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Antioxidant Properties of Water and Aqueous Ethanol Extracts and Their Crude Saponin Fractions from a Far-eastern Sea Cucumber, *Stichopus japonicus*

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Abstract Water and 70% ethanol extracts obtained from a sea cucumber (*Stichopus japonicus*) body wall by heat reflux or pressurized solvent extraction showed 2,2-diphenyl-1-picrylhydrazyl (DPPH˙) and 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) radical cation ($ABTS^{\cdot+}$) scavenging activities comparable to those of fruits and vegetables. The highest activities were observed for the water extract from heat reflux extraction. Crude saponins exhibited higher radical scavenging activities than the soluble matters in the extracts. However, they were responsible for only about 3 to 15% of the scavenging activities of the extracts. Total phenolic contents showed a significant correlation with DPPH˙ scavenging activities, suggesting a significant contribution of phenolic constituents to the antioxidant properties of the extracts. However, total flavonoid contents showed little correlation with the radical scavenging activities. The results suggest that the water or 70% ethanol extract obtained from sea cucumber body wall by simple heat reflux extraction could provide considerable antioxidant benefits.

Keywords: antioxidant, *Stichopus japonicus*, radical scavenging activity, saponin, phenolic

Introduction

Sea cucumber, a marine echinoderm of the class Holothuroidea, has long been used as an outstanding health-promoting food as well as a traditional medicine in many Asian countries due to its multiple biological properties such as antifungal, antivirus, antitumor, anticoagulant, anti-angiogenic, neuritogenic, and immunomodulatory activities (1-7). Triterpene glycosides, commonly known as sea cucumber saponins or holothurins, are known to be primarily responsible for such properties. Other important active components of sea cucumber include chondroitin sulfate, eicosapentaenoic acid, and ganglioside (2,4,8).

In spite of many studies on the biological properties, only a few reports are available on the antioxidant property of sea cucumber. Recently, *Cucumaria frondosa*, a sea cucumber species commonly found in coastal waters of the North Atlantic Ocean, has been demonstrated to have antioxidant activity (7,8). The activity varied depending on the part of the body of sea cucumber as well as the solvent used for extraction, but mostly comparable to the values reported for fruits, vegetables, and medicinal/culinary herbs (7). Phenolic compounds, especially flavonoids, were suggested to be mainly responsible for the antioxidant activity (7). Until now, the antioxidant property of sea cucumber species other than *C. frondosa* has been rarely reported.

A sea cucumber species of *Stichopus japonicus* has been widely consumed as a traditional nutraceutical and medicine in China, Japan, and Korea and found to contain biologically active saponins called holotoxins A, B, and C (1). Therefore, the extracts of *S. japonicus* have a high potential to be used as an active component of various health-care products, such as nutraceuticals, supplements, and cosmeceutics. Due to safety and economic concerns, such extracts are desirable to be prepared with a generally recognized as safe (GRAS) and inexpensive solvent such as water and aqueous ethanol. To the best of our knowledge, however, there is no published report on the antioxidant property of water or aqueous ethanol extract of *S. japonicus*. Some plant-derived saponins have been reported to possess antioxidant properties (9-11). Saponins are the predominant secondary metabolites of sea cucumbers (12), however, little information is available on the antioxidant properties of sea cucumber saponins.

The objective of this study was to investigate antioxidant properties of the water and aqueous ethanol extracts of *S. japonicus* body wall as well as of the crude saponin fractions obtained from the extracts. Relationships between antioxidant properties and the contents of total phenolics and flavonoids were also investigated. Heat reflux extraction (HRE) and pressurized solvent extraction (PSE) were employed in the present study. HRE was adopted because it is one of the most routine techniques in natural product extraction, and PSE was used because it can be performed under an oxygen- and light-free environment with less solvent within a shorter time, compared to traditional extraction processes (13-16).

