Hypoglycemic and Laxative Activities of Crude Ethanolic Extracts of Brown Seaweed Sargassum Oligocystum

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Abstract

The quest for the sources of effective yet affordable bioactive compounds for drug development has been intensely done even nowadays. Experts in the field made many attempts by using various plant extracts. Aquatic organisms like seaweeds became their least priority; although there are studies conducted by using several species of algae. Hence, this study determined the anti-spasmodic, hypoglycemic and laxative activities of crude ethanolic extracts of Sargassum oligocystum (CEESO). Extraction was done using the standard laboratory protocols. Two pharmacological assays (antispasmodic and fasting blood sugar) were performed by following the established laboratory procedures. The first one tested if the extract showed anti-spasmodic or laxative activity. The second one detected the ability of the extract to decrease fasting blood sugar level. Mice toxicity test was done to determine the lethal dose of the extract. Findings revealed that CEESO possessed significant anti-diabetic and laxative properties while it failed to demonstrate significant antispasmodic activity. The mice toxicity assay showed that the extract was not toxic to the mice even at the highest dose (1000 ppm) tested. These activities can be associated to the bioactive compounds present in the algal material, which were observed by the previous reports. Seaweed S. oligocystum, therefore, can be a potential source of metabolites for laxative and anti-diabetic drug development. Further investigation should be done on isolation, purification, structure elucidation, and identification of the compounds responsible for laxative and anti-diabetic activities of S. oligocystum.

Keywords: Anti-spasmodic, ethanolic extracts, hypoglycemic, laxative, Sargassum oligocystum

Introduction

Ocean is the home of rich biodiversity. Seaweeds are one of the dominant organisms found in this aquatic community. They have greater economic value, which can be utilized for product development. One of the abundant seaweeds in the marine environment is Sargassum (Family Sargassaceae, Order Fucales). In terms of distribution, Sargassum represents the most common species of brown macroalgae that are widely distributed in tropical to warm temperate waters (Guiry and Guiry, 2013). This organism has a wide variety of forms and reproductive strategies (Mattio and Payri, 2011). These morphological characteristics and reproductive strategies provide important ecological and economic benefits such as in nutrient cycling. Ecologically speaking, intertidal and subtidal Sargassum beds serves as nursery grounds and habitats for various marine organisms. This bed also provides food items and bioactive compounds for the people (Belleme and Belleme 2007; Zhao et al. 2008; Xie et al. 2013).

Sargassum is rich in phycocolloids and bioactive compounds. One of the most common bioactive compounds found in this alga are alginic acid and fucoydan. In addition, polyphenols are also dominantly observed in this macro-alga, a substance which can be utilized for several nutraceutical uses and some medical applications (Nishizawa 2002; Gupta and Abu-Ghanam 2011; Namvar et al. 2013). Hou and Jin (2005) reported that Chinese medicinal practices used Sargassum as an expectorant for some upper respiratory tract infections, hypertension, fever, and goiter.

One of the popular species belonging to genus Sargassum is S. oligocystum. Sargassum oligocystum possesses a holdfast that appears small and discoid. In terms of leaf morphology, the leaves of this species are large, linear-lanceolate to lanceolate or spatulate, simple, with an acute apex, marginindentate with teeth or entire, midrib distinct, reaching near apex. S. oligocystum has cryptostomata that is scattered or arranged in rows on both sides of midrib (Noiraksar et al. 2006). When it comes to reproduction, this seaweed is inherently monoecious; meaning both male and female reproductive organs are found in one thallus. Receptacles are androgyanous, terete to slightly compressed at upper portion, solitary or forked two to three times, warty, without spines or with a few spines, and usually pseudozygocarpic with vesicle (Noiraksar et al., 2006).

Previous reports indicated that Sargassum oligocystum contains various bioactive compounds with potential...
pharmaceutical value (Nishizawa 2002; Namvar et al. 2013). Due to this popularity, it has been used as a subject for natural product development studies. In fact, Zandi et al. (2011) revealed that Sargassum oligocystum gathered from Persian Gulf seashore has an antitumor activity against K562 and Daudi human cancer cell lines. In addition, the extracts of this organism have also been known to possess antibacterial activity. Baleta et al. (2011) indicated that the methanol extract of this algal species showed strong antibacterial activity against three species of bacteria such as V. harveyi, S. faecalis, and P. aeruginosa. On the other hand, Zandi et al. (2011) revealed that the hot water extract of S. oligocystum exhibited antibacterial activity against Staphylococcus aureus, Staphylococcus epidermidis, and Pseudomonas aeruginosa while the coldwater extract and hot glycerin extract did not show antibacterial activity on any of the four test bacteria in the investigation.

