

Evaluation of Antioxidant and Antidiabetic Activities from Red Seaweed (*Eucheuma cottonii*)

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ABSTRACT

Diabetes mellitus is a chronic disorder of metabolic characterized by high blood sugar levels (hyperglycemia) and glucose secretion in urine caused by lack of insulin. Hyperglycemia can cause free radical molecules formation, which can be stabilized by antioxidants. Antioxidants can be found in seaweed (e.g. red seaweed, i.e. *Eucheuma cottonii*), which is available in large quantities in Indonesia. The purpose of this research is to determine the antioxidant and antidiabetic activities of *E. cottonii* extract in vitro and in vivo. Different solvents such as ethanol, distilled water, and warm distilled water were used for extraction. The extracts were subjected to phenolic screening, bioactive compound identification by GC-MS, antioxidant analysis using DPPH, and α -amylase activity assay in vitro and in vivo. The highest yield was obtained from warm distilled water solvent (6.8%). The phytochemical content of *E. cottonii* crude extract included alkaloid, terpenoid, phenolic, flavonoid, and saponin. The highest percent inhibition on DPPH free radical scavenging and α -amylase used ethanol

solvent (34.27%) and (59.33%), respectively. Male *Mus musculus* L. mice was used as an animal model in antidiabetic in vivo assay. Warm distilled water and ethanolic extract of *E. cottonii* showed the ability to decrease BGL in diabetic mice below 100 mg/dL. Therefore, it can be concluded that the extract of red seaweed *E. cottonii* has the potential as an antioxidant and antidiabetic agent.

Keywords: antioxidant, antidiabetic, *E. cottonii*, α -amylase, DPPH, phytochemical screening

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INTRODUCTION

The prevalence of diabetes mellitus (DM) worldwide in 2010 will increase ten-fold by 2030, where 143 million of them are women and 142 million are men (1). According to the World Health Organization (WHO), DM is the most lethal disease in Southeast Asia. DM is a chronic metabolic disorder characterized by high blood sugar levels (hyperglycemia) and glucose secretion in urine due to disruption of production, secretion or resistance to insulin (1). Sarianet *et al.* (2017) reported that antioxidant plays important role in the alleviation of DM (2).

As an archipelago country which is rich in natural resources, Indonesia has increased the use of natural materials as a large scale source of antioxidant. Beside its affordable price, the use of natural materials as an antioxidant also have smaller side effects compared to antioxidant derived from chemical materials (3). Seaweed is one type of natural material that is often used as a source of antioxidant. Red seaweed, i.e. *E. cottonii*, also known as *Kappaphycus alvarezii*, is potential to be used as an antioxidant and quite good antidiabetic activity in vitro (4). The presence of phenolics, flavonoids, saponins, terpenoids, and alkaloids may contribute to the antioxidant and antidiabetic activity of *K. alvarezii* (5, 6).

Studies have demonstrated that bioactive compound from *E. cottonii* crude extract and fastly lower blood glucose level (BGL) of white Wistar rats (7). Many researches were conducted on antioxidant activities of red seaweeds (8).

However, the studies of *Eucheuma* sp on the antioxidant and antidiabetic properties in vitro and in vivo have not been conducted yet which can be used as an alternative to natural medicine. Therefore, the objective of this study was to evaluate the antioxidant and antidiabetic activities of *E. cottonii* dry extracts *vitro* and *in vivo*.

MATERIALS AND METHODS

Materials and Equipment

Materials used for this research were dried red seaweed *E. cottonii* from Makassar (South Sulawesi, Indonesia), ethanol, chloroform, distilled water, FeCl₃, HCl 2 N, H₂SO₄, chloroform, methanol, Mayer reagent (Potassium Mercuric Iodide), Liebermann-Burchard reagent (acetic acid anhydride: concentrated H₂SO₄ ratio 1:1), 3,5-dinitrosalicylic acid (DNS), starch, Dragendorff reagent (solution of Potassium Bismuth Iodide), sucrose, DPPH, ascorbic acid, alloxantetrahydrate, and mice (*Mus musculus* L.). Equipment used for this research were glassware, vacuum oven, microplate reader, Gas Chromatography - Mass Spectrometer (GC-MS), rotary evaporator, and glucometer.

E. cottonii Sample Preparation

Sample was cleaned from sands and rocks with distilled water. After that, sample was dried using vacuum oven at 37°C for 3-4 days. Next, the dried sample was mashed to powder and ready to be used in the next step.

Phytochemical Screening

Alkaloids Test

Approximately 0.5 g extract of sample and 5 mL of ethanol were added into test tube. After that, filtrates were divided into 2 test tubes. Filtrate in tube 1 was treated with Dragendorff's reagent and tube 2 with Mayer's reagent. Formation of orange precipitate in tube 1 (9) and white precipitate in tube 2 (10) indicated the presence of alkaloids.

Triterpenoid and Steroids Test

Approximately 0.5 g of sample and 5 mL of chloroform were added into test tube. Filtrates were treated with Liebermann-Burchard's reagent. Formation of violet color indicated the presence of triterpenoid, while green or blue color indicated the presence of steroid (10).

Phenolics Test

Approximately 0.5 g extract of sample and 5 mL of ethanol were added into test tube. Filtrates were treated with 3-5 drops of FeCl_3 solution. Formation of blue color indicated the presence of phenols (11).