Materials and Methods

Materials Live specimens of sea cucumber *Stichopus japonicus* (average body weight 167 g) were purchased from a fishery market (Mokpo, Korea). Folin-Ciocalteu's

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reagent, aluminum chloride, ABTS diammonium salt, 2,2-diphenyl-1-picrylhydrazyl (DPPH$^{\cdot}$), 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), rutin, and gallic acid were obtained from Sigma-Aldrich (St. Louis, MO, USA). Ethanol, methanol, and diethyl ether were obtained from Fisher Scientific (Ottawa, ON, Canada). All chemicals used in the study were of either analytical or high performance liquid chromatography (HPLC) grade.

Sample preparation and extraction procedures The visceral organs and body fluid of fresh sea cucumber specimens were taken out and the body wall was washed with tap water. The body wall was cut into small pieces, freezed at –80°C for at least 12 hr, and freeze-dried at 30°C for 72 hr. The dried sample was ground into powder and sieved with a ≤600-μm sieve. The powder was stored in a closed dark bottle at 2°C before experiments.

Four types of sea cucumber extracts were prepared by performing 2 types of extraction, HRE and PSE, with 2 solvents, distilled water, and 70% aqueous ethanol. PSE was performed using a fully automated pressurized solvent extraction system (ASE 300; Dionex Co., Sunnyvale, CA, USA) equipped with a solvent controller unit. The powder sample (5 g) was filled in a 100 mL extraction cell, and the extraction was conducted under the following conditions: pressure, 10.34 MPa; temperature, 80°C; heating time, 5 min; extraction time, 5 min; extraction cycles, 3 times; flush volume, 60%; purge time, 60 sec. The final extract volumes were measured to be 150 and 162 mL for the extractions with distilled water and 70% ethanol, respectively. HRE was performed using an apparatus equipped with a cooling condenser, a rotating 1-L round-bottom flask, and a temperature-controlled water bath. For comparison purpose, the conditions for HRE were set based on those for PSE. A volume of 150 mL of distilled water or 162 mL of 70% ethanol was poured into the round-bottom flask and heated until the temperature reached 80°C, followed by dispersing 5 g of the powder sample in the solvent. After 20 min extraction, the extract was filtered by suction filtration using a No. 2 filter paper (Advantec Toyo Roshi International, Inc., Dublin, CA, USA). The obtained extracts were stored at 2°C until further analyses.

Recovery of soluble matter and crude saponins The soluble matter and crude saponins of each of the 4 extracts were recovered to be tested for their antioxidant activities. Soluble matter was recovered by evaporating the solvent of extract under reduced vacuum at 40°C using a rotary evaporator (Rotavor R-210; Buchi Labortechnik AG, Flawi, Switzerland). Crude saponins were recovered according to the method of Kwon *et al.* (17) with slight modifications. A volume of 250 mL of each of the 4 extracts was transferred to a round-bottom flask of 1-L and the solvent was evaporated under reduced vacuum at 55°C using the rotary evaporator. A volume of 125 mL of distilled water was poured into the flask to dissolve the residue. The aqueous solution was transferred to a separating funnel, treated twice with 125 mL of diethyl ether to remove lipid components, and then extracted 4 times with 125 mL of water-saturated *n*-butanol. The butanol fractions were collected and treated twice with 75 mL of distilled water to remove impurities, and crude saponins were obtained by evaporating butanol under reduced vacuum at 55°C. The procedure was repeated several times to obtain sufficient amounts of crude saponins for further analyses.

The yields of soluble matter and crude saponins were determined in separate experiments. Soluble matter and crude saponins were isolated from 5 and 20 mL of each extract, respectively, dried at 105°C for 6 hr, and weighed after cooling in a desiccator. The yields were calculated as follows: Yield (%)=100 W/W_0, where W is the dry weight of soluble matter or crude saponins and W_0 is the dry weight of sea cucumber powder sample used for extraction.

