Available online at www.sciencedirect.com
ScienceDirect
Food Chemistry 104 (2007) 1040–1047
Food Chemistry
www.elsevier.com/locate/foodchem

Quantification of phenolic contents and antioxidant capacity of Atlantic sea cucumber, *Cucumaria frondosa*

Jean Mamelona [a], Émilien Pelletier [a,*], Karl Girard-Lalancette [b], Jean Legault [b], Salwa Karboune [c], Selim Kermasha [c]

[a] *Institut des Sciences de la Mer de Rimouski, 310 des ursulines, Rimouski (Québec), Canada G5L 3A1*
[b] *Université du Québec à Chicoutimi, 555 de l'Université, Chicoutimi (Québec), Canada G7H 2B1*
[c] *McGill University, MacDonald Campus, 21, 111 Lakeshore, Ste. Anne de Bellevue (Québec), Canada H9X 3V9*

Received 5 May 2006; received in revised form 10 November 2006; accepted 8 January 2007

Abstract

The antioxidant activity (oxygen radical absorbance capacity, ORAC) and total phenols and flavonoids were determined in extracts from digestive tract, gonads, muscles and respiratory apparatus of sea cucumber, *Cucumaria frondosa*. Total phenols content varied from 22.5 to 236.0 mg of gallic acid equivalents/100 g dw, and flavonoids from 2.9 to 59.8 mg of rutin equivalents/100 g. ORAC values ranged from 140 to 800 μmol of Trolox equivalents/g dw. Among all extracts, best antioxidant potencies were observed in ethyl acetate extracts from digestive tract, and in acetonitrile-rich fractions obtained from mixed extracts using acetonitrile/TFA (trifluoroacetic acid) acidified water on muscles, gonads and respiratory apparatus. The weakest potencies were observed with water extracts from digestive tract and respiratory apparatus, and with water-rich fractions obtained from mixed extraction of gonads and muscles. A significant correlation was observed between ORAC values and total phenol content in extracts and fractions of gonads and muscles, but ORAC and phenols were not correlated in digestive tract and respiratory apparatus extracts. ORAC values were significantly correlated ($p < 0.05$) with total flavonoids in all extracts. Successive eluates obtained from solid-phase extraction of water-rich fractions using C_{18} cartridge showed ORAC values (105–500 μmol of TE/g) reaching up to 2.3 times the potency of their parent fractions. Flavonoids are suggested to be mainly responsible for observed activities. Our results provide a first quantitative evaluation of *C. frondosa* tissues as useful sources of antioxidants for human consumption.

Keywords: ORAC; Sea cucumber; Total phenols; Total flavonoids; Antioxidant; Peroxyl radicals

1. Introduction

Diets high in fruits and vegetables are recognized to reduce the risks of some chronic and degenerative illnesses related to the oxidation of vital biomolecules (DNA, proteins, lipids, etc.), including atherosclerosis, cancer and cardiovascular diseases, among others. Antioxidants found in food and supplements support human intrinsic antioxidative protection to maintain the internal oxidation status by various processes such as in situ regeneration of antioxidant molecules (vitamins and enzymes) or direct neutralization of oxidative compounds (Kohen & Nyska, 2002; Lee, Koo, & Min, 2004). Emerging concerns related to the synthetic chemicals during the last decades have led to an increasing interest in the development of supplements containing mixtures of naturally-occurring antioxidants. Up to now, most available supplements are formulated with natural antioxidants derived from terrestrial plants and fish oil. Although, seaweeds and marine invertebrates (cnidarians, bryozoans, mollusks, tunicates, echinoderms) are well recognized as sources of various natural molecules most often tested against cancer cell-lines (Jha & Zi-rong,

* Corresponding author. Tel.: +1 418 723 1986x1764.
E-mail address: emilien_pelletier@uqar.qc.ca (É. Pelletier).

0308-8146/$ - see front matter © 2007 Elsevier Ltd. All rights reserved.
doi:10.1016/j.foodchem.2007.01.016

2004), very little is known about antioxidant properties of crude extracts from their tissues and organs.

As an example, sea cucumbers are well known to exert beneficial effects on human health. These echinoderms are used in Asian traditional medicine to maintain fitness during long fishing travels or to prevent, reduce or cure several ailments like constipation, renal deficiency or arthritis. Several papers published in the last two decades came in support to these medicinal purposes showing multiple biological activities of sea cucumber extracts as wound healing promoter and exhibiting antimicrobial, anticancer, and immunomodulatory properties (Aminin, 2001; Fredalina et al., 1999; Kuznetsova et al., 1982; Ridzwan, Kaswandi, Azman, & Fuad, 1995; Tian et al., 2005). Their antioxidant properties have recently been reported from cœlomic fluid of three species (*Bohadschia marmorata vitiensis*, *Stichopus variegatus*, *S. badionotus*) collected in Malaysian coastal waters (Hawa et al., 1999). Authors concluded that sea cucumbers might be in the future an appropriate source of antioxidants for humans. To the best of our knowledge this study remains the only one focused on antioxidative properties of sea cucumbers and data about other tissues and organs of these echinoderms are still unavailable. These benthic organisms deserve much more interest from researchers in marine natural products as their antioxidant properties and potential application to nutraceutical and medical products need to be studied.

