Bionatura Journal
Ibero-American Journal of Biotechnology and Life Sciences

Article

Radical-Scavenging and Anti-inflammatory Activities of Fermented *Eucheuma cottonii* from Lombok

Agustina L. N. Aminin [1*], *Ella Anggraeni* [1], *Safira A. Ramoza* [1], *Meiny Suzery* [1], *Bambang Cahyono* [1], *Anoosh Eghdami* [2]

[1] *Department of Chemistry, Diponegoro University, Semarang 50275, Indonesia;*
[1] *Department of Chemistry, Diponegoro University, Semarang 50275, Indonesia; ramadhanianggraeni@yahoo.com*
[1] *Department of Chemistry, Diponegoro University, Semarang 50275, Indonesia; aphroditeramoza@alumni.undip.ac.id*
[1] *Department of Chemistry, Diponegoro University, Semarang 50275, Indonesia; meiny.suzery@live.undip.ac.id.*
[1] *Department of Chemistry, Diponegoro University, Semarang 50275, Indonesia; cahyono@live.undip.ac.id .*
[2] *Department of Biology, Islamic Azad University, Saveh Branch, Saveh, Iran; eghdami@iau-saveh.ac.ir*

* *Correspondence: agustina.aminin@live.undip.ac.id; Tel.: (+6224) 76480824*

ABSTRACT

Eucheuma cottonii, a widely cultivated seaweed from the Island of Lombok, Indonesia, contains various bioactive compounds. Its utilization, however, is mainly limited based on its high carbohydrate content. Nevertheless, plant fermentation using lactic acid bacteria is renowned for increasing bioactive compounds and enhancing bioactivities. This study unveiled the potential of Lactobacillus plantarum-fermented E. cottonii as a functional food. *E. cottonii* was fermented using *L. plantarum* for 24 h. The folin-Ciocalteau method was used to determine the total phenolic content. The antioxidant capacity was measured using a 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) assay. Anti-inflammatory activity was carried out using cyclooxygenase (COX) inhibition assay against cervical cancer (HeLa) and colon cancer (WiDr) cells. Overall, fermentation successfully enhanced the bioactivities of *E. cottonii*. Fermented products exhibited higher antioxidant capacity than unfermented ones. Interestingly, the bioactivities only showed a moderate correlation with total phenolic content. Regarding anti-inflammatory activity, fermented extracts exhibited higher cyclooxygenase inhibition against HeLa cells, whereas no significant differences were observed between the fermented and unfermented products in WiDr cells. These findings indicate that *L. plantarum*-fermented *E. cottonii* holds promise to be a profitable functional food and has the potential to be utilized as an additional food therapy for cancer treatment.

Keywords: *Eucheuma cottonii*; fermentation; *Lactobacillus plantarum*; antioxidant, anti-inflammation.

INTRODUCTION

The occurrence of oxidative stress phenomenon is attributed to the insufficiency of the defense mechanisms, such as antioxidants, to detoxify radicals in cells [1]. Free radicals, including reactive oxygen species, damage DNA, lipids, and proteins, playing a critical role in the emergence of life-threatening illnesses, such as Alzheimer's disease, Parkinson's syndrome, cancer, and diabetes [2]. Plants and algae are abundant sources of natural antioxidants [3]. Thus, consumption of plant-based ingredients prevents ROS-induced cellular impairment.

Studies on natural antioxidants have become a primary focus because natural antioxidants are considered relatively safer than synthetic ones, such as BHT (butylatedhydroxytoluene) and BHA (butylatedhydroxyanisole) [4]. However, it is also widely known that antioxidants derived from natural sources tend to exert lower activity than their synthetic counterparts [5]. Efforts have been extensively made to increase plant bioactive capacity, one of which is through fermentation. Seaweed fermentation-related studies were conducted through both oligosaccharide fermentation[6] and direct fermentation[7]. Wu, Wang [6] reported that oligosaccharide fermentation of *Gelidium* sp., *Gracilaria* sp., *Monostroma nitidum*, and *Porphyra dentate* algae by *L. rhamnosus* and *S. faecalis* bacteria improved antioxidant activity. In addition, a study by Gupta, Abu-Ghannam [7] on the fermentation of brown seaweeds (*L. digitata*, *L. Saccharina*, and *H. elongate*) using *L. plantarum* showed that the bacteria grew better in previously heated seaweed. In terms of phytochemical content, L. plantarum-fermented red seaweed (*Gracilaria fisheri*) was found to have an increase in γ-aminobutyric acid [8]. In addition, lactic acid bacteria fermentation increased the phenolic content and antioxidant activity of brown algae (*Sargassum* sp.) [9].

Eucheuma cottonii, a red seaweed widely cultivated in Lombok, exhibits various bioactivities, including antioxidant and anticancer activities [10, 11]. Previous studies on the fermentation of E. cottonii by *Lactobacillus plantarum* have explored the total phenolic content and antioxidant activity [12]. Hence, this study examined the bioactivities ─ antioxidant and anti-inflammatory ─ of *L. plantarum*-fermented *Eucheuma cottonii*. Findings in this study are expected to provide information about the potential of *L. plantarum*-fermented *E. cottonii* as a functional food that may be used as an additional therapy for cancer. Moreover, as a functional food, fermented *E. cottonii* might have higher economic value, which is expected to improve the livelihoods of seaweed farmers in Lombok who have relied only on selling seaweed.

MATERIALS AND METHODS

Materials

Lactobacillus plantarum was obtained from the School of Biology, Institut Teknologi Bandung, Indonesia. *Eucheuma cottonii* was obtained from Hamlet Ekas, Jerowaru sub-district, East Lombok, West Nusa Tenggara, Indonesia. Gallic acid, Folin-Ciocalteu reagent, sodium carbonate (Na_2CO_3), 2,2-azinobis (3-ethyl-benzothiazoline-6-sulfonic acid) (ABTS), and 2,4-dinitrophenylhydrazine (DNPH) were obtained from Sigma-Aldrich Co. Cervix (HeLa) and colon (WiDr) cancer cells were collected from the Integrated Research and Testing Laboratory, Universitas Gadjah Mada, Indonesia. A colorimetric COX inhibitor test kit was obtained from Cayman, Australia. All chemicals were of analytical grade.