DPPH radical scavenging activity assay DPPH$^{\cdot}$ scavenging activities of soluble matters and crude saponins were determined according to a modified method of Brand-Williams *et al.* (18) and Kim *et al.* (19). A DPPH$^{\cdot}$ solution of 100 μM was prepared in 80% aqueous methanol. Serial 2-fold dilutions of soluble matters and crude saponins were prepared with the solvents used for the extraction. An aliquot of 100 μL of each serial dilution was added to 2.9 mL of the DPPH$^{\cdot}$ solution. The mixture was shaken vigorously, allowed to stand at 23°C in the dark for 30 min, and then its absorbance (A_S) was measured at 517 nm using the spectrophotometer. The percentage of scavenged DPPH$^{\cdot}$ was calculated using the following equation (20, 21): Scavenged DPPH$^{\cdot}$ (%)=$100(1-(A_S-A_B)/A_C)$, where A_B is the absorbance of blank (100 μL of sample dilution plus 2.9 mL of 80% methanol) and A_C is the absorbance of control (100 μL of 80% methanol plus 2.9 mL of the DPPH$^{\cdot}$ solution). A standard curve was obtained using Trolox standards (0-80 μM) in distilled water, and DPPH$^{\cdot}$ scavenging activities were expressed as μmol Trolox equivalents (TE)/g of dry solids. The activities of soluble matters were also expressed as μmol TE/g of dry sea cucumber. The whole experiment was conducted in triplicate.

ABTS radical cation scavenging activity assay ABTS$^{\cdot+}$ scavenging activities of soluble matters and crude saponins were determined according to the method described by Re *et al.* (22) and Li *et al.* (23) with slight modifications. ABTS$^{\cdot+}$ was produced by reacting 7 mM aqueous ABTS solution with 2.45 mM potassium persulfate in the dark at room temperature for 16 hr and used within 2 days. The ABTS$^{\cdot+}$ solution was diluted with ethanol to an absorbance of 0.700±0.050 at 734 nm. Soluble matters and crude saponins were appropriately diluted with the solvents used for the extraction such that after introducing a 50 μL aliquot of each sample dilution into the assay, they produced between 20 and 80% inhibition of the control absorbance. A volume of 1.9 mL of diluted ABTS$^{\cdot+}$ solution was added to 50 μL of each sample dilution, and the absorbance at 734 nm was recorded exactly after 6 min at room temperature. The percentage of scavenged ABTS$^{\cdot+}$ was calculated as described for DPPH$^{\cdot}$ assay. A standard curve was obtained using Trolox standards (0-20 μM) in distilled water, and ABTS$^{\cdot+}$ scavenging activities were expressed as μmol TE/g of dry solids. The activities of soluble matters were also expressed as μmol TE/g of dry sea cucumber. The whole experiment was conducted in triplicate.

Determination of total phenolic content Total phenolic

contents of soluble matters and crude saponins were determined using Folin-Ciocalteu's method as described by Mamelona *et al.* (7). Soluble matters and crude saponins were appropriately diluted with the solvents used for the extraction. An aliquot of 100 μL of each dilution was transferred into a test tube and adjusted to a volume of 500 μL with distilled water, followed by the addition of 250 μL of Folin-Ciocalteu's reagent and 1.25 mL of 12.5% aqueous sodium carbonate solution. The mixture was vortexed and placed at 25°C for 40 min, and then, the absorbance at 750 nm was recorded against the control (500 μL of distilled water plus 1.5 mL of reagent mixture) and the blank (100 μL of sample dilution plus 1.9 mL of distilled water). A standard curve was prepared using gallic acid standards (10-50 μg/mL) in distilled water, and total phenolic contents were expressed as mg gallic acid equivalent (GAE)/g of dry solids. The values for soluble matters were also expressed as mg GAE/g of dry sea cucumber. All measurements were carried out in triplicate.

Determination of total flavonoid content Total flavonoid contents of soluble matters and crude saponins were determined using aluminum chelating method as described by Mamelona *et al.* (7) and Maksimović *et al.* (24). Soluble matters and crude saponins were appropriately diluted with the solvents used for the extraction. An aliquot of 50 μL each dilution was transferred into a test tube and adjusted to a volume of 750 μL with distilled water, followed by the addition of 250 μL of $AlCl_3$ reagent. The mixture was vortexed and held at 25°C for 30 min, and then, the absorbance at 405 nm was recorded against the control (750 μL of distilled water plus 250 μL of the reagent) and the blank (50 μL of sample dilution plus 950 μL of distilled water). A standard curve was prepared using rutin hydrate standards (10-50 μg/mL) in distilled water, and total flavonoid contents were expressed as mg rutin equivalent (RE)/g of dry solids. The values for soluble matters were also expressed as mg RE/g of dry sea cucumber. All measurements were carried out in triplicate.