The purpose of this work was to evaluate the antioxidant potential of Atlantic sea cucumber *C. frondosa* Gunnerus (*Cucumaridae*), a widespread species in coastal waters of the North Atlantic Ocean. Aqueous and organic solvent extracts of digestive tract, gonads, muscles and respiratory apparatus were investigated for their antioxidative properties and their content of total phenols and flavonoids.

2. Materials and methods

2.1. Chemicals and reagents

Gallic acid, rutin hydrate, fluorescein, Folin–Ciocalteau's phenol reagent, 2,2′-azobis(2-amidinopropane) dihydrochloride (AAPH), 6-hydroxy-2,5,7,8-teramethylchroman-2-carboxylic acid (Trolox) were purchased from Sigma–Aldrich Ltd., (Oakville, ON, Canada). All other chemicals and solvents were of highest commercial grade.

2.2. Biological materials

Sea cucumbers of about 25 cm long and 400 g of fresh weight were collected by SCUBA diving in August 2004 at about 6 m depth from a rocky shore population near Baie-Comeau (north shore of St. Lawrence Estuary, Canada). Specimens were transported to nearby ISMER marine station (Rimouski, Canada) for dissection and analysis. Tissues of digestive tract, gonads, muscles, and respiratory apparatus of about 20 specimens were dissected and pooled together in pre-cleaned glass containers. All biological samples were freeze-dried for 96 h, dried samples were ground to fine powder and kept frozen (−20 °C) until extraction.

2.3. Extraction procedures

Antioxidant compounds were extracted as a function of their polarity using water, organic solvent and a mixture of water/miscible organic solvent.

2.3.1. Aqueous extraction

About 3.0 g of dried sample were suspended in 100 ml of freshly deionized water and the mixture continuously stirred in the dark at 4 °C to avoid accidental oxidation of light and thermal sensitive compounds. After 24 h, the supernatant was removed, centrifuged at 10 000*g*, filtrated on 0.45 μm glass filter, and collected in a series of preweighted tubes. Collected water extracts were freeze-dried until a constant weight was reached and tubes stored in a freezer until biological and chemical analyses were carried out.

2.3.2. Organic extraction

About 2.0 g of dried sample were first suspended in 70 ml of deionized water with pH adjusted to 2 using 6N HCl. Extraction was carried out using successively 180 and 70 ml of ethyl acetate in sepearatory funnel. The recovered organic fractions (ethyl acetate extracts) were dried with anhydrous Na_2SO_4 and the solvent was removed under vacuum, using an Automatic Environmental Speed Vac system (Instruments de Savant Inc., Holbrook, NY, USA) at 20 °C.

2.3.3. Water/organic solvent extraction

The extraction technique was adapted from Haug et al. (2002). Briefly, about 5–10 g of dried sample were suspended in 10 volumes (v/w) of a mixture of acetonitrile and 0.1% aqueous trifluoroacetic acid (TFA) solution in proportion of 60:40 (v/v) and continuously stirred for 24 h in the dark at 4 °C. Supernatant was removed, centrifuged at 2000*g*, filtered trough 0.45 μm filter and kept at 4 °C. Extraction was repeated once again and both extracts were combined thereafter. After cooling the mixture at −20 °C for 1 h a two-layer separation is obtained, the aqueous rich layer in the bottom was named the water-rich fraction and the top organic layer designated as the acetonitrile-rich fraction. These two layers were collected separately in pre-weighted tubes, evaporated under nitrogen flux and freeze-dried until a constant weight was attained.

2.4. Solid-phase extraction of water-rich fractions

According to Haug et al. (2002), a sequential elution of the water-rich fractions trough a chromatographic column was applied to aqueous extracts in this work, using C_{18} Sep-Pak cartridges (Waters). Briefly, dried extracts were

resuspended (100 mg/ml) in TFA acidified water (pH 4.7). Columns were pre-conditioned with 0.05% aqueous TFA. Samples were thereafter washed with deionized water to remove sea salt. Three successive elutions were carried out with 10%, 40% and 80% (v/v) of the mixture of acetonitrile and 0.05% aqueous TFA. Successive eluates were collected on pre-weighted tubes, evaporated under nitrogen flux and freeze-dried until a constant weight was attained.

2.5. Antioxidant assays

Antioxidant properties of samples were measured using (oxygen radical absorbance capacity) (ORAC) assay that measures their scavenging capacity against peroxyl radicals. The procedure was adapted from the method described by Ou, Hampsch-Woodill, and Prior (2001). Briefly, the ORAC assay was carried out using 96-wells microplates and a Fluoroskan Ascent Fl^{TM} microplate reader (Labsystems, Milford, MA, USA). Trolox, a water-soluble analog of vitamin E, was used as a positive control standard. The assay was conducted at 37.5 °C and pH 7.4 with a blank sample in parallel. The spectrofluorimeter was programmed to record the fluorescence of fluorescein every minute after addition of 2,2′-azobis(2-amidinopropane) dihydrochloride (AAPH). Final results were calculated by comparing the net areas under fluorescein decay curves between blank and samples. ORAC values were expressed in μmol of Trolox equivalents/mg dry weight of sample (μmol TE/mg dw).

