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ANTIMICROBIAL, ANTIOXIDANT AND FREE RADICAL-SCAVENGING CAPACITY OF BROWN SEAWEED *HIMANTHALIA ELONGATA* FROM WESTERN COAST OF IRELAND

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ABSTRACT

Water, methanol and mixtures (20–80%) thereof have been used for the extraction of phenolic compounds from Irish brown seaweed *Himanthalia elongata* for studying its antimicrobial and antioxidant properties. The 60% methanolic extract exhibited significantly ($P < 0.05$) the highest value of yield (6.8 ± 0.24%), total phenol (286.0 ± 4.61 mg gallic acid equivalents/g), flavonoid (109.8 ± 2.68 mg quercetin equivalents/g) and condensed tannin content (35.6 ± 1.03 mg catechin equivalents/g). Antimicrobial activity of 60% methanolic extract tested from disc diffusion and broth dilution methods was effective against various food spoilage and pathogenic bacteria studied. The same extract exhibited statistically highest reducing power and antioxidant capacity against DPPH radical, metal ions, lipid peroxides and hydrogen peroxide radicals. The UV-visible spectroscopy showed absorption maxima at 205 and 260 nm and the presence of hydroxyl group (3,431 cm^{-1}) and an aromatic ring (around 1,465, 1,505 and 1,624 cm^{-1}) in Fourier Transform Infrared spectroscopy, suggested the presence of phenolic compounds in the extract.

PRACTICAL APPLICATIONS

In recent years, much attention has been focused on the use of natural preservatives to enhance the quality, safety and stability of ready-to-eat food products. These products also undergo gradual changes during storage, due to autoxidation, which releases reactive oxygen species including free radicals into the food. The consumption of seaweed as food and nutraceutical has been well known in the ~~Orient~~. Nowadays, increasingly seaweeds are being investigated for the biological activity of their extracts which are finding hundreds of applications in pharmaceuticals, biotechnology and food preservatives. The present study demonstrates that *H. elongata* contains excellent antimicrobial and antioxidant properties which can provide opportunities for the application of seaweed extract as natural food preservative or nutraceutical for possible application in food and dietary supplemental products for health promotion.

INTRODUCTION

The beneficial action of food to maintain or even improve our health is because of the well-known correlation between food and health. This fact has brought about a great interest for search of new edible or nutraceuticals products that can contribute to improve our health and well-being (Plaza *et al.* 2008). The use of phenolics in food has increased greatly because of their antioxidant and possible health-promoting role in human health. The available food products in the market are enriched with synthetic preservatives such as antimicrobials (to increase the shelf life) and antioxidants (to

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reduce the risk of developing chronic diseases) which are toxic to health. Nowadays, there is an increase in the demand for more natural preservatives due to the abuse of toxic synthetic food substances and the increasing microbial resistance of pathogenic microorganisms against antibiotics. Natural substances isolated from plants are considered as promising sources of food preservatives (Peschel *et al.* 2006). The potential contribution of marine organisms to the discovery of new bioactive molecules is increasingly challenging. At present, researchers are trying to extract and characterize new functional compounds of biological origin having various bioactive natures (Plaza *et al.* 2008).

Marine algae have a significant attraction as an important resource of bioactive compounds as they are able to produce a great variety of secondary metabolites characterized by a broad spectrum of biological behavior such as antibacterial, antioxidant, anticancer, anticoagulant and antiviral properties (Vairappan *et al.* 2001; Athukorala *et al.* 2007). Seaweeds or marine macroalgae are the renewable living resources which are also used as food, feed and fertilizer in many parts of the world. Seaweeds are of nutritional interest as they are low calorie food and are rich in vitamins, minerals proteins, polyphenols, polysaccharides and dietary fibers (Burtin 2003; MacArtain *et al.* 2007). A variety of *in vitro* studies have demonstrated that algal derived polyphenols and flavonoids exhibit antimicrobial and antioxidant activity (Heo *et al.* 2005; Chandini *et al.* 2008; Zaragoza *et al.* 2008). They have been consumed from time immemorial in the whole world, especially in Asia, where they constitute an alternative to vegetables in human food. In our diet, many products are manufactured with seaweeds or their derivatives, such as sauces, creams, toothpaste and milk shakes of fruits. According to the CE 258/97 Regulation, algae are considered as new foods, and they could also be considered as functional foods because they have what defines this kind of aliment (Crespo and Yusty 2004). This regulation, in addition to the potential nutritional properties of seaweeds, allows the food industry to include seaweeds as raw or semi-processed materials in the formulation of seafood products (Burtin 2003). Apart from functional food products, newer applications of seaweeds and their bioactive compounds in different sectors, such as nutraceuticals, cosmetics, biomedicine and biotechnology, are constantly under development. Therefore, the short-term goal of functional foods, nutraceuticals and dietary supplements is to improve the quality of life and enhance health status while its long-term goal is to increase lifespan while maintaining health.

Himanthalia elongata (also known as "sea spaghetti" or "haricot" or "buttonweed" or "thongweed") are abundant and largest on the western coast of Ireland, where it is limited to semi-exposed shores and usually occurs near the low watermark of spring tides, but in some locations it also grows subtidally (Moss *et al.* 1973). As the food value of *H. elongata* is high, commercial harvesting from the west coast of Ireland, similar to that in northern France, is under way on a small scale, and is likely to expand quickly (Rouxel and Crouan 1995; Plaza *et al.* 2008). This alga is highly valued in Europe and has been included in different foods due to an excellent nutritional and gastronomic value but the consumption of the complete alga continues to be limited in Europe (Crespo and Yusty 2004). The capacity of plant-derived food to reduce the risk of chronic diseases has been associated with the phytochemicals that have been shown to exert a wide range of biological behavior such as antimicrobial and antioxidant properties (Espin *et al.* 2007). Furthermore, these phytochemicals are also helpful to act as natural food preservatives because many food spoilage and pathogenic bacteria spoil foods, thus, reducing their shelf life and can also lead to food poisoning of consumers. In addition to the microbial contamination, all packed and refrigerated foods undergo gradual changes during storage, due to autoxidation, which releases reactive oxygen species, including free radicals into the food and causes various chronic diseases (Devi *et al.* 2008). Thus, the increasing demand for safe, healthy and minimally processed foods is creating an opportunity for seaweed products to be marketed as functional foods or nutraceuticals.

In the present work, an attempt has been made to screen the extracts of marine macroalgae *H. elongata* collected from the western coastline of Ireland for antimicrobial and antioxidant capacities. This study is part of a program on screening of seaweeds for a variety of biological activities, with the aim of identifying novel nutraceuticals which can further be explored for potentially useful food preservative activities.

MATERIALS AND METHODS

Chemicals and Reagents

All reagents, standard compounds and solvents were purchased from Sigma-Aldrich Chemical Co. (Steinheim, Germany) unless otherwise mentioned. All the microbial growth media such as tryptic soy agar (TSA) and tryptic soy broth (TSB) were purchased from Scharlau Chemie (Barcelona, Spain). All other chemicals used in the experiments were of analytical grade.

Plant Materials

Brown seaweed (*Phaeophyta*) used in the present study was *H. elongata*. The seaweed was purchased from Quality Sea Veg. (Co Donegal, Ireland). Samples were washed thoroughly with fresh water to remove epiphytes, sand, shells and debris and stored at −18C until analysis.