Statistical analysis SPSS for Windows (version 11.0, SPSS Inc., Chicago, IL, USA) was used for statistical analysis. All data were expressed as mean±standard deviation (SD). The significance of differences ($p<0.05$) among the corresponding mean values was determined by using one-way analysis of variance (ANOVA) followed by Duncan's new multiple-range test.

Results and Discussion

Antioxidant properties of extracts Soluble matters were recovered from the obtained sea cucumber extracts and evaluated for their antioxidant activities. Their yields (about 43%) for the 2 PSE extracts were higher than those (about 41%) for the 2 HRE extracts, indicating that PSE was more efficient than HRE in the extraction of soluble matter (Table 1). This is because pressure can increase the rate of mass transfer and significantly enhance the diffusion of solvent (25). The type of solvent (water or 70% ethanol), however, did not significantly influence the yield of soluble matter in both HRE and PSE, while it affected the antioxidant activity of soluble matter as discussed below.

All types of soluble matter exhibited notable antioxidant activities. Their radical scavenging activities against $DPPH^{\cdot}$ and $ABTS^{\cdot+}$ varied from 7.31 to 16.37 μmol TE/g and from 0.83 to 1.50 μmol TE/g, respectively, depending on the extraction conditions (Table 1). In both HRE and PSE, water-soluble matters exhibited higher $DPPH^{\cdot}$ and $ABTS^{\cdot+}$ scavenging activities than 70% ethanol-soluble matters, indicating that water is a better solvent than 70% ethanol for the antioxidants in sea cucumber (*S. japonicus*) body wall. When the same solvent was used, the soluble matters from HRE generally showed higher antioxidant activities than those from PSE. It seems that components other than antioxidants were more extracted during PSE, which resulted in the higher yield but lower scavenging activities of soluble matter. The highest antioxidant activity was observed for the water-soluble matter obtained from HRE.

For the purpose of comparison with literature values, the activities of soluble matters were also expressed based on the dry weight of sea cucumber in Table 1 (3.20-6.79 and 0.34-0.62 μmol TE/g of dry body wall against $DPPH^{\cdot}$ and $ABTS^{\cdot+}$, respectively). To the best of our knowledge, the antioxidant activity of sea cucumber body wall was only investigated for *C. frondosa* (7,8). Zhong *et al.* (8) reported that the $DPPH^{\cdot}$ scavenging activity of the methanol extract

Table 1. Antioxidant activities and yields of water- and 70% ethanol-soluble matters extracted from body wall of sea cucumber (*S. japonicus*)[1)]

	Solvent	$DPPH^{\cdot}$ scavenging activity (μmol TE/g)	$ABTS^{\cdot+}$ scavenging activity (μmol TE/g)	Yield (%)
HRE[2)]	Water	16.37 ± 1.70^{a} (6.79 ± 0.10^{a})	1.50 ± 0.11^{a} (0.62 ± 0.04^{a})	41.50 ± 0.58^{b}
	70% Ethanol	11.23 ± 1.04^{b} (4.59 ± 0.34^{b})	0.83 ± 0.10^{c} (0.34 ± 0.03^{c})	40.87 ± 0.23^{b}
PSE[3)]	Water	10.22 ± 1.23^{b} (4.43 ± 0.58^{b})	1.34 ± 0.13^{b} (0.58 ± 0.03^{b})	43.31 ± 0.38^{a}
	70% Ethanol	7.31 ± 0.80^{c} (3.20 ± 0.35^{c})	0.92 ± 0.10^{c} (0.35 ± 0.02^{c})	43.81 ± 0.24^{a}

[1)]Values are expressed as mean±SD ($n=15$ for scavenging activities and 3 for yields). Values are calculated based on the dry weight of soluble matter while those in parentheses are based on the dry weight of sea cucumber body wall. Values in the same column with different superscripts are significantly different at $p<0.05$.
[2)]Heat reflux extraction.
[3)]Pressurized solvent extraction.

