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Identification of Phytochemical Compounds from Seaweed Extracts *Eucheuma cottonii* and *Gracilaria verucossa* and Their Effect on Bacteria Growth *Escherichia coli*

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ABSTRACT

Seaweed is known to have several bioactive compounds that function as secondary metabolites and antioxidants. This study aims to determine the compounds in the seaweed *Eucheuma cottoni* and *Gracilaria verucossa* and their effects on *Escherichia coli* bacteria. This research was conducted from February to April 2022. The research method used was the experimental method by performing phytochemical tests and the disc method on bacteria. Conclusions can be drawn because of the results of the research that has been done. Seaweed *E.cottoni* and *G.verucossa* contain steroidal compounds. The effect on the growth of *E.coli* bacteria at concentrations (5%) 8.24 mm, (10%) 8.5 mm, and (15%) 8.62 mm was classified as moderate.

Keywords: Seaweed, *Eucheuma cottoni*, *Gracilaria verucossa*, Phytochemicals

1. INTRODUCTION

Seaweed is known to have various bioactive compounds, each species having its own uniqueness. Coastal communities, including as a medicine for coughs, inflammation, and intestinal worms, often use seaweed for treatment. The various uses of seaweed turn out to be in each class there are different compounds that have specific chemical and physical properties as well.

Seaweed is used as an anti-cancer, increases endurance, prevents aging, and can maintain skin health and moisture. This is because seaweed has bioactive compounds that function as antioxidants, such as alkaloids, flavonoids, terpenoids, tannins, and saponins (Soamole *et al.,* 2018). The content of secondary metabolites in seaweed can be determined by approaching methods that can provide information on the presence of secondary metabolites, one of which is the phytochemical test method (Setyowati *et al*., 2014).

Several previous studies have carried out many phytochemical tests of various types of seaweed. Pangestuti & Amalia (2017), stated that the phytochemical screening of Sargassum sp. seaweed. Extracted using three types of solvents, namely methanol, ethyl acetate, and n-hexane. The results showed that the methanol solvent has a high secondary metabolite content compared to other solvents. This is because the higher the polarity of a solvent, the more phytochemical compounds are produced, the more polar the solvent, the more effective the solvent is in attracting secondary metabolites

In addition, Maharany et al. (2017), testing secondary metabolites on *Padina australis* and *E. cottonii* which are used as raw materials for sunscreen creams, obtained secondary metabolites from *P.australis* seaweed, including flavonoids, triterpenoids, phenol hydroquinones, tannins, and saponins. Whereas *E.cottonii* seaweed produces secondary metabolites, including flavonoids, phenol hydroquinones, and triterpenoids.

The solvent used in this study was n-hexane because it has a low boiling point and is easily evaporated without using high temperatures, is inert, and can release nonpolar components from a plant tissue to obtain active compounds contained in seaweed.

In this study, information regarding the ability of seaweed extract to inhibit the growth of pathogenic bacteria based on the disc method has not been well documented, as well as safe concentration (safe concentration) from seaweed n-hexane extracts. *E.cottonii* and *G.verucossa* against *E.coli* bacteria also not

much researched.

Based on previous research by Iskandar et al. (2005), the bioactive compounds contained in macroalgae *E.cottoni* can act as anti-bacterial compounds that can inhibit the growth of pathogenic bacteria in humans, such as *B.subtilis*, so this is the rationale for conducting research related to seaweed phytochemical tests and antibacterial against pathogenic bacteria. The purpose of this study was to analyze the compounds contained in the extracts of *E.cottonii* and *G.verucossa* seaweeds and to determine the effect of the extracts on the growth of *E.coli* bacteria.

2. RESEARCH METHODS

Time and Place

This research was conducted in February - April 2022, to collect seaweed samples in Sugiem Village, Karimun Regency, and Kepulauan Riau Province. Seaweed extraction activities were carried out at the Pure Organic Chemistry Laboratory, the Phytochemical Test process was carried out at the Biochemistry Laboratory, Faculty of Mathematics and Natural Sciences, Universitas Riau, and antibacterial observations were carried out at the Marine Microbiology Laboratory, Faculty of Fisheries and Marine, Universitas Riau.

