], Eka Astuty[1], Morgan Ohiwal[2], Sailful Alimudi[2]

[1]Faculty of Medicine, Universitas Pattimura, Indonesia
[2]Faculty of Fishery and Forestry, Universitas Muhammadiyah Maluku, Indonesia

[*] Corresponding author: meldayunita22@gmail.com

Article Info

Article History:
Received 02 February 2024
Revised 14 March 2024
Accepted 16 April 2024
Published 30 April 2024

Keywords:
Clear Zone
Escherichia coli
Inhibition
Red Algae
Staphylococcus aureus

ABSTRACT

Red algae *Eucheuma cottonii* is one of the potential marine biodiversity that provides added value in the pharmaceutical and cosmetic fields and is known to have antibacterial compounds. Research focusing on the potential of *E. cottonii* as an antibacterial is still limited, particularly when it comes to the Aru Islands. This study aimed to measure the antibacterial activity of *E. cottonii* against pathogenic bacteria including *E. coli* and *S. aereus* at several concentrations and to determine the best concentration for inhibiting these two pathogenic bacteria. The study was true experimental laboratory research with a post-test-only controlled group design. Antibacterial tests using the disc diffusion method and phytochemical tests were carried out. This study revealed that *E. cottonii* could be able to inhibit *S. aureus* ranging from 0.27±0.03 – 2.1±0.14 at a concentration of 50%-100%, and 0.17±0.05 – 0.45 ± 0.03 against *E. coli* at a concentration of 80-100%. Meanwhile, saponins and flavonoids were two bioactive compounds found through phytochemical testing. The study concluded that the n-hexane extract of *E. cottonii* was able to inhibit the growth of *E. coli* and *S. aureus,* even with a weak inhibition category. This study recommends that improvements are needed in the *E. cottonii* extraction process to maximize the antibacterial properties of *E. cottonii.*

Citation: Yunita, M., Warella, J.C., Astuty, E., Ohiwal, M., & Alimudi, S. (2024). Antibacterial activity in vitro investigation of eucheuma cottonii extract from aru islands against pathogenic bacteria. *JPBIO (Jurnal Pendidikan Biologi), 9*(1), 66-73. DOI: https://doi.org/10.31932/jpbio.v9i1.3241

INTRODUCTION

Indonesia is a maritime country with 70% of its territory consisting of seas or oceans. The ocean is a habitat for various organisms, both plants and aquatic animals (Andriani et al., 2015).

Because they are exposed to such a wide range of marine conditions—including variations in salinity, temperature, chlorophyll content, and water quality—marine organisms can synthesize novel chemicals. Even though marine areas constitute ¾ of the Earth, they are still one of the underutilized biological resources. One of the potential biological resources from Indonesian marine waters is macroalgae with various types. The distribution of macroalgae is found in almost all Indonesian waters (Andriani et al., 2015).

Macroalgae, known to the community as seaweed, is one of the potential marine biodiversity that provides added value in the pharmaceutical and cosmetic fields, including the hepatoprotective effect (Wardani et al., 2017) and antioxidant activity so it is widely used as an additional ingredient in making facial creams or sunscreens (Nurjanah et al., 2019). Research shows that marine algae have many uses in various fields, such as nutraceuticals, pharmaceuticals, especially cosmeceuticals (Syad et al., 2013; Andriani et al., 2016; Teo et al., 2020). However, the use of macroalgae in the pharmaceutical sector is still limited, while the potential of macroalgae in Indonesia, especially Maluku, as an archipelagic country is very large to be developed as a raw material for medicine. Several researchers have studied the potential of macroalgae on Maluku Island, but it is limited to Seram Island, namely Kotania Bay, West Seram (Arfah & Patti et al., 2014), and East Seram waters (Rugebregt et al., 2021). However, this research focused more on describing biodiversity and conservation efforts.