Table 2. Antioxidant activities and yields of crude saponins extracted from body wall of sea cucumber (*S. japonicus*)[1)]

	Solvent	DPPH· scavenging activity (μmol TE/g)	$ABTS^{\cdot+}$ scavenging activity (μmol TE/g)	Yield (%)
HRE[2)]	Water	30.91±3.55[a]	5.23±0.39[a]	1.85±0.07[b]
	70% Ethanol	17.77±1.51[b]	1.04±0.09[c]	1.54±0.20[bc]
PSE[3)]	Water	11.33±1.17[c]	2.24±0.11[b]	2.76±0.21[a]
	70% Ethanol	12.58±1.02[c]	0.98±0.08[c]	1.14±0.19[c]

[1)]Values are expressed as mean±SD (n=15 for scavenging activities and 3 for yields). Values are calculated based on the dry weight of crude saponins and those in the same column with different superscripts are significantly different at $p<0.05$.
[2)]Heat reflux extraction.
[3)]Pressurized solvent extraction.

from its body wall was 4.51 μmol TE/g of dry body wall, which is exactly in the range of our results. Mamelona *et al.* (7) showed that the oxygen radical absorbance capacity (ORAC) values of the body wall extracts prepared with water, ethyl acetate, or acetonitrile/0.1% aqueous trifluoroacetic acid were generally comparable to those of fruits, vegetables, and many medicinal/culinary herbs. Therefore, the body wall of *S. japonicus*, like that of *C. frondosa*, could be consumed as a useful source of antioxidant. It is also implied that its water or 70% ethanol extract obtained by simple HRE could deliver considerable antioxidant benefits to desired health-care products, such as nutraceuticals, supplements, and cosmeceutics, together with the other well-known biological profits of sea cucumber.

Antioxidant properties of crude saponins Crude saponins were also recovered from the obtained sea cucumber extracts to evaluate their antioxidant activities. Our preliminary experiments showed that the recovered crude saponins were responsible for more than about 70% of the antifungal activities of the extracts (data not shown), indicating that crude saponins were successfully recovered in the current study. Higher crude saponin yields were observed when the extraction was performed with water rather than 70% ethanol, and the highest yield (2.76%) was obtained when PSE was used with water (Table 2). The radical scavenging activities of crude saponins against DPPH· and $ABTS^{\cdot+}$ varied from 11.33 to 30.91 μmol TE/g and from 0.98 to 5.23 μmol TE/g, respectively, depending on the extraction conditions (Table 2). As observed for soluble matter, the crude saponins from HRE or water extraction generally exhibited higher scavenging activities compared to those from PSE or 70% ethanol extraction. The highest scavenging activity was observed for the water-extracted crude saponins from HRE.

All types of crude saponins showed higher radical scavenging activities than the corresponding types of soluble matter (Table 1 and 2). Considering the yields, however, crude saponins were responsible for only about 3 to 15% of the scavenging activities of soluble matters, indicating that saponins are not the primary antioxidant components of the obtained sea cucumber extracts. Our preliminary experiments also showed that the obtained crude saponins were able to scavenge only 1.78 to 5.29% of DPPH· at 100 μg/mL (data not shown). These scavenging activities are significantly lower than the values (15.81-91.03%) reported for the same amount of crude saponins derived from 11 traditional Chinese medicinal plants (11) and those (47.5% at 30 μg/mL) reported for a triterpenoid glycoside isolated from the berries of *Hedera colchita* (10), indicating that the saponins of *S. japonicus* body wall are weak antioxidant.