Flavonoids Test

Approximately 0.5 g extract of sample and 5 mL ethanol were added into test tube. Filtrates were treated with 1 mL of NaOH solution and 3-5 drops of HCl. Formation of yellow color change into clear indicated the presence of flavonoids (10).

Saponins Test

Approximately 0.5 g extract of sample in test tube was shaken with 5 mL of distilled water. If foam produced persisted for ten minutes, which did not disappear after HCl was added, it indicated the presence of saponins (9).

Extraction of Bioactive Compounds from *E. cottonii*

The method of extraction was done using maceration method with 3 solvents such as ethanol, distilled water, and warm distilled water. *E. cottonii* seaweed was mashed into small pieces with food processor and then inserted into Erlenmeyer. The solvent was added with the ratio 1:5 (w/v) for ethanol and 1:10 (w/v) for distilled water and warm distilled water (50°C). Sample was macerated with solvent, then covered with plastic wrap and wrapped in aluminum foil. Sample was macerated overnight. The obtained extract solution was filtered with Whatman filter paper to separate the filtrate and the residue (12).

Antioxidant Activity with DPPH (1, 1-Diphenyl-2-picrylhydrazyl) Radical Scavenging Assay

Antioxidant activity of *E. cottonii* sample was measured with various concentration such as 1000, 500, 250, and 125 ppm. Approximately 50 microliter of *E. cottonii* extract was added with 50 microliter of the ethanol and pipetted into microplate. Approximately 80 microliter of DPPH 0.1 mM was added into microplate. Solution in the microplate was incubated in dark room with room temperature at 30 min. to optimize the reaction and read with microplate reader at 517 nm wavelength (13). Blank used is 50 microliter methanol and 80 microliter DPPH 0.01 mM which pipetted into microplate.

Ascorbic acid was used as positive control (14). The percentage inhibition of antioxidant activity was calculated by following the formula:

$$\% \text{ Inhibition} = \frac{\text{abs. positive control} - \text{abs. sample}}{\text{abs. positive control}} \times 100\%$$

Alpha-Amylase Activity Assay

E. cottonii extract was diluted using buffer phosphate with various concentration as follow 1000, 500, 250, and 125 ppm, respectively. *E. cottonii* extract and alpha-amylase (1:1) were added to microplate and incubated at 25 °C for 10 mins. Approximately 250 microliter of starch 1 % in 0.02 M buffer phosphate (pH 6.9) was added to the mixture and incubated at 25°C for 10 mins. Approximately 500 pL of DNS added into microplate and vial tubes were heated 10 minutes and chilled at room temperature for around 5 minutes. DNS function is to stop the reaction between sample and enzyme. Approximately 150 pL mixture of enzyme and sample were added to microplate well and read with microplate reader at 540 nm wavelength (15). The percentage inhibition of alpha-amylase activity was calculated by following formula below:

$$\% \text{ Inhibition} = \frac{\text{abs. positive control} - \text{abs. sample}}{\text{abs. positive control}} \times 100\%$$

GC-MS Analysis

GC-MS analysis was performed with Thermo Scientific Single Quadrupole 5050A mass spectrometer coupled with Thermo Scientific Gas Chromatography and fitted with Ultra Alloy+ 5 capillary column (30 m length x 0.25 mm i.d., 0.25 pm film thickness). Helium was used as carrier gas at flow rate 1 mL/min. Oven temperature was set from 50 °C to 280 °C and held for 5 minutes at 280 °C. Interface temperature was kept at 250 °C. Ionization mode was set electron impact ionization with the range of analysis was 40-400 amu. The interval time of mass spectra was obtained at 0.5 second. The results were compared with library compounds presented in National Institute Standard and Technology (NIST) database (16).

Reduction of Blood Glucose Level (BGL) in Vivo Assay Animals

Healthy male white mice (*Mus musculus* L.) (with body weight were body weight 25-30 g) at the age 2-3 months old were obtained from Department of Nutrition, Faculty of Medicine, Universitas Indonesia, Jakarta were used in this research. The mice were maintained under standard conditions in the cage at room temperature ($\pm 25^\circ\text{C}$), humidity ($\pm 60\%$), and normal photo period (12 h dark/12 h light) for the experiment. The mice were acclimatized to the cage conditions three days prior to experiment (17).

Experimental Protocol

The mice were divided into six groups of five rats in each group (Table 1). The groups were blank, negative control, ethanol extract (EE), distilled water extract (DWE), and warm distilled water extract (WDWE) (18).

Table 1. Experimental design of the study

Group	Mice Model	Treatment
Blank	Normal	Untreated mice, normal mice were given water and normal pellet
Negative Control	Diabetes	Diabetic mice were given distilled water and normal pellet
Positive Control	Diabetes	Diabetes mice were given acarbose, distilled water and normal pellet
EE	Diabetes	Diabetic mice were given ethanol extract of <i>E. cottonii</i> , distilled water and normal pellet
DWE	Diabetes	Diabetic mice were given distilled water extract of <i>E. cottonii</i> , distilled water and normal pellet
WDWE	Diabetes	Diabetic mice were given warm distilled water extract of <i>E. cottonii</i> , distilled water and normal pellet

*Ethanol extract (EE), distilled water extract (DWE), warm distilled water extract (WDWE)

