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Biochemical composition and antioxidant potential of the edible Mediterranean sea cucumber *Holothuria tubulosa*

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SUMMARY: The sea cucumber or *holothurian* is a marine species which has been prized in some Asian countries for its nutritional qualities. The purpose of this work was to study the biochemical composition and free radical scavenging and antioxidant activities of *Holothuria tubulosa* tegument from the Bizerta lagoon in northern Tunisia. The obtained data demonstrated that the extract of sea cucumber teguments exhibited high biochemical levels (such as moisture 80.77%, protein 7.07%, lipids 10.21%, energy value 13.64 Kcal/g ww), and an important nutritional value (including n-3/n-6: 2.11, EPA+DHA: 20.96, AI: 1.38 and TI: 0.54). High antioxidant activities were recorded in the integument by the radical scavenging tests of ABTS and DPPH as well as by the total antioxidant capacity and the FRAP in comparison with the BHT standard. Our results showed that *H. tubulosa* tegument has high nutritional value with high antioxidant activities and could be considered a nutraceutical product.

KEYWORDS: *Antioxidants activities; Fatty acids;* Holothuria tubulosa*; Nutritional value; Proximate composition; Teguments*

RESUMEN: ***Composición bioquímica y potencial antioxidante del pepino del mar Mediterráneo comestible* Holothuria tubulosa**. El pepino de mar o la *holoturia* es una especie marina apreciada en algunos países asiáticos por sus cualidades nutricionales. El propósito de este trabajo fue estudiar la composición bioquímica y las actividades antioxidantes y de eliminación de radicales libres del tegumento de *Holothuria tubulosa* de la laguna de Bizerta, en el norte de Túnez. Los datos obtenidos demuestran que el extracto de tegumentos de pepino de mar mostró altos niveles bioquímicos (como humedad 80,77%, proteína 7,07%, lípidos 10,21%, valor energético 13,64 Kcal/gww) y un valor nutricional importante (incluyendo n-3/ n-6: 2,11, EPA+DHA: 20,96, AI: 1,38 y TI: 0,54). Se registraron altas actividades antioxidantes en el tegumento mediante las pruebas de eliminación de radicales de ABTS y DPPH, así como por la capacidad antioxidante total y el FRAP, y esto, en comparación con el estándar BHT. Nuestros resultados mostraron que el tegumento de *H. tubulosa* tiene un valor nutricional importante con una alta actividad antioxidante y podría considerarse un producto nutracéutico.

PALABRAS CLAVE: *Ácidos grasos; Actividades antioxidantes; Composición proximal;* Holoturia tubulosa*; Tegumentos; Valor nutricional*

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1. INTRODUCTION

Seafood is among the most appreciated foods worldwide and is in high demand by consumers. Its production has great economic value in coastal countries. Several marine organisms such as sea cucumbers are considered healthy seafood and have received an increase in interest by nutritionists and pharmacologists due to their health benefits (Santos *et al*., 2015; Bilgin and Tanrikulu, 2018). This marine organism contains high nutritional quality such as protein, fatty acids, especially ecosapentaeinoic acid (EPA; 20:5n-3), and docosahexaenoic acid (DHA; 22:6n-3), vitamins (A, C, B1, B2, and B3), aminoacids and essential elements such as Calcium, Magnesium, and Zinc (Barzkar *et al*., 2017; Bilgin and Tanrikulu, 2018). They also contain high levels of different physiologically active substances, including polysaccharides (chondroitin sulfate), and saponin glycosides along with a wide range of molecules endowed with high levels of biological activities. In Asia and in the Middle East, sea cucumbers are exported in large quantities to Asian markets and contribute significantly as a human food source (Taiyeb-Ali *et al*., 2003). On the Catalonian market (Spain), sea cucumber is one of the most expensive product among seafood (Maggi *et al*., 2015). The biological and beneficial effects of saponins from sea cucumber showed a wide spectrum of nutraceutical and pharmacological effects (Taiyeb-Ali *et al*., 2003; Ibrahim *et al*., 2017). In addition, Yaacob *et al*., (1994) showed similar anti-inflammatory properties of sea cucumber extract to indomethacin in rats. These authors also evaluated the antinociceptive activity of sea cucumber extract in mice and compared this activity to morphine, aspirin, and paracetamol. *In vivo* and *in vitro* tests showed that saponins extracted from sea cucumber species are effective in lowering body weight and it is suggested that holothurians secondary metabolites might represent a new source of anti-obesity drugs in diet therapy (Guo *et al*., 2016). Additionally, sea cucumber extracts possess potential activity to scavenge free radicals (Althunibat *et al*., 2009; Oh *et al*., 2017). In fact, Zhong *et al*. (2007) reported that *Cucumaria frondosa* exerted free radical scavenging properties using the ORAC (Oxygen Radical Absorbance Capacity) and DPPH (2,2-diphenyl-2-picrylhydrazyl) assays. In addition, Yu *et al*., (2014) demonstrated that the extracted molecules from *Thelenota ananas* had an inhibitor effect on super oxide radicals. Recently, Ibrahim *et al*., (2017) demonstrated that the Egyptian sea cucumber *Holothuria atra* possessed an important antioxidant activity to scavenge free radicals using the DPPH test. Despite several worldwide studies, particularly in Asian countries that have revealed the biological activities of several sea cucumber species, there is a lack of information about antioxidant activity and nutritional value of Southern Mediterranean species, especially those collected from lagoon ecosystems.