Correlations between antioxidant activities and contents of total phenolics and flavonoids Total phenolic contents

Table 3. Total phenolic and flavonoid contents in soluble matters and crude saponins extracted from body wall of sea cucumber (*S. japonicus*)[1)]

	Solvent	Soluble matter		Crude saponins	
		Phenolics (mg GAE/g)	Flavonoids (mg RE/g)	Phenolics (mg GAE/g)	Flavonoids (mg RE/g)
HRE[2)]	Water	22.77±3.02[a] (9.45±1.03[a])	3.66±0.59[ab] (1.50±0.30[ab])	40.99±7.16[a]	21.43±1.56[b]
	70% Ethanol	11.89±1.89[c] (4.86±0.62[c])	3.17±0.41[b] (1.29±0.18[b])	33.72±4.55[b]	29.86±3.22[a]
PSE[3)]	Water	17.63±1.86[b] (7.64±0.83[b])	3.34±0.42[b] (1.37±0.15[b])	18.65±8.08[c]	5.92±0.73[c]
	70% Ethanol	11.88±1.71[c] (5.21±0.70[c])	4.39±0.56[a] (1.80±0.23[a])	30.78±4.59[b]	30.38±3.58[a]

[1)]Values are expressed as mean±SD (n=15). Values are calculated based on the dry weight of soluble matter or crude saponins while those in parentheses are based on the dry weight of sea cucumber body wall. Values in the same column with different superscripts are significantly different at $p<0.05$.
[2)]Heat reflux extraction.
[3)]Pressurized solvent extraction.

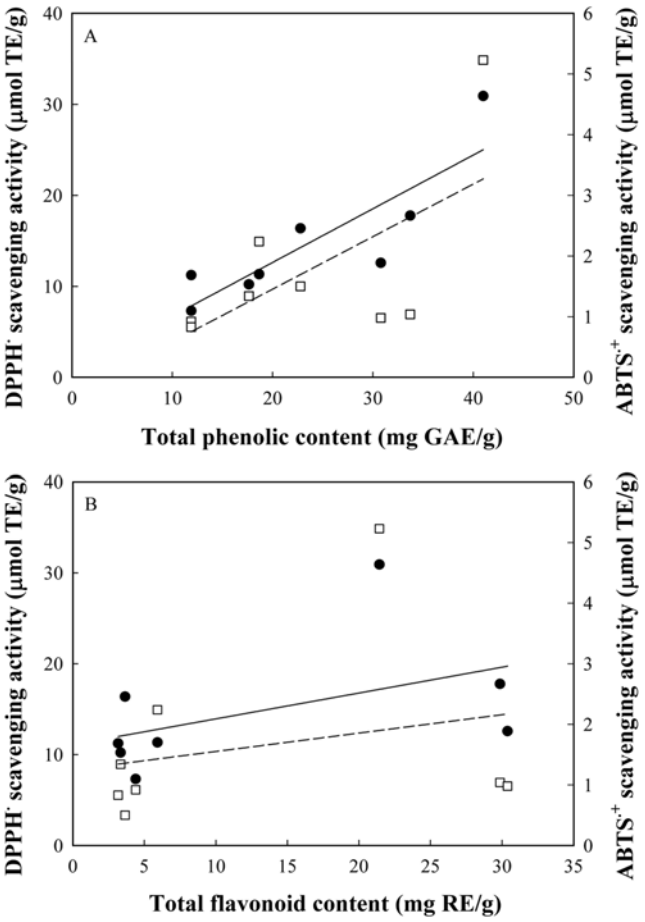

Fig. 1. Relationships of total phenolic contents (A) and total flavonoid contents (B) to DPPH· (●, —) and ABTS·+ (□, ---) scavenging activities of soluble matters and crude saponins extracted from sea cucumber (*S. japonicus*) body wall. GAE, RE, and TE stand for gallic acid equivalents, rutin equivalents, and Trolox equivalents, respectively.

