OPEN ACCESS

Saudi Journal of Medical and Pharmaceutical Sciences
Abbreviated Key Title: Saudi J Med Pharm Sci
ISSN 2413-4929 (Print) | ISSN 2413-4910 (Online)
Scholars Middle East Publishers, Dubai, United Arab Emirates
Journal homepage: https://saudijournals.com

Original Research Article | **Medical Laboratory Technology**

Antibacterial and Anticancer Activity of Protein from Red Algae (*Eucheuma cottonii*)

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DOI: 10.36348/sjmps.2021.v07i12.001 | **Received:** 29.10.2021 | **Accepted:** 02.12.2021 | **Published:** 10.12.2021

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Abstract

Protein is a major potential source of raw materials for new drugs. In this study, protein isolation of the red algae, *Eucheuma cottonii* was performed using a polar solvent (0.1 M Tris-HCl buffer). Purification of protein used fractionation method with ammonium sulfate, and then the last step of the isolation process performed protein dialysis. Determination of protein content was done using the Lowry method. Antibacterial activity test used agar diffusion method while for screening anticancer activity used the Brine Shrimp Lethality Test (BSLT) method. Anticancer effectiveness carried out against HeLa cancer cells. The results of antibacterial and BSLT tests on protein fractions F1 (0-20%) and F2 (20-40%) showed that these fractions were potential as anticancer agents, However, the test results of both fractions against HeLa cancer cells showed only the F2 protein fraction (20-40% fraction) was active in inhibiting the growth of HeLa cell cancer in the moderate category. The SDS PAGE electrophoresis test showed that the F2 protein fraction had a molecular weight of 26,76 kDa.

Keywords: Antibacterial, Anticancer, *Eucheuma cottonii*, *HeLa* cancer cells, Protein.

1. INTRODUCTION

At present, there is great interest in obtaining bioactive natural products from marine organisms. Many kinds of structural natural resources are found in the sea, such as secondary and primary metabolites [1]. Exploration of algae has done a lot to find new drugs around the world. Algae are a rich source of secondary metabolites that are useful as the main ingredients for pharmaceutical applications [2]. In addition to producing secondary metabolites, algae are also capable of producing primary metabolites that have various activities [3].

One component of algae's primary metabolites that has been explored is protein. The protein content of algae is varied, e.g. red algae contained the highest protein [maximum weight was found to be 47% (w/w) of dry weight], green algae contained a moderate protein [found around 9%-26% (w/w) of dry weight], whereas brown algae contained the lowest protein [3%-15% (w/w) of dry weight] [4].

Algal protein has biological activity so that it becomes a potential source of new natural medicinal ingredients as antibacterial, antifungal, and anticancer [5, 6]. The use of protein as a medicinal ingredient has the advantage that it is well accepted by the body and has few side effects [7]. Previous studies have shown that proteins from red algae (*Rhodophyceae*) have potential as anticancer drug agents [8], however, this study was still at the first stage as screening for anticancer activity used the Bhrine Shrimp Lethality Test (BSLT) method. In this study, the anticancer activity of bioactive proteins from red algae (*Rhodophyceae*) will be further tested in vivo against *HeLa* cancer cells.

2. MATERIALS AND METHODS

2.1. Materials

The tools used are analytical balance, incubator, UV-Vis spectrophotometer, autoclave, pH meter, blender, cold centrifuge, magnetic stirrer, petri dish, laminar flow, shaker incubator, vortex, Eppendorf tube, micropipette, aerator, incandescent lamp, water bath, light microscope, disk blank, Erlenmeyer and algae samples *Eucheuma cottonii (E. cottonii)* from Takalar sea waters, South Sulawesi.

2.2. Sample Preparation

Red algae samples were washed and rinsed with clean water, the function of this treatment was to remove residual salt. Samples were stored at 20^{O}C

before it was extracted. The samples were then identified by species [9]. The taxonomic identification of algae was carried out in the marine biology laboratory of FMIPA-UNHAS.

2.3. Protein Isolation

The samples were homogenized by blender using buffer solvent A (tris-HCl 0.1 M pH 8.3, NaCl 2 M, CaCl2 0.01 M, β-mercaptoethanol 1% Triton X-100 0.5). The homogenization results were filtered by a Buchner funnel to obtain the filtrate. The filtrate was freeze-thawed 2-3 times and then centrifuged at 12,000 rpm 4^{O}C, for 30 minutes. Furthermore, the supernatant from the centrifuge was fractionated by ammonium sulfate at saturation levels of 0-20%, 20-40%, 40-60%, 60-80%. After that, it continued with dialysis, and the protein fraction preparations were stored at 40C before being purified or further analyzed [10].