Optimization of Diabetic Level

The optimization of diabetic level in mice was administered by 21 mice *Mus musculus* L. (n=21). The assay was done twice. First, assay was divided into 3 groups which consist of three mice. All mice were fasted 8 h (water given) and then the BGL of mice were measured by glucometer. In each group of mice was injected by alloxan tetrahydrate with different concentrations (50, 60, and 100 mg/kg BW) via intraperitoneal route. Each group of mice was given 5% sucrose after injection. On day 3, BGL was measured and the result with BGL <126 mg/dL, were not considered as diabetes. Second, assay was divided into 3 groups which consist of four mice. In each group of mice was injected by alloxan tetrahydrate with different concentrations (100, 150, and 200 mg/kg BW) via intraperitoneal route. On day 3, BGL was measured and the result with BGL >126 mg/dL were observed as diabetes. The mice which were given 100 mg/kg BW showed the highest BGL (184 mg/dL) without showing any side effects. Alloxan tetrahydrate with 100 mg/kg BW was chosen for diabetes induction method (18, 19).

Induction of Diabetes

Diabetes induction was carried out for 3 days. All mice were fasted within 8 hours and then BGL of mice were measured by glucometer on day-0 and day 3 at 09.00 am. Blood samples of mice were taken via capillary route and tested using glucometer. Then, 100 mg/kg BW alloxan tetrahydrate was taken 0.2 mL and diluted in sterile double distilled water. Alloxan was injected in intraperitoneal for 3 days. During the induction, mice were given 10% sucrose solution

and normal pellet. On the day 3 day sampling, mice with BGL >126 mg/dL are considered as diabetic mice (19).

Diabetes Treatment

During treatment period, mice were given normal pellet and water. Positive control mice were given orally with 0.5 mL acarbose (150 mg/kg BW). According to treatment, mice (EE, DWE, WDWE) were given 0.5 mL *E. cottonii* extract (300 mg/kg BW). The treatment was given 2 times/day for 15 days (09.00 and 15.00). Mice were fasted for 8 hours before BGL measurement. BGL was measured on day-0, day 3, day 6, day 9, day 12, and day 15 with glucometer (19).

Statistical Analysis

Data was analyzed by using the IBM SPSS Statistics Data Editor software. One way ANOVA was used to analyze significant differences among solvent types and solvent concentration of *E. cottonii*. The significant value for data analyzed was set at $p < 0.05$ (19).

RESULTS

Phytochemical Screening

Based on qualitative phytochemical screening of red seaweed *E. cottonii* extract showed positive results for alkaloids, triterpeneoids, phenolic, flavonoids, and saponins as shown in Table 2. However, steroids were not detected.

Table 2. Phytochemical screening results of *E. cottonii* crude extract

Screening Test	Results	Observation
Alkaloid	+	Orange precipitate
	+	White precipitate
Terpenoid	+	Brownish violet
Steroid	-	Brownish precipitate
Flavonoid	+	Light yellow to clear
Phenolic	-	Yellow
Saponin	+	Foam more than 2 minutes

Extraction of Bioactive Compounds from *E. cottonii*

The mass of extracted compounds from 50 g of *E. cottonii* with solvents including ethanol, distilled water, and warm distilled water were compared in order to optimize the extraction condition.

The highest yield was obtained from warm distilled water extract (6.8%) out of three solvents. The mass of the extracted compounds (in %) using maceration method are summarized in Table 3.

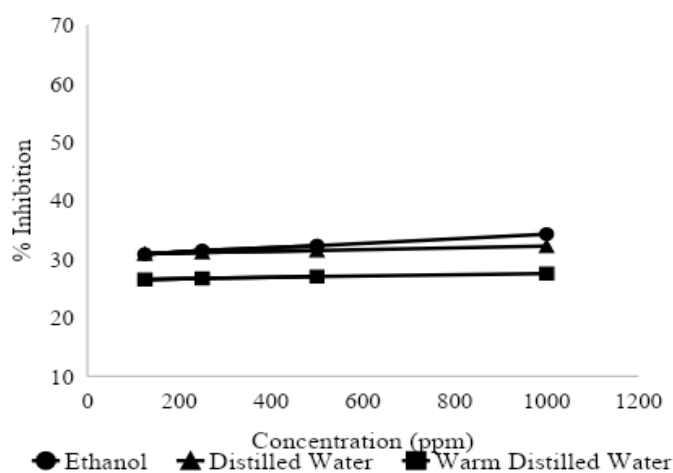
Table 3. The mass of *E. cottonii* using maceration method

Extract	Results
Warm Distilled Water	6.8%
Distilled Water	4.4%
Ethanol	2.2%

Antioxidant Activity with DPPH (1, 1-Diphenyl-2-picrylhydrazyl) Radical Scavenging Assay

E. cottonii crude extract demonstrated the capacity of DPPH free radicals with increasing concentrations, with ethanolic extract

exhibiting the strongest antioxidant activity values compared with the distilled water and warm distilled water extract. The difference in antioxidant activity between each treatment concentration of all three groups were statistically not significant ($p > 0.05$, $n = 3$) (Figure 1; Figure 2).