In view of the above information and in light of the fact that no studies are available in the literature regarding the biochemical and antioxidant activity of *H. tubulosa* from the Tunisian coastline, the present investigation aims to reveal the biochemical composition (lipids, proteins and fatty acids) of *H. tubulosa* teguments collected from the Bizerta lagoon, which is classed the second largest lagoon in Tunisia (Figure 1). As a second purpose, this study delivers new information about the capacity of both the free radical scavenging and antioxidant activities of the studied organism.

2. MATERIALS AND METHODS

2.1. Chemicals

The chemicals, reagents and solvents used were purchased from Sigma-Aldrich (Milan, Italy).

2.2. Sample collection

Specimens of sea cucumber were taken from the Bizerta lagoon (latitude: 37°8'–37°14'N, longitude 9°46'–9°56'E), located in the Northeast of Tunisia (Figure 1). Twenty-six individuals were collected by scuba diving at depths between 3 and 15 meters in July, 2014. The collected samples were transported immediately to the laboratory in an aerated seawater box. Upon arrival at the laboratory, the identification of the Holothuroidea species was based on the morphological and anatomical criteria as reported by several studies (Tortonese, 1965). For the biochemical analyses, ten individual sea cucumbers (n=10) were rinsed with ultrapure water and dissected on ice to obtain the teguments. Six (n=6) specimens were designated to fatty acid analysis and the other ones (n=10) were dissected and their portions were stored at -80 °C until analysis.

2.3. Proximate composition

The proximate chemical composition was determined with tegument samples from 10 animals. Thus, moisture content (%) was estimated by the weight change of 10 g of each sample at 105 ± 2 °C for 24h (AOAC, 2005). The protein content (%) of each sample (1 g) was determined according to the procedure of Lowry *et al*., (1951) using bovine serum albumin as the standard. Total lipids (%) for each sample (10 g) were obtained according to the procedure based on the Folch *et al*., (1957) method using the solvent mixture chloroform-methanol (2:1, v/v), which contains 0.01% butylated hydroxyl toluene (BHT), considered as an antioxidant.