2.6. Total phenols

Total phenols were determined using Folin–Ciocalteau's method. Aliquots of 40–100 μl of ethanol extracts were transferred into the test tubes and their volume adjusted to 500 μl with deionized water. After addition of 250 μl of Folin–Ciocalteau's reagent and 1.250 ml of 12.5% aqueous sodium carbonate solution, tubes were vortexed and held at room temperature for 40 min allowing complete reaction between reagent and phenols. Absorbance of the blue coloured solution was recorded at 750 nm (SpectraFluorPlus®, Tecan, Durham, NC, USA) against a blank containing 40–100 μl of ethanol. Total phenol content was calculated as a gallic acid equivalents using calibration curves prepared with gallic acid standard solutions. All measurements were carried out in triplicate.

2.7. Total flavonoids

Total flavonoids were determined using aluminum chelating method of Maksimovic, Malencic, and Kovacevic (2005). Aliquots of 50 μl of ethanol extracts were transferred into the test tubes and their volume completed to 750 μl with deionized water. After addition of 250 μl of $AlCl_3$ reagent, tubes were vortexed and held at room temperature for 30 min to allow the complete reaction between the reagent and flavonoids. Absorbance of the yellow coloured solution was recorded at 405 nm against blank containing 50 μl of ethanol. Total flavonoids content was calculated as a rutin equivalents using calibration curves prepared with rutin hydrate standard solutions covering a concentration range between 10 and 50 μg/ml.

2.8. Data analysis

Data were reported as mean values calculated from replicates ($n \geqslant 3$). Statistical treatments were performed using SigmaStat® software (Jandel Scientific) at 5% significance error level. Comparisons between groups were performed using *t*-test or 1-way ANOVA. When differences were detected we performed post hoc comparisons using the test of Student Newman Keuls (SNK). Relationship between ORAC values and total phenols and flavonoids were determined using linear regression.

3. Results

3.1. ORAC values

All samples showed antioxidative protection against peroxyl radicals. ORAC values varied greatly from 140 to 800 μmol of TE/g dw, depending upon tissues and extracts involved (Fig. 1). Digestive tract showed the best protection when considering water-rich fractions and ethyl acetate extracts. The best oxidative protection was obtained from gonads and muscles when considering acetonitrile-rich fractions and water extracts, respectively ($p < 0.05$). Among all extracts and fractions, acetonitrile-rich fractions were the most efficient ones with the highest ORAC values obtained for three (gonads, muscles and respiratory apparatus) out of four tissues of *C. frondosa*.

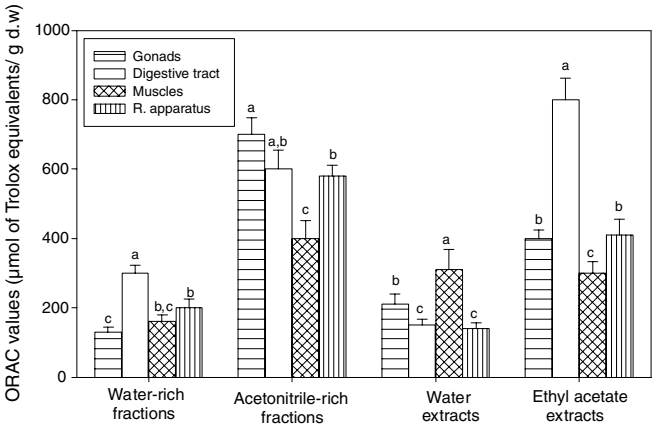

Fig. 1. ORAC values of extracts and fractions obtained from different tissues of *C. frondosa* using different solvents and extraction methods. Data are expressed as micromoles of Trolox equivalents per gram of dry extract. All assays were conducted in triplicate, and mean values are used. The vertical bars represent the standard deviation of each data point. Means within each group with different letters (a–d) differ significantly ($p < 0.05$) from each others. R. apparatus stands for respiratory apparatus.

Likewise, ORAC values of acetonitrile-rich fractions of digestive tract was relatively higher (600 ± 55 μmol of TE/g), but it was lower ($p < 0.05$) than ethyl acetate extracts (800 ± 62 μmol of TE/g) showing the highest values in this study (Fig. 1). The weakest ORAC values were obtained with water-rich fractions (gonads and muscles) and water extracts (digestive tract and respiratory apparatus). Higher ORAC values of the organic extracts over the aqueous ones were observed in both extraction types using water/organic mixture and single (water or organic) solvent. In water/organic solvent extraction, the ORAC ratio of the acetonitrile-rich/water-rich fractions ranged from 2.0 to 5.4, while in separated extractions, the ORAC ratio of extracts ethyl acetate/water varied between 1.0 and 5.3. An exception was found in muscle samples where ORAC values in ethyl acetate extracts (300 ± 34 μmol of TE/g) were comparable to the one in water extracts (310 ± 58 μmol of TE/g). The ORAC rank order for all four tissues was as follows: water-rich fractions ≈ water extracts < ethyl acetate extracts < acetonitrile-rich fractions.

ORAC values of eluates of water-rich fractions varied from 105 to 500 μmol of TE/g. Some eluates showed higher ORAC values than their parent fractions (Fig. 2). The ORAC ratio eluates/parent fractions varied greatly from 0.46 to 2.31 for all tissues. In muscles, all eluates showed ORAC values higher than the parent fraction ($p < 0.05$). In contrast, none of the three eluates from respiratory apparatus extract showed ORAC values higher than parent fraction. In eluates from gonads and digestive tract, only eluate 2 showed ORAC values higher than parent fractions. Comparison between eluates showed that the best ORAC response was obtained with eluates 2 and 3, with the exception observed for eluate #1 of respiratory apparatus.

