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Studies on *in−vitro* antioxidant activity of marine edible seaweeds from the east coastal region of Peninsular Malaysia using different extraction methods

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PEER REVIEW

Peer reviewer

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Comments

This study revealed that the seaweed of *E. cottonii* and *Padina* sp. were identified as the natural potential sources of antioxidant compounds with the maximum yield of phenolic content. The method in this study is one of the main pathways to determin some major beneficial antioxidant compounds from the seaweed extract for the future study.
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ABSTRACT

Objective: To determine the antioxidant activity of two edible marine seaweeds *Eucheuma cottonii* and *Padina* sp..
Methods: The two extraction methods such as conventional and soxhlet extraction were used to isolate the secondary metabolites using methanol as a solvent medium. Total phenolic content of crude seaweeds extract were analysed by standard FC method. The different antioxidant assays DPPH, ferric reducing antioxidant power and β−carotene bleaching assays confirmed the antioxidant activities.
Results: DPPH and ferric reducing antioxidant power assays showed the positive correlation with expressed higher total phenolic content in the seaweeds extract. Also β−carotene bleaching assay lower activity compare with BHT as reference control. Additionally IR spectra showed the phenolic related functional groups are present in the solvent extract. The phenolic related compounds are mainly responsible for higher rate of antioxidant activity.
Conclusions: The methanolic extracts of *Padina* sp. showed better radical scavenging and higher phenolic contents than the *Eucheuma cottonii*. And also the soxhlet extraction showed higher yield and better radical scavenging activity compared to conventional method. Moreover the studies confirmed both seaweeds are an effective candidate for the control the free radical scavenging activity.

KEYWORDS
Antioxidant activity, DPPH, Different extraction, Marine seaweeds

1. Introduction

Seaweeds are multicellular macroalgae used as potential renewable resource in the field of medical and commercial environment. The seaweed contains numerous pharmacologically important bioactive constituents such as flavanoids, carotenoids, dietary fiber, protein, essential fatty acids, vitamins and minerals. Nowadays seaweeds are used as dietary food supplements in daily life and it regulates the human health[1]. As well it enhances human health and controls the various pathogenic condition.

Eukaryotic system have a number of cellular defence systems which is includes enzymatic scavengers such as catalase, glutathione peroxide, and superoxide dismutase.

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As end of the metabolic pathways the excess free radicals were forming in the human cellular system. It is dangerous to human and animal system, damage the membrane of the cells. According to that naturally developed antioxidants such as seaweed, green algae and plants evolved to prevent the excess oxidative stress of cells[2]. Reactive oxygen species (ROS) and free radicals can only be eliminated efficiently under normal conditions but pathological conditions it induces various chronic diseases such as alzhemiers, cancer, diabetes and gastro. The balance between generation and elimination of ROS cannot be achieved under clammy condition[3]. Other than peroxidation of lipids, the ROS will also result in extensive oxidative damage which induce of serious human diseases including rheumatoid arthritis, muscular dystrophy, some neurological disorders, aging and cancer[4,5].

However, uses of the synthetic antioxidants such as butylated hydroxyanisol, butylated hydroxytoluene (BHT) have been suspected to be a possible cause for liver damage and carcinogenesis[6-8]. Consequently, nowadays most of the literatures are more publishing on finding alternative antioxidants from natural origin. According to that novel findings of marine seaweed is a valuable antioxidant source, it consists of high level of antioxidants compounds[9-11]. Based on that seaweeds and their extracts are beneficial to health and some even have been reported to retain biological activity of potential medicinal value. Hence, the present study investigated on the antioxidant activity of edible seaweeds and the studies on their percentage of phenolic content in the seaweed sample.

2. Materials and methods

2.1. Chemicals used

2,2-diphenyl-1-picrylhydrazyl (DPPH), BHT, Folin Ciocalteu's phenol reagent, 2,4,6-Tri(2-pyridyl)-1,3,5-triazine were purchased from Sigma, Malaysia. All other solvents and chemicals were of analytical grade used throughout the experiment.