Research focusing on the potential of macroalgae as antibacterial is still limited, particularly when it comes to the Aru Islands. Indeed, macroalgae, particularly red algae (*Eucheuma cottonii*), show promise for development as antibacterial agents. Research conducted by Julyasih et al., (2021) on ethanol extracts of red algae and green algae from Bali waters found that the highest antibacterial activity was respectively obtained by *E. cottonii* (11.1 mm), *Caulerpa* spp. (10.1 mm), *Gracilaria* spp. (6.0 mm), *E. spinosum* (4.2 mm). In the other investigation, it was revealed that the antibacterial activity of *E. cottonii* from Sumenep, Madura against *Escherichia coli* was 0.33 and there was no inhibition zone against *Staphylococcus aureus* (Andriani et al., 2015) Therefore, the present study tried to investigate the antibacterial activity in vitro against *S. aureus* and *E. coli* using *E. cottonii* extract from the Aru Islands on the growth of *E. coli* and *S. aureus* as representatives of gram-positive and negative bacteria. Apart from that, there is no specific study regarding any type of macroalgae in the waters of the Aru Islands, which is the reason for carrying out this research. The study was aimed to measure the antibacterial activity of the red algae *E. cottonii* against pathogenic bacteria including *E. coli* and *S. aereus* at several concentrations and to determine the best concentration for inhibiting these two pathogenic bacteria.

RESEARCH METHODS

Research Design

This study is a true experimental laboratory research with a post-test-only controlled group design. Post-test-only controlled group design is a design that uses two groups, where one group is used for the experiment (which is given treatment) and the other one is used as a control group (which is not given treatment). The study was conducted in July – September 2023. Sampling was carried out in the waters of Jabulenga Village, Aru Islands, Maluku with coordinates 5°46'52.82"S - 134°21'8.69"E. Meanwhile, laboratory tests will be carried out at FKIP Biology, Pattimura University, Ambon. The study site is presented in Figure 1. Determination of *E. cottonii* was carried out at the Department of Marine Science, Faculty of Fisheries and Marine Sciences.

Population and Samples

The population used in this study is the whole red algae *E. cottonii* which can be collected from the waters of Jabulenga Village, Aru Islands. Meanwhile, the sample used in this research was n-Hexana *E. cottonii* extract which was the independent variable. Meanwhile, *E. coli* and *S. aureus* are the dependent variables.