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Solvents Used for the Extraction

Seaweed extraction was carried out with water, methanol and thereof mixtures (20, 40, 60 and 80%) on the basis of polarity. The dielectric constant (ε) of mixed solvents is calculated on the basis of percentage (v/v) of each solvent used for the combinations. Among the solvents and their mixtures, water and methanol have the highest (80) and lowest (33) dielectric constant, respectively, while their mixtures have 70.6 (20% methanol), 61.2 (40% methanol), 51.8 (60% methanol) and 42.4 (80% methanol) dielectric constant.

Preparation of Seaweed Extract

Extraction of seaweed was carried out according to the method reported by Ganesan *et al.* (2008). In short, seaweed were crushed with liquid nitrogen using mortar and pestle; this powder and various solvents were then taken in flask in a ratio of 1:10 (w/v); the flasks were flushed with liquid nitrogen and kept in orbital incubator shaker (Innova 42, Mason Technology, Dublin, Ireland) at 40C and 100 rpm for 2 h under dark conditions. The samples were centrifuged at 9,168 × *g* and filtered with Whatman #1 filter paper (••, ••, ••). The resulting supernatant was evaporated to dryness in a multi-evaporator (Buchi Syncore Polyvap, Mason Technology). Dried extracts were collected and stored at −60C for further analysis.

Phytochemical Content Analysis

Total Phenolic Content. The amount of total phenolic compounds in the crude extracts was determined according to Ganesan *et al.* (2008) using Folin–Ciocalteu phenol reagent. Absorbance of all the samples and standard against reagent blank was determined at 720 nm with a spectrophotometer (Genesys 20, Thermo Spectronic, Madison, WI). The total phenolic content (TPC) was expressed as mg gallic acid equivalents (GAE)/g dry weight (dw) extract.

Total Flavonoid Content. Total flavonoid content (TFC) was determined by a colorimetric method described by Liu *et al.* (2009). The absorbance of samples and standard against the blank was recorded at 510 nm. TFC was expressed as mg quercetin equivalents (QE)/g extract (dw).

Total Condensed Tannin Content. Total condensed tannins were determined by a spectrometric method described by Liu *et al.* (2009). The absorbance of samples and standard against the blank was read at 500 nm, and results were expressed as mg (+)-catechin equivalents (ChE)/g extract (dw).

Antimicrobial Activity Analysis

Microbial Culture Preparation. All extracts were tested against various Gram positive and Gram negative food microorganism. Two species of common food pathogenic and food spoilage bacteria each, selected for this study were *Listeria monocytogenes* ATCC 19115, *Salmonella abony* NCTC 6017, *Enterococcus faecalis* ATCC 7080 and *Pseudomonas aeruginosa* ATCC 27853, respectively, obtained from Medical Supply Company (Dublin, Ireland). All cultures were maintained at −70C in glycerol stocks (20%) and grown in TSB at 37C; apart from *P. aeruginosa* which was incubated at 30C to obtain subcultures. Working cultures were prepared from subcultures and grown at optimal conditions for each bacterium for 18 h before analysis. Bacterial suspensions were then prepared in saline solution (NaCl 0.85%, BioMérieux, Craponne, France) equivalent to a McFarland standard of 0.5, using the Densimat photometer (BioMérieux) to obtain a concentration of 1×10^8 colony forming units (cfu)/mL. This suspension was then diluted in TSB to obtain a working concentration of 1×10^6 cfu/mL.

Antimicrobial Activity Assay

Disc Diffusion Assay. Preliminary identification of antimicrobial activity of various seaweeds extracts was tested by filter paper disc diffusion method. Agar plates made from TSA were spread with 100 µL of different bacterial cultures containing 1×10^8 cfu/mL. Water was used as a negative control and sodium nitrite and sodium benzoate were used as positive controls for this assay. The dried seaweed extracts and positive controls were dissolved in water to final concentrations in the range of 30–270 mg/mL. Sterile and dried filter paper discs (Whatman AA, Whatman International Limited, Maidstone, U.K.) 6 mm in diameter, impregnated with 30 µL of seaweed extract or standard, were placed on the TSA media plate on which bacteria had been spread. After incubation at 37C (*P. aeruginosa* inoculated plates were incubated at 30C) in the dark for 24 h, assessment was based on the absence or presence of bacterial growth in the contact zone between the agar and the samples and on the eventual appearance of an inhibition zone (IZ) which was calculated from Eq. (1):

$$H = \frac{D - d}{2} \quad (1)$$

where H is the IZ in mm, D is the total diameter of the paper disc and IZ in mm and d is the diameter of filter paper disc in mm (Tomšič *et al.* 2009). The values were recorded with the average (mm) of two diameter measurements per disc taken in perpendicular directions.

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Broth Micro-Dilution Assay. The antibacterial assay was carried out through broth dilution method according to the protocol reported in our earlier study (Gupta *et al.* 2010). The influence of varying concentrations of extract on antimicrobial efficacy was assessed against the same bacteria using 96-well microtiter plates (Sarstedt Limited, Leicester, U.K.). Seaweeds extracts were dissolved in TSB media and 200 µL was added to the first row of plate (concentration: 60 mg/mL). Serial dilutions along each column were made with the addition of 100 µL TSB to achieve concentrations of 30, 15, 7.5, 3.75, 1.88 and 0.94 mg/mL. A 100 µL of bacterial suspension containing 1×10^6 cfu/mL was added to the wells. Total volume in each well was 200 µL. Pure TSB and bacterium were used as controls (O) while sample blanks were prepared without adding any bacterial culture in the extracts. Absorbance readings were taken at 0 and 24 h at 600 nm using a microtiter plate spectrophotometer reader (Powerwave, Biotek, Winooski, VT) with 20 s agitation before each optical density (OD) measurement. Sodium benzoate and sodium nitrite were used as positive controls. Percentage inhibition was calculated according to Eq. (2):

$$\textit{Antimicrobial activity}\,(\%) = \frac{O-E}{O} \times 100 \qquad (2)$$

where O is (blank free OD of the organism at 24 h – blank free OD of the organism at 0 h) and E is (blank free OD of the organism in the presence of test extract at 24 h – blank free OD of the organism in the presence of test extract at 0 h).

Antioxidant Capacity Analysis

DPPH Radical-Scavenging Assay. This assay was carried out according to the method standardized in our laboratory (Rajauria *et al.* 2010). Briefly, the assay was performed in a 96-well round-bottomed microtiter plate with 1:1 (v/v) ratio of 100 µL of DPPH radical solution (165 µM) and 100 µL of sample. Different concentrations were tested for each sample in order to get EC_{50} value. The DPPH solution was freshly prepared for each experiment in methanol. The reaction mixtures were incubated for 30 min at 25C in dark conditions, and absorbance measured at 517 nm in a microtiter plate reader (Powerwave, Biotek). The ability to scavenge the DPPH radical was calculated using the following equation:

$$\text{Scavenging capacity}\,(\%) = \left[1 - \left(\frac{A_{\text{sample}} - A_{\text{sample blank}}}{A_{\text{control}}}\right)\right] \qquad (3)$$

where $A_{control}$ is the absorbance of the control (DPPH solution without sample), A_{sample} is the absorbance of the test sample (DPPH solution plus test sample) and $A_{sample\ blank}$ is the absorbance of the sample only (sample without any DPPH solution). Calculated EC_{50} values indicate the concentration of sample required to scavenge 50% DPPH radicals. The lower the EC_{50} value of the sample, the higher the antioxidant capacity.