2.4. Determination of Protein Content

The content of bioactive protein in buffer A (Tris-HCl 0.1 M pH 8.3, NaCl 2 M, CaCl2 0.01 M, β-mercaptoethanol 1% Triton X-100 0.5%) was determined by the Lowry method. The standard protein solution used bovine serum albumin (BSA) and a blank solution used distilled water. The absorbance was measured using a UV-Vis spectrophotometer at the maximum wavelength [11].

2.5. Atibacterial Test

The antibacterial test used the agar diffusion method to follow Kirby-Bauer. At an agar medium were inoculated by the test bacteria *Escherichia coli* (*E. coli*) and *Staphylococcus aureus* (*S. aureus*) with the swap method. A sterile paper disk measuring 6.2 mm was dipped into the test sample and placed on the agar medium. The test dish was incubated at 37^{O}C for 1-2 days. The inhibition of growth of microorganisms by antibacterial substances will be seen as a clear area around the paper disk. The clear area is an indication of the sensitivity of microorganisms against antibacterial substances or compounds. The size of the inhibition zone is measured from the diameter inhibition zone or clear zone [12].

2.6. Toxicity Test

Artemia salina eggs were incubated in artificial seawater (38 g of salt in 1000 mL of water and must be free of iodine) then this water was irradiated with an 18 watt TL lamp. Egg hatching media is aerated with air. After 48 hours, the larvae hatch into *nauplii* and are ready to use. *Nauplii* were put in a vial containing a protein fraction solution with a concentration of 10, 100, and 1000 bpj with 3 replications. All vials were incubated at room temperature for 24 hours under 18 watt TL lamps. It was observed after 24 hours of incubation by looking at the quantity of dead *Artemia salina* at each concentration. Determination of the value of LC50 in mg/mL used probit analysis [8].

2.7. Antiproliferation Test

The cell suspension (5 x 104 cell/100µL) were distributed into plate 96 well and incubated with the test compound with various concentration series in DMSO for 24, 48, and 72 hours. At the end of incubation, each well was added 10 L MTT (3-(4,5-dimethylthiazol-2-yl)- 2,5diphenyl-tetrazolium bromide) at a concentration of 5 mg/mL, then it was incubated again overnight at 37^{O}C. Live cells would react with MTT to form a purple color. The MTT reaction was stopped with a 10% SDS stopper reagent in 0.01% HCl, then incubated again overnight at room temperature. Absorption was read with an ELISA reader at a wavelength of 550 nm [13].

2.8. Protein Characterization

The characterization of proteins that have potential as anticancer in this study was performed using the Sodium Dodecyl Sulfate - Polyacrylamide Gel Electrophoresis (SDS-PAGE) method. This characterization was carried out in several stages, namely protein sample preparation, polyacrylamide gel preparation, chamber and glass plate assembly, bacterial protein sample injection, SDS-PAGE running process, and polyacrylamide gel staining and washing process. Running electrophoresis was carried out at a voltage of 120 V and a current of 28 A for 90 minutes [14].

3. RESULT

2.1. Protein Content

Protein extract from the red algae Eucheuma cottoni consisted of four fractions with different concentrations (Table 1).

Table 1: Protein Content of Red Algae *Eucheuma cottoni*

Code Fraction	Protein Fraction	Protein Content (mg/mL)	Volume of each Fraction (mL)	Total protein (mg)
EK	Crude Extract	4,45	125	556,33
F1	0-20 %	10,39	20	207,83
F2	20-40 %	4,89	20	97,75
F3	40-60 %	10,27	20	205,44
F4	60-80 %	6,00	20	120,01

2.2. Screening for anticancer activity

As an initial test of anticancer activity before tested activity on HeLa cells, it was carried out a test with shrimp eggs (toxicity test). All fractions showed very toxic activity, except the crude extract just toxic (Table 2).