In spite of the aforementioned investigations, there are no studies, which have been focused on antispasmodic, laxative, and hypoglycemic properties of Sargassum oligocystum. Hence, it is timely to evaluate the potential biological activities of S. oligocystum from Guimaras Island, Western Philippines with emphasis on its antispasmodic, laxative, and hypoglycemic activities.

Generally, this study sought to evaluate the pharmacological activities of the crude ethanolic extract of Sargassum oligocystum. Specifically, it determined if the crude extract of Sargassum oligocystum has antidiabetic, anti-spasmodic, and laxative activities and determined the lethal dose of the crude ethanolic extract in mice.

Materials and Methods
Collection, Washing and Transport of Sargassum oligocystum
Approximately 20 kg (wet weight) of Sargassum oligocystum was collected in the intertidal zone of Isla Nadulao, Brgy. Igcawayan, San Lorenzo, Guimaras, Western Philippines. The organism was identified using the key to species as suggested by Trono (1997). Collection was done in intertidal zone of the ocean by following the method of Fantonalgo and Salubre (2016). This was carried using a motorized banca during low tide of the day. Collected seaweeds were washed thoroughly using tap water to remove the epiphytes, debris, salts and other unnecessary materials. The washed samples were later put in a clean table made of bamboo to drain excess water. These samples were packed in the polyethylene plastic prior to transport in the laboratory for processing.

Drying and Pulverizing of the Samples
Drying was done in the Research Laboratory, College of Arts and Sciences, Western Institute of Technology (WIT). Individual thallus was hanged in a wire to expose the algal body to the air. Semi-dried seaweeds were cut into smaller pieces using the scissors. After cutting, the seaweeds were dried again at room temperature for one week. Dried algae were pulverized using a blender (Osterizer Model 4172) at the Research Laboratory of the same institution. Semi-pulverized materials were sieved using a strainer with very fine mesh size. The refined materials were stored in a clean beaker (1 L) covered with aluminum foil.

Extraction of the Seaweeds
One (1) kg freeze-dried sample was soaked for 24 to 48 hours in 4 L 80 % analytical-reagent (AR) grade ethanol. The soaking ratio of 1g dried Sargassum sp: 4mL ethanol was adopted from Jaya Prakash Goud et al (2007). The algal material was filtered and the filtrate was collected. The entire process of filtration was repeatedly done until the filtrate of the algae became colorless. Using a rotary evaporator (Eyela), the combined filtrate was concentrated at the Freshwater Aquaculture Station (FAS) Laboratory, Institute of Aquaculture (IA), College of Fisheries and Ocean Sciences (CFOS), University of Philippines Visayas, Miagao, Iloilo. The concentration was done under reduced pressure of 60 mmHg (1.16 psi) and at temperature that ranges from 40°C to 50°C as suggested by Fantonalgo et al. (2009). The ethanolic extract was collected from the surface of the flask when around 5 mL mixture remained in the container. It was later transferred to watch glass for drying. The dried extract was stored to sterilized container for future use.

Pharmacological Activity Assays
The two pharmacological assays performed were antispasmodic assay and fasting blood sugar assay. The first one tested if the extract showed anti-spasmyolytic or laxative activity. The second one detected the ability of the extract to decrease the fasting blood sugar level.

Antispasmodic Assay
The mice weighing 20- 25 grams were divided into four treatments with thirty-six animals per treatment. The experimental animals were reared by the investigator himself in Brgy. Ticud, La Paz, Iloilo City, Philippines. That was done with help of the expert animal breeders. Crude methanolic extract of Sargassum oligocystum at a dose of 300 mg kg\(^{-1}\) BW was applied orally to the first group of mice. Loperamide (Imodium) and Domperidone (Motilium) were administered orally at a dose of 5 mg kg\(^{-1}\)WB to the second and third groups of mice,
respectively. The dosage for the three treatments was based from the study of Anil Kumar et al. (2009). A negative control, normal saline solution was given orally to the fourth group of mice at a dose of 10 mL kg\textsuperscript{-1} BW (Okunrobo et al. 2009). After an hour, the test animals were orally treated with 0.5 mL of 2 % charcoal suspension and sacrificed by cervical dislocation 30 minutes after its administration. The peritoneal cavity was opened and the propulsive movement of the intestine was observed and cut immediately. The entire length of the intestine and the distance traveled by the charcoal suspension in it was measured using a ruler. The percent traveled by charcoal suspension was calculated by using this formula;

\[
\text{% traveled by charcoal suspension} = \frac{\text{Length traveled by charcoal suspension}}{\text{Total length of the intestine}} \times 100
\]