Ferric Reducing Antioxidant Power Assay. Total antioxidant reducing power of various extracts of seaweed was measured using ferric reducing antioxidant power (FRAP) assay according to the method reported by Benzie and Strain (1996) with some modifications (Jaiswal *et al.* 2011). Briefly, the reaction was performed in a microplate reader of 96-well plates (Powerwave, Biotek with software Gene 5) at a temperature of 37C. Preheated 100 µL FRAP reagent at 37C (300 mM acetate buffer, pH 3.6; 10 mM TPTZ in 40 mM HCl and 20 mM $FeCl_3 \cdot 6H_2O$ in the ratio of 10:1:1, v/v/v) was dispensed in each well with 50 µL of samples or standard. The absorbance was read after 10 min at 593 nm with the help of microplate spectrophotometer (Powerwave, Biotek). Trolox was used as a standard and the results were expressed as mg of trolox equivalents (TE)/g extract (dw).

Metal Ion-Chelating Ability Assay. The chelating ability of ferrous ion (FIC) by brown seaweed was estimated by the original method of Decker and Welch (1990) with minor modifications. This assay is based upon the formation of blue colored ferrous ion-ferrozine complex which has a maximum absorbance at 562 nm. Briefly, 100 µL of varying concentrations of different extract samples and standard were mixed with 100 µL of deionized water and 25 µL of ferrous chloride (0.5 mM) in a microtiter plate. The reaction was initiated by the addition of 25 µL of ferrozine (2.5 mM), and the reaction mixture was shaken vigorously. The absorbance was recorded at 562 nm with a microtiter plate reader, after 10 min of incubation at ambient temperature. Ethylenediaminetetraacetic acid (EDTA) was used as a standard compound. The percentage of inhibition of ferrozine-Fe^{2+} complex formation was calculated using Eq. (3).

Lipid Peroxidation in a Hemoglobin-Induced Linoleic Acid System (LPO)

The antioxidant activity was principally determined by a colorimetric assay reported by Kuda *et al.* (2005) with slight modifications (in the estimation of coloring compounds using the ferric-thiocyanate method), which is based upon the degree of inhibition of the hemoglobin-catalyzed peroxidation of linoleic acid. The sample (100 µL) of seaweed extracts and standard (ascorbic acid [AA]) were mixed in a test tube together with 25 µL of 0.1 M linoleic acid/ethanol and 75 µL of 0.2 M phosphate buffer (pH 7.2). The autoxidation of linoleic acid in the above reaction mixture was initiated by adding 50 µL hemoglobin (0.08% in water) and incubated for 60 min at 37C. The peroxidation of linoleic acid

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was stopped by adding 5 mL of HCl (0.6% in ethanol). The peroxidation value of samples in the reacted mixture was calculated using the ferric-thiocyanate method, in which red color appeared upon addition of 50 µL of ferrous chloride (20 mM) and ammonium thiocyanate (30%) each, in that order. For the estimation of peroxide value, 200 µL of the above colored reacted mixture (A_{sample}) was taken in triplicate in a microtiter plate, and absorbance was recorded at 490 nm using a microplate reader (Powerwave, Biotek). Ascorbic acid was used as a standard. The antioxidant capacity of the samples was calculated using Eq. (3).

Hydrogen Peroxide Scavenging Capacity Assay

The hydrogen peroxide (H_2O_2) scavenging capacity of seaweed extract was estimated by the method of Ruch *et al.* (1989). The concentration of hydrogen peroxide was determined spectrophotometrically (Agilent 8453 with photodiode array (PDA) detector, Waldbronn, Germany) by measuring absorption with molar extinction coefficient for H_2O_2 of 43.6/M/cm at 230 nm. Hydrogen peroxide solution (40 mM) was prepared in phosphate buffer (100 mM, pH 7.4). A total of 3.4 mL of different seaweed extracts (200–800 ppm) were added to 0.6 mL of hydrogen peroxide solution, and the absorbance was recorded at 230 nm after 10 min incubation, against reagent blank solution. BHT was used as a reference compound. The percentage scavenging of H_2O_2 was calculated according to Eq. (3).

Qualitative Identification of Phenolics in Seaweed Extract

UV-Visible Spectrophotometric Analysis. For qualitative analysis of phenolics in seaweed, the spectra of seaweed extract in water with different concentrations were measured on a UV-visible spectrophotometer coupled with PDA detector (Agilent 8453) and were recorded from 190 to 600 nm. Peaks assignments were made by comparing the UV-visible spectra of analytes with standard compounds such as gallic acid (5 ppm) and quercetin (15 ppm) (Avila *et al.* 2008).

Fourier Transform Infrared Spectroscopy. The Fourier Transform Infrared (FT-IR) spectra of dried seaweed extract and standard compounds were recorded in KBr pellet using an FT-IR spectrophotometer (AVATAR 360, Nicolet, Madison, WI) over the range 4,000–500 cm^{-1}. One milligram of dry sample was mixed with 100 mg of dry KBr, and the mixture was pressed into a disc (Lim *et al.* 2002). The samples were analyzed as KBr pellet and compared with quercetin and rutin standards.

Statistical Analysis. All the experiments were carried out in triplicate and replicated at least twice. Results are expressed as mean ± standard deviation. All statistical analyses were carried out using STATGRAPHICS Centurion XV (••, ••, ••). Statistical differences between extract activities were determined using ANOVA followed by Least Significant Difference testing. Differences were considered statistically significant when $P < 0.05$.

RESULTS AND DISCUSSION

Phytochemical Content of Seaweed Extracts

Extraction Yield. The secondary metabolites present in seaweed are not a qualitative or quantitative absolute or invariant characteristic of a species. On the contrary, the yields of secondary metabolites varies according to the extraction solvents used (Chandini *et al.* 2008; Wang *et al.* 2009). The yield (%) obtained from varying concentrations of aqueous methanolic extract of *H. elongata* is given in Table 1. Considerable variations in extraction yield were found among different concentrations of methanol. Among the various methanolic extracts of the *H. elongata*, the highest extraction yield was recorded for the 60% methanolic extract (6.8%) whereas the lowest extraction yield was observed for 100% methanolic extract (1.2%). Among all the tested methanol concentrations, the extraction yield increased from 20% methanol to 60% methanol and then reduced for 100% methanol (Table 1). However, with the exception of the 60% methanolic extract, the extraction yield of water (0% methanol) was higher than that obtained with other aqueous methanolic extracts. Interestingly, large differences in yield were observed between 60% methanolic extracts and other aqueous methanolic extracts which indicate that a solvent of optimum polarity is required for the extraction of most of the components from seaweed. Herrero *et al.* (2005) also suggested that the polarity of solvents significantly affects the yield of *Spirulina* microalgae wherein the highest yield was recorded with ethanol as compared with the other higher and lower polarity solvents. Similarly, Plaza *et al.* (2010) also documented that the polarity of the solvents had an impact on the extraction yield of seaweeds.

Total Phenolic Content. Phenolic compounds are commonly found in seaweeds and have been reported to have several biological activities including antimicrobial and antioxidant activity (Yuan *et al.* 2005; Duan *et al.* 2006; Rajasulochana *et al.* 2009). The TPC values are expressed as mg GAE/g dry weight (dw) extract and results are shown in Table 1. Among all the aqueous methanolic extracts, the extract obtained from 60% methanol resulted in the highest

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and significantly different ($P < 0.05$) yield of TPC (286.0 ± 4.61 mg GAE/g). However, 100% methanolic extract exhibited significantly lower value of TPC (59.8 ± 0.74 mg GAE/g). The reported value of TPC in *H. elongata* was much higher as compared with published reports on brown (Lim *et al.* 2002; Chandini *et al.* 2008) and red seaweeds (Duan *et al.* 2006; Ganesan *et al.* 2008). The recovery of TPC continuously increased as the concentration of methanol increased and reached a maximum at 60% and then was reduced. There was no significant difference observed for TPC of *H. elongata* between 20 and 80% methanol concentrations. A similar finding was reported for TPC from raisin extracts wherein the TPC increased from 0 to 60% methanol concentrations and then reduced until 100% methanol (Zhao and Hall 2008).