Procedure

Preparation of grass samples Marine *E.cottonii* and *G. verrucosa*

Collecting seaweed from Karimun waters, Sugie Village, Karimun Regency, Kepulauan Riau Province with a weight of 2 kg *E.cottonii* (wet weight), *Gracilaria* sp 2 kg (wet weight), then washed thoroughly with clean seawater to remove residual moss attached to the grass the sea. Furthermore, the sample is put into a plastic bag and stored in an ice box that has been given ice cubes, in order to maintain the freshness of the seaweed before further handling.

Seaweed Extraction

In this study, seaweed was dried at a stable room temperature to remove the water content contained in the seaweed. The process of separating the bioactive components is carried out by extraction by means of a seaweed sample that has been cleaned and weighed, then cut into small pieces and immersed in a sample bottle, extraction using the maceration method with n-hexane solvent. Seaweed was macerated using n-hexane for 24 hours with occasional stirring. Maceration was carried out for up to 3 × 24 hours, the marinade was filtered using filter paper and a Buchner funnel, the dregs were separated from the macerate, then the macerate was evaporated using a rotary evaporator to obtain a thick seaweed extract.

Phytochemical Test

Phytochemical tests carried out included; Test Flavonoids, Alkaloids, Saponins, Triterpenoids, and Steroids.

Purification of test bacteria

E.coli bacteria are inoculated into the agar medium by taking one looped needle aseptically and then inoculating by scraping the medium for slanting. Then incubated for 24 hours at 37°C until planting occurs. Preparation of a suspension of *E.coli* bacteria in oblique agar medium was taken aseptically in one ose, then put in 12 mL of NB medium and shaker until homogeneous. The number of *E.coli* cells in the suspension was measured until it reached 10^5 - 10^8 cells/mL using a hemocytometer.

Antibacterial Activity Test

Anti-bacterial activity test was carried out on *E.coli* bacteria. Antibacterial activity testing was carried out using the agar diffusion method. The workings of the agar diffusion method are that the test bacteria that have been rejuvenated are inoculated with as much as 200 µl in a petri dish that already contains media. Then it was kept for 1 hour at 37°C. After 1 hour, 6 mm disc paper was placed in the center of the media, and 20 µl/5 µg of 100% *E.cottonii* seaweed extract was dripped onto the test bacteria, then incubated at 37°C for 24 hours, then measured the diameter of the inhibition formed (Melki, 2010).

The criteria for antibacterial strength are classified based on the size of the inhibitory power as shown in Table 1.

Table 1. Inhibition Zone Strength Criteria

Inhibition Zone(mm)	Inhibitory Power
< 5	Weak
5 – 10	Currently
10 – 20	Strong
>20	Very strong

Determination of Minimum Inhibitory Concentration Value (MIC), the determination method used is the solid agar method. Extract samples were made with various concentrations ranging from large to small, namely 10%, 5%, 1%, and 0.05% (Purnama *et al.,* 2010).

Data analysis

The data obtained in this study will be presented in the form of tables and pictures explained descriptively referring to existing sources.

3. RESULT AND DISCUSSION

General Condition of Research Area

Sugie Village is one of the villages located in Moro District, Karimun Regency, Kepulauan Riau Province, with an area of approximately 62 km^2 with the boundaries of the area as follows: To the north, it is bordered by Keban Village, South is bordered by Moro Village, West is bordered by Pauh Village and The east is bordered by Belakang Padang District. Geographically, Sugie Village is located between 103° 43' E to 103° 45' E and 0° 48' N to 0° 51' N.

Water Quality

The results of water quality measurements in Sugie village waters are between 29-31^oC, brightness 2.5-7 m, current speed 20-30 cm/s, depth 8-15 m, and salinity 30-32 ppt where the quality of the waters is still low. The limits of good quality standards for the life of *E.cottoni* and *G.verucossa* can be seen in Table 2.

Table 2. Water Quality Measurement

No	Parameter	Laboratory Quality Standards	Measurement results
1	Temperature (^oC)	26–32	29 – 31
2	Brightness (m)	>1	2.5 – 7
3	Current Speed (cm/s)	20–40	20 – 30
4	Depth (m)	1–7	8 – 15
5	Salinity (ppt)	28–34	30 – 32

Extraction of Secondary Metabolites

As much as 2 kg of *E.cottonii* seaweed and 2 kg of dried *G.verucossa* were cut as small as possible to obtain 271 g of *E.cottonii* sample powder and 267 g of *G.verucosa* sample powder and then extracted by maceration using n-hexane solvent for 3 x 24 hours. A total extract of dark green n-hexane was obtained as much as 2.365 g of *E.cottonii* and a total extract of *G.verucossa* was as much as 2.146 g.

