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# An assessment of the antioxidant and antimicrobial activity of six species of edible Irish seaweeds

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**Abstract:** Six species of edible Irish seaweeds; *Laminaria digitata, Laminaria saccharina*, *Himanthalia elongata, Palmaria palmata, Chondrus crispus* and *Enteromorpha spirulina* were screened for potential bioactivity. Extraction of secondary metabolites was carried out using different solvents to determine antioxidant and antimicrobial properties of the dried extracts. The total phenolic contents of dried methanolic extracts were significantly different ($p < 0.05$). *H. elongata* exhibited highest phenolic content at 151.3 mg GAE/g of seaweed extract and also had the highest DPPH scavenging activity ($p < 0.05$) with a 50% inhibition ($EC_{50}$) level at 0.125µg/ml of extract. *H. elongata* also had the highest total tannin and total flavonoid contents ($p < 0.05$) of 38.34 mg CE/g and 42.5 mg QE/g, respectively. Antimicrobial activity was determined using a microtitre method which allowed detection of bacterial growth inhibition at low levels. All methanolic seaweed extracts inhibited the food spoilage and food pathogenic bacteria tested; *Listeria monocytogenes*, *Salmonella abony*, *Enterococcus faecalis* and *Pseudomonas aeruginosa,* except *C. crispus* extracts. It was found that dried methanolic extracts of red and green seaweeds had significantly lower antimicrobial activity than the brown species; *H. elongata* had the highest antimicrobial activity with up to 100% inhibition. In the studied work, the antimicrobial activity of red and green seaweed extracts significantly increased when ethanol and acetone were used as extraction solvents ($p < 0.05$). Inhibition of *E. faecalis* with *C. crispus* extracts increased from 39.28 to 100% when ethanol and acetone were applied as solvents.

**Keywords: Seaweeds, brown algae, red algae, green algae, antioxidant activity, antimicrobial activity**

## Introduction

Seaweeds belong to a group of plants known as algae. Seaweeds are classified as Rhodophyta (red algae), Phaeophyta (brown algae) or Chlorophyta (green algae) depending on their nutrient and chemical composition. Like other plants, seaweeds contain various inorganic and organic substances which can benefit human health (Kuda *et al.*, 2002). Seaweeds are considered as a source of bioactive compounds as they are able to produce a great variety of secondary metabolites characterised by a broad spectrum of biological activities. Compounds with antioxidant, antiviral, antifungal and antimicrobial activities have been detected in brown, red and green algae (Yuan *et al.*, 2005; Bansemir *et al.*, 2006; Chew *et al.*, 2008). The environment in which seaweeds grow is harsh as they are exposed to a combination of light and high oxygen concentrations. These factors can lead to the formation of free radicals and other strong oxidising agents but seaweeds seldom suffer any serious photodynamic damage during metabolism. This fact implies that seaweed cells have some protective mechanisms and compounds (Matasukawa *et al.*, 1997).

Reactive oxygen species (ROS) such as hydroxyl, superoxide and peroxyl radicals are formed in human cells by endogenous factors and result in extensive oxidative damage which can lead to age related degenerative conditions, cancer and a wide range of other human diseases (Reaven and Witzum, 1996; Aruoma, 1999). Phenolic compounds can act as antioxidants by chelating metal ions, preventing radical formation and improving the antioxidant endogenous system (Al-Azzawie and Mohamed-Saiel, 2006). The term "phenolic compound" describes several hundred molecules found in edible plants that possess on their structure a benzenic ring substituted by, at least, one hydroxyl group (Manach *et al.*, 2004). These phenolic compounds are commonly found in plants, including seaweeds (Duan *et al.*, 2006). Polyphenols represent a diverse class of compounds including flavonoids (i.e. flavones, flavonols, flavanones, flavononols, chalcones and flavan-3-ols), lignins, tocopherols, tannins and phenolic acids (Shukla *et al.*, 1997). Interest in new sources of natural antioxidants

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and antimicrobials has increased in recent years in order to reduce the use of synthetic forms such as Butylated Hydroxyanisole (BHA) and Butylated Hydroxytoluene (BHT). Natural antioxidants from plant origin can react rapidly with these free radicals and retard or alleviate the extent of oxidative deterioration (Akoh and Min, 1997). Furthermore, antioxidants from natural sources can also increase the shelf life of foods. Therefore, the consumption of antioxidant and/or addition of antioxidant to food materials could protect the body as well as the foods against these events (Chandini *et al.*, 2008).

Vegetable tannins are secondary plant metabolites subdivided into condensed and hydrolyzable compounds. Hydrolyzable tannins are gallic and/or egallic acid which easily hydrolyze in acidic media, and condensed tannins are polymeric flavonoids (Huang *et al.*, 2008).Tannins are defined as naturally occurring plant polyphenolic compounds and are widespread among terrestrial and marine plants (Haslam, 1989; Waterman and Mole, 1994). In contrast to terrestrial tannins, phlorotannins are tannin compounds which have been found only in marine algae. Phlorotannins are formed by the polymerization of phloroglucinol (1, 3, 5-trihydroxybenzene) monomer units and synthesized in the acetate-malonate pathway in marine alga (Ragan and Glombitza, 1986; Waterman and Mole, 1994; Arnold and Targett, 1998). Phlorotannins purified from several brown algae have been reported to possess strong antioxidant activity which may be associated with their unique molecular skeleton (Ahn *et al.*, 2007). Phlorotannins from brown algae have up to eight interconnected rings. They are therefore more potent free radical scavenger than other polyphenols derived from terrestrial plants, including green tea catechins, which only have three to four rings (Hemat, 2007).

Flavonoids, the largest group of phenolic compounds are known to contain a broad spectrum of chemical and biological activities including antioxidant and free radical scavenging properties (Kahkonen *et al.*, 1999). Flavonoids include flavonols, flavones, catechins, proanthocyanidins, anthocyanidins and isoflavonoids (Ndhlala *et al.*, 2007). Phenolic compounds are important in plant defence mechanisms against invading bacteria and other types of environmental stress, such as wounding and excessive light or ultraviolet (UV) radiation (Harborne, 1994; Herrmann, 1989; Wallace and Fry, 1994).

Many marine plants, including seaweeds, often carry significantly less macro and microepibionts on their thalli compared to co-occurring biofilms on inanimate substrata (Hellio *et al.*, 2001; Lam and Harder, 2007). Therefore it has been assumed that seaweeds defend themselves against bacterial fouling by production of secondary metabolites that prevent attachment and growth of bacterial colonizers (Maximilien *et al.*, 1998).

Recently, consumers are demanding foods which are fresh, natural and minimally processed along with the requirement for enhanced safety and quality. This perspective has put pressure on the food industry for progressive removal of chemical preservatives, and has fuelled research into alternative natural antimicrobials. Plant products with antimicrobial properties have obtained emphasis for possible application in food production in order to prevent bacterial and fungal growth (Lanciotti *et al.*, 2004).