Fasting Blood Sugar Assay
The mice were fasted 8 hours prior to the blood sample collection. A small drop of blood sample obtained from mouse’s was placed on a disposable test strip and was used to determine the fasting blood sugar level (mg dL\textsuperscript{-1}) using the glucometer (One Touch Horizon). The mice weighing 20-25 grams were divided into three treatments with thirty six (36) animals per treatment. The first group of mice was treated orally with the test extract at a dose of 800 mg kg\textsuperscript{-1} BW. A suspension of the oral hypoglycemic agent, Glipizide (Minidiab) in distilled water at a dose of 15 mg kg\textsuperscript{-1} BW was given orally to the positive control group. Distilled water, a negative control was administered orally at a dose of 10 mL kg\textsuperscript{-1} BW to the third group of mice. After 30 minutes, the glucose load was given orally to all treatments at a dose of 3 g kg\textsuperscript{-1} BW. The blood (0.2 mL) was drawn from the mouse’s tail 90 minutes after the glucose load administration. Only 90 minutes post glucose blood sugar level was determined according to WHO criteria since intermediate blood sampling is not necessary (Kumar and Clark, 1994). The % reduction in fasting blood sugar (FBS) level was calculated using this formula;

\[
\text{% Reduction in FBS level} = \left( \frac{\text{FBS level initial} - \text{FBS level final}}{\text{FBS level initial}} \right) \times 100
\]

Mouse Toxicity Assay
The Mouse toxicity assay was conducted following the protocol of Guevarra (2005) to determine the toxicity of the test extract. Healthy and properly labeled adult male and female white mice (7-8 week-old) were selected at random and maintained under standard laboratory conditions during the course of the assay. Five male and five female test organisms were used in every treatment. The treatments include the five doses (0, 10, 100, 1,000, and 10,000 mg kg\textsuperscript{-1} BW). All animals were starved for food and water for 16 hours prior to the test. Food and water were given 12 hours after the administration of the test extract. The crude ethanolic extract of \textit{S. oligocystum} of different doses was administered to the test animals. The mortality and the occurrence of their death were recorded after three days.

Statistical Analyses
One-Way Analysis of Variance (ANOVA) was used in comparing data among treatments set at alpha=0.01. Duncan’s Multiple Range Test (DMRT) (alpha=0.01) was used as post-hoc test.

Ethical Issue
The experimental animals were handled with care following the suggestions of Guevarra (2005). They were allowed to take in pain reliever (Mefenate acid) after the experiment. Antibiotic (Amoxicillin) was applied to the wounded area to prevent infection. The rights of the subject animals were respected by abiding to the articles stipulated in Republic Act 8485, which is also known as “The Animal Welfare Act of 1998.”

Results
Recovery of \textit{Sargassum oligocystum}
The present study was able to recovery 8.4 g of extract for every 1 Kg of dried powder of \textit{Sargassum oligocystum}. The ethanolic extract appeared greenish brown in color (Figure 1).

Activated Charcoal Suspension Assay
The distance traveled (%) by the activated charcoal suspension (2%) along the gastrointestinal tract of the mice in four treatments Loperamide, crude ethanolic extract of \textit{S. oligocystum}, normal saline solution, and Domperidone is shown in Figure 3. Findings of the study indicated that the highest percent distance traveled (94.52 %) by charcoal suspension were observed in Domperidone treatment while the lowest percent distance traveled (49.83 %) were noticed in Loperamide, an anti-diarrheal drug. The activity of the crude ethanolic extract of \textit{S. oligocystum} is the same (p> 0.01) as that of the Domperidone but significantly lower from that of Loperamide and normal saline solution (negative control) (p< 0.01).
Fasting Blood Sugar Assay
The mean reduction in fasting blood sugar (FBS) level (%) of the glucose-induced hyperglycemic white mice treated with Glipizide, crude ethanolic extract of *S. oligocystum*, and distilled water is displayed in Table 1. Findings of the investigation showed that Glipizide group (positive control) exhibited the highest percent decrease in fasting blood sugar level (25.52 %) after drug administration at a dose of 15 mg kg\(^{-1}\) BW and glucose loading at a dose of 3 g kg\(^{-1}\) BW. Using the Duncan’s Mutiple Range Test (p=0.01), the percent decrease in fasting blood sugar level of the mice that orally received the crude ethanolic extract of *S. oligocystum* (21.83 %) was found to be as effective as the positive control group. At a dose of 800 mg kg\(^{-1}\) BW, the crude ethanolic extract of *S. oligocystum* effectively reduced the fasting blood sugar level of the hyperglycemic mice the same way Glipizide did at a dose of 15 mg kg\(^{-1}\) BW. On the other hand, distilled water did show its capacity to lessen the fasting blood sugar level of the diabetic mice as indicated by its negative decrease in blood glucose level (-48.89 %).