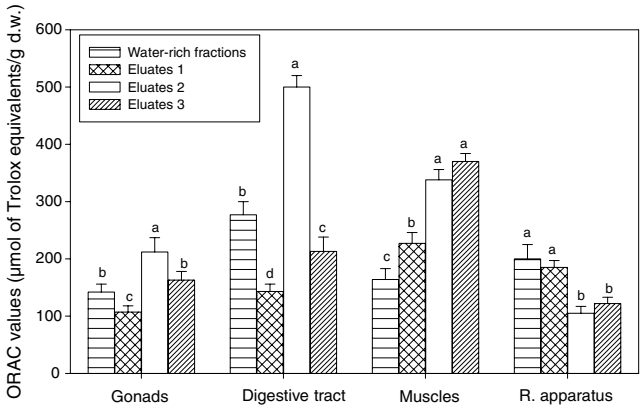

Fig. 2. ORAC values of eluates obtained from solid-phase extraction of water-rich fractions using different concentration of solvents. All tests were conducted in triplicate, and mean values are used. The vertical bars represent the standard deviation of each data point. Means within each group with different letters (a–d) differ significantly ($p < 0.05$) from each others. Eluates 1–3 were obtained from solvents ratios 10:90, 40:60 and 80:20 (v/v) using acetonitrile and 0.05% TFA acidified water, respectively.

3.2. Total phenols

Total phenols in samples varied from 22.5 to 236.0 mg of gallic acid equivalents/100 g dw, depending on tissues and extracts involved (Table 1). Digestive tract showed the highest level of total phenols when considering acetonitrile-rich fractions and ethyl acetate extracts, while the highest level was obtained from muscles and respiratory apparatus when considering water-rich fractions and water extracts, respectively ($p < 0.05$). Among fractions and extracts, acetonitrile-rich fractions showed the highest level of total phenols for all four tissues. Ethyl acetate and water extracts showed an intermediate level in total phenol content, while the lowest level was generally obtained for water-rich fractions. An exception was observed for respiratory apparatus where the lowest level was obtained from water extracts ($p < 0.05$). Total phenol contents of sea cucumber tissues followed order: water-rich fractions < water extracts ⩽ ethyl acetate extracts < acetonitrile-rich fractions.

Total phenols in eluates of water-rich fractions varied from 30.5 to 153.5 mg of GAE/100 g, depending on tissues and eluates involved (Table 2). Generally, there were more concentrated total phenols in eluates compared to their parent fractions, with the ratio eluates/parent fractions varying from 1.0 to 5.3. Comparison between eluates showed a general increase of total phenols as following: eluates 1 < eluates 2 < eluates 3. However, one exception was found in muscles where total phenol content was very similar between eluates 1 (43.7 ± 6.6 mg GAE/100 g) and eluates 2 (43.2 ± 7.9 mg of GAE/100 g).

3.3. Total flavonoids

The amount of total flavonoids in samples varied from 2.9 to 59.8 mg of rutin equivalents/100 g dw (Table 3).

Table 1
Total phenols (mg/100 g of dry weight)[a] in extracts and fractions obtained from digestive tract, gonads, muscles and respiratory apparatus of *C. frondosa* using separated and mixed extractions

Extracts/fractions	Gonads	D. tract[b]	Muscles	R. apparatus[c]
Mixed extractions				
Acetonitrile-rich fractions	130.2 ± 22.7	236.0 ± 36.3	194.1 ± 32.0	200.1 ± 33.7
Water-rich fractions	22.5 ± 4.0	30.7 ± 4.1	29.9 ± 5.5	41.2 ± 7.5
Separated extractions				
Ethyl acetate extracts	84.4 ± 12.9	177.6 ± 34.3	90.6 ± 13.4	75.6 ± 10.8
Water extracts	85.2 ± 14.1	67.0 ± 12.7	111.8 ± 17.2	26.1 ± 4.2

[a] Data expressed as gallic acid equivalent, mean ± SD ($n = 3$).
[b] D. tract, digestive tract.
[c] R. apparatus, respiratory apparatus.

Table 2
Total phenols (mg/100 g of dry weight)[a] in eluates obtained from solid-phase extraction of water-rich fractions of digestive tract, gonads, muscles and respiratory apparatus of *C. frondosa* using different proportions of solvents

Eluates[b]	Gonads	D. tract[c]	Muscles	R. apparatus[d]
1	30.5 ± 4.1 (22.5)	31.3 ± 3.6 (30.7)	43.7 ± 6.6 (29.9)	51.1 ± 8.2 (41.2)
2	61.5 ± 8.9 (22.5)	49.6 ± 7.3 (30.7)	43.2 ± 7.9 (29.9)	62.7 ± 8.1 (41.2)
3	75.6 ± 10.7 (22.5)	68.2 ± 9.9 (30.7)	153.5 ± 12.3 (29.9)	95.2 ± 10.3 (41.2)

[a] Data expressed as gallic acid equivalent, mean ± SD ($n = 3$). Total phenol contents of water-rich fraction of each tissue are given in parentheses.
[b] Eluates 1–3, obtained from solid-phase extraction of water-rich fractions using 10:90, 40:60 and 80:20 v/v of acetonitrile and 0.05% TFA acidified water, respectively.
[c] D. tract, digestive tract.
[d] R. apparatus, respiratory apparatus.