E. cottonii fermentation

Fresh *E. cottonii* was cleaned from sand with flowing water before hanging it dry for five days. The dried seaweed was then cut and powdered with a blender. The fermented seaweed product was prepared by adding 2% (w/v) seaweed powder with 2% (w/v) glucose in aqua dest, autoclaving, and allowed to cool afterward. The sterilised mixture was subsequently inoculated with 0.1% (v/v) *L. plantarum* starter (6.7 x 10^5 CFU/mL) and standardized by measuring its optical density at λ = 600 nm (OD600 = 0.15). Fermentation was carried out at 37°C for 6 h (FSE-6), 12 h (FSE-12), and 24 h (FSE-24). The non-fermented seaweed product (SE) was prepared by dissolving 2% (w/v) seaweed powder in aquadest. The products were then extracted with methanol for 24 h, dried to evaporation with a rotary evaporator at 40°C, and freeze-dried.

Total phenolic content

Determining the total phenolic content (TPC) followed the method by Dey and Kuhad [13] with minor modifications. Each extract (1 mL) was added with Folin-Ciocalteu reagent (0.5 mL), 7.5% Na_2CO_3 solution (2 mL), and aquadest (2.4 mL). After proper mixing, the mixture was left at room temperature for 15 min, and the absorbance was measured at 765 nm. Methanol and gallic acid served as negative and positive controls, respectively. The blank consisted of all reagents and solvents without extracts. TPC was determined using a gallic acid calibration curve, and the results were presented as mg gallic acid equivalent (GAE) per g of dry extract.

ABTS radical cation-scavenging activity

The scavenging activity was performed based on the method conducted by Xiao, Xing [14]. $ABTS^{\bullet+}$ was prepared beforehand by reacting 7 mM $ABTS^{\bullet+}$ solution with 2.45mM $K_2S_2O_8$ solution in the dark at room temperature for 16 h. To reach an absorbance value of 0.70 (±0.02) at 734 nm, the $ABTS^{\bullet+}$ solution was diluted with ethanol and equilibrated at 30˚C. Extracts at different concentrations (1 mL) were mixed with 4 mL $ABTS^{\bullet+}$ ethanolic solution. The mixture was incubated in the dark for 6 min. A UV-Vis spectrophotometer was used to measure the absorbance at 734 nm. $ABTS^{\bullet+}$ without extracts was the negative control, and ascorbic acid was the positive control. The scavenging activity (SA) was calculated using the following equation:

$$SA\ (\%) = (A_{control} - A_{sample})/(A_{control}) \times 100\% \quad (1)$$

Cell culture

The HeLa and WiDr cells were collections of the Integrated Research and Testing Laboratory, Universitas Gadjah Mada, Indonesia. Both cells were cultivated in RPMI 1640 medium (Gibco, Scotland) and MDA-MB-231 cells. Cells were supplemented with 10% Fetal Bovine Serum (FBS, Gibco, Scotland).

COX-2 enzyme inhibition assay

A cell-free system using a colorimetric COX inhibitor screening assay kit was used to determine the COX-2 inhibitory activity. The assay was performed based on the manufacturer's guidelines. At first, assay buffer (160 µL) and heme (10 µl) were added to the background well. Subsequently, the 100% initially activated well was added with the assay buffer (150 µL), heme (10 µL), and COX-2 enzyme (10 µL). 10 µl of the sample (final concentrations of 50 M and 100 M) were added to the sample wells, with DMSO (10 µL) added to the background wells. The plate was thoroughly shaken and incubated for 5 min at 25˚C. All wells were then filled with 20 µl of colorimetric substrate solution and 20 µl of arachidonic acid. The plate was once

again shaken and incubated for 5 min at 25°C. A microplate reader was used to measure the absorbance at 590 nm. The percentage of viable cells (VC) was calculated as follows:

$$VC\ (\%) = (A_{sample} - A_{blank}) / (A_{control} - A_{blank}) \times 100\% \quad (2)$$

Statistical analysis

All samples were analyzed in triplicate (n = 3), and data were presented as mean ± standard deviation. The correlation between variables was determined using Pearson's correlation coefficient. One-way analysis of variance (ANOVA) and Tukey's HSD test were performed to determine significance at $p < 0.05$. All statistical analysis was performed using Microsoft Excel software.

RESULTS

Physical and chemical profiles of fermented *E. cottonii*

Fermentation was carried out at different durations to monitor the effect of fermentation time on the product's bioactive capacity. Observation of the fermented product showed a physical change in color and texture from red-brown to pink and gel-shaped (soft), a typical acid scent, and a pH of 5 (data not shown). Moreover, the total phenolic content of fermented *E. cottonii* extracts was significantly increased compared with unfermented extracts (Figure 1). Among the samples, FSE-24 exhibited the highest TPC (5.567 ± 0.244 mg GAE/g dry extract), followed by FSE-6, FSE-12, and SE, respectively. This result was following previous studies [15, 16].