Seaweeds are a plentiful renewable natural resource in Ireland; *Laminaria digitata*, *Laminaria saccharina, Himanthalia elongata, Palmaria palmata, Chondrus crispus* and *Enteromorpha spirulina* are common species of seaweeds found in abundance around the Irish coastline. Many researchers have reported on the antioxidant and antimicrobial activity of seaweeds (Gonzales del Val *et al.*, 2001; Ganesan *et al.*, 2008; Plaza *et al.*, 2009). However, reports on the antioxidant and antimicrobial activity of seaweed extracts from Ireland are very limited. Hence, the present study aimed to investigate the antioxidant and antimicrobial activity of six common species of seaweeds from the west coast of Ireland.

## Methods

### *Chemicals*

2, 2-Diphenyl-1-picrylhydrazyl (DPPH), ascorbic acid, Folin-Ciocalteu's phenol reagent, gallic acid, sodium carbonate ($Na_2CO_3$), sodium benzoate, vanillin, hydrochloric acid (HCl), (+)-catecin, aluminium chloride ($AlCl_3$) and quercetin were purchased from Sigma Aldrich Chemie (Steinheim, Germany). For antimicrobial tests Tryptic Soy Broth (TSB) was purchased from Sparks (Dublin, Ireland).

### *Seaweed material*

*Laminaria digitata*, *Laminaria saccharina, Himanthalia elongata* (Phaeophyta), *Palmaria palmata, Chondrus crispus* (Rhodophyta) and *Enteromorpha spirulina* (Chlorophyta) were purchased from Quality Sea Veg., Co Donegal, Ireland. Samples were collected in June and September 2008, washed thoroughly with fresh water to remove epiphytes and stored at -18°C until analysis.

*Preparation of dried seaweed extracts*

Frozen seaweed samples (5 g) were powdered in liquid nitrogen using a mortar and pestle, then extracted with 50ml of either methanol, ethanol or acetone (60%) under nitrogen atmosphere for 2 hours. The extraction was carried out at 40°C at 100 rpm in a shaker incubator (Innova 42, Mason Technology, Ireland). Samples were filtered and centrifuged at 10,000 rpm for 15 min (Sigma 2K15, Mason Technology, Ireland). Resulting extracts were evaporated to dryness using vacuum polyevaporator (Buchi Syncore Polyvap, Mason Technology, Ireland) at 60°C. A pressure gradient program was designed for evaporation of the solvents with vacuum conditions of 556, 337, 175 and 72 mbar for acetone, methanol, ethanol and water, respectively.

*Preparation of seaweed extract stock solutions*

Stock solutions of seaweed extracts were prepared for analysis by dissolving 1000µg of dried seaweed extract in 1ml of deionised water. For antimicrobial analysis dried seaweed extracts were dissolved in TSB.

*Total phenolic content*

The total phenolic concentration was measured using the Folin-Ciocalteau method (Taga *et al.*, 1984). In this procedure, 100 µl aliquot of stock sample (extract concentration 1000 µg/ml of water) was mixed with 2.0 ml of 2% $Na_2CO_3$ and allowed to stand for 2 min at room temperature. Then 100 µl of 50% Folin-Ciocalteau's phenol reagent was added. After incubation for 30 min at room temperature in darkness, the absorbance was read at 720nm using spectrophotometer (Milton Roy Spectronic 1201). The total phenolic contents of the samples were expressed as mg gallic acid equivalent per gram (mg GAE/g). Ascorbic acid was used as a positive control.

*DPPH radical scavenging activity*

Free radical scavenging activity was measured by 2, 2-Diphenyl-1-picrylhydrazyl (DPPH) according to the method of Yen and Chen (1995). Briefly, a 2.0 ml aliquot of test sample was added to 2.0 ml of 0.16 mM DPPH methanolic solution. The mixture was shaken vigorously then left to stand at room temperature for 30 min in darkness. Changes in the absorbance of the samples were measured at 517 nm using a spectrophotometer (Milton Roy Spectronic 1201).

The ability to scavenge the DPPH radical was calculated using the following equation given by Duan *et al.* (2006):

Scavenging effect (%) =

$$\left[1-\left(\frac{A_{sample}-A_{sampleblank}}{A_{control}}\right)\right]\times 100$$

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). Ascorbic acid was used as a positive control.

*Total condensed tannin content*

Total condensed tannin content was determined according to the method of Julkunen-Titto (1985). Briefly, a 50 µl aliquot of each extract was mixed with 1.5 ml of 4% vanillin (prepared with methanol) and then 750 µl of concentrated HCl was added. The solution was shaken vigorously and left to stand at room temperature for 20 min in darkness. The absorbance against blank was read at 500nm. (+)-catechin was used to prepare the standard curve and results were expressed as mg catechin equivalents (CE)/g extract.

*Total flavonoid content*

Total flavonoid content were determined according to the method of Zhishen *et al.* (1999). Briefly, a 250 µl aliquot of each extract was mixed with 1.25 ml of double distilled (dd) $H_2O$ and 75 µl of 5% $NaNO_2$ solution. After 6 min, 150 µl of 10% $AlCl_3.H_2O$ solution was added. After 5 min, 0.5 ml of 1 M NaOH solution was added and then the total volume was made up to 2.5 ml with dd $H_2O$. Following thorough mixing of the solution, the absorbance against blank was determined at 510 nm. Quercetin was used to prepare the standard curve and results were expressed as mg Quercetin equivalents (QE)/g extract.

*Antimicrobial activity*

*Microbial culture*

Two species of common food pathogenic and

**Table 1.** Categories of growth inhibition in antimicrobial assays

| Classification criteria<br>Inhibition (%) | Classes<br>Inhibition intensity |
|---|---|
| 100 | Very strong |
| 90-100 | Strong |
| 50-90 | Moderate |
| <50 | Weak |

Source: Dubber and Harder (2008)

two species of food spoilage bacteria selected for this study were *Listeria monocytogenes* ATCC 19115, *Salmonella abony* NCTC 6017, *Enterococcus faecalis* ATCC 7080 and *Pseudomonas aeruginosa* ATCC 27853, respectively (Medical Supply Company, Dublin, Ireland). All cultures were maintained at -70°C in 20% glycerol stocks and grown in Tryptic Soy Broth (TSB) at 37°C; apart from *P. aeruginosa* which was incubated at 30°C to obtain sub-cultures. Working cultures were prepared from sub-cultures and grown at optimal conditions for each bacterium for 18 hours before analysis. Bacterial suspensions were then prepared in saline solution (NaCl 0.85%, BioMérieux, France) equivalent to a McFarland standard of 0.5, using the Densimat photometer (BioMérieux Inc, France) to obtain a concentration of $1x10^8$ colony forming units (CFU)/ml. This suspension was then diluted in TSB to obtain a working concentration of $1x10^6$ CFU/ml.

