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Evaluation of the biochemical and phytochemical components of green seaweed *Enteromorpha intestinalis* (Linnaeus) in Initao, Misamis oriental, Mindanao, Philippines

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Abstract

The green seaweed *Enteromorpha intestinalis* that occurred along the coast of Iligan Bay was analyzed for biochemical and phytochemical components. Powdered samples of *E. intestinalis* were analyzed for carbohydrate, protein, fat, ash and moisture content. Likewise, the presence of reducing sugar, tannins, phenols, saponins, anthraquinones, flavonoids, steroids, terpenoids and alkaloids were determined using the standard methods. The amount of carbohydrate, protein, fat, ash and moisture content was in considerable quantities and within the values specified for seaweeds. The phytochemical analysis revealed the presence of saponins, steroids and terpenoids as well as alkaloids. This study further suggested that *E. intestinalis* possesses both nutritional and pharmaceutical potential.

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Introduction

Seaweed is a term encompassing for macroscopic, multicellular or benthic marine algae. Seaweeds attached to the bottom in relatively shallow coastal waters. They are abundantly found in solid substrates and commonly presenting onto depths of 30 - 40 meters (Pal *et al.,* 2014). They have been used for a variety of purposes like food, medicines and other uses. People in the Philippines and Japan use *Enteromorpha* spp. as food (Hoppe, 1966; Tamura, 1970; Velasquez, 1972). They are valuable food source because they contain high amount of carbohydrates, protein, lipid, vitamins, amino acid and minerals, as well as important bioactive compounds (Chapman and Chapman, 1980; Norziah and Ching, 2000; Ruperez, 2002; Aguilera-Morales *et al.,* 2005; Ortiz *et al.,* 2006; Marsham *et al.,* 2007; Dawczynski *et al.,* 2007). Thus, they have been recognized as being beneficial for human and animal health (Fleurence, 1999). Aside from the nutritional value, it also contains phytochemicals that are extensively used in the confectionary, textile, dairy, paper and pharmaceutical industries (Seenivasan *et al.,* 2012). Phytochemicals are non-nutrient bioactive substances that are responsible for the protection of plants against infestations and microbial infections (Abo *et al.,* 1999; Liu, 2004; Nweze *et al.,* 2004; Doughari *et al.,* 2009).

Some of these phytochemicals are alkaloids, anthraquinones, reducing sugars, tannins, phenols, saponins, flavonoids, steroids, terpenoids, etc. (Tresina and Mohan, 2014). Several seaweeds including *Enteromorpha* have been investigated for their nutritional and biochemical potentials (Haroon, 2000; Saranya *et al.,* 2013; Manivannan *et al.,* 2008; Kasimala *et al.,* 2015). Among the species of *Enteromorpha* which have high economic value is *Enteromorpha intestinalis* (Haroon, 2000). This species has great variety of essential biochemical components (Sauze, 1981) and contains potential bioactive compounds (Manimala and Rengasamy, 1993). However, there is lack of information concerning the biochemical studies and biopotential of *Enteromorpha intestinalis* in Iligan Bay.

With this knowledge, the present study was aimed to analyze the biochemical composition and phytochemical components of *Enteromorpha intestinalis.*

Materials and methods

Collection of Enteromorpha intestinalis

Samples of *E. intestinalis* were collected randomly in the coastal area of Barangay Tubigan, Initao, Misamis Oriental (08° 32.0' North Latitude 124° 18.7' East Longitude) during low tide between January and April 2016. The intertidal and subtidal areas of Barangay Tubigan are made up of rocky substrates which favor the growth of seaweeds. *E. intestinalis* was found growing on rocks and appears to be highly concentrated within 15-25 meters seaward with depth of 33cm - 53cm during low tide and decreases as it goes offshore. The collected samples of *E. intestinalis* were placed in an ice bucket and brought to the laboratory where they were sorted and cleaned with water.

Protein, fat, carbohydrate, ash and moisture analysis

The collected samples of *E. intestinalis* were oven dried at 60°C for 24 hours. After drying, the samples were ground into powder and were brought to the Chemical Testing Laboratory of the Department of Science and Technology, Cagayan de Oro City, Mindanao, Philippines for analysis. Analysis of crude protein was done using Kjeldahl method, total fat by hydrolysis and solvent extraction method, ash content by gravimetric method, moisture content by air oven method, and total carbohydrate content by computational method [Carbohydrate = 100% - (% protein + % fat + % ash + % moisture)]. The method used for the analysis of crude protein, total fat, ash content and moisture content was based on OMA AOAC (2008) and the values were expressed as percentage on dry weight basis.