Phytochemical Test

Phytochemical tests are used to detect secondary metabolites in plants based on their group as initial information in knowing the chemical compounds in a plant. From the research that has been done, it was found that extracts of seaweed *E.cottonii* and *G.verucossa* have secondary metabolites of the terpenoid/steroid class. The results of the identification of the chemical content of these plants can be seen in Table 3.

Table 3. Screening results of phytochemical tests *E.cottonii* seaweed extract

Phytochemical Test	Positive Results According to Readers (Rivai *et al.*, 2013)	Results
Alkaloids	An orange precipitate (Dragendrof reagent) is formed	-
	A white precipitate (Meyer's reagent) is formed	-
Flavonoids	An orange or red solution is formed	-
Saponins	Stable foam formed ± 5 minutes	-
Terpenoids / Steroids	Brownish or violet ring	+
Phenolic	A bluish green solution was formed	-

Note: (+) indicates the presence of compounds; (-) no compound content

The results obtained from the *E.cottonii* seaweed extract phytochemical test screening were the absence of alkaloid compounds, flavonoids, phenolic saponins, and positive (+) containing steroid compounds (Table 4).

Table 4. Screening results of phytochemical tests *G. verucosa* seaweed extract

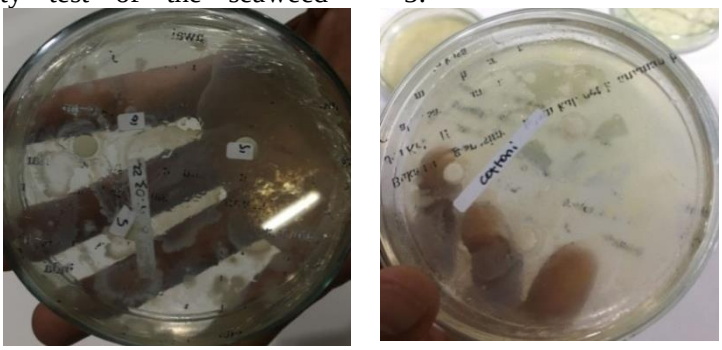
Phytochemical Test	Positive Results According to Readers (Rivai *et al.*, 2013)	Results
Alkaloids	An orange precipitate (Dragendrof reagent) is formed	-
	A white precipitate (Meyer's reagent) is formed	-
Flavonoids	An orange or red solution is formed	-
Saponins	Stable foam formed ± 5 minutes	-
Terpenoids / Steroids	Brownish or violet ring	+
Phenolic	A bluish green solution was formed	-

Note: (+) indicates the presence of compounds; (-) no compound content

Antibacterial Activity Test of *E. cottonii* and *G.verucossa* Seaweed Extracts

The results of the antibacterial activity test of *E.cottonii* and *G.verucossa* seaweed extracts against *E.coli* bacteria with a concentration of 5%, 10%, 15% can be seen in Figure 1. Based on the results of the antibacterial activity test of the seaweed extracts *E.cottonii* and *G.verucossa* with a concentration of 5%, 10%, and 15% has moderate inhibition, because the diameter formed on the disc paper in this trial has the same results which range in numbers 8-9 mm.

Testing the concentration of seaweed extract on the test bacteria can be seen in Table 5.



(a) *G. verucossa* (b) *E. cottonii*

Figure 1. Antibacterial extract activity of *E.coli*

Table 5. Test results for the minimum inhibitory concentration of seaweed extract against the test bacteria

Bacteria	Seaweed	Concentration (%)	Diameter of Clear Zone(mm)
Escherichia coli	*E.cottonii*	5	8,24
		10	8,50
		15	8,62
	G.verucossa	5	8,24
		10	8,50
		15	8,62

Based on the results of testing the minimum inhibitory concentration of *E.cottonii* the results obtained from the diameter of the clear zone for concentration (5%) 8.24 mm, (10%) 8.5 mm, (15%) 8.62 mm. The results of testing the minimum inhibitory concentration of *G.verucossa* seaweed extract produced clear zone diameters of concentration (5%) 8.24 mm, (10%) 8.5 mm, (15%) 8.62 mm. Research on anti-bacterial compounds used the agar diffusion method, namely paper discs. From the results, it was found that the compounds of seaweed extract *E.cottoni* and *G.verucossa* had moderate inhibition on the growth of E.coli bacteria. Based on research, conducted by (Dwyana & Johannes, 2012) *E.cottonii* seaweed extract can inhibit bacterial growth, both gram-negative and gram-positive bacteria, and the bioactivity of the red algae *E.cottonii* extract tends to be bacteriostatic.