Total Flavonoid Content. Flavonoids are the largest class of polyphenols, and are thought to exert beneficial health effects through their antioxidant and chelating properties, and are the major contributor to the antioxidant capacity of plants. They act either by blocking the generation of hypervalent metal forms, by scavenging free radicals, or by breaking lipid peroxidation chain reactions (Zaragoza *et al.* 2008). In this study, total flavonoid content was estimated in *H. elongata* and expressed as mg ChE/g dw extract. Among all the concentrations of methanol, the value of TFC increased (as the polarity decreased) from 0% methanol to 60% methanol and then was reduced until 100% methanol. The maximum and a significantly different ($P < 0.05$) recovery of TFC (109.8 ± 2.68 mg QE/g) was estimated with the 60% methanolic extract while lowest value of TFC (44.0 ± 1.47 mg QE/g) was observed from 100% methanolic extract (Table 1). The previous values of TFC were either similar or much higher as compared with the methanolic extracts of lychee flower and different varieties of mushrooms, respectively (Gursoy *et al.* 2009; Liu *et al.* 2009). Among the solvent systems studied, the extracts obtained from most and least polar solvents (100% water and methanol, respectively) had lowest values of TFC as compared with the other extracts. There was no significant difference observed in the values of TFC between the extracts obtained from 20 and 80% and 0 and 100% methanol concentrations.

Total Condensed Tannin Content. Total condensed tannin content (TTC) in the extracts of *H. elongata* was studied and expressed as mg QE/g dw extract. The TTC was observed in the range of 9.8 ± 0.83 to 35.6 ± 1.03 mg ChE/g with varying concentrations of aqueous methanol (Table 1). Among all the concentrations of aqueous methanol tested, extracts obtained from 60% methanol exhibited maximum and significantly different ($P < 0.05$) yield of TTC while 100% methanolic extracts showed significantly lowest value of TTC. Furthermore, similar trend as in TPC and TFC was also observed in TTC wherein tannin content increased as the concentration of methanol in water increased (as polarity decreased) up to 60% and then reduced. Results from a study (Liu *et al.* 2009) also showed that the dielectric constant of extractants significantly affect the TTC in lychee flower. However, methanolic extract of the same fruit showed slightly higher TTC as compared with the level of tannin content obtained in the present study (Liu *et al.* 2009).

From the above findings, it is evident that the recovery of phenolic compounds was dependent on the solvents used and their polarity. This indicates that phenolic compounds are more soluble in organic polar solvents than in water (Wang *et al.* 2009). Methanol and water mixtures are commonly used for the extraction of phenols from seaweeds. This is due to the wide range of phenols that can get dis-

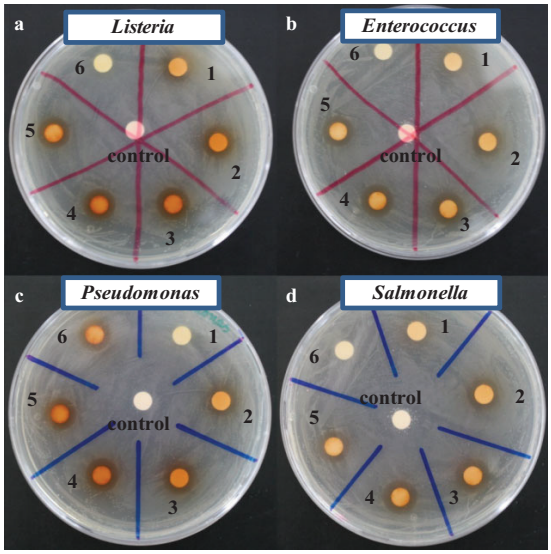
TABLE 1. EFFECT OF VARIOUS CONCENTRATIONS OF AQUEOUS METHANOL ON YIELD, PHYTOCHEMICAL CONTENT AND RADICAL-SCAVENGING CAPACITY OF *HIMANTHALIA ELONGATA* SEAWEED

Extraction Solvents	Extraction Yield (%)	TPC mg GAE/g	TFC mg QE/g	TTC mg ChE/g	DPPH EC_{50} (ppm)	FRAP mg TE/g
Water	1.8 ± 0.08[a]	116.5 ± 3.08[a]	46.7 ± 2.45[a]	14.6 ± 0.87[a]	105.4 ± 1.88[a]	6.8 ± 0.28[a]
20% Methanol	1.5 ± 0.09[b]	193.6 ± 4.03[b]	70.2 ± 2.00[b]	21.7 ± 0.92[b]	92.0 ± 1.07[b]	8.7 ± 0.12[b]
40% Methanol	1.7 ± 0.17[a]	259.1 ± 6.21[c]	96.5 ± 2.01[c]	27.2 ± 1.70[c]	78.7 ± 0.71[c]	10.3 ± 0.40[c]
60% Methanol	6.8 ± 0.24[c]	286.0 ± 4.61[d]	109.8 ± 2.68[d]	35.6 ± 1.03[d]	57.2 ± 0.48[d]	11.7 ± 0.21[d]
80% Methanol	1.4 ± 0.08[b]	193.9 ± 3.65[b]	66.0 ± 1.85[b]	22.4 ± 2.16[b]	87.0 ± 0.77[e]	8.3 ± 0.11[e]
Methanol	1.2 ± 0.12[d]	59.8 ± 0.74[e]	44.0 ± 1.47[a]	9.8 ± 0.83[e]	297.7 ± 2.99[f]	4.7 ± 0.08[f]

Values are expressed as mean ± standard deviation.
Values with different letters (a–f) in columns are significantly different ($P < 0.05$), $n = 6$.
Extraction yield (%) is calculated in terms of g of dry extracts/100 g of fresh weight.
TPC, TFC, TTC and FRAP are expressed as mg gallic acid equivalents/g (dw), mg quercetin equivalents/g (dw), mg catechin equivalents/g (dw) and mg trolox equivalent/g (dw), respectively.
TPC, total phenolic content; TFC, total flavonoid content; TTC, total condensed tannin content; FRAP, ferric reducing antioxidant power; GAE, gallic acid equivalent; QE, quercetin equivalent; ChE, catechin equivalent; TE, trolox equivalent.

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solved in the aqueous methanol mixtures. At the same time, methanol–water mixtures are considered good solvent systems for the extraction of polar antioxidants (Chandini *et al*. 2008). This study as well as other previously reported publications (Naczk and Shahidi 2004; Zhou and Yu 2004) clearly illustrates that it is essential to systematically evaluate and optimize the extraction solvent composition for accurate and reproducible estimation of structurally diverse phenolic compounds from different food matrices. Phenolic extracts of plant materials are always a mixture of different classes of phenolics that are soluble in the solvent system used. They may also exist as complexes with carbohydrates, proteins and other plant components; some high-molecular weight phenolics and their complexes may be quite insoluble. Solubility of phenolic compounds is governed by the type of solvent (polarity or dielectric constant) used, degree of polymerization of phenolics, as well as interaction of phenolics with other food constituents and formation of insoluble complexes (Naczk and Shahidi 2004). In the present study, the highest recoveries of hydrophilic polyphenols from seaweed samples were obtained from 60% methanol. It is also anticipated that the polarity or dielectric constant of 60% methanol was more selective to extract a range of phenolic compounds with varying biological properties from *H. elongata*.