**Figure 1. Antioxidant activity of *E. cottonii* extract by DPPH assay**

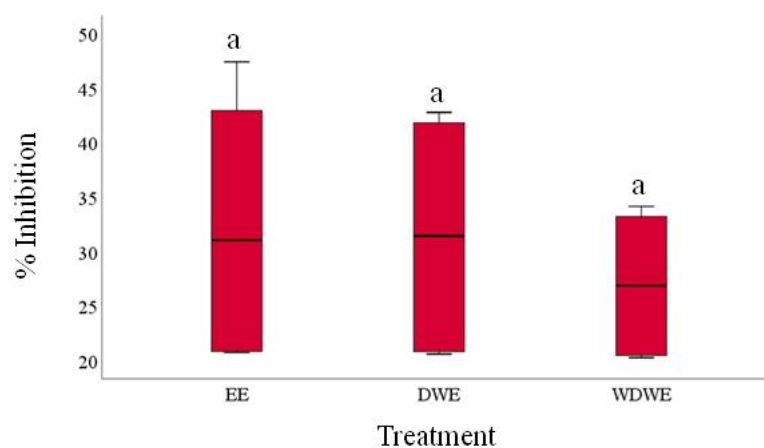


Figure 2. Boxplot of DPPH inhibition by *E. cottonii* extract. Ethanol extract (EE), distilled water extract (DWE), and warm distilled water extract (WDWE).

Enzyme (α-Amylase) Activity Assay

All extracts of red seaweed *E. cottonii* were found to possess α-amylase inhibitory activity. The inhibition of α-amylase by *E. cottonii* extract with 3 solvents are summarized in Figure 3 and Figure 4. The *E. cottonii* ethanolic extract inhibit the strongest α-amylase

inhibitory activity compared with the distilled water and warm distilled water extract. The difference in α-amylase inhibitory activity between each treatment concentration of all three groups were statistically significant ($p < 0.05$).

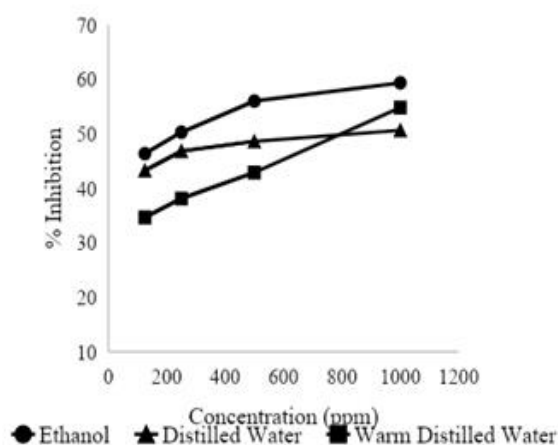


Figure 3. Alpha-amylase inhibitory activity of *E. cottonii* extract

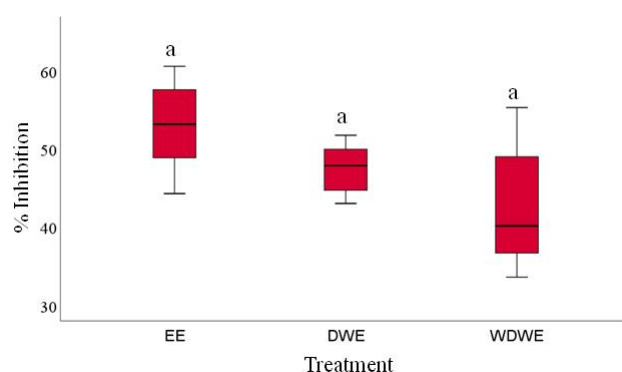


Figure 4. Boxplot of a-amylase inhibition by *E. cottonii* extract. Ethanol extract (EE), distilled water extract (DWE), and warm distilled water extract (WDWE).

GC-MS Analysis

The GC-MS profiling analyses of *E. cottonii* ethanolic, distilled water, and warm distilled water extracts indicated the presence of 12 compounds (Figure 5; Figure 6; Figure 7). Ethanolic extract showed the presence of many steroid derivatives (Table 4), while distilled and warm distilled water showed the presence of many fatty acids (Table 5 and Table 6).