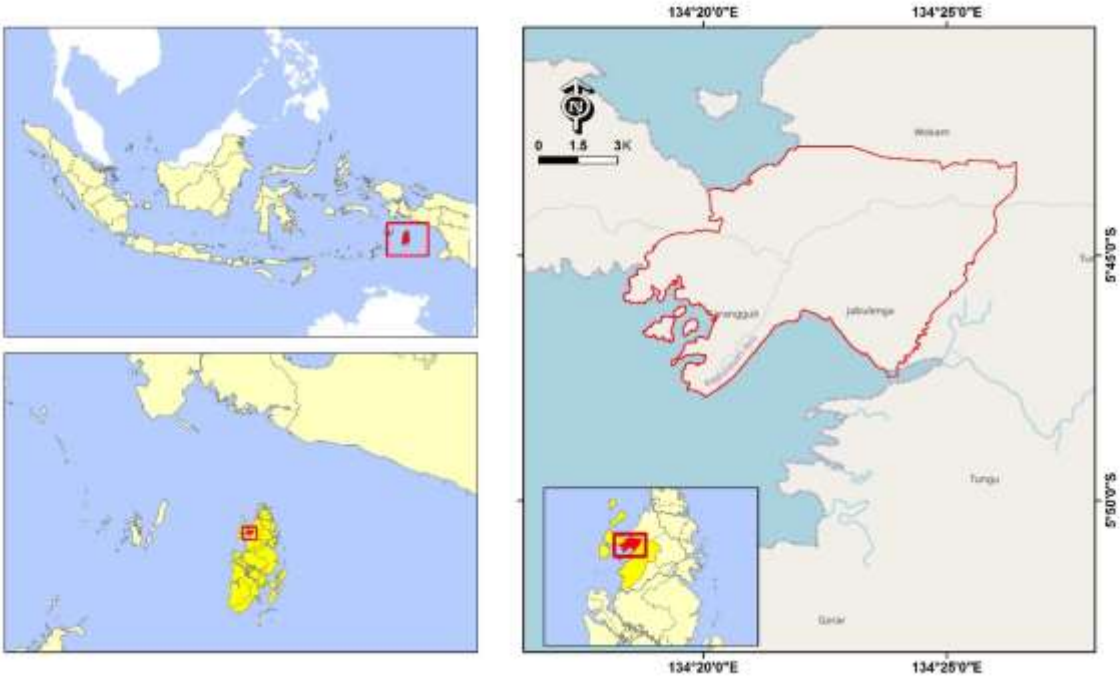

Figure 1. Sampling site of *Eucheuma cottonii*

Instruments

The following equipment was used in this study: sterile gauze, 100 ml measuring cups, 250 ml and 500 ml Erlenmeyer, bunsen lamps, micropipettes, magnetic stirrers, spatulas, autoclaves, oven, analytical balances, vortex, petric dishes, ose needles, paper disks, tweezers, label paper, cotton, and thread. Meanwhile, *E. cottonii* extract, pure cultures of pathogenic bacteria (*E. coli* and *S. aureus*), n-hexane solvent, Nutrient Agar (NA), disc paper, 70% and 96% alcohol, 5% sodium hypochlorite solution, distilled water as the negative control, and ampicillin as the positive control were the materials used for this investigation.

Procedures

Sample preparation was performed as follows: Red algae (*Eucheuma cottonii*) obtained from the waters of Jabulenga Village, Aru Islands were collected in zip plastic. Wet sorting was carried out by cleaning *E. cottonii* from adhering impurities and then washing thoroughly with running water. The *E. cottonii* sample was then doused with 5% sodium hypochlorite solution for surface sterilization. The samples were then stored in a cool box to be taken to the laboratory for further tests (Andriani et al., 2015).

The extraction process was performed by air drying *E. cottonii* samples for ± 4 days. The dried sample was cut into pieces and then ground using a blender until it became dry simplicia powder. A total of 50 g of simplicia powder was weighed and put into an Erlenmeyer. Maceration was carried out by soaking simplicia powder in n-hexane solvent in a ratio of 1:3 for 2x24 hours. Soaking functions to draw out the organic compounds contained in simplicia. The solution was filtered using ordinary filter paper and concentrated with an evaporator until a thick extract was formed. This extract was diluted and made into serial concentrations, namely 10%, 30%, 50%, 80%, and 100% (Teo et al., 2020).

Pathogenic bacterial culture preparation was carried out by subculturing *Escherichia coli* and *Staphylococcus aureus* obtained from the Maluku Province Health and Equipment Calibration

Laboratory in new Nutrient Agar media. The isolates were incubated at room temperature for 24 hours. The bacterial colonies that appeared were then transferred to a slant agar medium to be used as test isolates (Yunita et al., 2016; Yunita et al., 2022).

The antibacterial test was carried out using the Kirby Bauer disk diffusion method by testing the n-hexane extract of *E. cottonii* with serial concentrations against the growth of *E. coli* and *S. aureus*. Pathogenic bacteria are streaked or swabbed on Nutrient Agar media. Furthermore, 6 mm sterile paper disk that had been soaked in 200 µl of *E. cottonii* extract was placed on top of the pathogenic bacterial culture. The Petri dishes were wrapped in plastic wrap and stored in an incubator at 37^{0}C for 1-3 days. The positive control was tested using the antibiotic Amphicilin while the negative control was tested using sterile distilled water. Inhibition of the growth of pathogenic bacteria was visible as a clear zone around the paper disc. The size of the clear zone was an indication of the sensitivity of pathogenic bacteria to antibacterial compounds. The inhibition zone was calculated by subtracting the diameter of the inhibition zone obtained from the diameter of the paper disc, which was 6 mm. The diameter of the inhibition zone was measured using a caliper and the treatment was repeated 2 times (Kiriwenno et al., 2020).