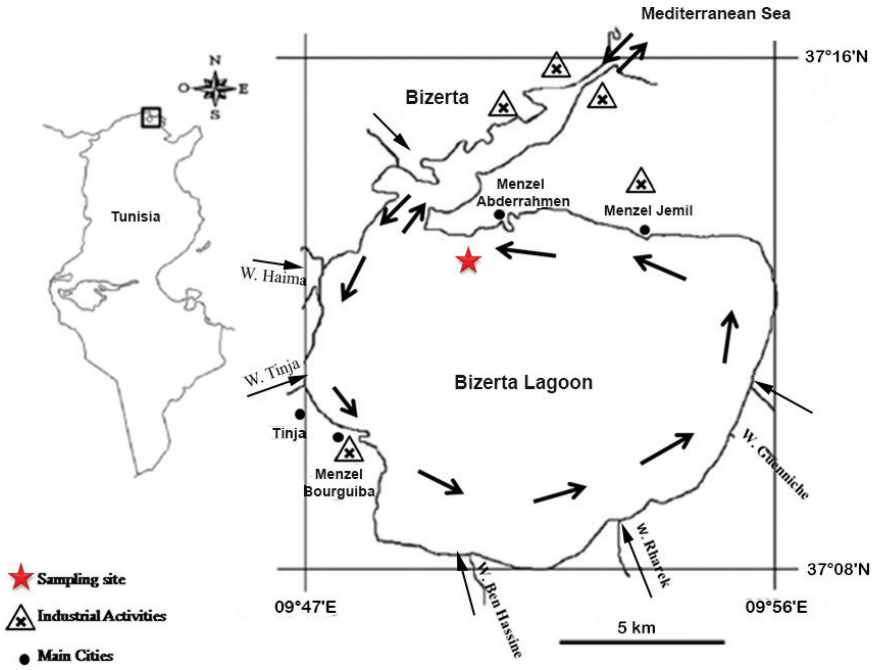

FIGURE 1. Schematic map of the Bizerta lagoon indicating the sampling area.

2.4. Energy value

This value is known as the caloric content and was determined on the base of the dry weight of the biochemical substrates by means of Rubner's coefficients, e.g., lipids 9.45, carbohydrates 4.20, and proteins 5.65 (Winberg, 1971). The energy value was expressed as Kcal/g DW.

2.5. Fatty acid analysis

Fatty acid extractions were trans-methylated from total lipids and the samples (n=10) were extracted with hexane: diethyl ether (1:1, v/v) and nonadecanoic acid (C19:0) was added as an internal standard in order to obtain fatty acid methyl-esters (FAMEs). The FAMEs were then determined by gas chromatography (HP, 6890 GC) using a split/splitless injector (30m HP In a no-wax capillary column, with a diameter of 250 mm and 0.25 mm film thickness) equipped with a flame ionization detector and Nitrogen gas (Bejaoui *et al.*, 2017). The injector temperatures were programmed to rise from 50 to 180 °C at a rate of 4 °C/min, from 180 °C to 220 °C at 1.33 °C/min and to stabilize at 220 °C for 7 min. FAMEs were determined in each sample according to the retention times of the commercial standard methyl esters (SUPELCO PUFA-3). The amount of FA was expressed as a percent of the total amount of the analyzed sample (Bejaoui *et al.*, 2017).

2.6. Nutritional quality analysis

According to Marque *et al.*, (2010), the nutritional quality analysis such as n-3 PUFA/n-6 PUFA and EPA+DHA, were determined in the edible tegument of *H. tubulosa*. Also, atherogenic and thrombogenic indices (AI and TI) were assessed using the Ulbricht and Southgate, (1991) method.

AI and TI indices were calculated using the following equations:

$$AI = (12{:}0 + 4 \times 14{:}0 + 16{:}0)/[\Sigma MUFA + \Sigma PUFA\ (n\text{-}6) \text{ and } (n\text{-}3)]$$

$$TI = (14{:}0 + 16{:}0 + 18{:}0)/[0.5 \times \Sigma MUFA + 0.5 \times \Sigma PUFA\ (n\text{-}6) + 3 \times \Sigma PUFA\ (n\text{-}3) + (n\text{-}3)/(n\text{-}6)]$$

With MUFA: monounsaturated fatty acids and PUFA: polyunsaturated fatty acids.

2.7. Determination of in vitro antioxidant activity

2.7.1. DPPH free radical scavenging activity

The Holothurian extracts were tested for their ability to scavenge 1,1-Diphenyl-2-picrylhydrazyl free radical (DPPH). Briefly, the principle of this test was to evaluate the antiradical species of the extract of sea cucumber teguments and the DPPH chemical radical, which was dissolved in methanol at different concentrations. The mixture of sample and DPPH solution were incubated in the dark for 30 min in

order to react, and the optical density was measured by spectrophotometer at 517 nm. Methanol was used as a blank and butylated hydroxytoluene (BHT) was used as the standard reference antioxidant. Results are expressed as percentage of activity and calculated following the decrease in the color of the mixture according to the followed equation:

$$I\ \% = [(Abs\ cont - Abs_{test\ sample})/Abs\ cont]*100$$

Where: **Abs cont:** absorbance of control; **Abs test sample:** absorbance of reacting mixture with the test sample.