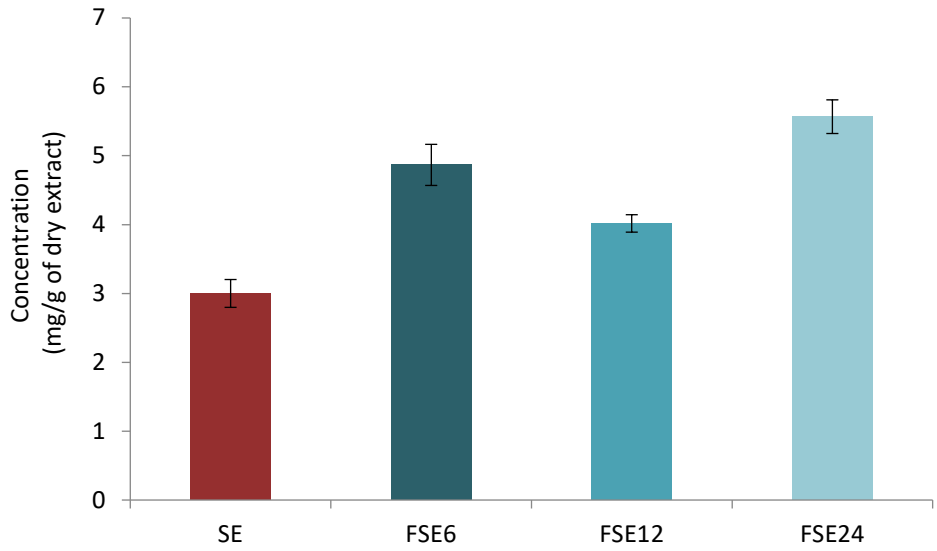

Figure 1. Total phenolic content of SE, FSE-6, FSE-12, and FSE-24.

Antioxidant activity

All fermented extracts exhibited significantly higher antioxidant activity than the unfermented extract, as shown in Figure 2 ($p < 0.05$). The inhibition percentage of all samples was in line with the increase in concentration. FSE-12 exhibited the best performance in scavenging $ABTS^{\bullet+}$, with an inhibition percentage of 83.36% at the same concentration, followed by FSE-6, FSE-24, and SE, respectively.

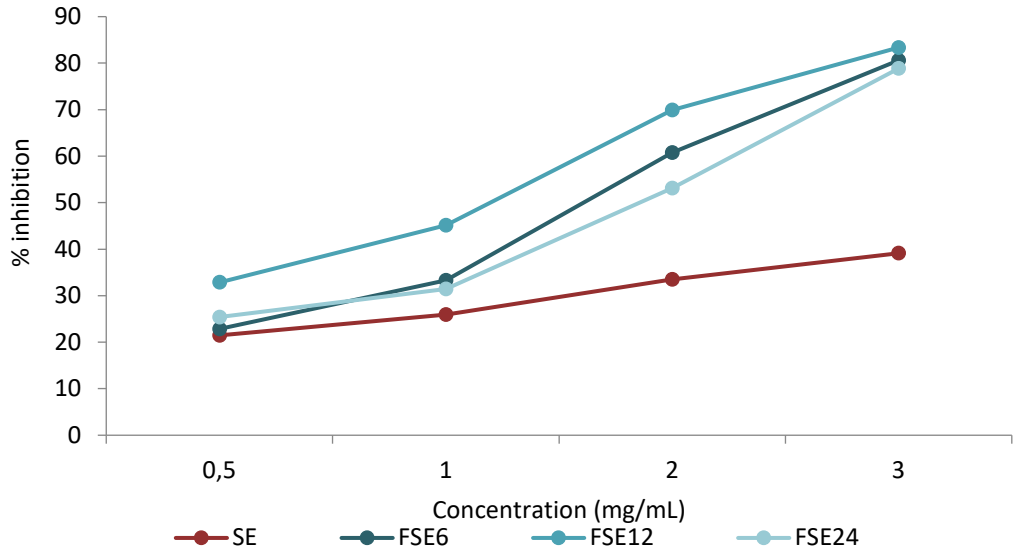

Figure 2. Antioxidant activity of SE, FSE-6, FSE-12, FSE-24.

Anti-inflammation activity

The in vitro anti-inflammatory assay was performed against HeLa and WiDr cancer cells based on inhibiting the cyclooxygenase isoenzyme (COX-2). Fermented *E. cottonii* extracts exhibit more anti-inflammatory activity than unfermented ones (Figure 3). The IC50 for the cyclooxygenase inhibitory activity of FSE-24 was the lowest among all extracts, indicating that it possessed the highest anti-inflammatory activity against HeLa and WiDr cancer cells. This result follows that of TPC. Furthermore, a significant decrease in IC50 was observed in HeLa cells ($p < 0.05$) but not in WiDr cells ($p > 0.05$). This demonstrated that fermented *E. cottonii* was more effective in treating HeLa cells than WiDr. Nonetheless, fermentation increased the anti-inflammatory activity of E. cottonii.

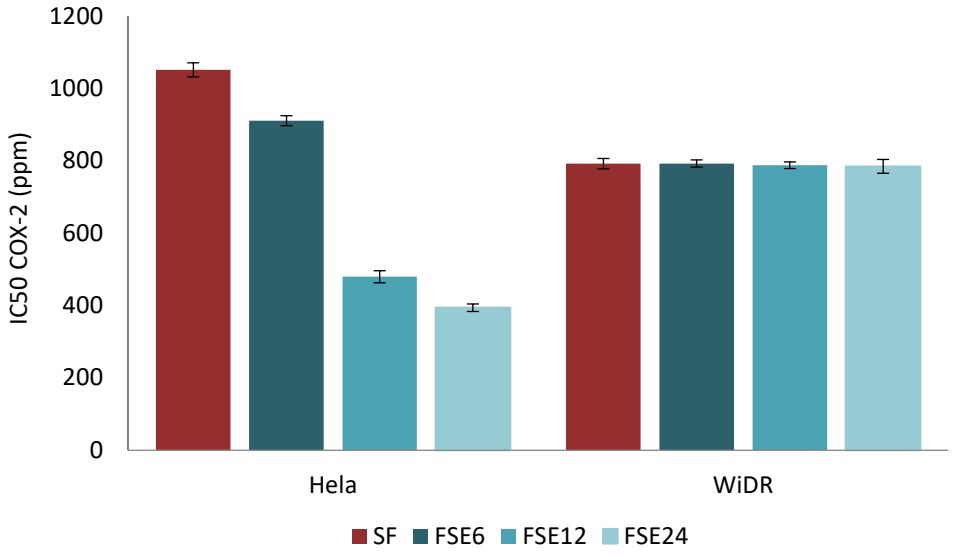

Figure 3. Anti-inflammatory activity of SE, FSE-6, FSE-12, FSE-24.