Qualitative standard screening through phytochemical tests was performed to determine the presence of secondary metabolites, including tannins, terpenoids, alkaloids, phenolics, flavonoids, and saponin, in the crude extract of *E. cottonii*. The presence/positive reaction (+) and absence/negative reaction (-) of phytochemical were used to express the qualitative results (Yunita et al., 2023).

Data Analysis

Data were analyzed descriptively and presented with tabulations and figures. All data are expressed as the mean and calculated using Ms. Excel. Standard deviation was calculated between extract concentrations. Data processing was performed by measuring the diameter of the inhibitory zone of the independent variables (*E. cottonii* extract) on the growth of *E. coli* and *S. aureus*. The inhibitory zone results were then divided into four categories based on their size: extremely strong (>20 mm), strong (11-20 mm), moderate (6-10 mm), and weak (<5 mm) (Davis & Stout, 1971).

RESULTS

Pathogenic bacteria (*E. coli* and *S. aureus*) from the Maluku Province Health and Equipment Calibration Laboratory were subculturing into new NA media to initiate the study stage. The pathogenic bacterial isolates utilized in this study are displayed in Figure 1.

Figure 1. Isolate *S. aureus* (left) and *E. coli* (right) used in this study

The growth of pathogenic bacteria was shown to be inhibited by the n-hexane extract from *E. cottonii*, with varied inhibition zones, according to antibacterial testing. The inhibition zone formed against *S. aureus* ranged from 0.27±0.03 – 2.1 ± 0.14 at a concentration of 50%-100%, and 0.17±0.05 – 0.45±0.03 against *E. coli* at a concentration of 80-100%. Meanwhile, a very large zone of inhibition was formed in the control treatment using ampicillin, namely 19±0.41 for *S. aureus* and 21±0.00 for *E. coli*. The results of measuring the inhibition zone of *E. cottonii* on the growth of the two pathogenic bacteria can be seen in Table 1.

Table 1. Results of measuring the inhibition zone of *E. cottonii* extract on the growth of *S. aureus* and *E. coli*

Extract Concentration of *Eucheuma cottonii*	Inhibitory response (mm)			
	S. aureus	Category	*E. coli*	Category
10%	00±00	-	00±00	-
30%	00±00	-	00±00	-
50%	0.27±0.03	Weak	00±00	-
80%	1.25±0.35	Weak	0.17±0.05	-
100%	2.1±0.14	Weak	0.45±0.03	-
K+	19±0.41	Strong	21±00 mm	Very Strong
K-	-	-	-	-

When comparing the effect of the extract on the growth of the two pathogenic bacteria, it was known that the n-hexane extract could inhibit the growth of *S. aureus* better than *E. coli*. The *E. cottonii* extract was able to inhibit the growth *of S. aureus* at a concentration of 50%. Meanwhile, *in E. coli*, a very small inhibition zone was only formed at a concentration of 80%. A comparative visualization of antibacterial activity against the two pathogenic bacteria is presented in Figure 3.