2.7.2. ABTS free radical scavenging activity

The purpose of this test was to assess the ability of the antioxidant substance of sea cucumber to scavenge the ABTS radical cation ($ABTS^{\bullet+}$) which was generated in the aqueous phase. Briefly, ABTS (7.4 Mm dissolved in water) was mixed with potassium persulfate (2.6 Mm dissolved in water) and incubated in the dark for 12 hours. Then the working solution was diluted with 60% methanol. *H. tubulosa* extracts were prepared at different concentrations and mixed with 3mL of the previous solution of ABTS, then incubated in the dark for 2 hours. The absorbance was determined at 734 nm. Methanol was used as a blank. Reaction was calculated according to the followed equation and expressed as percentage of scavenge ABTS radicals:

$$\text{ABTS radicals scavenged activity (\%)} = [(A\ cont - A\ test) / A\ cont] * 100$$

Where: **A cont:** absorption of control; **A test:** absorption of the tested sample.

2.7.3. Ferric reducing antioxidant power (FRAP) assay

This method was used to quantify the reducing power of antioxidant compounds. The ability of holothurian extract reduced the ferric component (Fe^{3+}) to the ferrous component (Fe^{2+}); the latter formed a blue complex ferrictripyridyl triazine (Fe^{2+}/TPTZ) at low pH, which was accompanied by the formation of a blue color. This reduction was monitored by measuring the absorption at 700 nm. This method consisted of mixing the holothurian extract at different concentrations with 2.5 ml of FRAP reagent (0.2 M phosphate buffer at pH 6.6 and 2.5 ml of a 1% solution of $K_3Fe(CN)_6$, (w/v). The resulting mixture was incubated for 20 minutes at 50 °C and then 2.5 ml of 10% trichloroacetic acid were added to stop the reaction. The mixture was centrifuged at 1000 g for 10 minutes at room temperature and 2.5 ml of the supernatant were added with 2.5 ml of distilled water and 0.5 ml of 0.1% $FeCl_3$ (w/v). The EC_{50} value ($\mu g.ml^{-1}$) was the effective concentration of the extract at which the absorbance was 0.5.

2.7.4. Total antioxidant capacity (T-AOC) assay

This test was based on the reduction of molybdenum (VI) to molybdenum (V) by the holothurian extract. At acidic pH, this reduction induced the formation of the green phosphate/Mo (V) complex. An aliquot of 0.1 ml of extract was combined in a tube with 1 ml of solution composed of sulfuric acid (0.6 N), sodium phosphate (28 mM) and ammonium molybdate (4 mM). The tubes were incubated at 95 °C for 90 minutes. After standing for 6 minutes at room temperature, the absorbance was measured at 695 nm against a blank instead of the extract. The total antioxidant activity was expressed in mg of gallic acid equivalent per gram of dry holothurian extract ($\mu g\ EAG.mg^{-1}$ DW).

2.8. Statistical analysis

The results are presented as means ± standard deviation (S.D.) of three replicates. For each biological parameter, the data were tested for normality and homogeneity of variance, respectively by the Kolmogorov-Smirnov and Levene tests. Comparisons between two samples were performed by the Student's parametric t-test. In addition, the comparisons of more than two samples were conducted through one-way analysis of variance (ANOVA) followed by Tukey's *post hoc* HSD test. The median inhibitory concentration, IC_{50}, was evaluated from dose-response curves fitted by nonlinear analysis using the GraphPad Prism® v.6.0 statistical software package (from GraphPad Software). Antioxidant activity is expressed as IC_{50} and its 95% CI (95% confidence interval) of at least three independent experiments. IC_{50} represents the geometric mean, which is the best measure of the central tendency parameter for determining IC_{50} values due to the fact that they follow log-normal distribution. Data analysis was carried out using GraphPad InStat v.3.0a for MacIntosh (GraphPad Software, San Diego, CA, USA). All statistical tests were two-sided and a p-value ≤ 0.05 was regarded as statistically significant.