*Antimicrobial activity assay*

The influence of varying concentrations of extract on efficacy was assessed against *L. monocytogenes*, *S. abony*, *E. faecalis* and *P. aeruginosa* using 96-well microtitre plates (Sarstedt Ltd., UK). Extracts achieved from 5 g of fresh seaweed were dissolved in 2.5 ml of TSB and 200 µl was added to the first row of each plate. All other wells were filled with 100 µl of TSB and 100 µl from the first well was serial diluted two-folds along each column. Finally, 100 µl of bacterial suspension containing $1x10^6$ CFU/ml was added to the wells. The last column was used for bacterium and media controls and sample blanks were prepared for all of the extracts. Absorbance readings were then taken at 0 and 24 hours at 600 nm using a microplate spectrophotometer (Powerwave, Biotek) with 20 seconds agitation before each optical density (OD) measurement. Analysis of growth over time was also performed on most effective extracts. OD measurements were taken every three hours for 24 hours. Sodium benzoate and sodium nitrite were used as controls. Percentage inhibition was calculated as follows:

$$\text{Bacterial inhibition (\%)} = \left[\left(\frac{O-E}{O}\right)\right]\times 100$$

Where: $O$ is (OD of the Organism at 24h - OD of the Organism at 0h) and $E$ is (OD of the Extract at 24h – Blank at 24h) – (OD of the Extract at 0h – Blank at 0h) .

Results were interpreted by categorising percentage inhibitions based on inhibition intensity as given in Table 1.

*Statistical analysis*

All experiments were performed in duplicate and replicated at least three times. All statistical analyses were carried out using STATGRAPHICS Centurion XV. Statistical differences between extract activities were determined using ANOVA followed by Least Significant Difference (LSD) testing. Differences were considered statistically significant when $p < 0.05$.

## Results and Discussion

*Total phenolic content of methanolic seaweed extracts*

Natural antioxidants are not limited to terrestrial sources and reports have revealed seaweeds to be rich sources of natural antioxidant compounds (Lim *et al.*, 2002; Duan *et al.*, 2006; Kuda *et al.*, 2007). Phenolic compounds are commonly found in plants, including seaweeds, and have been reported to have a wide range of biological activities including antioxidant properties (Duan *et al.*, 2006; Kuda *et al.*, 2007; Wang *et al.*, 2009). The Folin-Ciocalteu method was applied to study the total phenolic content of the seaweeds. Folin-Ciocalteu reagent determines total phenols, producing blue colour by reducing yellow heteropolyphosphomolybate-tungstate anions

**Table 2.** Total phenolic content and antioxidant activity of methanolic extracts of *L. digitata, L. saccharina*, *H. elongata, P. palmata, C. crispus* and *E. spirulina*

| Species | Total phenolic content (mg GAE/g) | Antioxidant activity $EC_{50}$[a] |
|---|---|---|
| *L. digitata* | 37.66±0.00a | 1.0±0.00a |
| *L. saccharina* | 66.75±3.72b | 10.0±0.16b |
| *H. elongata* | 151.33±6.75c | 0.125±0.14c |
| *P. palmata* | 42.83±3.26d | 25.0±0.06d |
| *C. crispus* | 62.33±1.04b | 5.0±0.04e |
| *E. spirulina* | 49.75±0.41e | 50.0±0.04f |
| AscA | 178.75±2.58f | 1.0±0.09a |

Each value is presented as mean ± SD (n = 6). Means within each column with different letters (a-f) differ significantly ($p < 0.05$).
[a]refers to µg/ml of extract required to reduce DPPH radical by 50% ($EC_{50}$)

(Athukorala *et al.*, 2006). The total phenolic contents of dried methanol extracts of six Irish seaweeds as obtained in this study are presented in Table 2. The total phenolic content of studied seaweeds ranged from 37.66 to 151.33 mg GAE/g of extract. Brown seaweed, *H. elongata* exhibited highest phenolic content; as compared to other brown species, *L. digitata* and *L. saccharina* which had total phenolic contents of 37.6 and 64.75 mg GAE/g extract, respectively. Red and green seaweeds contained less than half the phenolic content of *H. elongata*, but similar to that of the brown *L. digitata* species; *C. crispus*, *E. spirulina* and *P. palmata* contained 62.33, 49.75 and 42.83 mg GAE/g extract, respectively.

Reports have revealed that phenolic compounds are one of the most effective antioxidants in brown algae (Nagai and Yukimoto, 2003). The total phenolic content results of *H. elongata* and *L. saccharina* obtained in this study were higher than some reports for other brown seaweeds. Chandini *et al.* (2008) reported that brown seaweed extracts had a phenolic content of 24.61 and 49.16 mg GAE/g of seaweed extract. Ganesan *et al.* (2008) reported crude methanolic extracts of red seaweeds to yield results in the range of 1.5 to 4.1 mg GAE/g, which is lower phenolic contents than the red species studied in this work.

Wang *et al.* (2009) reported the total phenolic content in different Icelandic seaweeds ranging from 4 to 242 mg PGE/g extract; *P. palmata* had the lowest total phenolic content of the seaweeds tested, which is in agreement with the results obtained for red seaweeds in the present study. However, the total phenolic content of *P. palmata* was over ten times lower in the Icelandic seaweeds (Wang *et al.*, 2009). The results of the present study are promising as algal polyphenolic compounds are effective antioxidants in delaying oil rancidity, therefore the seaweed extracts could have potential in food applications (Yan *et al.*, 1996).

*DPPH radical scavenging activity of methanolic seaweed extracts*

DPPH reagent has been used extensively for investigating the free radical scavenging activities of compounds. In the DPPH test, the dried extracts are potentially able to reduce the stable DPPH radical to the yellow coloured diphenylpricrylhydrazine. The assay is based on the reduction of alcoholic DPPH solution in the presence of a hydrogen-donating antioxidant due to the formation of the non-radical form DPPH-H by the reaction (Shon *et al.*, 2003). DPPH results are often interpreted as the "efficient concentration" or $EC_{50}$ value, which is defined as the concentration of substrate that causes 50% loss of the DPPH activity (Molyneux, 2004).

The $EC_{50}$ values of DPPH radical scavenging activity from dried methanolic extracts of seaweeds are presented in Table 2. The antioxidant activities of all six seaweeds were significantly different ($p < 0.05$), and $EC_{50}$ levels ranged from 0.125 to 50 µg/ml. Brown seaweed, *H. elongata* showed highest activity achieving 50% inhibition of DPPH radical at a concentration as low as 0.125 µg/ml of extract. Other brown seaweeds also achieved good inhibition at $EC_{50}$ levels of 1 and 10 µg/ml of extract (*L. digitata* and *L. saccharina*, respectively). Red and green seaweeds, *P. palmata* and *E. spirulina* had significantly lower antioxidant activity with $EC_{50}$ levels at 25 and 50 µg/ml, respectively ($p < 0.05$). *C. crispus* had an $EC_{50}$ level of 5 µg/ml which was the best among the red seaweed species studied in this present work.