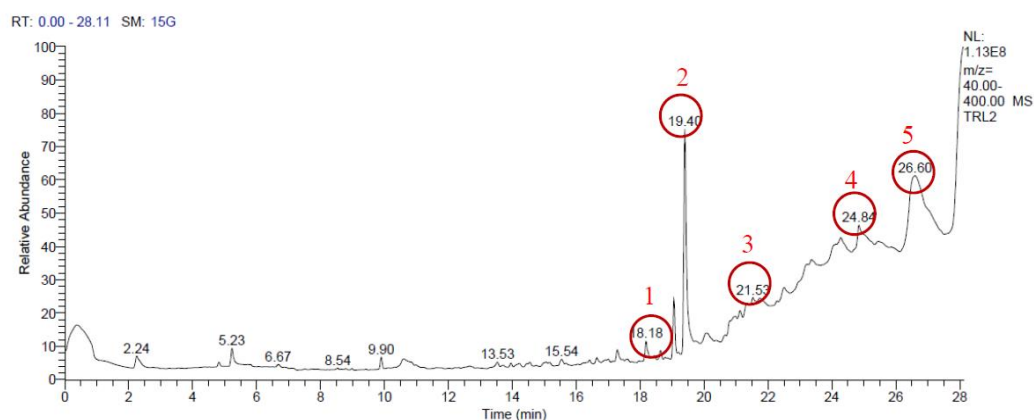


Figure 5. The result of *E. cottonii* ethanolic extract chromatogram

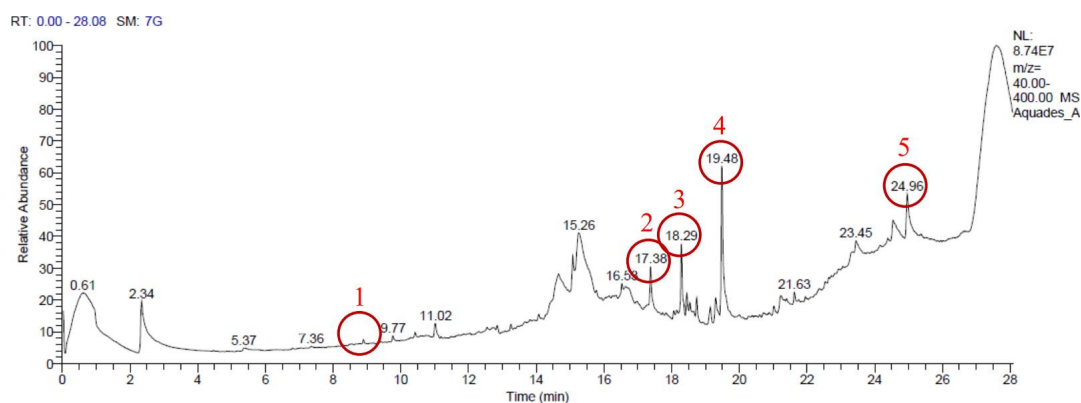


Figure 6. The result of *E. cottonii* distilled water extract chromatogram

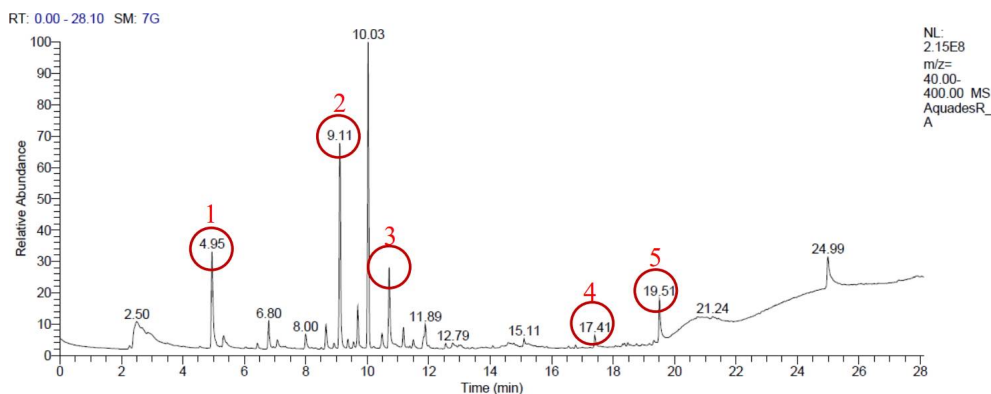


Figure 7. The result of *E. cottonii* warm distilled water extract chromatogram

Table 4. Bioactive components in ethanol extract revealed in chromatogram

No	Compound Name	Compound Nature	MW	Molecular Formula	Biological Properties	References
I	Hexadecanoic Acid, Methyl Esther	Palmitic Acid, Methyl Esther	270	$C_{17}H_{34}O_2$	Antioxidant, Hypocholesterolemic, Nematicide, Pesticide, Antiandrogenic, flavor, Hemolytic, Alphareductase inhibitor	(20)
2	n-Hexadecanoic Acid	Palmitric Acid (Saturated Fatty Acid)	256	$C_{16}H_{32}O_2$	Antioxidant, Hypocholesterolemic, Antiandrogenic, Hemolytic, 5-alpha reductase inhibitor, Antipsychotic	(21)
3	Ethyl Iso-Allocholate	Steroid Derivative	436	$C_{26}H_{44}O_5$	Antimicrobial Activity, Anti-inflammatory, Antiasthma	(22, 23)
4	Ergosta-5,22-dien-3-ol, acetate, (3a,22E)	Steroid	440	$C_{30}H_{48}O_2$	Anti tumor activity and immunomodulatory activity	(24)
5	Sesamin	Lignan	354	$C_{20}H_{18}O_6$	Antioxidant	(25)

Table 5. Bioactive components in distilled water extract revealed in chromatogram

No	Compound Name	Compound Nature	MW	Molecular Formula	Biological Properties	References
1	Ergosta-5,22-dien-3-ol, acetate, (3a, 22E)	Steroid	440	C ₃₀ H ₄₈ O ₂	Antitumor activity and immuno modulatory activity	(24)
2	Tetradecanoic Acid	Acid	228	C ₁₄ H ₂₈ O ₂	Antioxidant, Anti-cancer	(22)
3	Ethyl Iso-Allochololate	Steroid Derivative	436	C ₂₆ H ₄₄ O ₅	Antimicrobial Activity, Anti-inflammatory, Antiasthma	(22, 23)
4	Hexadecanoic Acid	Acid	568	C ₃₅ H ₆₈ O ₅	Antioxidant, Hypocholesterolemic	(22)
5	Hexasiloxane	Silicon based polymer	597	C ₁₂ H ₃₈ O ₅ S	Antimicrobial, Antiseptic	(26)