Gonads showed the highest level of flavonoids when considering water-rich and acetonitrile-rich fractions ($p < 0.05$). The highest level was obtained in digestive tract when considering water extracts and, in muscles ≈ digestive tract when considering ethyl acetate extracts. Flavonoid contents in acetonitrile-rich fractions and water extracts were generally comparable, being higher than the ones in ethyl acetate extracts and water-rich fractions ($p < 0.05$). However, a remarkably higher content of total flavonoids was found in acetonitrile-rich fractions (59.8 ± 6.3 mg of RE/100 g) compared to water extracts (8.1 ± 1.6 mg of RE/100 g) for gonad tissues ($p < 0.01$). The overall rank order for total flavonoids of the four tissues is given by the order: water-rich fractions < ethyl acetate extracts < water extracts ≈ acetonitrile-rich fractions.

Table 3
Total flavonoids (mg/100 g of dry weight)[a] in extracts and fractions obtained from digestive tract, gonads, muscles and respiratory apparatus of *C. frondosa* using separated and mixed extractions

Extracts/fractions	Gonads	D. tract[b]	Muscles	R. apparatus[c]
Mixed extractions				
Acetonitrile-rich fractions	59.8 ± 10.6	44.1 ± 8.2	21.8± 3.9	9.6 ± 1.0
Water-rich fractions	4.0 ± 0.9	2.9 ± 0.6	3.3 ± 0.6	3.2 ± 0.7
Separated extractions				
Ethyl acetate extracts	6.2 ± 1.0	10.0 ± 1.9	11.3 ± 2.1	4.2 ± 0.9
Water extracts	8.1 ± 1.3	44.8 ± 6.2	25.9 ± 3.8	11.7 ± 1.5

[a] Data expressed as rutin equivalent, mean ± SD ($n = 3$).
[b] D. tract, digestive tract.
[c] R. apparatus, respiratory apparatus.

Total flavonoids in eluates of water-rich fractions showed a high variability from 0.87 to 33.09 mg of RE/100 g (Table 4). Some eluates contained a much higher concentration of flavonoids than their parent fractions, with eluates/parent fractions ratio varying from 0.22 to 10.31. In gonads and digestive tract, eluates 2 and 3 showed total flavonoids contents higher than parent fractions ($p < 0.05$). In muscles and respiratory apparatus, eluates 3 showed total flavonoids higher than parent fractions ($p < 0.05$). Eluates 1 and 2 from muscles showed total flavonoids similar to and higher than parent fractions, respectively. Comparisons between eluates showed the general following pattern: eluates 1 ≈ eluates 2 < eluates 3.

3.4. ORAC values vs. phenolic compounds

Correlations between ORAC values of the whole of fractions and extracts and their content in total phenols and flavonoids are given in Fig. 3. ORAC values did not significantly correlate with total phenol contents ($r^2 = 0.13$, $p = 0.17$, $n = 16$), but total flavonoids and ORAC values showed a significant correlation ($r^2 = 0.729$, $p < 0.01$, $n = 16$). When analyzing data by individual tissues and organs significant correlations existed between ORAC values and total phenols for gonads ($r^2 = 0.79$, $p = 0.047$) and muscles ($r^2 = 0.92$, $p = 0.042$), but not for digestive tract and respiratory apparatus. Total flavonoids and ORAC values showed significant correlations for all fractions of the sea

Table 4
Total flavonoids (mg/100 g of dry weight)[a] in eluates obtained from solid-phase extraction of water-rich fractions of digestive tract, gonads, muscles and respiratory apparatus of *C. frondosa* using different proportions of solvents

Eluates[b]	Gonads	D. tract[c]	Muscles	R. apparatus[d]
1	0.87 ± 0.1 (3.97)	2.21 ± 0.5 (2.94)	5.09 ± 1.3 (3.32)	3.41 ± 0.7 (3.21)
2	10.54 ± 2.7 (3.97)	7.97 ± 1.8 (2.94)	3.53 ± 0.7 (3.32)	1.37 ± 0.3 (3.21)
3	12.15 ± 1.6 (3.97)	13.85 ± 2.1 (2.94)	20.91 ± 3.7 (3.32)	33.09 ± 5.7 (3.21)

Total flavonoid concentrations of water-rich extracts obtained of each tissue are given in parentheses.
[a] Data expressed as rutin equivalent, mean ± SD ($n = 3$).
[b] Eluates 1–3, obtained from solid-phase extraction of water-rich fractions using ratios 10:90, 40:60 and 80:20 v/v of acetonitrile and 0.05% TFA acidified water, respectively.
[c] D. tract, digestive tract.
[d] R. apparatus, respiratory apparatus.