The results of the present study are in line with Wang *et al.* (2009) and Yan *et al.* (1999), who also found that brown algae contained higher amounts of polyphenols and DPPH radical scavenging activity than red and green algae. However, Chandini *et al.* (2008) reported low levels of DPPH radical scavenging activity in brown seaweeds, in the range of 17.79 to 23.16% at an extract concentration of 1000 µg/ml. Also, Duan *et al.* (2006) reported the DPPH radical scavenging activity of crude extract of red alga, *Polysiphonia urceolata* to be half that of the red seaweed species utilised in the present study. Wang *et al.* (2009) reported on the antioxidant activities of Icelandic seaweeds and found that brown species exhibited the most effective scavenging ability on

**Table 3.** Total condensed tannins and total flavonoid content of methanolic extracts of *L. digitata, L. saccharina*, *H. elongata, P. palmata, C. crispus* and *E. spirulina*

| Species | Total condensed tannins (mg CE/g) | Total flavonoids (mg QE/g) |
|---|---|---|
| *L. digitata* | 5.44±0.23a | 7.66±0.60a |
| *L. saccharina* | 6.17±0.32b | 9.66±0.25b |
| *H. elongata* | 38.34±0.91c | 42.50±0.86c |
| *P. palmata* | 3.19±0.35d | 6.83±0.25d |
| *C. crispus* | 4.35±0.55e | 7.41±0.49ad |
| *E. spirulina* | 3.21±0.50d | 19.05±0.73e |

Each value is presented as mean ± SD (n = 6). Means within each column with different letters (a-e) differ significantly ($p < 0.05$).

DPPH radicals with an $EC_{50}$ of 10.7 g/ml. These results are substantially less effective than that of all six species of seaweeds in the present study which shows that the Irish seaweeds have good potential as sources of natural antioxidants.

*H. elongata* had significantly more activity than the ascorbic acid control ($p < 0.05$) at the $EC_{50}$ level; however at higher concentrations the activity of ascorbic acid rapidly increased to 97.11% (10 µg/ml ascorbic acid). The ability of seaweed extracts to quench free radicals is known to take place over longer period of time than rapid acting synthetic antioxidants such as BHA. This may have benefits for extending the shelf life of processed foods during distribution and storage which may be a potential application for the extracts used in this study. The three brown seaweed extracts in this study had very effective DPPH radical scavenging activity as they had $EC_{50}$ values at low levels (below 10 µg/ml) of extract. This would be beneficial for industrial application as low quantities of seaweed extracts could be utilised as effective antioxidants.

In the present study, *H. elongata* had the best DPPH activity and also the highest total phenolic content. However, as previously discussed, *L. saccharina* had a significantly higher phenolic content than *L. digitata* ($p < 0.05$), but *L. digitata* had higher DPPH scavenging activity than *L. saccharina*. Also, *L. saccharina* and *C. crispus* had similar phenolic content ($p < 0.05$) yet there was a significant difference in their antioxidant activity. Brown seaweed *L. digitata* had a lower phenolic content than the red and green seaweeds, however it had significantly higher antioxidant activity ($p < 0.05$). This result implies that DPPH radical scavenging activity may not be directly related to total phenolic content which indicates the role of other compounds in the antioxidant activity of seaweeds. Antioxidant activity of marine algae may arise from pigments such as chlorophylls and carotenoids, vitamin and vitamin precursors including α-tocopherol, β-carotene, niacin, thiamine and ascorbic acid, phenolics such as polyphenolics and hydroquinones and flavonoids, phospholipids particularly phosphatidylcholine, terpenoids, peptides, and other antioxidative substances, which directly or indirectly contribute to the inhibition or suppression of oxidation processes (Shahidi, 2008).

*Total flavonoids and total condensed tannins of methanolic seaweed extracts*

The total flavonoid content of the dried seaweed extracts are presented in Table 3. Total flavonoids in the seaweeds ranged from 7.66 to 42.5 mg QE/g of extract. Brown species, *H. elongata* contained significantly higher total flavonoid contents ($p < 0.05$) than the other brown seaweeds studied in this work. Red seaweeds, *P. palmata* and *C. crispus* had the lowest total flavonoid contents at 6.83 and 7.41 mg QE/g extract, respectively ($p < 0.05$). Kahkonen *et al.* (1999) stated that flavonoids are probably the most important natural phenolics due to their broad spectrum of chemical and biological activities, including antioxidant and free radical scavenging properties. Flavonoids have been reported as antioxidants, scavengers of a wide range of reactive oxygen species and inhibitors of lipid peroxidation, and also as potential therapeutic agents against a wide variety of diseases (Ross and Kasum, 2002; Williams *et al.*, 2004).

Total condensed tannin content of dried methanolic extracts of *L. digitata, L. saccharina*, *H. elongata, P. palmata, C. crispus* and *E. spirulina* can be seen in Table 3. Condensed tannins of the studied seaweeds ranged from 3.19 to 38.34 mg CE/g of extract. The content of tannins from the brown seaweed, *H. elongata* was significantly higher than the other species ($p < 0.05$). Brown species *H. elongata*, *L. saccharina* and *L. digitata* contained 38.34, 6.17 and 5.44 mg CE/g extract, respectively. The red and green species contained lower total condensed tannins than the brown seaweeds, in the range 3.19 to 4.35 mg CE/g of seaweed extract. Phlorotannins are a group of phenolic compounds which are restricted to polymers of phloroglucinol and have been identified from several brown algae. Many studies have shown that phlorotannins are the only phenolic group detected in brown algae (Jormalainen and Honkanen, 2004; Koivikko *et al.*, 2007). Phlorotannin concentrations vary greatly among different species of brown seaweeds, as well as among different geographical areas. Concentration of phlorotannins are generally

**Table 4.** Percentage inhibition of most effective concentrations of methanolic extracts of *L. digitata, L. saccharina*, *H. elongata, P. palmata, C. crispus* and *E. spirulina* against selected food pathogenic [(a)] and food spoilage [(b)] bacteria

| Species | *L. monocytogenes*[a] | *S. abony*[a] | *E. faecalis*[b] | *P. aeruginosa*[b] |
|---|---|---|---|---|
| *L. digitata* | 100.00±00a | 86.36±3.50a | 75.35±3.65a | 72.16±2.34a |
| *L. saccharina* | 93.49±2.00b | 98.16±2.07b | 97.07±2.67bc | 93.66±3.84b |
| *H. elongata* | 100.00±0.00a | 100.00±0.00b | 100.00±0.00c | 98.40±2.49c |
| *P. palmata* | 62.09±4.11c | 2.21±0.87c | 39.28±4.36d | 19.22±3.60d |
| *C. crispus* | -3.88±1.36d | -10.70±0.96d | -66.08±3.89e | -31.72±1.52e |
| *E. spirulina* | 44.72±1.77e | 21.21±3.69e | 24.13±1.18f | 12.45±3.72f |
| Sodium benzoate | 96.55±4.07ab | 97.34±3.07b | 89.42±3.72g | 99.04±0.58c |
| Sodium nitrite | 96.19±2.93ab | 98.45±2.26b | 93.75±2.16gb | 97.27±2.69bc |

Each value is presented as mean ± SD (n = 6). Means within each column with different letters (a-g) differ significantly ($p < 0.05$).