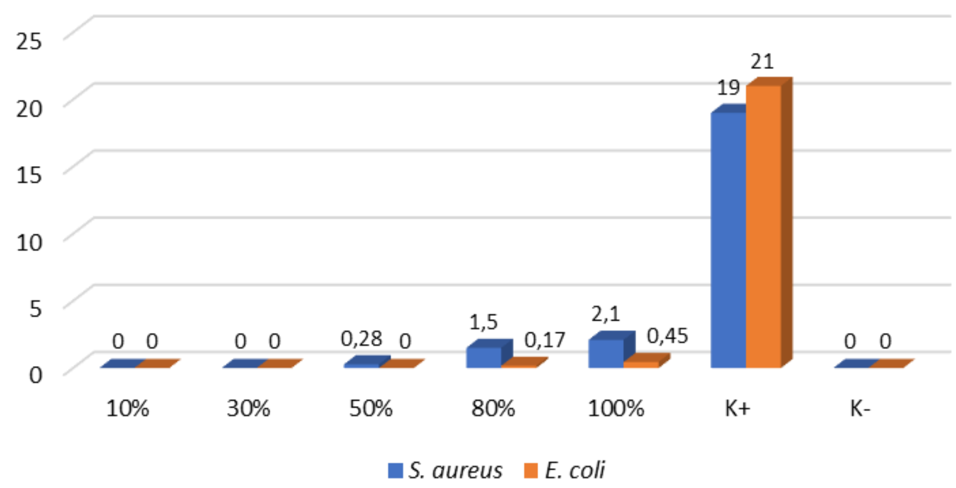

Figure 3. A comparison of antibacterial activity against pathogenic bacteria

The results of the phytochemical test were carried out to see the content of bioactive compounds contained in the hexane extract of *E. cotonii*. In the seven bioactive compounds tested, it is known that the n-hexane extract of *E. cottonii* contains flavonoids, phenolics, and saponins. In detail, the results of the phytochemical tests are presented in Table 2.

Table 2. Phytochemical test results of *E. cottonii* extract

No	Parameter	Indicator	Result
1	Alkaloid	Yellow	-
2	Flavonoid	Greenish yellow	+
3	Terpene	Red ring	-
4	Steroid	Yellow ring	-
5	Phenolic	Blue	-
6	Saponin	*Foam*	+
7	Tannin	Orange	-

DISCUSSION

Plant extraction is a process of separating active plant materials or secondary metabolites including alkaloids, flavonoids, terpenes, saponins, steroids, saponin, and glycosides from inert or inactive materials using an appropriate solvent and standard extraction procedure (Bhuiya et al., 2020). In this study, we extracted the red algae *E. cottonii* using n-hexane as a solvent. N-hexane is a solvent utilized in plant extraction because of its characteristics, which include easy recovery, non-polarity, low latent heat of vaporization (330 kJ/kg), and strong solvent selectivity. N-Hexane is quite good for the extraction process because of its low boiling point and high evaporation rate. However, the antibacterial activity of *E. cottonii* extract shown in this research was still in the weak category due to the small clear zone visible around the paper disc (Agboke & Attama, 2016). A clear zone that forms around the disc paper is an indication that the *E. cottonii* extract is inhibiting the growth of *E. coli* and *S. aureus*. Variations in the concentration of bioactive antibacterial chemicals within the inhibition zone as well as the rate that which the antibacterial material diffuses into the agar medium can account for variations in its size. The sensitivity of antibacterial growth, the interaction between the active ingredient and the medium, and the incubation temperature are other parameters that are thought to affect the formation of the inhibitory zone (Yusvantika, 2021).

In this study, flavonoids and saponins were known to be present in the *E. cottonii* hexane extract, out of the five components examined in the phytochemical test. This is consistent with the research findings of Fahrul et al. (2021), which also indicated that the red algae *E. cottonii* obtained from Karimun Regency, Riau province, solely contained flavonoids, saponins, and phenolic compounds. The flavonoids found in *E. cottonii* have antibacterial properties because of their ability to block the formation of nucleic acids, energy metabolism, and cytoplasmic membrane function. The antibacterial activity can be considered an effective indicator for *E. cottonii* to synthesize bioactive secondary metabolites. Another compound found in this study is saponin. Saponin is commonly found in plant extract (Agboke & Attama, 2021). High polarity, instability in both chemical and thermal processes, lack of volatility, and low concentrations are typical characteristics of saponins. Therefore, even when non-polar solvents such as n-hexane were used in plant extraction, saponins produced the highest yields (Majinda, 2012).