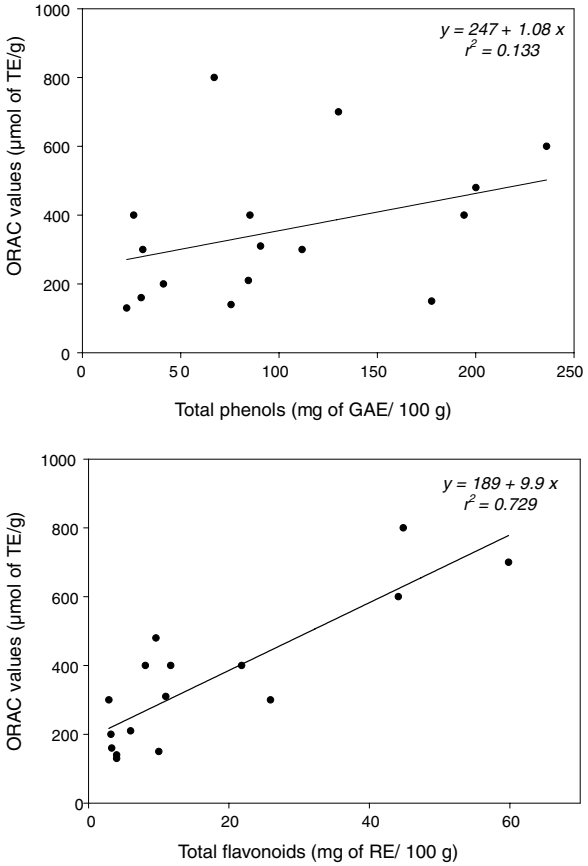

Fig. 3. Relationship between ORAC values of all extracts and fractions and their content in phenolic compounds. Top panel -total phenols, expressed as mg of gallic acid equivalents/100 g dw. Bottom panel -Total flavonoids, expressed as mg of rutin equivalent/100 g dw. Solid lines represent linear regression curves.

cucumber ($r^2 = 0.67 - 0.85$, $p < 0.05$; $n = 4$ for each tissue). Finally, no significant correlation was found between ORAC values of eluates of water-rich fractions and their content in total phenols ($r^2 = 0.03$; $p > 0.05$; $n = 12$, for the four tissues) and total flavonoids ($r^2 = 0.003$; $p > 0.05$; $n = 12$).

4. Discussion

The detailed study here on *C. frondosa* tissues and organs is a first to report a quantitative evaluation of the antioxidant properties of sea cucumber extracts. This anti-peroxyl radical activity screening of sea cucumber extracts is part of our research effort to explore the potential health benefit of echinoderms of St. Lawrence Estuary. The initial objective was to obtain some extracts showing suitable ORAC values that might rival the ones previously observed from natural extracts from plants. All ORAC values observed for *C. frondosa* (140–800 μmol of TE/g) are considered low when compared to very strong natural antioxidant fractions obtained from grape seeds (12 000 μmol of TE/g) and grape skin (15 000 μmol of TE/g) (Ou et al., 2001), or from medicinal plant leaves (16 000 μmol of TE/g) (Dominguez et al., 2005). However, most extracts and fractions showed ORAC values comparable to or exceeding those reported in the literature for several natural fractions obtained from plant materials, including medicinal (20–200 μmol of TE/g) and culinary herbs (20–800 μmol of TE/g) (Zheng & Wang, 2001), dried comestible (30–125 μmol of TE/g) and fresh fruits (20–600 μmol of TE/g), and vegetables (30–250 μmol of TE/g) (Wu et al., 2004).

Anti-peroxyl radical properties of natural extracts are generally attributed to redox reactions with some bioproducts present in extracts, notably phenolic compounds including flavonoids, anthocyanins or anthocyanidins (Ehlenfeldt & Prior, 2001; Prior et al., 1998). These compounds may act as rapid donators of a hydrogen atom to peroxyl radicals, before the latter react with biological molecules or, fluorescein for ORAC assays. Their presence in *C. frondosa* tissues was expected because these natural molecules are relatively easy to assimilate (Bravo, 1998; Karakaya, 2004) and main food sources of this suspensivorous benthic invertebrate are phytoplankton and particles derived from degrading marine macro-algae, rich in phenolic compounds. Total phenol and total flavonoid contents in marine algae might reach up to 20% and 12% of dry mass, respectively (Lim, Cheung, Ooi, & Ang, 2002; Van Alstyne, McCarthy, Hustead, & Duggins, 1999; Yoshie-Stark, Hsieh, & Suzuki, 2003). Levels of total phenols and flavonoids from sea cucumber tissues are reported for the first time in this study. Concentrations observed in *C. frondosa* extracts are consistently lower compared to those generally observed in extracts from plants known for their high antioxidant properties. However, their range in total phenols (23–236 mg of GAE/100 g) is similar to values observed in anti-peroxyl radical plant extracts with low phenolic contents including those of some comestible fruits (59–262 mg/100 g), vegetables (24–244 mg/100 g) and nuts (68–274 mg/100 g) (Toor & Savage, 2006; Wu et al., 2004). Likewise, their content of total flavonoids (3–60 mg/100 g) approached or exceeded values observed in plants with low content of flavonoids including some fruits (10–96 mg/100 g) (Kim, Chun, Kim, Moon, & Lee, 2003; Kuti, 2004; Luximon-Ramma, Bahorun, & Crozier, 2003) and vegetables (21–94 mg/100 g) (Luximon-Ramma et al., 2003).

A good correlation existed between the ORAC values and total phenols in gonads and muscles, but an apparent lower contribution of total phenols in digestive tract and respiratory apparatus. This apparent discrepancy might be the result of a significant difference in phenolic constituents between tissues. Previous studies reported that contribution of individual phenols to anti-peroxyl radicals varies among chemical species and depends upon their concentration (Zheng & Wang, 2003). When examining the relationship between total flavonoids and ORAC values we observed a highly significant correlation of this subgroup of phenols with anti-peroxyl radical activities. This result shows that sea cucumber extracts present some similarities in their anti-peroxyl radical activities with plant materials showing a probable high contribution of flavo-

noids to the redox reactions. This correlation also suggests that among phenolic constituents present in *C. frondosa* tissues, flavonoids contributed a major part to anti-peroxyl radical activities observed in digestive tract and respiratory apparatus extracts. As well, they seem to contribute extensively to the redox activity in extracts and fractions from gonads and muscles, but a significant contribution from other phenols is also expected because flavonoids measured in *C. frondosa* extracts represented in average less than 20% of total phenols. The presence of easy assimilated antioxidant phenols like anthocyanins, anthocyanidins, tannins and others in the food sources of *C. frondosa* suggests their potential occurrence within its tissues.