Phytochemical analysis

Fifteen (15) grams of powdered sample of *E. intestinalis* was soaked with enough volume of 90% ethanol for 24 hours. The resulting mixture was then filtered using an ordinary filter paper to separate the ethanol extract solution from the rest of the powdered material.

The extract was concentrated through water bath to obtain the crude ethanol extract to be used for phytochemical tests. A portion of the crude extract was dissolved in ethanol as material for the different phytochemical analysis such as reducing sugar, tannins and phenols, saponins, anthraquinones, flavonoids, steroids, terpenoids and alkaloids. All analysis was done following the method of Guevara (2005).

Results and discussion

Carbohydrate, protein, fat, ash and moisture contents

Table 1 shows the levels of carbohydrate, protein, fat, ash and moisture contents in *E. intestinalis*. The carbohydrate content was 37.28±1.37% and lower than the range specified for green seaweeds [42-46% DW (Levring *et al.*, 1969; Arasaki and Arasaki, 1983; Nisizawa *et al.*, 1987; Indergaard and Minsaas, 1991)].

Table 1. Levels of carbohydrates, protein, total fat, ash and moisture content measured in *Enteromorpha intestinalis* (mean ± SD). Values are presented as percent (%) dry weight.

Biochemical Constituents	Result of this Study	Range Values for Green Seaweeds (% DW)	Source
Carbohydrate	37.28±1.25	42 - 46	Levring *et al.*, 1969 Arasaki and Arasaki, 1983 Nisizawa *et al.*, 1987 Indergaard and Minsaas, 1991
Protein	5.57±0.06	5 - 20	Wong and Cheung, 2001
Total Fat	0.43±0.02	0.6 - 0.7	Levring *et al.*, 1969 Arasaki and Arasaki, 1983 Nisizawa *et al.*, 1987 Indergaard and Minsaas, 1991
Ash	51.38±1.54	13 - 22	Levring *et al.*, 1969 Arasaki and Arasaki, 1983 Nisizawa *et al.*, 1987 Indergaard and Minsaas, 1991
Moisture	4.93±0.43	16 - 19	Pádua *et al.*, 2004

This low carbohydrate content implies that *E. intestinalis* in the sampling area is in the growing phase as it experienced extensive growth of its thallus. Also, the biochemical constituents of seaweeds can be affected by the change in season such as northeast (amihan) and southwest (habagat) monsoon that is occurring in the country. The environmental conditions as well as various phases of plant growth and development could also affect the change in the amount of biochemical components in the seaweeds.

The present value of carbohydrate was comparable for those reported by some authors working on chemical constituents of *E. intestinalis* [23.84% (Manivannan *et al.*, 2008); 30.58% (Parthiban *et al.*, 2013); 30.58% (Chakraborty and Santra, 2008)].

On the other hand, the protein content was 5.57±0.06% and the value was within the range specified for green seaweeds [5-20% dry weight (Wong and Cheung, 2001)]. Comparable value of protein (6.15%) was obtained by Chakraborty and Santra (2008) for the same species collected in the coast of Sunderban, India.

The total fat content was 0.43±0.02% and the value was lower than the range specified for green seaweeds [0.6-0.7% DW (Levring *et al.*, 1969; Arasaki and Arasaki, 1983; Nisizawa *et al.*, 1987; Indergaard and Minsaas, 1991)]. Generally, seaweeds have very low total fat contents but the essential fatty acids especially present in green seaweeds are still higher than those found in land plants (Dharmananda, 2002).

Table 2. The phytochemical components (secondary metabolites) found in the ethanol extracts of *Enteromorpha intestinalis* and its uses.

Phytochemical Components	Result	Uses	Source
1. Reducing Sugars	-	Precursors for biofuel and biochemical production	Vanegas and Bartlett, 2013
2. Tannins and Phenols	-	Tannins: Healing agents in inflammation and burns Antidote, anti-ulcer and antioxidant Phenols: Antioxidant	Kolodziej and Kiderlen, 2005 Kumar *et al.*, 2008
3. Saponins	+	Antimicrobial Anti-inflammatory Antifeedent Antihemolytic	Xu *et al.*, 1996 Francis *et al.*, 2002
4. Anthraquinones	-	As raw material for manufacturing vat dyes	HSBD, 2010
5. Flavonoids	-	Antitumor and antioxidant	Cody *et al.*, 1988
6. Steroids	+	Insecticidal Antimicrobial Antiparasitic Cardiotonic	Okwu, 2001
7. Terpenoids	+	Antimalarial Anticancer Chemical defense against herbivory Antioxidant	Schnitzler *et al.*, 2001 Martin *et al.*, 2003 Chakkaravarthy and Kumar, 2009
8. Alkaloids	+	Antimicrobial Cytotoxic properties Antioxidant Antiplasmodic	Nobori *et al.*, 1994 Omulokoli *et al.*, 1997 Stray, 1998 Cowan, 1999 Banerjee and Maulik, 2002 Okwu and Okwu, 2004 Srivastava *et al.*, 2010

Legend: (-) negative; (+) positive.