**Table 5.** Percentage inhibition of methanolic extract of *L. digitata* against selected food pathogenic [(a)] and food spoilage [(b)] bacteria

| Concentration (mg/ml) | *L. monocytogenes*[a] | *S. abony*[a] | *E. faecalis*[b] | *P. aeruginosa*[b] |
|---|---|---|---|---|
| 8.00 | 100.00±0.00a | 86.36±3.50a | 75.35±3.65a | 72.16±2.34a |
| 4.00 | 98.28±2.75a | 63.13±3.11b | 51.52±3.79b | 47.65±4.87b |
| 2.00 | 75.34±2.74b | 51.48±1.67c | 38.55±2.27d | 37.11±2.75cd |
| 1.00 | 66.29±3.74c | 48.02±1.88c | 43.94±2.98cd | 40.88±1.39bc |
| 0.50 | 62.29±3.90c | 45.94±4.22c | 45.33±3.15bc | 30.89±4.48d |
| 0.250 | 60.48±4.53c | 48.01±3.10c | 44.63±4.01cd | 35.13±3.74cd |
| 0.125 | 60.86±3.80c | 49.67±2.48c | 45.17±2.88c | 34.65±0.79cd |

Each value is presented as mean ± SD (n = 6). Means within each column with different letters (a-d) differ significantly ($p < 0.05$).

greater in fucoid species than in kelps, which is in line with the results of the present study as *H. elongata* (fucoid species) contained higher condensed tannins than the kelps, *L. digitata* and *L. saccharina* (Pavia and Toth, 2000). *H. elongata* was the most potent DPPH and hydroxyl radical scavenger, and also contained the highest levels of phenols, tannins and flavanoids. This suggests that algal polyphenols including tannins and flavonoids may be the principal constituents responsible for the antiradical properties of extracts from this species.

*Antimicrobial activity of methanolic seaweed extracts*

Food poisoning is a concern for both consumers and the food industry despite the use of various preservation methods. Food processors, food safety regulators and regulatory agencies are continuously concerned with the high and growing number of illness outbreaks caused by some pathogenic and spoilage microorganisms in foods. Recently, consumers are demanding foods which are fresh, natural and minimally processed. Along with this, consumers are also concerned about the safety of foods containing synthetic preservatives. This has put pressure on the food industry and has fuelled research into the discovery of alternative natural antimicrobials (Shan *et al.*, 2007). In the majority of reports on antimicrobial activities of seaweed extracts, bacterial growth inhibiting activities were investigated by standard agar disc diffusion assays (Bansemir *et al.*, 2006; Kuda *et al.*, 2007; Shanmughapriya *et al.*, 2008). There have been few reports on quantitative methods being utilised for seaweed extracts such as the 96-well method which was applied in this study. Percentage inhibition of each seaweed extract was calculated over a 24 hour period and the assays revealed different susceptibilities of the bacteria under investigation to the seaweed extracts.

The entire yield of dried extract from 5g of each of the fresh seaweeds was dissolved in 2.5ml TSB and utilized in the assay. The percentage inhibition of the highest concentration of methanolic seaweed extracts against food spoilage and food pathogenic bacteria are presented in Table 4. Generally, all of the common food spoilage and food pathogenic bacteria under investigation (*L. monocytogenes*, *S. abony*,

**Table 6.** Percentage inhibition of methanolic extract of *L. saccharina* against selected food pathogenic [(a)] and food spoilage [(b)] bacteria

| Concentration (mg/ml) | *L. monocytogenes*[a] | *S. abony*[a] | *E. faecalis*[b] | *P. aeruginosa*[b] |
|---|---|---|---|---|
| 6.20 | 93.49±2.00a | 98.16±2.07a | 97.07±2.67a | 93.66±3.84a |
| 3.10 | 86.15±2.50b | 87.38±3.17b | 78.26±3.77b | 76.21±1.76b |
| 1.55 | 76.47±3.24c | 71.94±3.99c | 78.56±3.07b | 72.97±3.90bc |
| 0.775 | 74.66±2.38c | 77.13±2.05d | 81.63±3.60b | 77.12±3.97bc |
| 0.387 | 76.35±1.38c | 73.84±2.05cd | 86.38±2.81c | 77.10±2.19bc |
| 0.194 | 76.54±2.15c | 77.80±2.18d | 86.97±3.37c | 81.24±3.18cd |
| 0.097 | 76.17±2.48c | 77.48±1.80d | 88.49±2.77c | 82.31±2.22d |

Each value is presented as mean ± SD (n = 6). Means within each column with different letters (a-d) differ significantly ($p < 0.05$).

*E. faecalis* and *P. aeruginosa*) were susceptible to the seaweed extracts, causing substantial growth inhibition of the test strains, with the exception of *C. crispus* extracts which enhanced the growth. The entire spectrum of inhibitory effects is reported as outlined in Table 1.

At the highest dried extract concentrations (8, 6.4, 6.2, 5, 4.8 and 5.8 mg/ml for *L. digitata*, *H. elongata*, *L. saccharina*, *P. palmata, C. crispus* and *E. spirulina,* respectively), all of the brown seaweeds had above 70% inhibition. *H. elongata* had the highest antimicrobial activity achieving strong to very strong activity at 6.4mg/ml of extract against *L. monocytogenes*, *S. abony* and *E. faecalis*, and *P. aeruginosa* (100, 100, 100 and 98.4%, respectively). This was followed by *L. saccharina* which had strong to very strong activity against *S. abony*, *E. faecalis*, *P. aeruginosa* and *L. monocytogenes* (98.16, 97.07, 93.66 and 93.49%, respectively). *L. digitata* displayed very strong activity against *L. monocytogenes* (100%) and moderate activity against *S. abony*, *E. faecalis* and *P. aeruginosa* (86.36, 75.35 and 72.16%, respectively).

In the case of antimicrobial activity against *L. monocytogenes*; there was no significant difference between the activity of brown seaweed extracts of *L. digitata* and *H. elongata*; however, *L. saccharina* had significantly lower activity ($p < 0.05$) compared to the other two brown seaweed species studied. When tested against *S. abony*, *E. faecalis* and *P. aeruginosa*; brown seaweeds, *H. elongata* and *L. saccharina* had significantly stronger activity than *L. digitata* ($p < 0.05$). The antimicrobial activities of the red and green seaweed extracts were significantly lower against the four strains of food spoilage and food pathogenic bacteria than the brown seaweed species ($p < 0.05$). *P. palmata* had moderate antimicrobial activity against *L. monocytogenes* (62.09%) and weak activity against *E. faecalis*, *P. aeruginosa* and *S. abony* (39.28, 19.22 and 2.21%, respectively). Methanolic extracts of *C. crispus* were the only seaweed extracts which had no antimicrobial activity against the four strains tested as it enhanced the growth of the food spoilage and food pathogenic bacteria. A possible explanation for this may be the presence of certain sugars and proteins in this seaweed species which could have enhanced the growth of the bacteria (Galland-Irmouli *et al.*, 1999). Green seaweed, *E. spirulina* had weak activity against *L. monocytogenes*, *E. faecalis*, *S. abony* and *P. aeruginosa* with inhibitions of 44.72, 24.13, 21.21 and 12.45%, respectively.