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FIG. 1. ANTIMICROBIAL ACTIVITY OF THE *HIMANTHALIA ELONGATA* EXTRACT OBTAINED FROM VARIOUS CONCENTRATIONS OF METHANOL IN WATER (0% METHANOL [1]; 20% METHANOL [2]; 40% METHANOL [3]; 60% METHANOL [4]; 80% METHANOL [5]; 100% METHANOL EXTRACT [6])

Antimicrobial Activity of Seaweed Extracts

Disc Diffusion Assay. Most of the studies done up to date, use the agar disc diffusion method for describing the antimicrobial activities of seaweeds (Bansemir *et al*. 2006; Rajasulochana *et al*. 2009); however, very few reports are available on the antimicrobial activity of *H. elongata* (Plaza *et al*. 2010). This disc diffusion assay is good and produces predominantly qualitative results. In order to screen the qualitative antimicrobial activity of various methanolic extracts of *H. elongata*, the disc diffusion method was quite useful to obtain preliminary information. Various food spoilage (*E. faecalis* and *P. aeruginosa*) and pathogenic bacteria (*L. monocytogenes* and *S. abony*) were used to determine the antibacterial activities of brown seaweed aqueous methanolic extracts (30–90 mg/mL) and synthetic compounds (30–270 mg/mL) by the disc diffusion assay. Clear IZs were found after 24 h incubation at 37C (Fig. 1). The zone of inhibition of more than 4 mm is considered to be excellent while more than 3, 2 and 1 mm are considered as very good, good and moderate, respectively. However, the IZ of less than 1 mm is not considered acceptable for antimicrobial activity. The IZ of seaweed extracts was measured taking the reference of the inhibition exhibited by synthetic food antimicrobials. The diameter of the paper disc was not included in the measurements of IZ (Table 2). For the preliminary analysis, the amount of extract (10–50 µL) impregnated on the paper disc was also optimized (data not shown), and it was observed that 30 µL was optimum to get the clear IZ. Although, there was a bigger zone of inhibition observed with 40 and 50 µL extract but it was not distinct due to the overflow of extract from the paper disc. Among all of the aqueous methanolic extracts, the extract obtained from 60% methanol showed the highest antimicrobial activity against all the microorganisms studied. However, 100% methanolic extract did not show any antimicrobial activity against any microorganism. The 60% methanolic extract, at a concentration of 60 mg/mL (1.8 mg extract per disc), exhibited excellent (IZ > 4 mm) antimicrobial activity against Gram positive bacteria such as *L. monocytogenes* and *E. faecalis* while good activity was observed (IZ > 2 mm) against Gram negative bacteria such as *P. aeruginosa* and *S. abony* (Fig. 1). The extracts obtained from 40 and 80% methanol and 0 and 20% methanol showed very good (IZ > 3 mm) and good (IZ > 2 mm) antimicrobial activity against Gram positive bacteria while these extracts exhibited moderate (IZ > 1 mm) antimicrobial activity against Gram negative bacteria (Table 2). Antimicrobial activity of studied seaweed was much higher as compared with the activity in seaweeds reported by Rajasulochana *et al*. (2009) wherein 50 µL methanolic extract showed only 2 mm IZ against various microorganisms. However, our results are almost comparable to the data reported by Bansemir *et al*. (2006) wherein 2 mg

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TABLE 2. ANTIMICROBIAL ACTIVITY OF DIFFERENT AQUEOUS METHANOLIC EXTRACTS OF *HIMANTHALIA ELONGATA* AT 60 mg/mL CONCENTRATION AGAINST VARIOUS FOOD SPOILAGE AND PATHOGENIC BACTERIA OBTAINED FROM AGAR DISC DIFFUSION TESTS

Extraction Solvents	*L. monocytogenes* Size of inhibition zone (mm)	*E. faecalis*	*P. aeruginosa*	*S. abony*
Water	2.36[a]	2.29[a]	0.77[a]	0.56[a]
20% Methanol	2.67[b]	2.42[b]	1.06[b]	1.08[b]
40% Methanol	3.74[c]	3.33[c]	1.71[c]	1.64[c]
60% Methanol	4.58[d]	4.10[d]	2.29[d]	2.14[d]
80% Methanol	3.34[e]	3.02[e]	1.56[e]	1.53[e]
Methanol	0.98[f]	0.78[f]	0.47[f]	0.36[f]

The diameter of the paper disc is not included. Values are expressed as average of three replicates. Statistical analysis for antimicrobial activity was performed among the various solvent extracts against each individual bacteria.
Values with different letters (a–f) in each column are significantly different ($P < 0.05$).
The zone of inhibition around the disc was calculated from H = (D-d)/2; where H is the inhibition zone in mm, D is the total diameter of the paper disc and inhibition zone in mm and d is the diameter of the paper disc in mm.
Antimicrobial effect: inhibition zone considered excellent (>4 mm); very good (>3 mm); good (>2 mm), moderate (>1 mm) and no activity (<1 mm).

of seaweed extract exhibited similar antimicrobial activity against various microorganisms.

Sodium benzoate and sodium nitrite are listed as GRAS (generally recognized as safe) by the U.S. Food and Drug Administration (U.S. FDA 21 CFR) when used as an antimicrobial agent and preservative for fish and meat products, respectively. In contrast to the seaweed extracts, synthetic food grade antimicrobials such as sodium benzoate and sodium nitrite showed only good (IZ > 2 mm) antimicrobial activity against *L. monocytogenes* and *S. abony* at a concentration of 180 mg/mL. However, moderate activity (IZ > 1 mm) was obtained against *E. faecalis*, while no activity was seen against *P. aeruginosa* at similar concentration (Table 2).

Broth Micro-Dilution Assay. Preliminary analysis by disc diffusion assay showed that among various concentrations of methanol, extract obtained from 60% methanol showed the highest antimicrobial activity against all the tested microorganisms. Furthermore, to check the efficacy of this extract quantitatively, the antibacterial activity of the brown seaweed was assessed using the microtiter-plate-based dilution method against the same microorganisms. This method is robust, quantitative and free from mathematical inaccuracies. The inhibition obtained with the methanolic extract was concentration dependent. The crude methanolic (60%) extract obtained from *H. elongata* had strong antimicrobial activity against all the four bacterial strains studied. The crude extracts of *H. elongata* showed a very strong antimicrobial activity against *L. monocytogenes* (98.7%), *P. aeruginosa* (98.7%), *E. faecalis* (98%) and *S. abony* (95.6%) at a concentration of 60 mg/mL (Fig. 2). Generally, antibiotics are less effective against Gram negative bacteria because of their more complex multilayered cell wall structure with additional lipopolysaccharides on the outer cell surface, which makes it more difficult for the active components to penetrate (Rang and Dale 1987). However, the extract obtained from *H. elongata* showed an excellent antibacterial activity against Gram negative (more than 95%) as well as Gram positive bacteria (around 98%) at a concentration of 60 mg/mL. Additionally, the antimicrobial activity was retained around 80% against all the tested microorganisms even after the concentration of the extract was reduced to half (30 mg/mL). Furthermore, comparisons were also carried out with the commonly used chemical food preservatives such as sodium benzoate and sodium nitrite. In the present study, sodium benzoate and sodium nitrite were used at a concentration (60 mg/mL) similar to that of the seaweed extract. Methanolic extracts of *H. elongata* showed significantly higher inhibition ($P < 0.05$) against *L. monocytogenes* (98.7%) and *E. faecalis* (98.1%) (Gram positive), while sodium benzoate and sodium nitrite showed 96.5% and 96.2% against *L. monocytogenes* and