DISCUSSION

Fermentation was successful as it released an acidic scent, supported by a pH decrease of 5. This acidic smell was beneficial because it eliminated the fishy aroma. The texture of the seaweed post-fermentation was soft, which might correlate with the breakdown of tenacious complex carbohydrates, such as fibers, into a more straightforward form. Zhang, Hu [17] reported that fermented seaweed increased crude protein, but a significant decrease in fiber supported this argument. Meanwhile, other physical changes, such as the acidic smell and the reduction in pH after fermentation, might be caused by the production of organic acids during fermentation [18]. Another study on the fermentation of seaweed, *Gracilaria fisheri* by *L. plantarum*, reported that the fermentation produced lactic acid and acetic acid [8], which confirmed the findings of this study. The production of organic acids resulted from polysaccharide hydrolysis by *L. plantarum* to provide energy and nutrients for growth.

Furthermore, fermentation also influenced the total phenolic content (TPC) of *E. cottonii* (Figure 1). The Folin-Ciocalteu reagent used to determine TPC is not specific and detects all phenolic groups contained in the extract [19], which may include phenolic acids, flavonoids, polyphenols, tannins, and quinones [15]. The increase in TPC in the earlier fermentation product, FSE-6, might be attributed to the hydrolysis of glycosides by *L. plantarum*-excreted enzymes, such as glucosidases and esterases, to its corresponding aglycones [20]. In contrast, the release of insoluble-bound phenolics [21] and their modification by *L. plantarum* might explain the increase in TPC in FSE-24. Conversely, the decrease in TPC in FSE-12 might have resulted from the degradation of total soluble phenolics into small molecules by *L. plantarum* [22].

In this study, the antioxidant activity determined using the $ABTS^{\bullet+}$ assay showed a significant increase in FSEs compared to SE, with the highest antioxidant capacity observed in FSE-12 ($p < 0.05$) (Figure 2). This result confirmed findings 23, which revealed that *E. cottonii* exhibited $ABTS^{\bullet+}$ scavenging activity. The $ABTS^{\bullet+}$ assay applies to hydrophilic and lipophilic antioxidant systems and is based on the reduction of the generated blue/green $ABTS^{\bullet+}$ [24]. This indicates that the antioxidants in SE and FSEs were both hydrophilic and lipophilic. In various studies, the antioxidant activity results were mainly in line with the TPC results. The fermentation of *Gelidium sp.* and *E. cottonii* by lactic acid bacteria [12] and Betaphycus gelatinum by Lactobacillus brevis 25 increased TPC, which was highly correlated with an increase in antioxidant capacity. Interestingly, in this study, antioxidant capacity was moderately correlated with the total phenolic content (TPC) result ($r = 0.766$) (Figure 1). Therefore, the antioxidant activity of fermented *E. cottonii* was allegedly exhibited not only by phenolics but also by other bioactive compounds. *E. cottonii* contains 9.76% protein and 26.49% polysaccharides [26], possibly biotransformed into bioactive peptides and polysaccharides by *L. plantarum* during fermentation [27]. Bioactive peptides might exert antioxidant activity by inhibiting peroxidation and scavenging free radicals through their amino acids. Amino acids such as histidine and tyrosine neutralize free radicals by donating protons, whereas aromatic amino acids (phenylalanine, tryptophan, and tyrosine) stabilize free radicals by giving them electrons [28]. Turgeon, Gauthier [27] found that low-molecular-weight short peptides from the hydrolysis of proteins are much easier to absorb by the digestive system and have higher antioxidant capacity than combinations of amino acids in similar concentrations.

A study by Muhialdin, Rani [29] concluded that an increase in low-molecular-weight peptides during the fermentation of bitter beans caused an increase in antioxidant activity. Furthermore, the fermentation of goat milk with *L. plantarum* produced three antioxidative peptides [30]. In that study, it was explained that certain proteolytic enzymes within bacterial strains influenced the antioxidant peptides' radical scavenging activity

rather than the proteolytic state of fermented products. In addition, *E. cottonii* contains Kappa carrageenan, which exhibits radical scavenging capacity towards superoxide radicals, hydroxyl radicals, and lipid peroxidation with an IC50 of 0.112 ± 0.003, 0.335 ± 0.016, 0.323 ± 0.011 respectively, as reported in the study by Rocha de Souza, Marques [31]. A study by Zhao, Li [32] on the fermentation of polysaccharides from *Ganoderma lucidum* by *L. plantarum* resulted in a significant decrease in the molecular weight of the polysaccharides and changes in monosaccharide contents, which were concluded to be the reason behind the increase in its antioxidant activity. Hence, fermentation might also modify kappa carrageenan and other polysaccharides in *E. cottonii*, which is speculated to increase antioxidant activity in FSEs. Thus, it can be concluded that fermentation successfully increased the antioxidant activity of *E. cottonii*.

Reactive oxygen species (ROS) play a crucial role in the progression of inflammatory disorders [33], one of which is activating cyclooxygenase (COX-2) [34]. COX-2 is an enzyme that catalyzes the conversion of arachidonic acid (AA) to prostaglandins (PGHs) [35]. PGHs mediate inflammation, which is a chronic state that is associated with a high likelihood of carcinogenesis. In addition, COX-2 is expressed in various cancers, including cervical cancer, and is related to their development, metastases, and prognosis [36]. Thus, inhibiting COX-2 activity is a promising strategy to reduce and prevent inflammation. As shown in Figure 3, *E. cottonii* exerted COX-2 inhibition activity, and fermentation significantly lowered the IC50, or in other words, increased the anti-inflammatory activity of *E. cottonii* in HeLa cells ($p < 0.05$), whereas not much difference was observed in WiDr cells. This implied that bioactive compounds in SE and FSEs, which exert anti-inflammatory activity, successfully inhibited COX-2. However, although FSEs exhibited COX-2 inhibition in WiDr cells, fermentation did not change that activity. Thus, FSEs demonstrated better efficacy in treating HeLa cells than WiDr cells via COX-2 inhibition. Nevertheless, this result follows an in vitro study by Arsianti, Aziza [11], which found that *E. cottonii* extracts demonstrated cytotoxic solid activity against Hela cells.