Sodium benzoate and sodium nitrite were utilised as controls; sodium benzoate is a common food preservative used in salad dressings, carbonated drinks and condiments. Sodium nitrite is a preservative used in meats and fish. According to the European Food Directive 95/2/EC on "Food Additives other than Colours and Sweeteners", the maximum permitted level of sodium benzoate is 0.15-2 g/kg depending on the food product. Sodium nitrite is permitted at 0.3 g/kg in meat products. Sodium benzoate and sodium nitrite had similar bacterial growth inhibition to the brown seaweed extracts tested. This implies that the seaweed extracts examined in this study have activity in the same line as commercially applied antimicrobials.

Food spoilage due to the presence of bacteria causes economic losses on a global scale. Since many marine plants have evolved potent defence mechanisms against bacteria; there is interest in seaweeds as a source of natural antifouling and antimicrobial sources. In this context, the results of the present study show that extracts of brown seaweeds could have potential as a source for new antimicrobial agents equal to that of commercially applied synthetic antibacterial agents.

Since the brown seaweeds were the most effective antimicrobials, significant differences between the different brown seaweed extract concentrations tested were found against food pathogenic and food spoilage bacteria and results are presented in Tables 5, 6 and

**Table 7.** Percentage inhibition of methanolic extract of *H. elongata* against selected food pathogenic [(a)] and food spoilage [(b)] bacteria

| Concentration (mg/ml) | *L. monocytogenes*[a] | *S. abony*[a] | *E. faecalis*[b] | *P. aeruginosa*[b] |
|---|---|---|---|---|
| 6.40 | 100.00±0.00a | 100.00±0.00a | 100.00±0.00a | 98.40±2.49a |
| 3.20 | 97.37±0.65ab | 95.40±1.42a | 88.41±2.13b | 91.16±1.52a |
| 1.60 | 96.14±2.26ab | 72.99±4.57b | 100.00±2.33a | 76.98±3.30b |
| 0.80 | 92.10±4.96b | 53.53±1.92c | 86.71±3.17b | 50.74±2.95c |
| 0.40 | 90.67±3.01b | 36.70±0.81d | 55.14±3.98c | 43.68±3.45c |
| 0.20 | 79.76±1.90c | 5.10±1.46e | 34.32±2.09d | 33.84±3.77d |
| 0.10 | 54.41±4.91d | 0.55±0.85e | 10.36±4.31e | 8.42±1.92e |

Each value is presented as mean ± SD (n = 6). Means within each column with different letters (a-e) differ significantly ($p < 0.05$).

7 as they were the most effective antimicrobials. Percentage inhibition of *L. monocytogenes* was equally as effective when using 4mg of *L. digitata* extract as double the concentration at 8 mg/ml ($p > 0.05$). However, the antimicrobial activities from 1 to 0.125 mg/ml extract were significantly lower ($p < 0.05$) achieving moderate activity (< 66.29% inhibition). When tested against *S. abony*, *E. faecalis* and *P. aeruginosa*; *L. digitata* extracts at concentration of 8mg/ml were significantly higher than all other concentrations tested ($p < 0.05$) and had weak activity (< 50% inhibition at 1 mg/ml and lower).

*L. saccharina* extracts (Table 6.) exhibited strong inhibition activity at the highest concentration (6.20 mg/ml) against *S. abony*, *E. faecalis*, *P. aeruginosa* and *L. monocytogenes*, (98.16, 97.07, 93.66 and 93.49%, respectively). This was significantly higher than all other concentrations of extracts tested ($p < 0.05$). However, even at the lowest concentration of extract tested (0.097 mg/ml) the activity against all tested bacteria was above 76%, which was stronger than that of the other brown seaweeds. This could possibly be due to the high concentration of polysaccharides in *L. saccharina* which are known to have antimicrobial properties (Yamashita *et al.*, 2001). There was no significant difference ($p > 0.05$) in the activity of *H. elongata* extracts at concentrations of 6.4 and 3.2 mg/ml against the tested bacteria (Table 7). Starting from a concentration of 1.60mg/ml and lower, there were significant differences in inhibition activity ($p < 0.05$). At concentrations of 0.4, 0.2 and 0.1mg/ml; *H. elongata* extracts had weak activity against *S. abony*, *E. faecalis* and *P. aeruginosa* (< 50%).

The results of the present study indicated that the antimicrobial activity of seaweed extracts were concentration dependant. At higher extract concentrations, *H. elongata* had the strongest activity ($p < 0.05$) compared to the other two brown seaweeds; however at lower concentrations, *L. saccharina* was most effective against the food spoilage and food pathogenic bacteria tested.

Analysis of the most effective concentration of extracts against food spoilage and food pathogenic bacteria was performed over time. Figure 1 illustrates antimicrobial activity of the three brown seaweed extracts which were most effective over a 24 hour period. The seaweed extracts inhibited growth of each of the bacteria tested from the first hour resulting in lag phase extension. All three methanolic brown seaweed extracts displayed inhibition activity similar to that of the commercial controls.

In previous reports investigating the effects of marine macroalgae extracts; antimicrobial compounds were detected in extracts of red and brown algae including *L. digitata* (Hellio *et al.*, 2001; Bansemir *et al.*, 2006; Dubber and Harder, 2008). *L. saccharina* and *H. elongata* have been less studied with regard to antimicrobial activity. Ely *et al.* (2004) analysed the antimicrobial activity of a brown seaweed *Sargassum marginatum* against two species of fish pathogenic bacteria and found its activity weak at 5 mg/ml. Comparing this to the results in the present study; at approximately similar concentration, all brown seaweeds had higher activity with moderate inhibition intensity against the four species of bacteria tested.

The phenolic compounds of the dried seaweed extracts may be responsible for their antimicrobial properties. Other researchers have also reported that phenolic compounds from different plant sources could inhibit various foodborne pathogens (Smid and Gorris, 1999; Prashanth *et al.*, 2001; Kim *et al.*, 2005; Plaza *et al.*, 2009). Polyphenols, such as tannins and flavonoids, are important antibacterial substances, for example ellagitannin from pomegranates is the active substance responsible for its antimicrobial activity (Machado *et al.*, 2002).