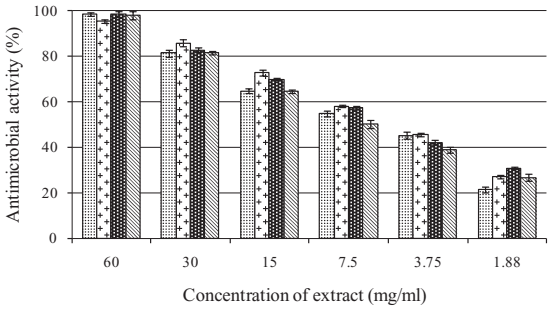


FIG. 2. ANTIMICROBIAL ACTIVITY ANALYSES OF 60% METHANOLIC EXTRACT OF *HIMANTHALIA ELONGATA* AGAINST FOOD PATHOGENIC AND FOOD SPOILAGE BACTERIA (▦: *L. MONOCYTOGENES*; ⊡: *S. ABONY*; ▩: *P. AERUGINOSA*; ▧: *E. FAECALIS*)

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89.4% and 93.7% against *E. faecalis*, respectively (data not presented). Inhibition against the other two microorganisms (Gram negative) obtained by *H. elongata* extracts and both the positive controls was comparable. In general, the percentage inhibition for the positive controls was in the range of 89–99% which was almost similar to the activity (95–98%) given by the seaweed extract in the present study. Plaza *et al.* (2010) also reported that the *H. elongata* extract obtained from different polarity solvents had potential antimicrobial activity against different bacteria and fungi. Antibacterial activity of extracts from brown seaweeds has been studied and found to be active against a range of bacterial species. A high antimicrobial activity from seaweed extracts has been reported against Gram positive and Gram negative bacteria (Rajasulochana *et al.* 2009). The powerful antimicrobial activity of *H. elongata* brown seaweed suggests that the seaweed extract could be a potential alternative to chemical preservatives in foods. The exact mechanism and the specific compound responsible for the antimicrobial activities are currently unclear. Though, it can be anticipated that antimicrobial activity may involve complex mechanisms, like the inhibition of the synthesis of cell walls and cell membranes, nucleic acid and protein, as well as the inhibition of nucleic acid metabolism. It also seems likely that substances in the extracts may act separately or synergistically to exert these effects.

Antioxidant Capacity of Seaweed Extracts

DPPH Radical-Scavenging Capacity. DPPH method measures the radical-scavenging capacity of antioxidants toward DPPH radical in organic systems and has been used extensively as a prescreening method for new antioxidants from natural resources due to its stability, simplicity, rapidity and reproducibility. The DPPH radical in fact may be neutralized by either direct reduction via single electron transfer (SET) or by radical quenching via hydrogen atom transfer (HAT). Upon reduction, the color of the solution fades from purple to yellow and the reaction progress is conveniently monitored by a spectrophotometer (Huang *et al.* 2005). The percentage of remaining DPPH is proportional to the antioxidant concentration, and the concentration that causes a decrease in the initial DPPH radical concentration by 50% is defined as EC_{50}. The lower the EC_{50} value of the sample, the higher its antioxidant capacity.

The DPPH radical-scavenging capacity of water and different concentrations of methanolic extracts increased in a concentration dependent manner up to 60% and after that started to decline. As pointed out by some researchers, change in extractant polarity alters its efficacy in extracting a specific group of antioxidant compounds and influences the antioxidant properties of the extracts (Zhou and Yu 2004). At a concentration of 100 ppm, 60% methanolic extract showed the highest scavenging capacity (87.4%) while the water extract (0% methanol) exhibited the lowest scavenging capacity (16.6%). However, synthetic antioxidant compounds such as AA and BHT showed 98.9 and 100% scavenging capacity against DPPH radical at the same concentration, respectively. At a concentration of 700 ppm, all the extracts obtained from 0–100% aqueous methanol showed complete scavenging (100%) capacity; however, at the concentration of 200 ppm, the extract obtained from 60% methanol showed 100% scavenging capacity against DPPH radicals (Fig. 3). The DPPH radical-scavenging capacity of all the methanolic extracts of Irish seaweed was much higher as compared with methanolic extracts and other solvent fractions of Indian brown (Chandini *et al.* 2008) and red seaweeds (Ganesan *et al.* 2008). Further, in terms of EC_{50}, the results from Table 1 also suggest that the lowest and significantly different ($P < 0.05$) EC_{50} value was recorded for the extract of 60% methanol (EC_{50} 57.2 ± 0.48 ppm).

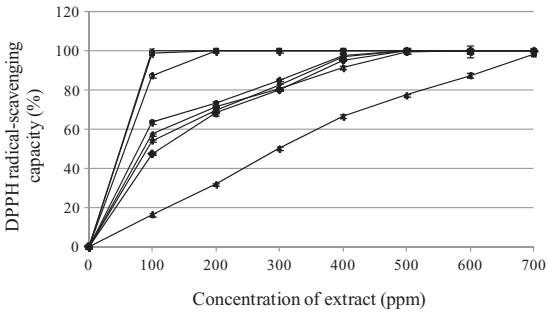


FIG. 3. DPPH RADICAL-SCAVENGING CAPACITY (%) ANALYSIS OF *HIMANTHALIA ELONGATA* EXTRACTS USING VARIOUS CONCENTRATIONS OF METHANOL IN WATER (0% METHANOL [–◆–]; 20% METHANOL [–◇–]; 40% METHANOL [–●–]; 60% METHANOL [–○–]; 80% METHANOL [–△–]; 100% METHANOL [–▲–]; ASCORBIC ACID [–□–]; BHT [–×–])

FRAP. The FRAP mechanism is electron transfer rather than mixed SET and HAT; thus, FRAP cannot detect compounds that act by radical quenching (HAT) (Ou *et al.* 2002). Reducing power appears to be related to the degree of hydroxylation and extent of conjugation in polyphenols. The ferric reducing power of *H. elongata* brown seaweed was studied and results were expressed as mg TE/g dw extract (Table 1). The reducing power of brown seaweeds was in the range of 4.7 ± 0.08 to 11.7 ± 0.21 mg TE/g in the extracts obtained from various concentrations of methanol in water. Among all the tested concentrations of methanol from 0–100% in water, extracts from 60% methanol exhibited the highest and significantly different ($P < 0.05$) reducing power. However, extracts

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obtained from 100% methanol gave the lowest reducing power. The value of FRAP obtained from 60% methanolic extract was 1.1 to 2.5-fold higher than the values obtained from different methanolic extracts. Reducing power of methanolic extracts of *H. elongata* was much higher as compared with the result reported in other brown seaweeds study (Chandini *et al.* 2008; Ganesan *et al.* 2008).