The anti-inflammatory activity against Hela cells was moderately correlated with TPC (r = -0.622) and ABTS cation radical-scavenging activity (r = -0.706) results. Previous studies have proven that antioxidants play a significant role in protecting and preventing carcinogenesis [37]. Furthermore, *E. cottonii* contains 2-(methylthio)pyrimidine-5- carboxaldehyde, 2,3-Dihydropyrazolo[5,1-b][1,3]thiazole6-carbaldehyde [15], and ethyl iso-allocholate [38] which exerted anti-inflammatory activities [39-41]. The steroid ethyl iso-allocholate formed strong interactions with COX-2 via hydrogen bonds with Arg120 and Ser119; π-π interactions with Val89, Tyr115, Ile112; and hydrophobic interactions. In addition, ethyl iso-allocholate exhibited strong interaction not only with COX-2 but also with all protein targets, especially caspase-1 [42]. These interactions and possible modulation of the protein targets were presumed to cause its anti-inflammatory activity. A study on the fermentation of brown seaweed *Laminaria japonica* using *Bacillus subtilis* resulted in the enhancement of its anti-inflammatory activity through the inhibition of ROS production, inactivation of NF-κB phosphorylation, and subsequent inhibition of nitric oxide (NO) production. Meanwhile, fermentation using kimchi-derived *Lactobacillus* sp. increased the anti-inflammatory activity of *Sargassum thunbergii* through the reduction of NO and suppression of iNOS and COX-2 expression due to the modification of bioactive compounds via fermentation [43]. Thus, the structural modification, change in contents, and difference in mechanism-of-action of the bioactive compounds in fermented *E. cottonii* might explain the increase in the anti-inflammatory activity of FSEs compared to SE in HeLa cells. The highest activity was displayed by FSE-24 (Figure 3). Studies in this area have found that polyphenols exerted inhibition activity against COX-2 in cervical cancer [44]. Hence, the release of insoluble-bound secondary metabolites [21] and biotransformation of glycosides into their corresponding aglycones [20] might explain this activity.

A study by [42] discovered that each bioactive compound exhibited different binding affinities toward anti-inflammatory protein targets. Isoledene interacted strongly with the protein targets – interleukin-1-beta converting enzyme (Caspase-1), beta-2 adrenergic receptor (ADRB2), cyclooxygenase-2 (COX-2), and tumor necrosis factor-alpha (TNF-α), especially at the pralnacasan binding site of caspase-1. In contrast, cholest-22-ene-21-ol, 3,5-dihydro-6- methoxy-, and pivalate bound firmly with ADRB2 and TNF-α, whereas alpha-cadinol exhibited highest binding affinity with COX-2. Furthermore, inflammation pathways may differ (e.g., NF-κB, MAPKs, and JAK-STAT); therefore, responses to inflammation may also be different, one of which is through the expression of various types and quantities of inflammatory proteins – transcription factors (e.g., NF-κB), cytokines (e.g., TNF-α and IL-6), and enzymes (e.g., COX-2 and caspase-1), with each exhibiting their molecular mechanisms [45]. A study by [46] reported that fucoxanthin, a carotenoid found in abundance in seaweeds, inhibited the proliferation of HepG2 (liver cancer) and DU145 (prostate cancer) cells. On the one hand, fucoxanthin inhibited p38 MAPK, enhancing the induction of *gadd45a* expression and G1 arrest in HepG2 cells. On the other hand, fucoxanthin inhibited SAPK/JNK, suppressing the induction of *gadd45a* expression and G1 arrest in DU145 cells. Their findings suggest that the pattern of MAPK involvement in the induction of gadd45a and G1 arrest by fucoxanthin differs depending on the cell type. Therefore, presumably, the bioactive compounds in FSEs favored binding with COX-2 compared with other inflammatory proteins expressed in HeLa cells, resulting in increased COX-2 inhibition. Meanwhile in WiDr cell lines, those compounds exhibited higher affinity with inflammatory proteins other than COX-2. In addition, compounds present in SE, which exerted higher binding affinity with COX-2, might not be biotransformed during fermentation, resulting in the indefference of COX-2 inhibiton activity between SE and FSEs against WiDr cells. Hence, the difference in molecular mechanisms of bioactive compounds in FSEs and the numerous inflammatory pathways in cell lines might be the plausible reasoning for the difference in anti-inflammatory activities against HeLa and WiDr cells (Figure 3). Thus, according to the findings in this study, it may be proposed that the presence of antioxidant phenolics in fermented *E. cottonii* partly contributes to its anti-inflammatory activity.

In addition, studies on *E. cottonii* revealed that this seaweed exhibited various anticancer activities, such as lung carcinoma SK-Lu-1, colorectal HCT-116 cells [47], breast MCF-7, and colorectal HCT-116 cells [48]. An LC/MS analysis on the aqueous extract of *E. cottonii* by [15] resulted in the discovery of flavonoids and saponins. Among the flavonoids discovered, 2-thienyl acetic acid [49] and 4-chloro-1H-pyrazolo [3,4-b]pyridine [50] exhibited antioxidant and anticancer activities, while most of the rest exhibited antimicrobial activities. Furthermore, bioactive compounds, especially the abundantly identified terpenoids in the ethanolic extract of dried *E. cottonii,* inhibited the α-amylase enzyme and reduced blood glucose levels in diabetic mice [38]. E. cottonii also possesses in vivo wound healing abilities and antibacterial activity against *Staphylococcus aureus*, allegedly exhibited by its flavonoids, phenolics, and *k*-carrageenan [10]. Nonetheless, although in vitro findings in this study unveiled significant hidden potentials of fermented *E. cottonii*, using a single bacterial strain might limit the potency of bioactive compounds in E. cottonii to be fully biotransformed and explored. Thus, further investigation using different bacterial strains and employing in vivo and in silico approaches must be implemented to uncover other bioactivities and details on their molecular mechanisms and understand their implications for human health.