Finally, this study utilised a substantially more sensitive detection method to quantify antimicrobial effects of seaweed extracts as compared to previous studies, which mainly relied on rather insensitive

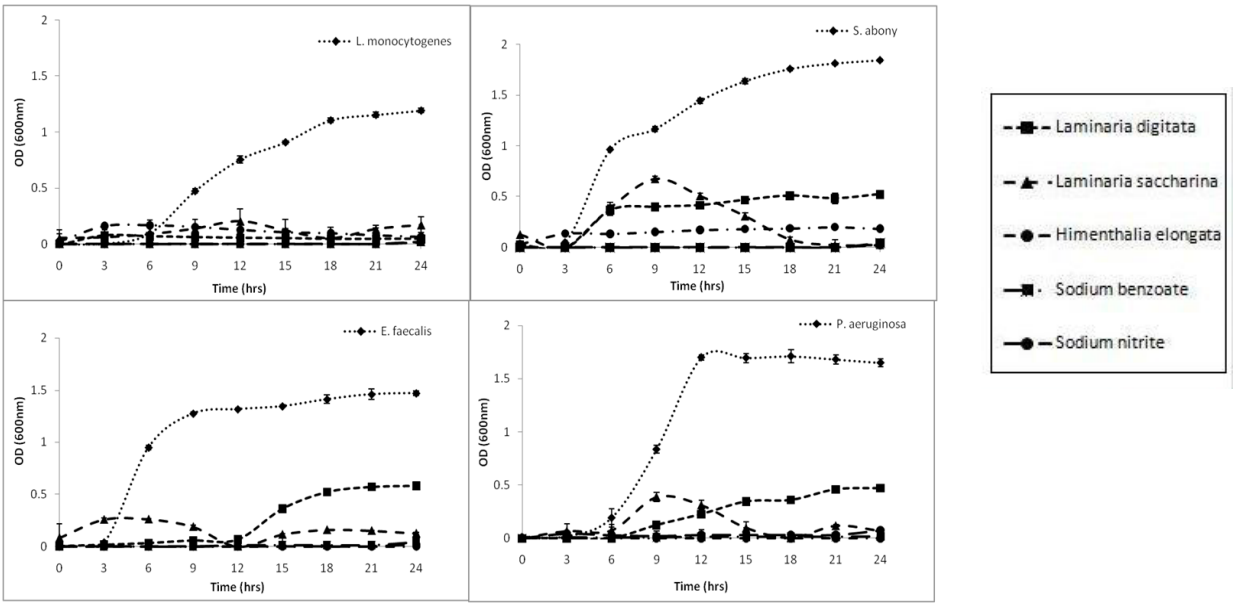


**Figure 1.** Growth inhibition analysis of the highest effective concentration of brown seaweed extracts against common food spoilage and food pathogenic bacteria

**Table 8.** Extraction efficiencies of methanol, ethanol and acetone on total phenolic content and antioxidant activity of *P. palmata* (8a.), *C. crispus* (8b.) and *E. spirulina* (8c.)

(8a.)

| Solvent | Total phenolic content (mg GAE/g) | Antioxidant activity $EC_{50}$ [a] |
|---|---|---|
| 60% methanol | 42.83±3.26a | 25.00±0.06a |
| 60% ethanol | 30.00±0.00b | 25.00±0.35a |
| 60% acetone | 38.00±2.82c | 25.00±1.12a |

(8b.)

| Solvent | Total phenolic content (mg GAE/g) | Antioxidant activity $EC_{50}$ [a] |
|---|---|---|
| 60% methanol | 62.33±1.04ab | 5.00±0.04a |
| 60% ethanol | 61.00±2.82a | 10.00±0.64b |
| 60% acetone | 62.00±2.13b | 10.00±0.88b |

(8c.)

| Solvent | Total phenolic content (mg GAE/g) | Antioxidant activity $EC_{50}$ [a] |
|---|---|---|
| 60% methanol | 49.75±0.41a | 50.00±0.04a |
| 60% ethanol | 24.50±0.54b | 50.00±0.19a |
| 60% acetone | 24.50±0.07b | 50.00±0.72a |

Each value is presented as mean ± SD (n = 6). Means within each column with different letters (a-c) differ significantly ($p < 0.05$).

[a]refers to µg/ml of extract required to reduce DPPH radical by 50% ($EC_{50}$)

standard agar disc diffusion assays. The microtitre assay applied in this study allowed us to detect antimicrobial effects of algal metabolites at and below algal tissue level concentrations which would otherwise have been undetected in the agar disc diffusion assay (Dubber and Harder, 2008).

*Extraction efficiencies of methanol, ethanol and acetone on antioxidant and antimicrobial activity*

The dried methanolic extracts of three brown species of seaweeds were found to have good antioxidant and antimicrobial activity. Although a variety of solvents have been employed in screening algae for antioxidant and antimicrobial activity, it is still uncertain what kind of solvent is the most effective and suitable for extraction from seaweeds (Zheng *et al.*, 2001). In order to potentially enhance the antioxidant and antimicrobial content from red and green species, ethanol and acetone were utilised as extraction solvents. Phenolic compounds are generally more soluble in polar organic solvents than in water, therefore the effective extractants recommended are aqueous mixtures of methanol, ethanol and acetone (Waterman and Mole, 1994). With such polar solvents, phenolic compounds which are attached to sugar or protein, saponins, glycosides, organic acids, tannins, salts, and mucus can be extracted (Cho *et al.*, 2007). The extraction efficiencies of methanol, ethanol and acetone on total phenolic content and antioxidant activity of the red and green seaweed species are presented in Table 8. The total phenolic content of methanol extracts of *P. palmata* at a concentration of 1mg/ml extract was 42.83 mg GAE/g (Table 8a.) which was significantly higher than that of acetone (38 mg GAE/g) and ethanol (30 mg GAE/g extract). However, there was no significant difference in the antioxidant activity of methanol, ethanol and acetone extracts of *P. palmata*, which were all 25 µg/ml ($p < 0.05$).

Methanol was also the best solvent for extraction from *C. crispus* (Table 8b.), obtaining a total phenolic content of 62.33 mg GAE/g and an $EC_{50}$ level for antioxidant activity at 5 µg/ml which was half that of the ethanol and acetone extracts ($p < 0.05$). *E. spirulina* (Table 8c.) had a significantly higher total phenolic content from methanol extracts (49.75 mg GAE/g) than ethanol and acetone extracts which was 24.5 mg GAE/g extract for both ($p < 0.05$). Also there was no significant difference in the antioxidant activity with all solvents giving $EC_{50}$ levels at 50 µg/ml ($p < 0.05$).

As discussed, methanol extracts of red and green seaweeds had higher or the same total phenolic content and DPPH radical scavenging activity as ethanol and acetone extracts. This implies that the bioactive metabolite in the algae might be readily soluble in methanol and slightly more sparingly soluble in ethanol and acetone. Extracts of red seaweeds have been reported to exhibit weak DPPH radical quenching activity when obtained using water, ethanol or methanol as solvents (Matsukawa *et al.*, 1997; Han *et al.*, 1999), while chloroform, ethyl acetate and acetone extracts from several red seaweeds have been reported to exhibit strong DPPH quenching activity (Yan *et al.*, 1998). Therefore, the particular solvent used to extract the seaweed material will have a dramatic effect on the compounds in the extract recovered. For example, the non-polar fraction from chloroform: methanol extraction of *Porphyra tenera* exhibited a significant protective effect against lipid oxidation associated with phospholipids such as, phosphatidyl choline and phosphatidyl ethanolamine (Kaneda and Ando, 1971). Whereas, Nakayama *et al.* (1999) reported that hexane, chloroform and methanol extracts of red seaweed, *Porphyra yezoensis* exhibited antioxidant activities attributed to the presence of β-carotene, chlorophyll analogues and amino compounds. Overall, methanol was the most effective solvent for extraction of antioxidant properties from seaweeds, which may be due to methanol having a higher dielectric constant than ethanol and acetone.