4. CONCLUSION

The results of the research on the phytochemical test of extracts of *E.cottonii* and *G.verucosa* seaweed with n-hexane solvent can be concluded that the seaweeds *E.cottonii* and

G.verucossa contain the same phytochemical compounds in the form of steroids. The results of measuring the diameter of the clear zone with concentrations of 5%, 10%, and 15% for *E.cottoni* and *G.verucossa* seaweed extracts against *E.coli* bacteria had concentration results (5%) 8.24 mm, (10%) 8, 5mm, (15%) 8.62 mm.

It is hoped that in future studies it will be necessary to add several types of bacteria in order to obtain more varied comparative data.

REFERENCES

Dwyana, Z., & Johannes, E. (2012). *Uji Efektivitas Ekstrak Kasar Alga Merah Eucheuma cottonii Sebagai Antibakteri Terhadap Bakteri Patogen. Skripsi*. Makassar: Jurusan Biologi Fakultas Matematika dan Ilmu Pengetahuan Alam Universitas Hasanuddin.

Iskandar, Y., Rusmiati, D., & Dewi, R.R. (2005). *Uji aktivitas Antibakteri Ekstrak Etanol Rumput Laut (Eucheuma cottonii) terhadap Bakteri Escherichia coli dan Bacillus cereus. Skripsi.* Sumedang: Jurusan Farmasi Fakultas Mipa Universitas Padjadjaran Jatinangor.

Maharany, F., Nurjanah., Suwandi, R., Anwar, E., Hidayat, T. (2017). Kandungan Senyawa Bioaktif Rumput Laut *Padina australis* dan *Eucheuma cottonii* Sebagai Bahan Baku Krim Tabir Surya. *Jphpi*. 20(1):10–17.

Melki. (2010). *Efektivitas Ekstrak Mangrove Sebagai Antibiotik pada Penyakit Vibrosis Udang Windu. Tesis*. Bogor: Sekolah Pascasarjana Institut Pertanian Bogor.

Pangestuti, I.E., & Amalia, U. (2017). Skrining Senyawa Fitokimia Rumput Laut *Sargassum* sp. dan Aktivitasnya Sebagai Antibakteri Terhadap *Staphylococcus aureus* dan *Eschericia coli. Sainstek Perikanan*. 12(2): 98–102.

Purnama, R., Melki., Putri, W.A.E., Rozirwan. (2010). Potensi Ekstrak Rumput Laut *Halimeda renchii* dan *Eucheuma cottonii* sebagai Antibakteri *Vibrio parahaemolitycus, Vibrio alginolyticus*, dan *Vibrio charcariae.* Indralaya. *Jurnal Maspari.* 5(2): 82-88.

Rivai, H., Putra, R.Y., & Krisyanella. (2013). Penentuan Pengaruh Jenis Pelarut Pengekstrak Terhadap Perolehan Kadar Senyawa Fenolat dan Aktifitas Antioksidan dari Daun Jambu Biji (*Psidium guajava L*). *Jurnal Farmasi Higea*, 4(1):16-23.

Setyowati, W.A.E., Ariani, S.R.D., Ashadi, B., Mulyani, C.P., Rahmawati. (2014). Skrining Fitokimia dan Identifikasi Komponen Utama Ekstrak Metanol Kulit Durian (*Durio zibethinus* mur.) Varietas Petruk. *Seminar Nasional Kimia dan Pendidikan Kimia VI*. Surakarta: Prodi Pendidikan Kimia Jurusan FMIPA FKIP Universitas Surakarta.

Soamole, H.H., Sanger, G., Harikedua, S.D., Dotulong, V., Mewengkang, H.W., Montolalu, R.I. (2018). Kandungan Fitokimia Ekstrak Etanol Rumput Laut Segar (*Turbinaria sp., Gracilaria sp*., dan *Halimeda macroloba*). *Media Teknologi Hasil Perikanan.* 6(3).