CONCLUSION

Lactobacillus plantarum-fermented Eucheuma cottonii (FSEs) generally exhibited higher bioactivities than unfermented *E. cottonii* (SE). FSE-12 exhibited the highest antioxidant activity. On the contrary, the highest activity was shown by FSE-24 for anti-inflammatory activities, which was moderately correlated with the total phenolic content. Interestingly, the anti-inflammatory activity of SE and FSEs against WiDr cells showed insignificant differences. In summary, fermentation successfully enhanced the bioactivities of *E. cottonii* and thus demonstrated great potential as an additional functional food therapy to treat cancer. Nonetheless, future in vivo studies and the determination of bioactive compounds contained using LC/MS-MS may be considered to deepen our understanding of the biotransformation and bioactivities of fermented *E. cottonii* seaweed.

Author Contributions: Conceptualization, A.A.; methodology, A.A. and E.A..; validation, A.A., A.E., and M.S.; formal analysis, E.A. and S.R.; resources, A.A.; data curation, B.C. and A.E.; writing—original draft preparation, A.A.; writing—review and editing, S.R.; visualization, B.C. and S.R.; supervision, A.N.; funding acquisition, AA. All authors have read and agreed to the published version of the manuscript.

Funding: This research was supported financially by DITJEN DIKTI KEMENDIKBUD of Indonesia (DITLITABMAS DITJEN DIKTI KEMENDIKBUD No 146-03/UN7.5.1/PG/2015).

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Acknowledgments: We thank our colleagues from the UNESCO Chair on Interdisciplinary Research in Diabetes, the Institute of Biochemistry and Biophysics (IBB), and the University of Tehran, who provided expertise and partial funding that greatly assisted the research.

Conflicts of Interest: The authors declare no conflict of interest.

REFERENCES

1. Pizzino G, Irrera N, Cucinotta M, Pallio G, Mannino F, Arcoraci V, et al. Oxidative stress: harms and benefits for human health. *Oxidative medicine and cellular longevity*. **2017**, *2017*, 1-13. https://doi.org/10.1155/2017/8416763
2. Lee H-H, Lin C-T, Yang L-L. Neuroprotection and free radical scavenging effects of Osmanthus fragrans. *Journal of Biomedical Science*. **2007**, *14*, 819-827. https://doi.org/10.1007/s11373-007-9179-x
3. Duan X-J, Zhang W-W, Li X-M, Wang B-G. Evaluation of antioxidant property of extract and fractions obtained from a red alga, Polysiphonia urceolata. *Food chemistry*. **2006**, *95*, 37-43. https://doi.org/10.1016/j.foodchem.2004.12.015
4. Kulisic T, Radonic A, Katalinic V, Milos M. Use of different methods for testing antioxidative activity of oregano essential oil. *Food chemistry*. **2004**, *85*, 633-640. https://doi.org/10.1016/j.foodchem.2003.07.024
5. Moure A, Cruz JM, Franco D, Domínguez JM, Sineiro J, Domínguez H, et al. Natural antioxidants from residual sources. *Food chemistry*. **2001**, *72*, 145-171. https://doi.org/10.1016/s0308-8146(00)00223-5
6. Wu S-C, Wang F-J, Pan C-L. The comparison of antioxidative properties of seaweed oligosaccharides fermented by two lactic acid bacteria. *Journal of Marine Science and Technology*. **2010**, *18*, 1-11. https://doi.org/10.51400/2709-6998.1908
7. Gupta S, Abu-Ghannam N, Scannell AG. Growth and kinetics of Lactobacillus plantarum in the fermentation of edible Irish brown seaweeds. *Food and Bioproducts Processing*. **2011**, *89*, 346-355. https://doi.org/10.1016/j.fbp.2010.10.001