Solid-phase extraction (SPE) technique was used in an attempt to increase the antioxidative potency of water-rich fractions that generally showed low ORAC values. This technique is useful to discard some water-soluble and non-antioxidative constituents that might exert a dilution effect lowering weight based ORAC values. However, some anti-peroxyl radical constituents could be lost in washing solvent or retained within SPE column during elution steps (Kähkönen, Hopia, & Heinonen, 2001; Zheng & Wang, 2003). In most of our eluates, the relative concentration of total phenols increased compared to their parent fractions. This result suggests a significant removal of some water-soluble constituents like salt, sugars, ascorbic acid, glutathione, peptides, and other water-soluble compounds during SPE process that have little antioxidant activities. Yellow to reddish color of the washing eluates observed during SPE process suggests the removal of some water-soluble pigments. There was probably a loss of flavonoid constituents in washing eluates since total flavonoids in some eluates remained stable or decreased after SPE treatment (Tables 1 and 2). Finally, results obtained from correlation analysis suggest a relative increase of the presence of some other water-soluble anti-peroxyl radical constituents following SPE. In fact, neither total phenols, nor total flavonoids present in these eluates seem to contribute significantly to the response of ORAC assays. In light of these results, improving ORAC values by using SPE is possible, but further studies are needed to learn more about the changes in anti-peroxyl radical constituents during successive elution steps.

Our results come in support to the antioxidant potential previously observed in cœlomic fluid of three sea cucumber species collected from coastal waters of Terengganu, Malaysia (Hawa et al., 1999). Cœlomic fluid in echinoderms shows a chemical composition close to seawater surrounding the animal and contents amoebocytes devoted to the immune defense system of the animal. Authors observed an important activity of enzymatic antioxidants (superoxide dismutase = 5–9 $\times$ 10^5 IU/g protein) and low to moderate scavenging activity of DPPH (9–52%). This antioxidative potential of sea cucumber cœlomic fluid comes in support to its believed health benefit by Asian people and suggests its usefulness in preventing oxidation of vital biomolecules. As observed from our study, the edible part of *C. frondosa* (muscles, mainly exported to Asian market) could also provide to consumers an appropriate anti-peroxyl radical protection. By-products including gonads, digestive tract and respiratory apparatus showed suitable anti-peroxyl radical activities suggesting the moderate antioxidant potential of *C. frondosa* and a possible valorization of its tissues as an additional source of antioxidants for human diet.

5. Conclusions

Results from this study show that tissues of *C. frondosa* contain natural bioproducts able to prevent oxidative reactions, notably the ones initiated by peroxyl radicals. Active extracts examined here might exhibit beneficial effects in prevention of polyunsaturated fatty acids oxidation and the alteration of biological membranes often linked with numerous severe diseases. Among the anti-peroxyl radical bioproducts, flavonoids are suggested to be mainly responsible of observed activities, but a significant contribution of other phenolic compounds is expected. Further, research is needed to identify and characterize chemicals contributing to antioxidant properties of the extracts.

Acknowledgements

This study has been supported by the Canadian Research funds FQRNT-Actions concertées and Quebec government partners. Authors thank Oursins Nordiques Inc. of Baie-Comeau (Québec, Canada) for providing sea cucumbers. This paper is a contribution of Quebec-Ocean network.

References

Aminin, D. L. (2001). Immunomodulatory properties of Cucumariosides from edible far-eastern holothurian *Cucumaria japonica*. *Journal of Medicinal Food, 4*, 127–135.

Bravo, L. (1998). Polyphenols: chemistry, dietary sources, metabolism, and nutritional significance. *Nutrition Reviews, 56*, 317–333.

Dominguez, M., Nieto, A., Marin, J. C., Keck, A. G., Jeffrey, E., & Céspedes, C. L. (2005). Antioxidant activities of extracts from *Barkleyanthus salicifolius* (Asteracea) and *Penstemon gentianoides* (Scrophulariacea). *Journal of Agricultural and Food Chemistry, 53*, 5889–5895.

Ehlenfeldt, M. K., & Prior, R. L. (2001). Oxygen radical absorbance capacity (ORAC) and phenolic and anthocyanin concentrations in fruit and leaf tissues of highbush blueberry. *Journal of Agricultural and Food Chemistry, 49*, 2222–2227.

Fredalina, B. D., Ridzwan, B. H., Zainal Abidin, A. A. Z., Kaswandi, M. A., Zaiton, H., Zali, I., et al. (1999). Fatty acid compositions in local sea cucumber, *Stichopus chloronotus*, for wound haling. *General Pharmacology, 33*, 337–340.