Table 2: Toxicity test results protein fraction from red algae *Eucheuma cottoni*

Code Fraction	C (ppm)	RLH	RLM	RKH	RKM	TL	M(%)	LC_{50} (ppm)	Toxicity Category
EK	100	0,67	9,33	9,67	0,33	10,00	90	27,645	Toxic
	10	6,33	3,67	9,67	0,33	10,00	33		
	1	9,67	0,33	9,67	0,33	10,00	0		
F4	100	0,00	10,00	9,67	0,33	10,00	97	16,864	Very Toxic
	10	8,67	1,33	9,67	0,33	10,00	10		
	1	9,33	0,67	9,67	0,33	10,00	3		
F3	100	0,67	9,33	9,67	0,33	10,00	90	16,867	Very Toxic
	10	7,33	2,67	9,67	0,33	10,00	23		
	1	9,00	1,00	9,67	0,33	10,00	7		
F2	100	0,00	10,00	9,67	0,33	10,00	97	7,330	Very Toxic
	10	7,00	3,00	9,67	0,33	10,00	27		
	1	7,33	2,67	9,67	0,33	10,00	23		
F1	100	0,00	10,00	9,67	0,33	10,00	97	9,686	Very Toxic
	10	6,00	4,00	9,67	0,33	10,00	37		
	1	9,00	1,00	9,67	0,33	10,00	7		

Evidence. C (ppm) = Solution Concentration, RLH = Average of Live Larvae, RLM= Average of Deadh Larvae, RKH= Average of Live Control Larva, RKM= Average of Dead Control Larva, TL=Total Larva, M(%)=Mortality (% Death)

2.3. Antibacterial activity

All protein extracts of red algae *E. cottonii* were tested for their antibacterial activity against *E. coli* and *S. aureus* (Table 3). The positive control used in this test is chloramphenicol 30µg.

Table 3: Inhibition diameter of protein fraction against *E. coli* and *S. aureus*

Code	Protein Fraction	Average of inhibition diameter (mm)			
		E. coli		*S. aureus*	
		24 hour	48 hour	24 hour	48 hour
F1	0-20 %	12,2	6,50	6,50	6,50
F2	20-40 %	17,5	8,20	6,50	6,50
F3	40-60 %	6,50	6,50	6,50	6,50
F4	60-80 %	6,50	6,50	8,30	6,50
EK	Crude Extract	6,50	6,50	8,40	8,20
(-)	buffer	6,50	6,50	6,50	6,50
(+)	Chloramphenicol 30µg	33,8	13,9	27,5	14,9

2.4. Antiproliferation activity

The two fractions with the highest toxicity (Fraction F1 and F2) were tested for their anticancer activity against HeLa cells (Table 4).

Table 4: IC_{50} value from Protein fraction of red algae (*E. cottonii*)

Code	IC_{50} (µg/mL)	Category
F1	926,32	Weak
F2	234,28	Medium

2.5. Protein Characterization

The protein fraction with the best activity was characterized by molecular weight by SDS-PAGE. In this study, the highest activity was the protein fraction of 20-40% (Figure 1).

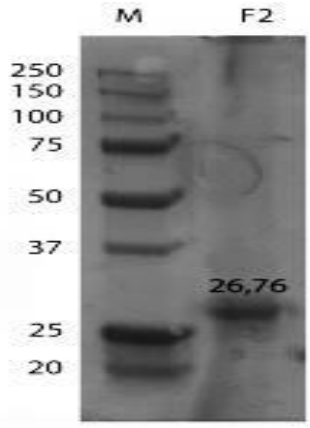

Figure 1: Chromatogram SDS PAGE from protein fraction 20-40% (F2) and marker (M)

4. DISCUSSION AND CONCLUSION

The protein sample of *E. cottonii* was isolated with tris buffer solvent pH 8.3. At the time of protein isolation, the pH was maintained because a slight change in pH could change the structure and activity of important compounds such as enzymes (proteins) present in algae [15]. The crude extract was then fractionated using ammonium sulfate salt and obtained several fractions, that is Fraction 0-20% (F1), Fraction 20-40% (F2), Fraction 40-60% (F3), and Fraction 60-80% (F4).

The fractions obtained were determined by their protein content. The results of the measurement of protein content can be seen in Table 1; this data shows that the highest protein content of the fraction is in the 0-20% fraction, which is 10,39 mg/ml and the lowest protein content is in the crude extract, which is 4.45 mg/ml. Protein in each fraction is not too far apart; this means that the solubility of each protein in water is not much different.

Protein fraction that has been dialyzed and the protein content have been calculated. The fraction was then tested for toxicity using the BSLT method. BSLT is a preliminary test for screening for anticancer activity because it is easy and inexpensive [16]. The test was carried out using *Artemia* larvae; the number of larval deaths was then analyzed to obtain the LC50 value.