Activated Charcoal Suspension Assay
The distance traveled (%) by the activated charcoal suspension (2%) along the gastrointestinal tract of the mice in four treatments Loperamide, crude ethanolic extract of *S. oligocystum*, normal saline solution, and Domperidone is shown in Figure 3. Findings of the study indicated that the highest percent distance traveled (94.52 %) by charcoal suspension were observed in Domperidone treatment while the lowest percent distance traveled (49.83 %) were noticed in Loperamide, an anti-diarrheal drug. The activity of the crude ethanolic extract of *S. oligocystum* is the same (p> 0.01) as that of the Domperidone but significantly lower from that of Loperamide and normal saline solution (negative control) (p< 0.01).
Values not sharing a common superscript letter differ significantly at $p<0.01$ (DMRT).

Table 1. Percent reduction in fasting blood sugar (FBS) level (%) of the glucose-induced hyperglycemic white mice treated with crude ethanolic extract of 
*S. oligocystum* (CEESO), Glipizide, and distilled water ($p<0.01$).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose</th>
<th>Mean reduction in FBS level (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CEESO</td>
<td>800 mg kg$^{-1}$ BW</td>
<td>21.83 ± 4.43$^a$</td>
</tr>
<tr>
<td>Glipizide (Minidiab)</td>
<td>15 mg kg$^{-1}$ BW</td>
<td>25.52± 3.27$^a$</td>
</tr>
<tr>
<td>Distilled water</td>
<td>10 mL kg$^{-1}$ BW</td>
<td>-48.89± 6.67$^b$</td>
</tr>
</tbody>
</table>

Values not sharing a common superscript letter differ significantly at $p<0.01$ (DMRT).

Discussion

Prior to the emergence of modern sophisticated medicine, people were used to utilize plants as remedy for many ailments. The use of extracts for curing or healing various diseases has been part of the history of humankind. The old practice of using herbal plants for this purpose has been developed as the time goes by. In this light, studies of the novel compounds in the quest for modern affordable drugs have drastically increased; however, these are concentrated mostly on the terrestrial plants as manifested by the studies of Chandrika et al. (2006), Pinent et al. (2004), Vessal et al. (2003) and Ahmed et al. (2010). Only few studies have been conducted using marine organisms especially seaweeds such as the investigations of Lee et al. (2010) and Moon et al. (2011).

In the case of this investigation, findings revealed that at a dose of 800 mg kg$^{-1}$ BW, CEESO effectively reduced the blood sugar level (21.83 %) of the hyperglycemic mice in the same manner ($p>0.01$) as Glipizide (25.52 %) did at a dose of 15 mg kg$^{-1}$ BW. The anti-diabetic property of the CEESO may be linked to its insulinogenic activity. The insulinogenic activity of the said extract may be associated to the hypoglycemic mechanisms of the phytochemical fucoxanthin present in the *Sargassum oligocystum*. Maeda *et al.* (2009) observed that fucoxanthin significantly reduced the blood glucose and plasma insulin levels among diabetic/obese KK-Ay mice. Hosokawa *et al.* (2010) and Miyashita *et al.* (2010) provided mechanisms by which the fucoxanthin improved insulin resistance and decreased blood glucose level. It performed the said biological activity by down-regulating adipokines such as tumor necrosis factor-$\alpha$, monocyte chemo-attractant protein-1, interleukin-6, and plasminogen activator inhibitor-1 via down regulating their mRNA expression by directly acting on adipocytes and macrophages in white adipose tissue and up-regulation of glucose transporter 4 in skeletal muscle in KK-Ay mice (Hosokawa *et al.*, 2010; Miyashita *et al.*, 2010). Hosokawa *et al.* (2010) also indicated that fucoxanthin attenuated hyperglycemia in KK-Ay mice, but did not affect blood glucose levels in lean C57BL/6J mice. Maeda *et al.* (2009) observed that fucoxanthin significantly lowered the fasting blood glucose concentration, the plasma insulin level, and the insulin resistance index in diet-induced obese mice. Fucoxanthin might improve alterations in lipid metabolism and insulin resistance induced by a high fat diet, at least in part, through reducing visceral fat mass, hyperinsulinemia, hepatic glucose production, and hepatic lipogenesis, and altering hepatic glucose-regulating enzymes activities (Park *et al.*, 2011).