Haug, T., Kjuul, A. K., Styrvold, O. B., Sandsdalen, E., Olsen, Ø. M., & Stensvåg, K. (2002). Antibacterial activity in *Strongylocentrotus droebachiensis* (Echinoidea), *Cucumaria frondosa* (Holothuroidea), and *Asterias rubens* (Asteroidea). *Journal of Invertebrate Pathology, 81*, 94–102.

Hawa, I., Zulaikah, M., Jamaludin, M., Zainal Abidin, A. A., Kaswandi, M. A., & Ridzwan, B. H. (1999). The potential of the coelomic fluid of sea cucumber as an antioxidant. *Malaysian Journal of Nutrition, 5*, 55–59.

Jha, R. K., & Zi-rong, X. (2004). Biomedical compounds from marine organisms. *Marine Drugs, 2*, 123–146.

Kähkönen, M. P., Hopia, A. I., & Heinonen, M. (2001). Berry phenolics and their antioxidant activity. *Journal of Agricultural and Food Chemistry, 49*, 4076–4082.

Karakaya, S. (2004). Bioavailablity of phenolic compounds. *Critical Reviews in Food Science and Nutrition, 44*, 453–464.

Kim, D-O., Chun, O. K., Kim, Y. J., Moon, H-Y., & Lee, C. Y. (2003). Quantification of polyphenolics and their antioxidant capacity in fresh plums. *Journal of Agricultural and Food Chemistry, 22*, 6509–6515.

Kohen, R., & Nyska, A. (2002). Oxidation of biological systems: oxidative stress phenomena, antioxidants, redox reactions, and methods for their quantification. *Toxicologic Pathology, 30*, 620–650.

Kuti, J. O. (2004). Antioxidant compounds from four *Opuntia cactus* pear fruit varieties. *Food Chemistry, 85*, 527–533.

Kuznetsova, T. A., Anisimov, M. M., Popov, A. M., Baranova, S. I., Afiyatullov, S. S., Kapustina, I. I., et al. (1982). A comparative study in vitro of physiological activity of triterpene glycosisdes of marine invertebrates of echinoderm type. *Comparative Biochemistry and Physiology C, 73*, 41–43.

Lee, J., Koo, N., & Min, D. B. (2004). Reactive oxygen species, aging, and antioxidative nutraceuticals. *Comprehensive Reviews of Food Science and Food Safety, 3*, 21–33.

Lim, S. N., Cheung, P. C. K., Ooi, V. E. C., & Ang, P. O. (2002). Evaluation of antioxidative activity of extracts from a brown seaweed, *Sargassum siliquastrum*. *Journal of Agricultural and Food Chemistry*, 3862–3866.

Luximon-Ramma, A., Bahorun, T., & Crozier, A. (2003). Antioxidant activities and phenolic and vitamin C contents of common Mauritian exotic fruits. *Journal of the Science of Food and Agriculture, 83*, 496–502.

Maksimovic, Z., Malencic, D., & Kovacevic, N. (2005). Polyphenol contents and antioxidant activity of *Mayadis stigma* extracts. *Bioresource Technology, 96*, 873–877.

Ou, B., Hampsch-Woodill, M., & Prior, R. L. (2001). Development and validation of an improved oxygen absorbance capacity assay using fluorescein as the fluorescent probe. *Journal of Agricultural and Food Chemistry, 49*, 4619–4626.

Prior, R. L., Cao, G., Martin, A., Sofic, E., McEwen, J., O'Brien, C., et al. (1998). Antioxidant capacity as influenced by total phenolic and anthocyanin content, maturity, and variety of *Vaccinum* species. *Journal of Agricultural and Food Chemistry, 46*, 2686–2693.

Ridzwan, B. H., Kaswandi, M. A., Azman, Y., & Fuad, M. (1995). Screening for antibacterial agents in three species of sea cucumbers from coastal areas of Sabah. *General Pharmacology, 26*, 1539–1543.

Tian, F., Zhang, X., Tong, Y., Yi, Y., Zhang, S., Li, L., et al. (2005). A new sulfated saponin from sea cucumber, exhibits anti-angiogenic and anti-tumor activities in vivo and in vitro. *Cancer Biology and Therapy, 4*, 874–882.

Toor, R. K., & Savage, G. P. (2006). Effect of semi-drying on the antioxidant components of tomatoes. *Food Chemistry, 94*, 90–97.

Van Alstyne, K. L., McCarthy, J. J., III, Hustead, C. L., & Duggins, D. O. (1999). Geographic variation in polyphenolic levels of Northeastern Pacific kelps and rockweeds. *Marine Biology, 133*, 371–379.

Wu, X., Beecher, G. R., Holden, J. M., Haytowitz, D. B., Gebhardt, S. E., & Prior, R. L. (2004). Lipohilic and hydrophilic antioxidant capacities of common foods in the United States. *Journal of Agricultural and Food Chemistry, 52*, 4026–4037.

Yoshie-Stark, Y., Hsieh, Y-P., & Suzuki, T. (2003). Distribution of flavonoids and related compounds from seaweeds in Japan. *Journal of Tokyo University of Fisheries, 89*, 1–6.

Zheng, W., & Wang, S. Y. (2001). Antioxidant activity and phenolic compounds in selected herbs. *Journal of Agricultural and Food Chemistry, 49*, 5165–5170.

Zheng, W., & Wang, S. Y. (2003). Oxygen radical absorbing capacity of phenols in blueberries, cranberries, chokeberries, and lingonberries. *Journal of Agricultural and Food Chemistry, 51*, 502–509.