It was observed that the extracts containing a high level of TPC were also potent DPPH radical scavengers and showed maximum FRAP value, suggesting that algal polyphenols may be the principal constituents responsible for the antiradical properties of the extracts. A correlation analysis indicated a high and significant ($P < 0.05$) correlation between DPPH scavenging capacity and phytochemical content (TPC: $r^2 = 0.9515$; TFC: $r^2 = 0.9339$ and TTC: $r^2 = 0.9608$). We also observed a strong and statistically significant relationship between reducing antioxidant power (FRAP) and phytochemical content (TPC: $r^2 = 0.9919$; TFC: $r^2 = 0.9882$ and TTC: $r^2 = 0.9856$) of seaweed extracts. Similar observations in the seaweeds were also reported by Wang *et al.* (2009) and Matanjun *et al.* (2008). Furthermore, high and significant correlation between antimicrobial activity and radical-scavenging capacity (DPPH: $r^2 = 0.9741$ and FRAP: $r^2 = 0.9647$) of seaweed extracts was also observed. These findings also suggest that yield and phytochemical content (TPC, TFC and TTC) may be a good indicator of potential antimicrobial and antioxidant capacity (DPPH and FRAP) of the extracts based on the fact that phytochemical content, antimicrobial activity and antioxidant capacity were highest in the 60% methanolic extracts wherein yield (%) was also estimated to be at maximum level (Table 1). On the basis of yield, phytochemical content and antioxidant capacity (DPPH and FRAP), the polarity of 60% methanol was optimum to extract maximum bioactive polyphenols from the brown seaweeds studied. Thus, free radical-scavenging capacity analysis was further carried out with 60% methanolic extract.

Metal Ion Chelating Ability. Metal chelating ability in terms of ferrous ion chelating capacity is claimed as one of the important mechanisms of antioxidant activity. Ferrous ions are the most powerful prooxidants among various species of transition metals present in food systems. These ions react with hydrogen peroxide via the Fenton reaction and produce dangerous hydroxyl radicals (Halliwell 1991). However, dietary antioxidants (nutrients) having metal chelating ability may act as preventive or secondary antioxidants as they form σ-bonds with metal ions and reduce the redox potential thereby stabilizing the oxidized form of the metal ions (Gordon 1990). Furthermore, these antioxidants prevent the formation of hydroxyl radicals by either deactivating free metal ions through chelation or converting H_2O_2 to other

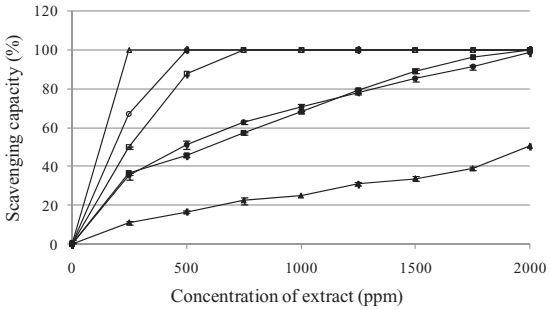


FIG. 4. METAL ION CHELATING (FIC) ABILITY, LIPID PEROXIDATION (LPO) SCAVENGING CAPACITY AND HYDROGEN PEROXIDE (H_2O_2) SCAVENGING CAPACITY OF *HIMANTHALIA ELONGATA* AND SYNTHETIC ANTIOXIDANT COMPOUNDS FIC ABILITY: *H. ELONGATA* (–▲–) AND EDTA (–△–); LPO SCAVENGING: *H. ELONGATA* (–■–) AND ASCORBIC ACID (–□–); H_2O_2 SCAVENGING: *H. ELONGATA* (–●–) AND BHT (–○–)

harmless compounds (such as water and oxygen) (Huang *et al.* 2005). In this study, results obtained as shown in Fig. 4 reveal that *H. elongata* demonstrated a moderate capacity for iron binding (EC_{50} 1,982.0 ± 8.73 ppm). At a concentration of 1,000 ppm, 60% methanolic extract of *H. elongata* showed 25% iron chelating ability. However, the extracts were not as strong as the positive control such as EDTA which showed 100% ferrous ion chelating ability at 50 ppm concentration. Nonetheless, significant activities were exhibited by the crude methanolic extract of *H. elongata* as compared with the activities of crude extracts of Icelandic seaweeds. The extracts from Icelandic seaweeds showed 95% scavenging capacity at a concentration of 5,000 ppm (Wang *et al.* 2009) while in case of Irish seaweed, around 50% scavenging obtained only at the concentration of 2,000 ppm. The iron chelating ability of seaweeds may be attributed to the presence of endogenous chelating agents, mainly phenolics because certain phenolic compounds have properly oriented functional groups, which can chelate metal ions (Wang *et al.* 2009).

Lipid Peroxidation Scavenging Capacity. In the biological system and foods, transition metals like iron can stimulate lipid peroxidation by the Fenton reaction, and also accelerates peroxidation by decomposing lipid hydroperoxides into peroxyl and alkoxyl radicals that can themselves abstract hydrogen and perpetuate the chain reaction of lipid peroxidation. Antioxidants bind with these transition metals and reduce the concentration of the catalytic transition metal in lipid peroxidation (Halliwell 1991). In the present study, lipid peroxidation scavenging was estimated with the help of hemoglobin induced lipid peroxidation in the linoleic acid system, and results were presented in Fig. 4. The seaweed extract obtained from 60% methanol exhibited effective

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lipid peroxide scavenging antioxidant capacity at all concentrations. The antioxidant capacity of the seaweed extract increased with increasing concentration of extract. The percentage inhibition of peroxidation on linoleic acid system by 250 to 1,500 ppm concentrations of methanolic extract was found to be 36.5 to 89.2%. On the other hand, AA, a synthetic peroxide scavenger, exhibited 75.7% inhibition at a concentration of 500 ppm. The lipid peroxides scavenging capacity was significantly higher in AA (EC_{50} 332.9 ± 5.79 ppm) as compared with *H. elongata* (EC_{50} 546.3 ± 7.61 ppm).

Hydrogen Peroxide Scavenging Capacity. Hydrogen peroxide is generated *in vivo* by several oxidase enzymes, and in biological systems it is only a mildly reactive species by itself. However, when it reacts with metal ions such as Fe (II) and Cu (II) via Fenton reaction, it produces hydroxyl radicals which are very dangerous free radicals for biomolecules (Halliwell 1991). Thus, the removal of H_2O_2 is very important for antioxidant defense in cell or food systems. Hydrogen peroxide scavenging capacity of brown seaweed was studied, and results are presented in Fig. 4. The 60% methanolic extract obtained from *H. elongata* exhibited potential (EC_{50} 361.7 ± 6.30 ppm) hydrogen peroxide scavenging capacity. At the concentration of 200 to 800 ppm, the H_2O_2 scavenging capacity ranged from 39.1 to 77.4%. The percentage inhibition of hydrogen peroxide scavenging capacity by 200 ppm concentration of methanolic extract of *H. elongata* was found as 39.1% which was almost 35% less as compared with synthetic hydrogen peroxide scavenger such as BHT (60.5%) at the same concentration. These results indicated that the methanolic extract of *H. elongata* have a noticeable effect on hydrogen peroxides scavenging and the scavenging capacity also increased with increasing concentration of the extract.