On the other hand, the phytochemical test findings of *E. cottonii* in this study did not reveal the presence of alkaloids or other more complex chemicals. Fahrul (2021) also found no alkaloids in the phytochemical test results, confirming the same findings. Teo et al., (2020) state that alkaloids are mostly found in higher plants (Angiosperms), especially in dicotyledonous plants. Many kinds and quantities of phytochemical substances that are found in plants can be impacted by environmental factors. Increased production and accumulation of secondary chemicals inside the plant can result from several conditions, including increased sunlight exposure, nutrient-deficient soil, pest infestation, and drought-induced stress (Andriani et al., 2015).

In summary, our study illustrates that the *E. cottonii* extract has potential as a natural antibacterial source, particularly when it comes to inhibit the growth of *E. coli.* This study provides preliminary findings that point to the need for more antibacterial research to enhance, optimize, and identify the bioactive compounds for possible use in complementary and alternative medicine to treat a range of diseases, especially those caused by *E. coli.* These results will also be helpful to others as a guide for the development of medicinal products

CONCLUSION

The study concludes that the n-hexane extract of the red algae *Eucheuma cottonii* was able to inhibit the growth of *Escherichia coli* and *Staphylococcus aureus* with a weak inhibition category. While phytochemical test indicates that n-hexane extract of E. cottonii contained flavonoids, phenolics, and saponins. This study implies that improvements are needed in the *E. cottonii* extraction process to maximize the antibacterial properties of *E. cottonii*, such as using different solvents that have polar properties.

ACKNOWLEDGMENT

We sincerely thank the Faculty of Medicine, Pattimura University, which has funded this research through PNBP funds with contract number: 13/UN1366/SK/2023. We also thank the staff of the basic biology laboratory of FKIP Biology, Pattimura University.

REFERENCES

Agboke, A. A., & Attama, A. A. (2016). Bioactive components and antibacterial activities of n-hexane extract of Moringa oleifera root bark on clinical isolates of methicilin resistant Staphylococcus aureus. *International Journal Current Research Chemical Pharmaceutical Science*, *3*(3), 1-9. Retrieved from https://www.researchgate.net/profile/Akeem-Agboke-2/publication/

Andriani, Z., Fasya, A. G., & Hanapi, A. (2015). Antibacterial activity of the red Algae Eucheuma cottonii extract from Tanjung Coast, Sumenep Madura. *Alchemy: Journal of Chemistry*, *4*(2), 93-100. Retrieved from http//doi.org/10.18860/al.v4i2.3197

Arfah, H., & Patty, S. I. (2014). Keanekaragaman dan biomassa makro algae di perairan Teluk Kotania, Seram Barat. *Jurnal Ilmiah Platax*, *2*(2), 63-73. Retrieved from https://doi.org/10.35800/jip.2.2.2014.7150

Bhuiya, M. M. K., Rasul, M., Khan, M., Ashwath, N., & Mofijur, M. (2020). Comparison of oil extraction between screw press and solvent (n-hexane) extraction technique from beauty leaf (Calophyllum inophyllum L.) feedstock. *Industrial crops and products*, *144*, 112024. Retrieved from https://doi.org/10.1016/j.indcrop.2019.112024

Davis, W., & Stout, T. (1971). Disc plate method of microbiological antibiotic assay. I. factors influencing variability and error. Applied Microbiology, 22(4), 659–665. Retrieved from https://tinyurl.com/4hc4c8d5

Fahrul, M., Sari, I., & Iriani, D. (2021). Antibacterial effectiveness of seaweed (eucheuma cottonii) extract with different solvent. *Jurnal Agroindustri Halal*, *7*(1), 001-008. Retrieved from https://doi.org/10.30997/jah.v7i1.3253

Julyasih, K. S. M., Ristiati, N. P., & Arnyana, D. I. B. P. (2020). Potensi alga merah dan alga hijau untuk menghambat pertumbuhan bakteri eschericia coli. Agrotrop, *10*(1), 11-17. Retrieved from https://doi.org/10.24843/AJoAS.2020.v10.i01.p02