2.2. Collection and processing of seaweeds

The sample *Eucheuma cottonii* (*E. cottonii*) and *Padina* sp. was collected from Pulau Besar, Mersing, Malaysia in September 2013. The seaweeds were washed thoroughly with running tap water to remove undesired particles and epiphytes. Then the samples were dried in oven at 45 °C for 24 h. The dried seaweeds were cut into smaller pieces and grind into powder. The fine powder was used for the extraction studies.

2.3. Extraction of seaweeds

Conventional method: A 10 g of seaweed powder were weighted and 100 mL of methanol was added into the clean conical flask and kept in shaker at 120 r/min in room temperature for 72 h. The extracted sample were filtered in a Whatman filter paper No.1. The solvent were removed by using rotary evaporator under vacuum at 40 °C. The method was followed by Souza *et al*.[12], with some modification.

Soxhlet method: A 20 g of seaweed powder was weighted put in a thimble and placed into soxhlet apparatus and extracted using 200 mL of methanol at 60 °C for continually repeating 12 cycles. The solvent was evaporated by using a rotary evaporator under vacuum at 40 °C. Both the extracts obtained were left at air dried in a fume hood to make a dried sample. The dried extracts were stored at -20 °C for further use to control the microbial contamination.

2.4. Measurement of extraction yield

The yield of the extracts obtained from different extraction methods. The found dry weight of the seaweed samples was calculated the total weight[13].

2.5. Determination of total phenolic content

The total phenolic contents of methanolic seaweed extract were performed by Taegu *et al*[14]. Sample (10 mg/mL) of 100 μL was added to 2 mL of 2% Na_2CO_3. The mixture was then left to stand for 2 min for incubation at room temperature. Further the reaction mixture adding with 100 μL of 50% Folin-Ciocalteau's phenol reagent and incubated the reaction mixture at room temperature in the dark for 30 min. After 30 min by read the absorbance of all the sample at 720 nm. A gallic acid calibration curve was constructed to determine the phenolic contents in term of gallic acid equivalents per gram (GAE/g) of extract.

$C=c\times(V/m)$

Where C=Total phenolic content of sample extract in mg GAE/g, c=concentration of gallic acid established from calibration curve (mg/g), V=volume of the extract (mL) and m=weight of sample extract (g).

2.6. DPPH free radical scavenging assay

DPPH radical scavenging ability of the seaweed extracts was monitored by Nurul *et al*[15] method. Initially, the 0.16

mmol/L DPPH solution was prepared freshly in methanol. A volume of 100 μL of DPPH solution was added to 100 μL of samples with different concentrations in 96-well plates and incubated at 37 °C for 30 min. The methanol used as negative control and while positive control was BHT with the addition of DPPH solution respectively. After incubation, the absorbance of the samples were measured using the Tecan Infinite M200 PRO microplate reader at 515 nm. The scavenging activity of the samples was determined by following formula.

DPPH scavenging activity (%)=[1-((A$_{sample}$-A $_{blank}$)/A$_{control}$)]×100

Where, A$_{sample}$ is the absorbance of test samples, A$_{blank}$ is the absorbance of samples and A$_{control}$ is the absorbance of control (BHT).

2.7. Feeric reducing antioxidant powder (FRAP) assay

FRAP assay was measured the scavenging activity of the seaweed extract according to modified method of Benzie *et al*.[16]. The FRAP reagent solution was freshly prepared with 300 mmol/L acetate buffer (pH 3.6), 10 mmol/L 2,4,6-Tri(2-pyridyl)-1,3,5-triazine, 40 mmol/L HCl and 20 mmol/L $FeCl_3.6H_2O$ in the ratio of 10:1:1. The reagent solution was warmed to 37 °C before use. Briefly, 200 μL of the reagent solution was dispensed into the 96-well plates followed by adding 20 μL crude extracts to initiate the reaction. The reaction mixtures were left for 10 min and the absorbance was read at 593 nm using a Tecan Infinite M200 PRO microplate reader. Ascorbic acid with concentration of 2 mg/mL was used as positive control.