Qualitative Identification of Phenolics in Seaweed Extract

UV-Visible Spectrophotometry Analysis. Each group of phenolic compounds is characterized by one or several UV/visible light absorption maxima. Simple phenolics have absorption maxima in the region between 200–220 and 250–280 nm; however, closely related phenolics show quite wide variations in molecular absorptivity (Shahidi and Naczk 2004). Furthermore, the absorption spectra of total phenolics extracts can be used to identify the presence of groups of predominant phenolic compounds in the extracts. However, the absorption is affected by the nature of solvents, pH and the material to be analyzed (Shahidi and Naczk 2004). According to the UV-visible spectral data obtained from Fig. 5A, the methanolic extract of *H. elongata* contained absorption maxima at 205 and 260 nm. However, phenolic compounds also had absorption maxima in this region. The absorption spectrum obtained from the seaweed was compared with the standards of gallic acid (λ_{max} 212 and 262 nm) and quercetin (λ_{max} 218 and 255 nm) with the maximum absorption at similar regions (Chen *et al.* 2007), which indicated that flavonoids could be the predominant phenolic compounds in seaweed studied (Avila *et al.* 2008). Harbone (1984) also identified the characteristic UV/DAD spectra of flavonoids due to their characteristic UV spectral pattern (λ_{max} around 220–280 nm). This UV pattern allows for the selection of flavonoid peaks for quantitative analysis; hence, UV/DAD is an important alternative in the absence of a mass detector. These findings indicate that *H. elongata* contained different types of phenolic compounds which had different absorption maxima in UV-visible spectral regions (Lim *et al.* 2002). Preliminary identification showed that the compounds present in methanolic extracts are of phenolic nature.

FT-IR Spectroscopy. The FT-IR technology provides rapid, reproducible, nondestructive, multiconstituent analysis of food sample, with minimal or no sample preparation (Versari *et al.* 2009). In seaweeds, this technique is majorly used for the analysis of polysaccharides, and very few reports are available for polyphenols (Lim *et al.* 2002; Duan *et al.* 2006). The FT-IR spectrum of brown seaweed showed the characteristic "fingerprint" region in the range between 4,000 and 500 cm^{-1} (Fig. 5B). In general, hydroxyl group have maximum transformation in 3,300 to 3,500 cm^{-1} region and aromatic rings are generally detected in the range of 1,400 to 1,680 cm^{-1} region. The presence of hydroxyl group (3,431 cm^{-1}) and an aromatic ring (around 1,465, 1,505 and 1,624 cm^{-1}) in *H. elongata* in the same regions also suggested the presence of phenolic compounds in the extract. Similar findings are also reported for polyphenols in seaweeds wherein a hydroxyl group and aromatic ring lied in the same region (Duan *et al.* 2006). Furthermore, these spectra were also compared with standard compounds such as rutin and quercetin and a similar pattern was observed. These findings also support the results of UV-visible spectra that the extract from all the three seaweeds contained phenolic compounds with the dominancy of flavonoids.

CONCLUSION

Brown seaweeds contained high levels of hydrophilic components, such as polyphenols and soluble polysaccharides which were easily extracted by polar solvents. Overall results of this study showed that Irish brown seaweed *H. elongata* has strong antimicrobial activity and antioxidant capacity. These seaweed contained diverse class of compounds such as

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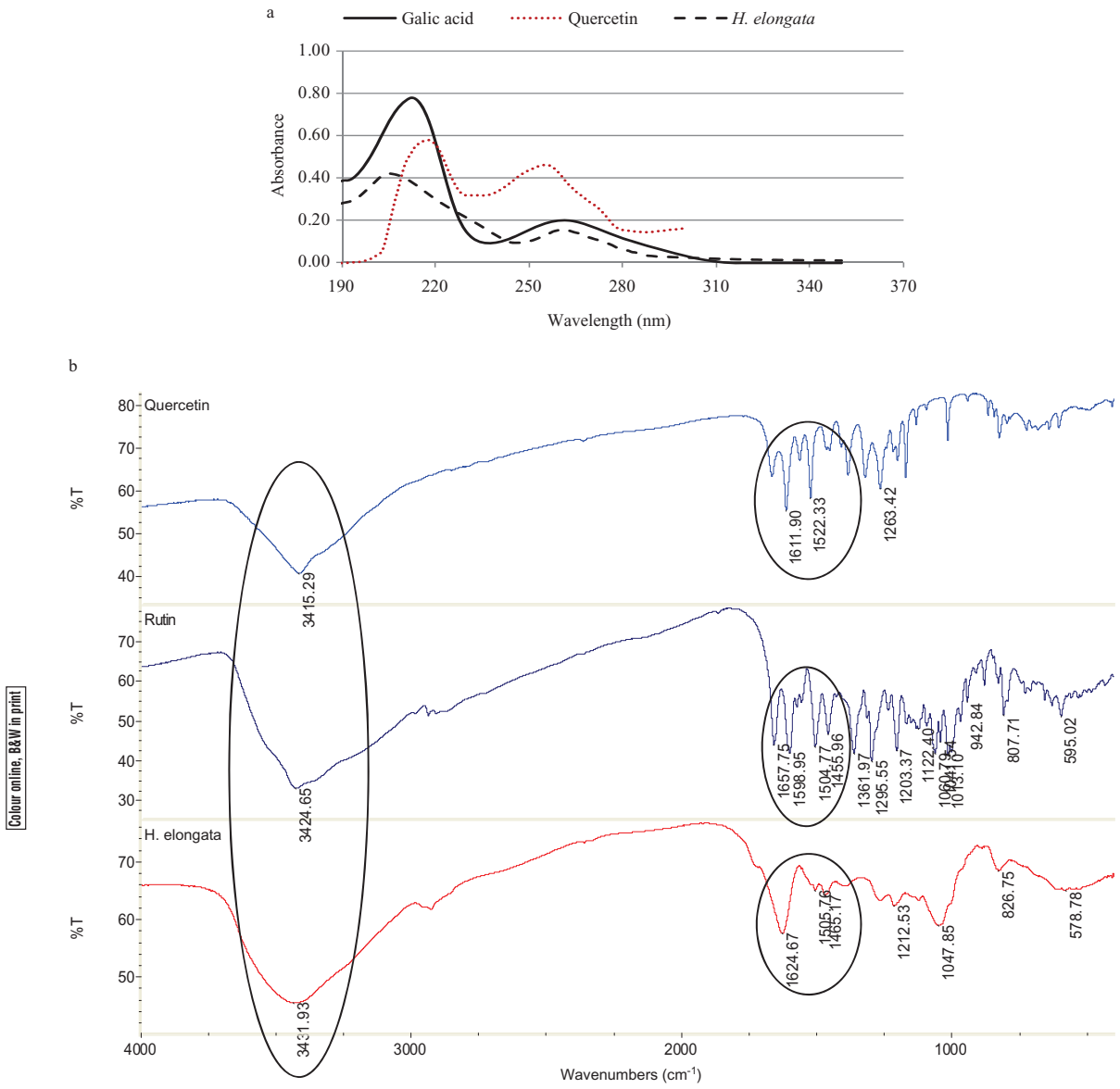


FIG. 5. UV-VISIBLE ABSORPTION SPECTRA OF GALLIC ACID, QUERCETIN AND 60% METHANOLIC EXTRACT OF *HIMANTHALIA ELONGATA* BROWN SEAWEED RECORDED FROM 190 TO 400 nm SPECTRAL REGION (a). A CHARACTERISTIC FT-IR SPECTRA OF QUERCETIN, RUTIN AND 60% METHANOLIC EXTRACT OF *H. ELONGATA* BROWN SEAWEED RECORDED FROM 4,000 TO 500 cm^{-1} SPECTRAL REGION (b)

phenols, flavonoids and tannins. However, the phytochemical content and their activities were majorly affected by solvents polarity or dielectric constant. This suggests that algal polyphenols including tannins and flavonoids may be the principal constituents responsible for the antimicrobial and antiradical properties of extracts from this species. These findings suggested that there may be a potential to utilize such seaweed extracts in food products to act as antioxidant which could enhance the quality and nutritive value of foods. In addition, the antimicrobial properties of seaweeds would have promising applications in enhancing the food safety.

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