Kiriwenno, J. V., Yunita, M., & Latuconsina, V. Z. (2020). Perbandingan aktivitas antibakteri antara ekstrak daun katang-katang (ipomoea pes-caprae l.) dan minyak seith terhadap pertumbuhan staphylococcus aureus. *Majalah Farmaseutik, 17*(1), 122-131. Retrieved from https://doi.org/10.22146/farmaseutik.v17i1.58292

Majinda, R. R. (2012). Extraction and isolation of saponins. *Natural products isolation*, 415-426. Retrieved from https://doi.org/10.1007/978-1-61779-624-116

Nurjanah, Luthfiyana, N., Hidayat, T., Nurilmala, M., & Anwar, E. (2019). Utilization of seaweed porridge Sargassum sp. and Eucheuma cottonii as cosmetic in protecting skin. In *IOP Conference Series: Earth and Environmental Science* (Vol. 278, No. 1, p. 012055). IOP Publishing. Retrieved from https://iopscience.iop.org/article/10.1088/1755-1315/278/1/012055/meta

Rugebregt, M. J., Pattipeilohy, F., Matuanakott, C., Ainarwowan, A., Abdul, M.S. & Kainama, F. (2021). Potensi rumput laut perairan pulau keffing, seram bagian timur, maluku. *Jurnal Ilmu Lingkungan, 19*(3), 497-510. Retrieved from https://doi.org/10.14710/jil.19.3.497-510

Syad, A. N, Shunmugiah, K. P., & Kasi, P. D. (2013). Seaweed as nutritional supplements: analysis of nutritional profle, physicochemical properties and proximate composition of G. acerosa and S. wightii. *Biomedicine and Preventive Nutrition, 3,* 139-144. Retrieved from https://doi.org/10.1016/j.bionut.2012.12.002.

Teo, B. S. X., Gan, R. Y., Abdul Aziz, S., Sirirak, T., Mohd Asmani, M. F., & Yusuf, E. (2020). In vitro evaluation of antioxidant and antibacterial activities of eucheuma cottonii extract and its in vivo evaluation of the wound healing activity in mice. *Journal of Cosmetic Dermatology*. Retrieved from https://doi.org/10.1111/jocd.13624

Wardani, G., Farida, N., Andayani, R., Kuntoro, M., & Sudjarwo, S. A. (2017). The potency of red seaweed (Eucheuma cottonii) extracts as hepatoprotector on lead acetate-induced hepatotoxicity in mice. *Pharmacognosy research, 9*(3), 282-286. Retrieved from https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5541486/

Yunita, M., Mubarik, N.R., & Solihin, D.D. (2016). Isolation and identification of chitinolytic bacteria as biocontrol agent of pathogenic fungi on gold silkworm cocoon Cricula trifenestrata. *Malaysian Journal of Microbiology, 12*(1), 69-75. Retrieved from https://doi.org/10.21161/mjm.74415

Yunita, M., Ohiwal, M., Dirks C.S., Wibriyono, O.A., Sukmawati, S., & Ilsan, N.A. (2022). Endophytic bacteria-associated with Myristica fragrans: improved media, bacterial population, preliminary characterization, and potential as antibacterials. *Biodiversitas Journal of Biological Diversity, 23*(8), 4047-4054. Retrieved from https://doi.org/10.13057/biodiv/d230824

Yunita, M., Ohiwal, M., Elfitrasyah, M. Z., & Rahawarin, H. (2023). Antibacterial activity of Paederia foetida leaves using two different extraction procedures against pathogenic bacteria. *Biodiversitas Journal of Biological Diversity, 24*(11), 5920-5927. Retrieved from htps://doi.org/10.13057/biodiv/d241110

Yusvantika, N. (2021). Antibacterial activity of crude extract red algae eucheuma spinosum against staphylococcus epidermidis bacteria growth. *Fisheries Product Technology, 1*(1), 1-10. Retrieved from https://repository.unair.ac.id/111348/1