8. Ratanaburee A, Kantachote D, Charernjiratrakul W, Penjamras P, Chaiyasut C. Enhancement of γ-aminobutyric acid in a fermented red seaweed beverage by starter culture Lactobacillus plantarum DW12. *Electronic Journal of Biotechnology*. **2011**, *14*, 1-14. https://doi.org/10.2225/vol14-issue3-fulltext-2
9. Shobharani P, Halami P, Sachindra N. Potential of marine lactic acid bacteria to ferment Sargassum sp. for enhanced anticoagulant and antioxidant properties. *Journal of Applied Microbiology*. **2013**, *114*, 96-107. https://doi.org/10.1111/jam.12023
10. Teo BSX, Gan RY, Abdul Aziz S, Sirirak T, Mohd Asmani MF, Yusuf E. 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*. **2021**, *20*, 993-1001. https://doi.org/10.1111/jocd.13624
11. Arsianti A, Aziza YAN, Kurniasari KD, Mandasari BKD, Masita R, Zulfa FR, et al. Phytochemical test and cytotoxic activity of macroalgae Eucheuma cottonii against cervical HeLa cells. *Pharmacognosy Journal*. **2018**, *10*, 1012-1017. https://doi.org/10.5530/pj.2018.5.172
12. Sumardianto R, PH A, AD R, Rianingsih L. Phenol content and antioxidant activity in seawed fermented with lactic acid bacteria. *Food Res*. **2021**, *5*, 7-13. https://doi.org/10.26656/fr.2017.5(s3).006
13. Dey TB, Kuhad RC. Enhanced production and extraction of phenolic compounds from wheat by solid-state fermentation with Rhizopus oryzae RCK2012. *Biotechnology Reports*. **2014**, *4*, 120-127. https://doi.org/10.1016/j.btre.2014.09.006
14. Xiao Y, Xing G, Rui X, Li W, Chen X, Jiang M, et al. Enhancement of the antioxidant capacity of chickpeas by solid state fermentation with Cordyceps militaris SN-18. *Journal of functional foods*. **2014**, *10*, 210-222. https://doi.org/10.1016/j.jff.2014.06.008
15. Purbosari N, Warsiki E, Syamsu K, Santoso J. The potential of Eucheuma cottonii extract as a candidate for fish anesthetic agent. *Aquaculture and Fisheries*. **2022**, *7*, 427-432. https://doi.org/10.1016/j.aaf.2021.06.003
16. Purbosari N, Warsiki E, Syamsu K, Santoso J. Effect of Harvest Age and Solvents on the Phenolic Content of Eucheuma cot-tonii Extract. *Makara Journal of Science*. **2020**, *24*, 141-147. https://doi.org/10.7454/mss.v24i3.1177
17. Zhang S, Hu X, Ma J, Ma Z, Liu X, Cui L. Study on feed fermented from seaweed waste. *African Journal of Microbiology Research*. **2012**, *6*, 7610-7615. https://doi.org/10.5897/ajmr12.2439
18. Wang Y, Wu J, Lv M, Shao Z, Hungwe M, Wang J, et al. Metabolism characteristics of lactic acid bacteria and the expanding applications in food industry. *Frontiers in bioengineering and biotechnology*. **2021**, 1-19. https://doi.org/10.3389/fbioe.2021.612285
19. Naczk M, Shahidi F. Extraction and analysis of phenolics in food. *Journal of chromatography A*. **2004**, *1054*, 95-111. https://doi.org/10.1016/s0021-9673(04)01409-8
20. Rodríguez H, Curiel JA, Landete JM, de las Rivas B, de Felipe FL, Gómez-Cordovés C, et al. Food phenolics and lactic acid bacteria. *International journal of food microbiology*. **2009**, *132*, 79-90. https://doi.org/10.1016/j.ijfoodmicro.2009.03.025
21. Shahidi F, Yeo J. Insoluble-bound phenolics in food. *Molecules*. **2016**, *21*, 1-22. https://doi.org/10.3390/molecules21091216
22. Kaprasob R, Kerdchoechuen O, Laohakunjit N, Sarkar D, Shetty K. Fermentation-based biotransformation of bioactive phenolics and volatile compounds from cashew apple juice by select lactic acid bacteria. *Process Biochemistry*. **2017**, *59*, 141-149. https://doi.org/10.1016/j.procbio.2017.05.019
23. Saputri RK, Setiawan B, Nugrahenny D, Kania N, Wahyuni ES, Widodo MA. The effects of Eucheuma cottonii on alveolar macrophages and malondialdehyde levels in bronchoalveolar lavage fluid in chronically particulate matter 10 coal dust-exposed rats. *Iranian Journal of Basic Medical Sciences*. **2014**, *17*, 541-545.
24. Kim D-O, Lee KW, Lee HJ, Lee CY. Vitamin C equivalent antioxidant capacity (VCEAC) of phenolic phytochemicals. *Journal of Agricultural and food chemistry*. **2002**, *50*, 3713-3717. https://doi.org/10.1021/jf020071c
25. Wang Z, Zhao C, Guo Z, Li S, Zhu Z, Grimi N, et al. Fermentation of Betaphycus gelatinum Using Lactobacillus brevis: Growth of Probiotics, Total Polyphenol Content, Polyphenol Profile, and Antioxidant Capacity. *Foods*. **2023**, *12*, 1-16. https://doi.org/10.3390/foods12183334
26. Matanjun P, Mohamed S, Mustapha NM, Muhammad K. Nutrient content of tropical edible seaweeds, Eucheuma cottonii, Caulerpa lentillifera and Sargassum polycystum. *Journal of Applied Phycology*. **2009**, *21*, 75-80. https://doi.org/10.1007/s10811-008-9326-4