The ash content of *E. intestinalis* was 51.38±1.54%. This result was comparatively higher than those specified for green seaweeds [13-22% DW (Levring *et al.,* 1969; Arasaki and Arasaki, 1983; Nisizawa *et al.,* 1987; Indergaard and Minsaas, 1991)] and higher than those specified for terrestrial counterparts with only 5% - 10% dry weight (USDA, 2001).

The high ash content in seaweeds indicates high amount of mineral components (Mantanjun *et al.,* 2009). In fact, seaweeds are known to contain more than sixty trace elements and this is due to the high ability of seaweeds to absorb elements present in seawater (Chapman and Chapman, 1980; Seenivasan *et al.*, 2012). Moreover, the moisture content which is an important criterion in determining the shelf-life and quality of processed seaweed meals was 4.93±0.43%.

Phytochemical component

Table 2 shows the result of the phytochemical analysis of *E. intestinalis*. It shows that the extract of *E. intestinalis* contained saponins, steroids, terpenoids, and alkaloids, which are known to influence several biological activities in microorganisms and animals, including humans.

Saponins are considered as a key ingredient in traditional Chinese medicine and are responsible for most of the observed biological effects. Saponins are known to produce inhibitory effect on inflammation. There is tremendous, commercially driven promotion of saponins as dietary supplements and nutriceuticals. Saponin possesses specific physical, chemical and biological activities that make them useful as drugs. Some of these biological properties include antimicrobial, anti-inflammatory, antifeedent, and hemolytic effects (Xu *et al.,* 1996; Francis *et al.,* 2002).

Steroids of plant origin are known to be important for insecticidal, antimicrobial, antiparasitic and cardiotonic properties. Steroids also play an important role in nutrition; herbal medicine and cosmetics (Okwu, 2001).

Terpenoids, a class of isoprenoids often isolated from plants, have attracted commercial interest as pharmaceuticals or nutraceuticals (Eisenreich *et al.,* 2001). They are used as commercial flavour and fragrance compounds, as antimalarial or anticancer drugs (Martin *et al.,* 2003), and as chemical defenses against herbivores (Schnitzler *et al.,* 2001). Also, terpenoids have antioxidant properties (Chakkaravarthy and Kumar, 2009).

Alkaloids possess a wide variety of chemical structures and have been identified as responsible for many of the pharmacological properties of medicinal plants (Barbosa *et al.,* 2000a, 2000b; Conserva *et al.,* 2005; de Sousa Falcão *et al.,* 2008; Cunha *et al.,* 2009). Alkaloids are commonly found to have an antimicrobial (Omulokoli *et al.,* 1997; Cowan, 1999; Srivastava *et al.,* 2010) and cytotoxic properties (Nobori *et al.,* 1994). It also produced an antioxidant (Banerjee and Maulik, 2002) and antiplasmodic properties (Stray, 1998; Okwu and Okwu, 2004).

Saponins, steroids and alkaloids were also present in the phytochemical screening of *E. intestinalis* conducted by Kumbhar *et al.* (2014) in Sindhudurg District of Maharashtra as well as in the coastal area of Mumbai, India (Shankhadarwar, 2015). *E. intestinalis* collected from the western coast of Gujarat was positive for saponins and steroids (Nair *et al.,* 2009). Other species of green seaweed like *Ulva lactuca* collected from the Gulf of Mannar Biosphere Reserve (Thinakaran and Sivakumar, 2012) and Abu Kir Beach, Alexandria coast, Egypt (Elmegeed *et al.,* 2014) also contains saponin, alkaloids, steroids and terpenoids. *Ulva fasciata, Chaetomorpha media and Chaetomorpha anteninna* from Sindhudurg District of Maharashtra tested positive on steroids, alkaloids and saponins (Kumbhar *et al.,* 2014).

Enteromorpha compressa and *Enteromorpha prolifera* from the western coast of Libya were also positive for alkaloids, saponins and terpenoids (Alghazeer *et al.,* 2013).

Conclusion

Based from the result of the study, it is concluded that *Enteromorpha intestinalis* is a potential health food in human diet and maybe of use in food industry as a source of ingredients with high nutritional value.

Likewise, the phytochemical analysis revealed the presence of constituents which have medicinal merit. The present study confirmed the presence of saponins, steroids and terpenoids as well as alkaloids. These phytochemicals can be further screened for different kinds of biological activities depending on their therapeutic uses.

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