Table 2. Survival rate of the mice treated with crude ethanolic extract of *S. oligocystum* in five treatments (0, 10, 100, 1000, and 1000 mg kg$^{-1}$ BW).

<table>
<thead>
<tr>
<th>Treatment/Dose (%)</th>
<th>Number of mice treated</th>
<th>Number of mice died</th>
<th>Survival rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 mg kg$^{-1}$ BW (distilled water)</td>
<td>10</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td>10 mg kg$^{-1}$ BW</td>
<td>10</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td>100 mg kg$^{-1}$ BW</td>
<td>10</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td>1,000 mg kg$^{-1}$ BW</td>
<td>10</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td>10,000 mg kg$^{-1}$ BW</td>
<td>10</td>
<td>0</td>
<td>100</td>
</tr>
</tbody>
</table>

Apart from fucoxanthin, another potential substance that can account for anti-diabetic activity of the brown seaweeds is phlorotannin. Recently, it was discovered that dieckol, a type of phlorotannin from *Ecklonia cava* possesses hypoglycemic activity by performing some mechanisms. It has the capacity to inhibit $\alpha$-Glucosidase (Lee *et al.*, 2009), show postprandial hyperglycemia-lowering effect (Lee *et al.*, 2010), elevate glucose uptake effect skeletal muscle (Guan 2011) and inhibit PTP 1B (Moon *et al.*, 2011). Diphlorethohydroxycarmalol, another type of phlorotannin derived from brown alga *Ishige okamurae* also showed hypoglycemic activity. It has the ability to lower the blood sugar level by inhibiting the $\alpha$-Glucosidase (Heo *et al.*, 2009), demonstrating protective effect against diabetes complication (Heo *et al.*, 2010), and showing postprandial hyperglycemia-lowering effect
This study also indicated that the activity of CEESO was the same as that of Domperidone but significantly different from that of Loperamide and NSS. With this observation, it can be inferred that the crude ethanolic extract of *S. oligocystum* does not possess antispasmodic property however, it shows laxative property. The findings of Gutierrez and Solis (2009) are synonymous to the results of the present investigation. Their findings showed that at doses of 50, 100, 200, and 300 μg mL⁻¹, methanolic extract of microalgae *Blennothrix ganeshii* and *Microcoleus lacustris* failed to display a significant antispasmodic activity on the contraction of the rat ileum induced by three spasmogens: acetylcholine, histamine, and barium chloride. On the other hand, it was also observed that the hexane extract of the two microalgal species possessed a significant antispasmodic activity in rats using the same spasmogens. Likewise, the findings of Fantonalgo et al. (2009) support the results of the current study that the crude methanolic *N. paleacea* did not show significant anti-spasmodic activity. The laxative activity of the extract can be associated to the ability of the metabolites in promoting intestinal contraction (Fantonalgo et al. 2009). The possible intestinal contraction may potentially allow the fast movement of the substances along the intestinal tract of the mice.

It was established in the toxicity assay (current study) that the crude ethanolic extract of *S. oligocystum* was not toxic even at highest dosage (10,000 mg kg⁻¹ BW) of the treatment because no single occurrence was observed during the duration of the observation. The 100% survival rate in all mice treated with the said extracts is a proof that CEESO does not contain substances that may possibly serve as threats to the physiological processes of the mice. Despite with this inference, there is still a need to test the blood chemistry of the subject animals and hepato-pancreatic parameters in order to ensure the safety of the extracts for animal and human consumption. Further clinical trials need to be conducted intensely to assure public safety.

**Conclusion and Recommendations**

Current investigation revealed that crude ethanolic extract of *S. oligocystum* possessed a significant anti-diabetic and laxative properties while it failed to demonstrate a significant antispasmodic activity. These activities can be associated to the bioactive compounds present in the algal material. Seaweed *S. oligocystum*, therefore, can be a potential source of metabolites for laxative and anti-diabetic drug development.

Present study suggests that further investigation will be done on the isolation, purification, structure elucidation, and identification of the compounds responsible for the laxative and anti-diabetic activities of *S. oligocystum*. The pharmacological properties of the extract could further be evaluated by using various concentrations and performing sequential extraction using solvents of different polarities.

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