FRAP scavenging (%)=[(Abs Sample-Abs Control)/Abs Sample]×100

2.8. β-carotene bleaching assay

The total antioxidant activity of seaweed extracts and standards (BHT) was measured according to the method of Velioglu *et al*[17]. A 1 mL of β-carotene solution (0.2 mg/mL chloroform) was dispersed into a round-bottom flask containing 0.02 mL of linoleic acid and 0.2 mL of 100% Tween 20. The mixture solution chloroform was evaporated at 40 °C for 10 min using rotary evaporator. Once the evaporation was completed the 100 mL of distilled water was immediately added to the mixture. The mixture mix well and it was formed like foam. The BHT and sample solutions prepared in 2 mg/mL were added with 5 mL aliquots of the emulsion. The tubes were mixed gently and placed in a water bath at 45 °C for 2 h. A blank, consisting of an emulsion without β-carotene and methanol in 5 mL of the above emulsion as control were also prepared. The changes of absorbance was measured at 470 nm. The assay was calculated by using the following formula:

$$\beta\text{-carotene bleaching assay}=1-\frac{(A0-At)}{(A°0-A°t)}\times 100$$

where A0 and A°0 are the absorbance values measured at initial time of the incubation for samples and control respectively, while At and A°t are the absorbance values measured in the samples or standards and control at t=120 min.

2.9. Fourier transform infra-red spectroscopy (FT-IR)

The dried crude extract of seaweed samples was mixed with potassium bromide to prepare as a pellet. The disc prepared was analyzed in Perkin Elmer, Spectrum 100 FT-IR-Spectrometer between 4000-400 cm^{-1}.

2.10. Statistical analysis

All the assays were carried out in triplicate and the values are expressed in the mean±standard error. SPSS 20.0 was used for the experiment One-way analysis of variance (ANOVA) to compare the mean values of the intensity. Then the analysis was proceed through Turkey's multiple range test statistical significance at $P\leq 0.05$.

3. Results

Table 1 shows the extraction yield of the methanolic crude extracts of *E. cottonii* ranged from 2.76% to 5.03% while the *Padina* sp. crude extracts total yield was 1.21% to 2.87% in conventional and soxhlet extraction respectively. For both methods, the methanolic extracts of *E. cottonii* showed higher yield as compared with the *Padina* sp. From the result, it showed an increased of extraction yield by using different extraction method with same solvent. The yield increased at around 50% when using soxhlet extraction method compared to conventional extraction method. Table 2 shows the total phenolic contents of *Padina* Sp. in conventional and soxhlet extraction were (14.58±0.12) mg GAE/g and (15.28±0.11) mg GAE/g respectively. However *E. cottonii* were (8.71±0.09) mg GAE/g and (9.04±0.05) mg GAE/g respectively.

Table 1

The extraction yields of the methanolic extracts of *E. cottonii* and *Padina* sp. from conventional and soxhlet extraction method.

Seaweeds	Yield (%) w/w	
	Conventional	Soxhlet
E. cottonii	2.76	5.03
Padina sp.	1.21	2.87

Table 2
Total phenolic content of seaweed extracts in different extraction methods.

Seaweed	Total phenolic content (mg GAE/g)	
	Conventional	Soxhlet
E. cottonii	8.71±0.09[a]	9.04±0.05[b]
Padina sp.	14.58±0.12[a]	15.28±0.11[b]

Comparision of conventional extract[a] with Soxhlet extract[b].