The extract efficiencies of methanol, ethanol and acetone on antimicrobial activity of red and green seaweeds are outlined in Table 9. Methanolic extracts of *P. palmata* (Table 9a.) had double the antimicrobial activity against *L. monocytogenes* (62.09%) than acetone and ethanol extracts ($p < 0.05$). In the case of *S. abony*, acetone extracts of *P. palmata* had the highest antimicrobial activity (23.39%), while for inhibiting *P. aeruginosa,* ethanol extracts were most effective achieving 93.89% inhibition which was significantly higher than that of methanol ($p < 0.05$). There was no significant difference in the antimicrobial activity of ethanol and acetone extracts of *P. palmata* against *E. faecalis* both achieving 100% inhibition, however, methanolic extracts were significantly lower with only 2.21% inhibition.

Acetone extracts of *C. crispus* (Table 9b.) had a significantly higher antimicrobial activity against *E. faecalis, P. aeruginosa* and *L. monocytogenes* than methanol extracts ($p < 0.05$). There was a much lower activity against *S. abony*; for all the extraction solvents tested, ethanol was the best extraction solvent providing only 0.8% inhibition. However, ethanol significantly increased the inhibition against *E. faecalis*, from a 66.08% increase in growth with methanol, to 100% with ethanol as solvent. This was

**Table 9.** Extraction efficiencies of methanol, ethanol and acetone on antimicrobial activity of *P. palmata* (9a.), *C. crispus* (9b.) and *E. spirulina* (9c.) against selected food pathogenic [(a)] and food spoilage [(b)] bacteria

(9a.)

| Solvent (60%) | *L. monocytogenes*[a] | *S. abony*[a] | *E. faecalis*[b] | *P. aeruginosa*[b] |
|---|---|---|---|---|
| methanol | 62.09±4.11a | 2.21±0.87a | 39.28±4.36a | 19.22±3.60a |
| ethanol | 37.78±1.70b | 0.05±1.99b | 100.00±2.77b | 93.89±3.87b |
| acetone | 37.94±2.93b | 23.39±4.13c | 100.00±4.01b | 78.34±2.30c |

(9b.)

| Solvent (60%) | *L. monocytogenes*[a] | *S. abony*[a] | *E. faecalis*[b] | *P. aeruginosa*[b] |
|---|---|---|---|---|
| methanol | -3.88±1.36a | -10.70±0.96a | -66.08±3.89a | -31.72±1.52a |
| ethanol | 50.27±2.26b | 0.80±2.87b | 100.00±0.38b | 61.51±5.34b |
| acetone | 56.13±2.85c | -4.70±2.02c | 89.74±2.68c | 81.74±4.96c |

(9c.)

| Solvent (60%) | *L. monocytogenes*[a] | *S. abony*[a] | *E. faecalis*[b] | *P. aeruginosa*[b] |
|---|---|---|---|---|
| methanol | 44.72±1.77a | 21.21±3.69a | 24.13±1.18a | 12.45±3.72a |
| ethanol | 81.95±4.30b | 63.36±3.19b | 97.58±1.45b | 65.63±2.09b |
| acetone | 100.00±2.53c | 98.26±3.53c | 94.36±1.79c | 91.30±2.65c |

Each value is presented as mean ± SD (n = 6). Means within each column with different letters (a-c) differ significantly ($p < 0.05$).

a significant finding and implies that antimicrobial properties are substantially affected by solvent selection.

Ethanol was the most effective solvent for extraction from *E. spirulina* (Table 9c.) against *E. faecalis* and *L. monocytogenes* (97.58 and 81.95% inhibition, respectively); and acetone was the most effective extraction solvent against *P. aeruginosa* and *S. abony* with 91.3 and 68.26% inhibition, respectively ($p < 0.05$). The use of ethanol and acetone increased the antimicrobial activity of the seaweed extracts significantly dependant on the food spoilage and food pathogenic bacteria being considered. *P. palmata* in particular had weak activity against *E. faecalis* and *P. aeruginosa,* however, with ethanol extraction inhibition of bacteria increased to 100 and 93.89%, respectively. Acetone was a significantly better solvent against *E. faecalis* and *P. aeruginosa* achieving 89.74 and 81.74% inhibition, respectively. This was an interesting finding as with methanol extraction *C. crispus* extracts were enhancing the growth of the bacteria up to 66.08%. Methanolic extracts of *E. spirulina* were also weak but with ethanol and acetone as solvents they were increased to moderate and strong. The results imply that the bioactive secondary metabolite in the red and green seaweeds might be more soluble in ethanol and acetone and sparingly soluble in methanol.

Previous reports on the most effective solvent for the extraction of antimicrobials have been varied; Gonzalez del Val *et al.* (2001) selected methanol as solvent for extraction of antimicrobial compounds from red, green and brown seaweeds. Shanmughapriya *et al.* (2008) found methanol:toluene (3:1) as the best solvent for extracting antimicrobials from fresh algae. The same author also reported that ethanolic extracts had no antibacterial activity. Whereas Parekh *et al.* (1984) reported that extracts obtained with acetone, ethyl alcohol and ether showed higher antimicrobial activity than chloroform extracts. Plaza *et al.* (2009) found significant differences in the antimicrobial activity depending on the solvent used. These authors found that extracts obtained using ethanol were more active than those obtained with hexane. Plaza *et al.* (2009) also identified several volatile compounds in ethanol extracts from brown seaweeds including *H. elongata*. The compounds which were characterized included fatty acids, alkanes, phenols and compounds such as phytol (2-hexadecen-1-ol,3,7,11,15-tretramethyl) and neophytadiene. Antimicrobial activity has usually been attributed to long-chain unsaturated fatty acids (C16-C20),

including palmitoleic, oleic, linoleic and linolenic acids, while long chain saturated fatty acids, including palmitic and stearic acids were less effective (Zheng *et al.*, 2005).

These results indicate that the extraction solvent has a definite effect on the isolation of bioactive principles. In the present study, the antimicrobial inhibitory activity was only observed in the extracts obtained with a particular solvent (ethanol and acetone) but not as high in those obtained with methanol. This suggests that a particular solvent is required to extract some antimicrobial substances within the algal plant; however ethanol and acetone had no significant effect on increasing the total phenolic content and antioxidant properties of the dried seaweed extracts.

## Conclusions

The results of the present study indicated that Irish seaweeds; *L. digitata, L. saccharina, H. elongata, P. palmata, C. crispus* and *E. spirulina*, successfully displayed antioxidant and antimicrobial activities and contained phenolics, condensed tannins and flavonoids. *H. elongata* had the highest antimicrobial and antioxidant content. The extraction of antimicrobials from the different species of seaweeds was solvent dependent. Methanol was a good solvent for extraction of antimicrobials from brown seaweeds whereas acetone was better for red and green species. The antioxidant content of the seaweeds did not vary significantly with different polar solvents. This is a promising finding, as there may be a potential to utilise such extracts in food products to act as antioxidants which could enhance food quality, and also as antimicrobial agents, which could potentially increase the shelf life and safety of a wide range of food products.

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