The LC_{50} (Lethal Concentration) value is the number of levels that cause the death of 50% of the animal's test at a certain time interval, the classification of the LC_{50} toxicity value is if LC_{50} < 20 g/mL is categorized as very toxic, if the LC_{50} is at 20-100 g/mL is categorized toxic, if the LC_{50} value is at 100-500 g/mL it is categorized as moderate, if the LC_{50} value is at 500-1000 g/mL it is categorized as weak and if the LC_{50} value > 1000 g/mL is categorized as non-toxic [17].

In the results of the BSLT toxicity test for protein fractions (Table 2), from the five protein fractions were tested the toxicity, four of them were in the very toxic category (F1, F2, F3, and F4), and one was in the toxic category (EK). The most toxic protein fraction was F2 with an LC50 value of 7,330 ppm. The extract tested by the BSLT method and can cause death of 50% of *Artemia* larvae within 24 hours with a concentration of LC50 <1000 ppm indicates that the sample has potential as anticancer, antibacterial, antifungal, and so on [18], This means that all the protein fractions tested have potential as anticancer, antibacterial, antifungal, and other activities.

The BSLT test is also known to be positively correlated with anticancer, antibacterial and other activities. Therefore, the research also continued on antibacterial tests against *E. coli* and *S. aureus* bacteria before proceeding to anticancer (antiproliferative) tests on *HeLa* cells. In this test, the method used is the agar diffusion method with a paper disc. The positive control was used chloramphenicol drug (30 g), while the negative control used a buffer.

The results of the measurement of the diameter of the inhibition zone of the red algae protein fraction against the two test bacteria after incubation for 24 hours and 48 hours obtained the results as listed in Table 3. The test results showed that the active protein fraction against bacteria was only the F1 and F2 fractions. Its inhibitory activity is only against gram-negative bacteria (*E. coli*) and is not active against gram-positive bacteria (*S. aureus*). Based on this information, only two fractions were continued for anticancer testing (Antiproliferation Test) on HeLa cells.

Measurement of anticancer activity from protein fractions F1 and F2 used the MTT test method with *HeLa* cells as a cervical cancer cell model. The results showed that both samples could inhibit the growth of *HeLa* cells. The inhibitory power of each fraction was determined based on its IC_{50} value. In the cytotoxicity test, the IC50 (Inhibitory Concentration) value indicates the concentration required to inhibit the growth of *HeLa* cancer cells by 50% of the total population. The determination of the IC_{50} value is calculated by linear regression. In this study, the two protein fractions tested had IC_{50} values which were in the medium and weak categories (Table 4).

The results of SDS-PAGE electrophoresis showed that the protein fraction (F2) had a molecular weight (BM) of 26,76 kDa (Figure 1). Based on the results of electrophoresis, it can be seen that the resulting band is still quite thick, possibly the fraction is less pure. Further research is expected to be purified and from the results of the purification it can increase its anticancer activity against HeLa cells.

In conclusion, in this study, the protein was successfully extracted from red algae E. cottoni with the protein fraction having the opportunity as an antibacterial and also anticancer agent is the protein fraction F2 (20-40%) with a molecular weight (BM) of 26,76 kDa.

ACKNOWLEDGEMENT

The author thanks to the Directorate of Research and Community Service (DPRM) of the Directorate General of Research and Development Strengthening, Ministry of Research, Technology and Higher Education who has funded this research with scheme the Beginner Lecturer Research (PDP) (The contract number: 1864/E4/AK.04/2021 dated 07 June 2021), number: 3100/L9/KU.03.00/2021 dated 19 July 2021, number 084/07.091056/VII/2021 dated 25 July

2021). The results of the isolation obtained crude extract.