Table 3 illustrates the DPPH activity of *E. cottanii* and *Padina* sp. of conventional and soxhlet extraction were (32.74±0.01) mg/mL and (39.41±0.00) mg/mL respectively. These readings were higher than BHT [(61.29±0.02) mg/mL], lower compared to *E. cottanii* of conventional [(29.74±0.16) mg/mL] and soxhlet extraction [(32.04±0.08) mg/mL]. Thus, this study showed that the antioxidant activity of *Padina* sp. is higher than *E. cottanii* lower than the positive control of BHT. Table 3 shows ferric reducing power of *E. cottonii* and *Padina* Sp. extracts with the increased concentration of the extract. At concentration 2 mg/mL, the antioxidant content of *Padina* sp. extract from conventional and soxhlet extraction (47.06±0.73)% and (56.03±0.22)% respectively. Compared with *E. cottanii* was showed effective values in both extraction such as 40.54±0.62 and 46.22±0.13 respectively. The ascorbic acid expressed (86.10±0.01)% higher than the *E. cottanii* and *Padiana* sp. The beta carotene scavenging activity of *Padiana* sp. shows (27.86±0.08)% and (34.72±0.28)% respectively. Similarly *E. cottanii* expressed moderate scavenging properties, the value is 21.45±0.24 and 28.69 ±0.12 for conventional and soxhlet methanolic seaweed extracts. The inhibition of the β-carotene bleaching activity of seaweed extracts and BHT at various time intervals were measured. The total antioxidant activity between seaweed extracts of *E. cottonii*, *Padina* sp. and BHT did not showed significant difference (P>0.05). At the same time different extraction methods did not affect the antioxidant activity of the seaweeds as well.

Table 3
Free radical scavenging activities of conventional and soxhlet extraction of *E. cottonii* and *Padina* sp.

Seaweed extract	Extraction methods	Antioxidant assays		
		DPPH	FRAP	β-carotene
E. cottonii	Conventional	32.74±0.16	40.54±0.62	27.86±0.08
	Soxhlet	31.44±0.08	46.22±0.13	34.72±0.28
Padina sp.	Conventional	29.64±0.05	47.06±0.22	21.45±0.24
	Soxhlet	32.45±0.01	56.03±0.37	28.67±0.02
Standard		61.29±0.02	86.10±0.01	66.84±0.37

Comparision of conventional extract[a] with soxhlet extract[b].

FTIR analysis shows the functional group of *E. cottanii* and *Padina* sp. shown in Table 4. The *E. cottanii* and *Padina* sp. strong peaks in the region of (3500-3200) cm^{-1} is (O-H stretch) indicates phenolic active compounds, (1500-1400) cm^{-1} (C-C stretch) of aromatics clusters, (1000-650) cm^{-1} (C-H bend-alkenes) and (900-675) cm^{-1} (C-H stretch-aromatic). Furthermore, the FT-IR spectrums *E. cottanii* and *Padina* sp. also showed moderate peaks in (1680-1640) cm^{-1} region it may be (-C=C stretch) alkanes.

Table 4
Studies on FT-IR functional groups of *E. cottonii* and *Padina* sp. extracts.

IR frequency (cm^{-1})	Bond	Functional groups	*E. cottonii*	*Padina* sp.
3500-3200	O-H stretch	alcohols, phenols	+	+
3100-3000	C-H stretch	aromatics	-	-
3300-2500	O-H stretch	carboxylic acids	+	+
1760-1665	C=O stretch	unknown	-	-
1500-1400	C-C stretch	aromatics	+	+
1320-1000	C-O stretch	alcohols, carboxylic acids, esters, ethers	+	+
1000-650	C-H bend	alkenes	+	+
900-675	C-H	aromatics	+	+

4. Discussion

Naturally seaweeds are contains novel antioxidant compounds which control the free radical formation from metabolic reaction. Some active therapeutic metabolites are identified in different marine seaweed such as *Sargassum* sp., *Padina* sp. and *Eisenia bicyclis*. These edible seaweeds contain phlorotannins, fucoxanthin, polyphenols and phylopheophylin. The bioactive metabolites might be involved in the metabolic regulation and regulate the normal mechanism. Even though it control and capture excess free radicals from the cells[18,19].

Kalpana *et al.*[20] stated that soxhlet extraction method that contributed to maximum yield among other extraction methods. Jiménez-Escrig *et al.*[21] reported that the brown seaweeds showed significantly higher phenolic content than red seaweeds. The total phenolic contents of seaweed extracts is different concentration were observed in different extraction methods, where the total phenolic content of soxhlet extract is higher than the conventional extract.