in the soluble matters and crude saponins obtained from *S. japonicus* body wall were determined, because phenolic compounds, including flavonoids, tannins, and phenolic acids, are relatively easy to assimilate and rich in main food sources of sea cucumber, such as phytoplankton and algal particles, and therefore, believed to play an important role for the antioxidant property of sea cucumber (7,8). The total phenolic contents determined for soluble matters were found to range from 11.88 to 22.77 mg GAE/g (Table 3). The contents expressed based on the dry weight of sea cucumber (values in parentheses in Table 3) ranged between 4.86 and 9.45 mg GAE/g of dry body wall, which were a bit higher than the values (0.91-1.11 mg GAE/g of dry body wall) reported for the water, methanol, and ethyl acetate extracts of *C. frondosa* body wall (7,8). The total phenolic contents in crude saponins ranged from 18.65 to 40.99 mg GAE/g (Table 3), showing that about 74-85% of the phenolic compounds in soluble matters was lost during the preparation of crude saponins. It was shown in Fig. 1A that total phenolic contents had a quite significant correlation with DPPH· scavenging activities (R^2=0.730, p=0.007), although their correlation with ABTS·+ scavenging activities was rather weak (R^2=0.390, p=0.098). Mamelona *et al.* (7) also reported a high correlation between total phenolic contents and ORAC values (R^2=0.92, p=0.042) for the water, ethyl acetate, and acetonitrile/trifluoroacetic acid extracts of *C. frondosa* body wall. It is suggested from the results that phenolic constituents contribute a significant part to the antioxidant properties of the extracts from the body wall of *S. japonicus*.

Total flavonoid contents were also determined because it was reported by Mamelona *et al.* (7) that flavonoids contributed extensively to the antioxidant activity of the extracts from the body wall of *C. frondosa*. The total flavonoid contents determined for soluble matters and crude saponins were found to range from 3.17 to 4.39 mg RE/g and from 5.92 to 30.38 mg RE/g, respectively (Table 3). The contents expressed based on the dry weight of sea cucumber (1.29-1.80 mg RE/g of dry body wall in parentheses in Table 3) were slightly higher than the values (0.11-0.26 mg RE/g of dry body wall) reported for the water and ethyl acetate extracts of *C. frondosa* body wall (7). Little correlation was observed between total flavonoid contents and the scavenging activities against DPPH· (R^2=0.227, p=0.233, n=8) and ABTS·+ (R^2=0.06, p=0.563, n=8) (Fig. 1B). The results suggest that phenolic components other than flavonoids, such as tannins and phenolic acids, may also contribute to the antioxidant properties of the extracts from *S. japonicus* body wall.

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References

1. Kitagawa I, Sugawara T, Yosioka I. Saponin and sapogenol. XV. Antifungal glycosides from the sea cucumber *Stichopus japonicus* SELENKA. (2) Structures of holotoxin A and holotoxin B. Chem. Pharm. Bull. 24: 275-284 (1976)
2. Mourão PAS, Pereira MS, Pavão MSG, Mulloy B, Tollefsen DM, Mowinckel MC, Abildgaard U. Structure and anticoagulant activity of a fucosylated chondroitin sulfate from Echinoderm. J. Biol. Chem. 271: 23973-23984 (1996)
3. Maier MS, Roccatagliata AJ, Kuriss A, Chludil H, Seldes AM, Pujol CA, Damonte EB. Two new cytotoxic and virucidal trisulfated triterpene glycosides from the Antarctic sea cucumber *Staurocucumis liouvillei*. J. Nat. Prod. 64: 732-736 (2001)
4. Kaneko M, Kisa F, Yamada K, Miyamoto T, Higuchi R. Structure of a new neuritogenic-active ganglioside from the sea cucumber *Stichopus japonicus*. Eur. J. Org. Chem. 6: 1004-1008 (2003)
5. Tian F, Zhang X, Tong Y, Yi Y, Zhang S, Li L, Sun P, Lin L, Ding J. PE, a new sulfated saponin from sea cucumber, exhibits anti-angiogenic and anti-tumor activities *in vitro* and *in vivo*. Cancer Biol. Ther. 4: 874-882 (2005)
6. Aminin DL, Pinegin BV, Pichugina LV, Zaporozhets TS, Agafonova IG, Boguslavski VM, Silchenko AS, Avilov SA, Stonik VA. Immunomodulatory properties of Cumaside. Int. Immunopharmacol. 6: 1070-1082 (2006)
7. Mamelona J, Pelletier É, Girard-Lalancette K, Legault J, Karboune S, Kermasha S. Quantification of phenolic contents and antioxidant capacity of Atlantic sea cucumber, *Cucumaria frondosa*. Food Chem. 104: 1040-1047 (2007)
8. Zhong Y, Khan MA, Shahidi F. Compositional characteristics and