Table 6. Bioactive components in warm distilled water extract revealed in chromatogram

No	Compound Name	Compound Nature	MW	Molecular Formula	Biological Properties	References
1	Furfural	Aldehyde Compound	96	C ₅ H ₄ O ₂	Antimicrobial preservative	(27)
2	Levogluconone	Fatty Acid	126	C ₆ H ₆ O ₃	Chiral Agent, Catalyst, Antiviral, Anti-cancer	(28, 29)
3	Ethyl Iso-Allochololate	Steroid Derivative	436	C ₂₆ H ₄₄ O ₅	Antimicrobial Activity, Anti-inflammatory, Antiasthma	(22, 23)
4	Tetradecanoic Acid	Acid	228	C ₁₄ H ₂₈ O ₂	Antioxidant, Anti-cancer	(22)
5	n-Hexadecanoic Acid	Palmitric Acid (Saturated Fatty Acid)	256	C ₁₆ H ₃₂ O ₂	Antioxidant, Hypocholesterolemic, Antiandrogenic, Hemolytic, 5-alpha reductase inhibitor, Antipsychotic	(21)

Reduction of Blood Glucose Level (BGL) *In Vivo* Assay

All treatments decreased mice BGL (Figure 8). The highest decreasing value of blood sugar level was ethanolic extract, followed by warm distilled water, and distilled water. Ethanolic extract showed the best activity for decreasing BGL (Figure 9; Figure 10). The difference in blood glucose showed declining activities between each treatment concentration of all three groups were significantly different ($p < 0.05$).

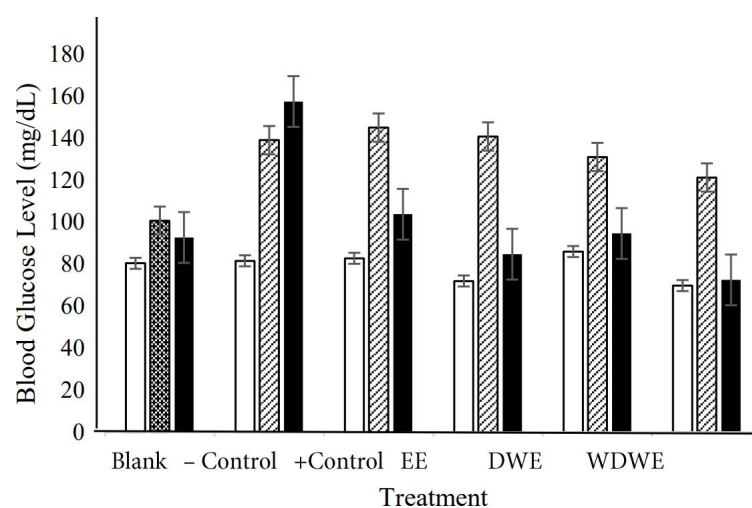


Figure 8. Alterations of BGL before DM Induction, after DM induction, and after treatment with *E. cottonii* extracts and acarbose for 1 day and acarbose for 14 days. Blank, negative control, positive control, ethanol extract (EE), distilled water extract (DWE), and warm distilled water extract (WDWE).

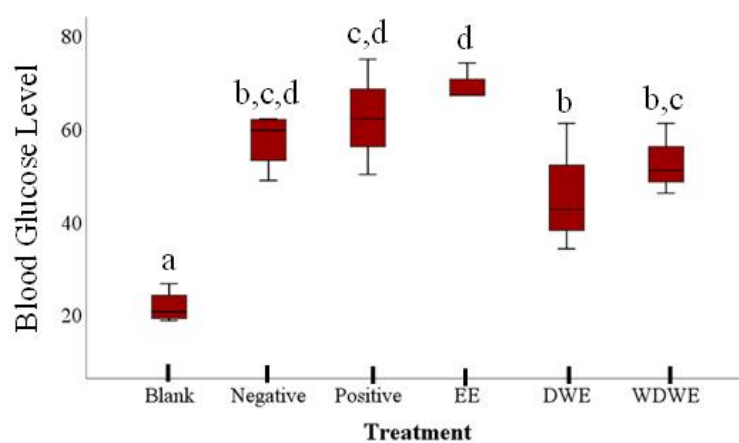


Figure 9. Diabetes induction with alloxan tetrahydrate. Blank, negative control, positive control, ethanol extract (EE), distilled water extract (DWE), and warm distilled water extract (WDWE).

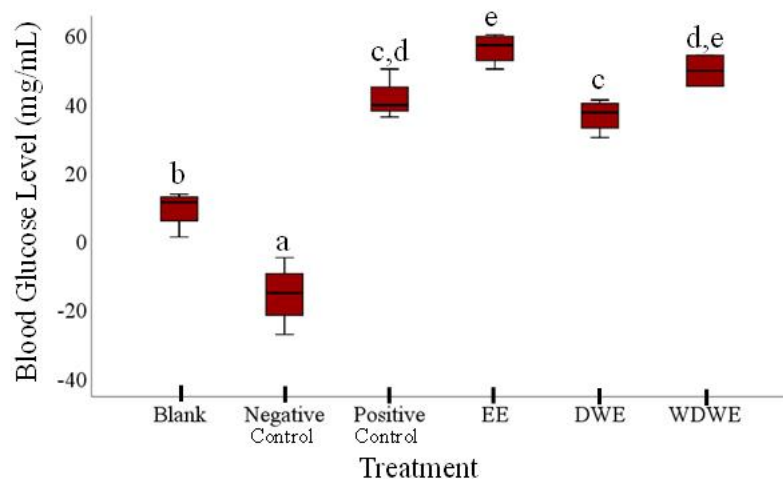


Figure 10. Reduction of blood glucose level. Blank, negative control, positive control, ethanol extract (EE), distilled water extract (DWE), and warm distilled water extract (WDWE).