According to Nakamura *et al.*[22] phaeophyta contains a kind of polyphenol called phlorotannins. This polyphenol had been reported as potential antioxidant, anticancer, antibacterial and antifungal compounds. Also the different extraction methods do not have significant influence in *Padina* sp. compare with *E. cottanii*. This happened might be due to extraction method and solvent system, sometimes the these physic-chemical factor may influence to produce higher antioxidant ranges[23]. Selvaraju *et al.*[24] reported the brown seaweed *Sargassum wightii* possessed higher

antioxidant content. The reducing power property indicated the *Padina* sp. and *E. cottanii* extracts shows the consist of higher antioxidant compounds in the seaweeds which are involved in electron donors and will be able to reduce the oxitative stress from lipid per oxidation process[25]. Likewise Devi *et al.*[26] stated that the presence of phenolic compounds in a seaweeds extract has the probably in effecting their antioxidant activity. Phenols one of the main plant essential chemical constituents are capability of scavenging of free radicals. Also type of solvents as a essential factor for the isolation of antioxidant compounds. Also polar solvents are extracted considerable crude metabolites including polyphenols and tannins from the plant origin. These natural compounds have been exhibiting considerable antioxidant potential[27]. Meenakshi *et al.*[28] the infrared peaks of aromatic and hydroxyl groups revealed that *E. cottanii* and *Padina* sp. probably consists of phenolic compounds.

This findings is more beneficial for the future study and also determining the major antioxidant compounds in the seaweed extract. Therefore, further analysis will be evaluate the different antioxidant assays and finding the active compounds that involved in the antioxidant activity.

In conclusion the methanolic crude extracts of *E. cottanii* and *Padina* sp. can be utilized as a source of natural antioxidant compounds, and it has possessed higher antioxidant activity was proved. The results indicate that the antioxidant activity of the seaweeds is related to the presence of phenolic compounds in the solvent extract. The soxhlet extraction method seems to be able to maximize the yield of extracts and more effective in extracting the phenolic compounds compared to conventional extraction method. Moreover, FTIR showed there are present of potential phenolic functional groups in the methanolic seaweeds extract. These seaweeds have potent bioactive compounds that important for the food and medical industries.

Conflict of interest statement

No conflict of interest in the studies

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Comments

Background

Marine seaweeds have become a major food ingredient in products and the processed seaweeds using as additives in the food preparation. Free radicals are responsible for aging and causing various human diseases. This study shows that antioxidant substances which scavenge free radicals play an important role in the prevention of free radical-induced diseases. The primary radicals are reduced to non-radical chemical compounds and are then converted to oxidize antioxidant radicals. This action helps in protecting the body from degenerative diseases.

Research frontiers

Antioxidant activity of *E. cottonii* and *Padina* sp. were determined by different radical scavenging methods.

Related reports

The antioxidant activity of commercial seaweeds to determine the antioxidant and free radical scavenging activities and also comparatively evaluate the antioxidant properties of these seaweeds with commercial antioxidants.

Innovations and breakthroughs

Seaweeds contain various kinds of inorganic and organic substances such as high levels of minerals, vitamins, essential amino acids, carbohydrates, flavonoids, carotenoids and dietary fiber. Seaweeds have been developed as raw or semi-processed food products; their antioxidant activity is higher than the other commercial potential benefits of seaweeds which can contribute to human health.

Applications

The present study reported the two edible seaweeds of *E. cottonii* and *Padina* sp. are commonly available in Malaysia. These seaweeds can be used as a potential antioxidant agent.

Peer review

This study revealed that the seaweed of *E. cottonii* and *Padina* sp. were identified as the natural potential sources of antioxidant compounds with the maximum yield of phenolic content. The method in this study is one of the main

pathways to determin some major beneficial antioxidant compounds from the seaweed extract for the future study.

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