antioxidant properties of fresh and processed sea cucumber (*Cucumaria frondosa*). J. Agr. Food Chem. 55: 1188-1192 (2007)
9. Sur P, Chaudhuri T, Vedasiromoni JR, Gomes A, Ganguly DK. Antiinflammatory and antioxidant property of saponins of tea [*Camellia sinensis* (L) O. Kuntze] root extract. Phytother. Res. 15: 174-176 (2001)
10. Gülçin İ, Mshvildadze V, Gepdiremen A, Elias R. The antioxidant activity of a triterpenoid glycoside isolated from the berries of *Hedera colchita*: 3-*O*-(β-D-glucopyranosil)-hederagenin. Phytother. Res. 20: 130-134 (2006)
11. Xi M, Hai C, Tang H, Chen M, Fang K, Liang X. Antioxidant and antiglycation properties of total saponins extracted from traditional Chinese medicine used to treat diabetes mellitus. Phytother. Res. 22: 228-237 (2008)
12. Kerr RG, Chen Z. *In vivo* and *in vitro* biosynthesis of saponins in sea cucumbers. J. Nat. Prod. 58: 172-176 (1995)
13. Kaufmann B, Christen P. Recent extraction techniques for natural products: Microwave-assisted extraction and pressurised solvent extraction. Phytochem. Analysis 13: 105-113 (2002)
14. Denery JR, Dragull K, Tang CS, Li QX. Pressurized fluid extraction of carotenoids from *Haematococcus pluvialis* and *Dunaliella salina* and kavalactones from *Piper methysticum*. Anal. Chim. Acta 501: 175-181 (2004)
15. Herrero M, Martín-Álvarez PJ, Señoráns FJ, Cifuentes A, Ibáñez E. Optimization of accelerated solvent extraction of antioxidants from *Spirulina platensis* microalga. Food Chem. 93: 417-423 (2005)
16. Wang L, Weller CL. Recent advances in extraction of nutraceuticals from plants. Trends Food Sci. Tech. 17: 300-312 (2006)
17. Kwon JH, Bélanger JMR, Paré JRJ. Optimization of microwave-assisted extraction (MAP) for ginseng components by response surface methodology. J. Agr. Food Chem. 51: 1807-1810 (2003)
18. Brand-Williams W, Cuvelier ME, Berset C. Use of a free radical method to evaluate antioxidant activity. LWT-Food Sci. Technol. 28: 25-30 (1995)
19. Kim DO, Lee KW, Lee HJ, Lee CY. Vitamin C equivalent antioxidant capacity (VCEAC) of phenolic phytochemicals. J. Agr. Food Chem. 50: 3713-3717 (2002)
20. Masuda T, Yonemori S, Oyama Y, Takeda Y, Tanaka T, Andoh T, Shinohara A, Nakata M. Evaluation of the antioxidant activity of environmental plants: Activity of the leaf extracts from seashore plants. J. Agr. Food Chem. 47: 1749-1754 (1999)
21. Maisuthisakul P, Suttajit M, Pongsawatmanit R. Assessment of phenolic content and free radical-scavenging capacity of some Thai indigenous plants. Food Chem. 100: 1409-1418 (2007)
22. Re R, Pellegrini N, Proteggente A, Pannala A, Yang M, Rice-Evans C. Antioxidant activity applying an improved ABTS radical cation decolorization assay. Free Radical Bio. Med. 26: 1231-1237 (1999)
23. Li HB, Cheng KW, Wong CC, Fan KW, Chen F, Jiang Y. Evaluation of antioxidant capacity and total phenolic content of different fractions of selected microalgae. Food Chem. 102: 771-776 (2007)
24. Maksimović Z, Malenčić Đ, Kovačević N. Polyphenol contents and antioxidant activity of *Maydis stigma* extracts. Bioresource Technol. 96: 873-877 (2005)
25. Shouqin Z, Ruizhan C, Changzheng W. Experiment study on ultrahigh pressure extraction of ginsenosides. J. Food Eng. 79: 1-5 (2007)