DISCUSSION

The crude extracts of seaweed *E. cottonii* were analyzed to determine the active component compound that acts as an antioxidant. The phytochemical compounds detected are known to have medicinal importance. For example, the biological function of flavonoids beside its antioxidant properties are protection from allergies, inflammation, and free radicals (Sheikh *et al.*, 2013). According to previous research, flavonoids play a role in the prevention of diabetic complications (31). Chen *et al.* (2014) proved that saponin from brown seaweed *Radix trichosanthis* acts as antioxidant potency both in vitro and in vivo (32). Alkaloids work as antioxidants. Balanquit and Fuentes (2015) proved that brown seaweed *Sargassum* sp showed antioxidant activity by DPPH assay and Fe^{2+} chelating ability (33). Triterpenoids work as antioxidant which play an important role in health such as carotenoids for vision and human immune function. Based on previous research, triterpenes are used as antidiabetic remedies in various countries (34). Polyphenol compounds work as free radical scavenging or antioxidants. Phenol can absorb free radicals and can chelate metal ions that could catalyze formation of ROS which promotes lipid peroxidation (35). Flavonoids are phenolic compounds and plant phenolics are a major group of compounds that act as primary antioxidants or free radical scavengers (36).

The extraction is a separation method of one or more solid or liquid material with the aid of solvents. Separation occurs on the basis of different solubility of the inner component mixture. Maceration is an extraction process using solvents with some time shaking or stirring in certain temperature like room temperature. Constant stirring can increase speed reaction of the extraction. Maceration has several advantages, such as low amount of solvent used and the temperature used is below the solvent boiling point in which prevent the degradation of oil component due to heat (37). Solvents, temperature, extraction time, the polarity of sample, and extraction methods will affect the results of secondary metabolites compound extracted. The principle used is "dissolve like dissolve", depending its polarity solution,

where the non-polar compounds will dissolve in non-polar solvents, while the polar compounds will dissolve in polar solvents (38, 39). Yield is an important parameter to determine the economic value and effectiveness of a product. Yield is the percentage share of the raw materials that can be utilized. The yield of *E. cottonii* using solvent ethanol, distilled water, and warm distilled water amounted to 2.2%, 4.4%, and 6.8%, respectively. Ethanolic solvents used in this research caused by ethanol is non-toxic solvent compared to methanol (40). Ethanol is a semi polar solvent while distilled water is a polar solvent.

The greatest yield obtained by warm distilled water solvent. This happened because seaweed can be extracted perfectly at high temperatures which are close to the boiling point of the solution (41). Increasing temperature can increase the permeability of cell wall which results in solubility and diffusion bioactive compound increase with decrease the viscosity of solvents to facilitate the extraction process. However, besides being able to increase the solubility of bioactive compounds, increasing temperature can also damage the bioactive compound contained in seaweed (42).

Antioxidant is capable to prevent oxidation by free radicals. DPPH is a free radical compounds which are stable at room temperature is often used for antioxidant assay. DPPH assay has several advantages, such as simple, easy, and using small amount of samples with a short time (43). Antioxidants worked against oxidative stress and many pathological conditions such as diabetes mellitus and cancer to protect body tissues by neutralizing harmful free radicals such as ROS. ROS causes cell death, skin ageing, and tissue injury from the action of oxidising biomolecules (44).

The ethanolic extract, distilled water extract, and warm distilled water extract of *E. cottonii* showed potential percentage of DPPH inhibition while ethanolic extract showed the greatest inhibition. Ethanol extract of *E. cottonii* is the greatest extract to inhibit DPPH caused by many phytochemical screening, dissolve in semi polar solvent. Based on the results obtained, the ethanolic extracts which are semi polar extracts, were more powerful and

effective antioxidants compared to the polar distilled water and warm distilled water extracts in DPPH assay (45). Based on previous research, the lowest antioxidant activity obtained by distilled water extract of *K. alvarezii*. The research indicated that non polar compound found in extract possesses strongest ability to scavenge DPPH radicals (46). All extracts showed weak inhibition of DPPH with IC₅₀ value >200ppm. It happens due to the use of dried seaweed. Heat and sunlight can reduce or damage bioactive component in the seaweed (47). But there are several advantages of using dried seaweed as sample, such as cheaper and reduce the chances of decay and mold growth.

Diabetes mellitus is a metabolic disorder caused by inherited and/or acquired deficiency in production of insulin by pancreas. The key player to regulate the metabolism of carbohydrate, fat, and protein is insulin. Insulin deficiency may affect to those metabolism. Reduction of post prandial hyperglycemia can occur when the α -amylase enzyme is inhibited (48). The ethanolic extract, distilled water extract, and warm distilled water extract of *E. cottonii* showed potential percentage of α -amylase inhibition while ethanolic extract showed the greatest inhibition.