27. Turgeon SL, Gauthier SF, Paquin P. Interfacial and emulsifying properties of whey peptide fractions obtained with a two-step ultrafiltration process. *Journal of Agricultural and Food chemistry*. **1991**, *39*, 673-676. https://doi.org/10.1021/jf00004a009
28. Elias RJ, Kellerby SS, Decker EA. Antioxidant activity of proteins and peptides. *Critical reviews in food science and nutrition*. **2008**, *48*, 430-441. https://doi.org/10.1080/10408390701425615
29. Muhialdin BJ, Rani NFA, Hussin ASM. Identification of antioxidant and antibacterial activities for the bioactive peptides generated from bitter beans (Parkia speciosa) via boiling and fermentation processes. *Lwt*. **2020**, *131*, 1-7. https://doi.org/10.1016/j.lwt.2020.109776
30. Makkar HP, Siddhuraju P, Becker K. *Plant secondary metabolites*. Springer, 2007; pp. 1-122.https://doi.org/10.1007/978-1-59745-425-4
31. Rocha de Souza MC, Marques CT, Guerra Dore CM, Ferreira da Silva FR, Oliveira Rocha HA, Leite EL. Antioxidant activities of sulfated polysaccharides from brown and red seaweeds. *Journal of applied phycology*. **2007**, *19*, 153-160. https://doi.org/10.1007/s10811-006-9121-z
32. Zhao Y, Li Q, Wang M, Wang Y, Piao C, Yu H, et al. Structural characterization of polysaccharides after fermentation from Ganoderma lucidum and its antioxidant activity in HepG2 cells induced by H2O2. *Food Chemistry: X*. **2023**, *18*, 1-13. https://doi.org/10.1016/j.fochx.2023.100682
33. Rizwan S, ReddySekhar P, MalikAsrar B. Reactive oxygen species in inflammation and tissue injury. *Antioxidants & redox signaling*. **2014**, 1126-1167. https://doi.org/10.1089/ars.2012.5149
34. Hu Y-P, Peng Y-B, Zhang Y-F, Wang Y, Yu W-R, Yao M, et al. Reactive oxygen species mediated prostaglandin E2 contributes to acute response of epithelial injury. *Oxidative medicine and cellular longevity*. **2017**, *2017*, 1-8. https://doi.org/10.1155/2017/4123854
35. Smith WL, DeWitt DL, Garavito RM. Cyclooxygenases: structural, cellular, and molecular biology. *Annual review of biochemistry*. **2000**, *69*, 145-182. https://doi.org/10.1146/annurev.biochem.69.1.145
36. Li Y, Pu D, Li Y. The expression of cyclooxygenase-2 in cervical cancers and Hela cells was regulated by estrogen/progestogen. *Journal of Huazhong University of Science and Technology*. **2007**, *27*, 457-460. https://doi.org/10.1007/s11596-007-0428-z
37. Ahmad N, Mukhtar H. Antioxidants meet molecular targets for cancer prevention and therapeutics. *Antioxid Redox Signal*. **2013**, *19*, 85-88. https://doi.org/10.1089/ars.2013.5299
38. Prasasty VD, Haryani B, Hutagalung RA, Mulyono N, Yazid F, Rosmalena R, et al. Evaluation of antioxidant and antidiabetic activities from red seaweed (Eucheuma cottonii). *Systematic Reviews in Pharmacy*. **2019**, *10*, 276-288.
39. Zhang Y, Luo L, Han C, Lv H, Chen D, Shen G, et al. Design, synthesis, and biological activity of tetrahydrobenzo [4, 5] thieno [2, 3-d] pyrimidine derivatives as anti-inflammatory agents. *Molecules*. **2017**, *22*, 1-21. https://doi.org/10.3390/molecules22111960
40. Kryshchyshyn A, Roman O, Lozynskyi A, Lesyk R. Thiopyrano [2, 3-d] thiazoles as new efficient scaffolds in medicinal chemistry. *Scientia Pharmaceutica*. **2018**, *86*, 1-24. https://doi.org/10.3390/scipharm86020026
41. Muthulakshmi A, Mohan V. GC-MS analysis of bioactive components of Feronia elephantum Correa (Rutaceae). *Journal of Applied Pharmaceutical Science*. **2012**, 69-74.
42. Johnson TO, Odoh KD, Nwonuma CO, Akinsanmi AO, Adegboyega AE. Biochemical evaluation and molecular docking assessment of the anti-inflammatory potential of Phyllanthus nivosus leaf against ulcerative colitis. *Heliyon*. **2020**, *6*, 1-10. https://doi.org/10.1016/j.heliyon.2020.e03893
43. Mun OJ, Kwon MS, Karadeniz F, Kim M, Lee SH, Kim YY, et al. Fermentation of Sargassum thunbergii by Kimchi-Derived Lactobacillus sp. SH-1 Attenuates LPS-Stimulated Inflammatory Response Via Downregulation of JNK. *Journal of Food Biochemistry*. **2017**, *41*, 1-9. https://doi.org/10.1111/jfbc.12306
44. Di Domenico F, Foppoli C, Coccia R, Perluigi M. Antioxidants in cervical cancer: chemopreventive and chemotherapeutic effects of polyphenols. *Biochimica et Biophysica Acta (BBA)-Molecular Basis of Disease*. **2012**, *1822*, 737-747. https://doi.org/10.1016/j.bbadis.2011.10.005
45. Chen L, Deng H, Cui H, Fang J, Zuo Z, Deng J, et al. Inflammatory responses and inflammation-associated diseases in organs. *Oncotarget*. **2018**, *9*, 7204-7218. https://doi.org/10.18632/oncotarget.23208
46. Satomi Y, Nishino H. Implication of mitogen-activated protein kinase in the induction of G1 cell cycle arrest and gadd45 expression by the carotenoid fucoxanthin in human cancer cells. *Biochimica et Biophysica Acta (BBA)-General Subjects*. **2009**, *1790*, 260-266. https://doi.org/10.1016/j.bbagen.2009.01.003

47. Lee J, Wang J, Ng K, Tan C, Rabina P, Teo S. In-vitro anticancer activity of Eucheuma cottonii extracts against HeLa cell line, humn lung carcinoma cell line (SK-LU-1), human colon carcinoma cell line (HCT-116), and fibroblast. *IJCMS*. **2015**, *1*, 69-73.
48. Arsianti A, Fadilah F, Fatmawaty Y, Wibisono L, Kusmardi S, Azizah N, et al. Phytochemical composition and anticancer activity of seaweeds Ulva lactuca and Eucheuma cottonii against breast MCF-7 and colon HCT-116 cells. *Asian J Pharm Clin Res*. **2016**, *9*, 115-119. https://doi.org/10.22159/ajpcr.2016.v9i6.13798
49. Singh N, Ranjana R, Kumari M, Kumar B. A review on biological activities of hydrazone derivatives. *Int J Pharm Clin Res*. **2016**, *8*, 162-166.
50. Karrouchi K, Radi S, Ramli Y, Taoufik J, Mabkhot YN, Al-Aizari FA, et al. Synthesis and pharmacological activities of pyrazole derivatives: A review. *Molecules*. **2018**, *23*, 1-86. https://doi.org/10.3390/molecules23010134

Received: 1 August 2024/ **Accepted:** 1 September 2024 / **Published:** 15 September 2024

Citation: Aminin A. L. N, Anggraeni E, Ramoza S A., Suzery M, Cahyono B., Eghdami A. Radical-Scavenging and Anti-inflammatory Activities of Fermented *Eucheuma cottonii* from Lombok. Bionatura Journal 2024; 1 (3) 1*8*. http://dx.doi.org/10.70099/BJ/2024.01.03.1*8*

Correspondence should be addressed to ***agustina.aminin@live.undip.ac.id***

Peer review information. Bionatura thanks anonymous reviewer(s) for their contribution to the peer review of this work using https://reviewerlocator.webofscience.com/

ISSN. 3020-7886