REFERENCES

1. Lakmal, H. C., Samarakoon, K. W., Lee, W., Lee, J. H., Abeytunga, D. T. U., Lee, H. S., & Jeon, Y. J. (2014). Anticancer and antioxidant effects of selected Sri Lanka marine algae. *Journal of the National Science Foundation of Sri Lanka*, *42*(4).
2. Samarakoon, K. W., Nam, O. K., Ko, J. Y., Lee, J. H., Kang, M. C., Kim, D., Lee, B. J., Lee, J. S., & Jeon, Y. J. (2013). Purification and identification of novel angiotensin-I converting enzyme (ACE) inhibitory peptides from cultured marine microalgae (Nannochoropsis oculata) protein hydrolysate. *Journal of Applied Phycology*, 25: 1595 − 1606.
3. Salehi, B., Sharifi-Rad, J., Seca, A. M., Pinto, D. C., Michalak, I., Trincone, A., Mishra, A.P., Nigam, M., Zam, W., & Martins, N. (2019). Current trends on seaweeds: Looking at chemical composition, phytopharmacology, and cosmetic applications. *Molecules*, *24*(22), 4182.
4. Harnedy, P. A., & FitzGerald, R. J. (2011). Bioactive Proteins, Peptides, and Amino Acids from Macroalgae. *Journal of Phycology*, *47*(2), 218-232.
5. Rodrigues, E. G., Dobroff, A. S., Taborda, C. P., & Travassos, L. R. (2009). Antifungal and antitumor models of bioactive protective peptides. *Anais da Academia Brasileira de Ciências*, 81(3), 503-520.
6. Zheng, S., Liu, Q., Zhang, G., Wang, H., & Ng, T. B. (2010). Purification and characterization of an antibacterial protein from dried fruiting bodies of the wild mushroom Clitocybe sinopica. *ACTA Biochimica polonica*, 57(1), 43-48
7. Yadav, D., & Negi, P. S. (2021). Bioactive components of mushrooms: Processing effects and health benefits. *Food Research International*, 110599.
8. Sugrani, A., Ahmad, A., Djide, M. N., & Natsir, H. (2019). Biofunctional protein fraction from red algae (Rhodophyta) Eucheuma spinosum as an antibacterial and anticancer drug agent. *International Research Journal of Pharmachy*, 10(3), 64-69.
9. Al-Saif, S. S. A., Abdel-Raouf, N, El-Wazanani, H. A., & Aref, I. A. (2014). Antibacterial substances from marine algae isolated from Jeddah coast of Red sea, Saudi Arabia. *Saudi Journal of Biological Sciences*, 21, 57–64.
10. Zodape, G. V., & Bhadekar, N. S. (2021). Biomedical Activities of Marine Sponge Suberites carnosus (Johnston) Collected from West Coast of Mumbai, India. *Saudi J Med Pharm Sci*, *7*(7), 307-319.
11. Martínez–Hernández, G. B., Castillejo, N., Carrión–Monteagudo, M. D. M., Artés, F., & Artés-Hernández, F. (2018). Nutritional and bioactive compounds of commercialized algae powders used as food supplements. *Food Science and Technology International*, *24*(2), 172-182.
12. Meroni, G., Soares Filipe, J. F., & Martino, P. A. (2020). In Vitro Antibacterial Activity of Biological-Derived Silver Nanoparticles: Preliminary Data. *Veterinary sciences*, *7*(1), 12.
13. Caamal-Herrera, I. O., Carrillo-Cocom, L. M., Escalante-Réndiz, D. Y., Aráiz-Hernández, D., & Azamar-Barrios, J. A. (2018). Antimicrobial and antiproliferative activity of essential oil, aqueous and ethanolic extracts of Ocimum micranthum Willd leaves. *BMC complementary and alternative medicine*, *18*(1), 1-9.
14. Machsun, I. R., & Zulaika, E. (2017). Profil Protein Bakteri Ureolitik. Jurnal Sains dan Seni ITS, 6(2), E56-E58.
15. Dobrinčić, A., Balbino, S., Zorić, Z., Pedisić, S., Bursać Kovačević, D., Elez Garofulić, I., & Dragović-Uzelac, V. (2020). Advanced technologies for the extraction of marine brown algal polysaccharides. *Marine drugs*, *18*(3), 168.
16. Hamburger, M., & Hostettmann. (1991). Bioactivity in plant: the link between phytochemistry and medicine. *Phytochemistry*, 30(12), 3864-3874.
17. Ibrahim, M. A., & El Bushra, E. (2015). Cytotoxicity study on Maerua pseudopetalosa (Glig and Bened.) De Wolf tuber fractions. *African Journal of Plant Science*, *9*(12), 490-497.
18. Mayer, B. N., Ferrigni, N. R., Putnam, J. E., Jacobsen, L. B., Nichols, D. E., & McLaughlin, J. L. (1982). Brine shrimp: a convenient general bioassay for active plant constituents, *Planta Medica*, 45, 31-34.