Inhibitory action of *E. cottonii* extracts increases with the increased concentration. Based on research that has been carried out, the greatest ability to inhibit α -amylase activity is the secondary metabolites which are known as polyphenol derivatives rather than primary metabolites, such as polysaccharides.

Carrageenan (polysaccharide) is easily soluble in water. This polysaccharides can form a lattice which capable to bind many water molecules. The lattice allows entrapment of substrates or water-soluble enzymes. Substrates and enzymes are trapped in the lattice and are enclosed in the polysaccharide so they do not react each other and there is no formation of simple sugars. Polyphenol inhibit α -amylase enzyme by delay carbohydrate hydrolysis, disaccharides and glucose absorption, and inhibit the metabolism of sucrose into glucose and fructose (49).

The inhibition of α -amylase by the secondary metabolites resulted in the failure of the carbohydrate breakdown process to become a monosaccharide form. This explains the effect of bioactive compounds on reducing BGL (50). All extracts showed weak inhibition of α -amylase with IC₅₀ value >200ppm. The low activity of α -amylase inhibitory is due to the use of dried samples. Heat and sunlight can damage the bioactive content in the sample. This result is supported by Sharoet al. (2013) (47), phytochemical screening from dry seaweed and extracted by ethanol solvents containing triterpenoid compounds only and not containing alkaloids, flavonoids, and steroids (51).

Nowadays, the GC-MS profiling analysis of *E. cottonii* extract with three solvents revealed the presence of 7 main classes of active compounds such as steroid, acids, esters, terpenoid, methyl ester, palmitic acid, and lignin. There were about 8 compounds in *E. cottonii* extract showed antioxidant activity. GC-MS analysis showed different results with phytochemical screening.

Alkaloids, terpenoids, saponins, and flavonoids are detected in phytochemical screening. Therefore, based on the GC-MS analysis there is steroid compound detected such as ethyl isoallocholate which play a role as antimicrobial activity (22).

Similar results were observed by various previous researches and the compounds were found to have various biological applications such as hypocholesterolemic, antiarthritic, anticoronary, antiandrogenic, antiinflammatory, and anticancer, and antioxidant activities (52).

The presence of bioactive compounds in this research have more potential medicinal importance such as antioxidant, anti-inflammatory, antiviral, anticancer, anti-asthma, antimicrobial, antipyretic, antiseptic, antiarthritic, anti-androgenic, antipsychotic, nematocide, and pesticide.

Alloxan is a compound that can damage the pancreatic beta cells which causes impaired insulin secretion and results in hyperglycemia. Hyperglycemia mice can be seen from the physical condition of mice that emit a lot of urine and drink a lot. *E. cottonii* extracts were able to reduced blood sugar levels of hyperglycemic mice. The higher concentration of seaweed extracts, the higher decrease in blood sugar levels in mice. In other words, the greater concentration of seaweed extract, the faster time needed to reduce the condition of hyperglycemia to normal blood sugar levels.

The ability of *E. cottonii* extracts in reducing blood sugar levels is thought to be related to the high fiber content contained in it.

According to previous research, *E. cottonii* contain 61.59% of carrageenan where carrageenan is a complex polysaccharides composed of galactose and anhydrogalactose. This indicates that dietary fiber in *E. cottonii* extracts is dominated by carrageenan. The ability of *E. cottonii* extracts in reducing blood sugar levels is also suspected because fiber is able to reduce the activity of digestive enzymes and reduce the rate of penetration of enzymes in food. In addition, fiber can slow down the absorption of glucose, which plays a role in regulating BGL (53).

Negative control in Figure 10 showed no correlation on decreasing BGL in diabetic mice (-15.7) otherwise increasing BGL in diabetic mice. It happened due to the absence of insulin. Pancreatic cannot secrete insulin which play a role to convert the sugar into glycogen when the sugar level are high (54). Warm distilled water and ethanolic extract of *E. cottonii* showed the ability of decreasing BGL in diabetic mice below 100 mg/dL. Based on previous research, ethanolic extract of *Sargassum polystum* can decrease BGL in diabetic rats caused by terpenoids and phenolic compound (55). All extracts of *E. cottonii* were statistically significant decreased the mice BGL. It happens due to the presence of phytochemical contents in ethanolic extract and polysaccharides in distilled and warm distilled water extract. Phytochemical contents inhibit the α -glucosidase intestinal also have hypoglycemic effect. Polysaccharides have hypoglycemic effect (49).

CONCLUSION

Among marine organisms, seaweeds have long been known as therapeutic entities. Due to their rapid growing nature, high content of proteins and diverse profile of bioactive compounds, seaweed can be well positioned as an alternative medicine. According to in vitro assay ethanolic extract of *E. cottonii* showed the highest antioxidant and α -amylase inhibitory activity. According to in vivo assay, ethanolic and warm distilled water extract of *E. cottonii* showed the ability of decreasing BGL in diabetic mice below 100 mg/dL. This study showed potential

antioxidant and possess ability to decrease BGL in diabetic mice. However, more comprehensive *in vitro* and *in vivo* studies are needed to elucidate the exact mechanism of its antidiabetic activity. Consequently, the findings of this study suggest that the use of these seaweed extracts may maximize the chances of developing medicine from nature. Further experiment will be needed to optimize the extraction condition of *E. cottonii* warm ethanolic extract.

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