3. RESULTS

3.1. Proximate composition and energy value

The data concerning the proximate composition of *H. tubulosa* are reported in table 1. The results revealed that lipid levels were the major compounds in the tegument (10.213 ± 0.372%) followed by proteins (7.077 ± 0.145%). Lipids and proteins have numerous imperative roles for human health,

depending on several factors (Çakli *et al*., 2008). Gianasi *et al*., (2016) found that nutritional reserves of sea cucumber varied, mostly during the reproductive cycle. However, Hudson *et al*., (2004) reported that the amount and the biochemical composition of *Amperima rosea*, *Deima validum* and *Bathyplotes natans* depended more on the availability of food in the sampled ecosystem. Similar trends were observed for lipids in *H. tubulosa* and *H. polii* from the Southern Adriatic Sea (Sicuro *et al*., 2012). Our data were more important in terms of lipids than those reported by Çakli *et al*., (2008) carried out on *H. fuscogilua*, *H. ananas*, *B. argus* and *H. tubulosa*.

The moisture level and energy value presented promising values in sea cucumber teguments, with 80.773 ± 0.676 and 13.649 ± 0.433%, respectively. In line with our findings, several reports carried on *H. tubulosa* collected from Turkey and Sri Lanka showed similar trends for moisture level (Aydin *et al*., 2011; Çakli *et al*., 2008) as did other sea cucumber species such as *H. polii* and *H. mammata* (Aydin *et al*., 2011). Barzkar *et al*., (2017) also found high moisture levels in *Holothuria arenicola* and *Stichopus horrens* from Oman. Our results indicated that *H. tubulosa* from the Bizerta lagoon are characterized by remarkable energy values due to the great lipid levels.

Table 1. Proximate composition (PC) and energy value (EV) of *H. tubulosa* teguments.

	Teguments
Moisture (%)	80.773 ± 0.676
Proteins (%)	7.077 ± 0.145
Lipids (%)	10.213 ± 0.377
EV (Kcallg ww)	13.649 ± 0.433

Data are expressed as mean ± SD (n = 10).

3.2. Fatty acid composition

The fatty acid composition of *H. tubulosa* teguments is shown in Table 2. The teguments were predominated by saturated fatty acids (SFA), followed by polyunsaturated (PUFA) and monounsaturated (MUFA) fatty acids with 48.85, 32.48 and 25.97%, respectively. Three SFA were identified. Among them, the major ones were stearic acid (C18:0) and palmitic acid (C16:0), followed by myristic (C14:0) acid. The results revealed that MUFA was dominated by oleic acid (C18:1n-9) with 12.86% and palmitoleic acid (C16:1) with 9.44%. Similar results were reported by Drazen *et al*., (2008) from several species such as *H. leucospilota*, *H. atra*,

Table 2. Fatty acid (%) composition of *H. tubulosa* teguments in the present study and as described in several bibliographic references.

Fatty acids	**The present study (Mediterranean sea)**	**Sicuro *et al*. (2012) (Southern Adriatic sea)**	**Bilgin *et al*. (2018) (Izmir Turkey)**	**Aydin *et al*. (2011) (Sakran Turkey)**
C14:0	1.14 ±1.00	3.14 ±1.00	1.65 ±0.05	1.38 ±0.26
C16:0	14.96 ±1.90	8.96 ±1.92	6.46 ±0.57	4.05 ±0.15
C18:0	19.28 ±1.63	9.28 ±1.63	7.20 ±1.01	3.54 ±0.09
UD SFA	3.47 ±2.03	7.25 ±0.00	4.42 ±0.54	7.39 ±0.61
Total SFA	**38.85 ±1.64**	**28.63±1.61**	**19.73±1.47**	**16.36 ±1.11**
C16:1	8.93 ±2.04	9.44 ±2.85	4.80 ±0.19	4.11 ±0.54
C18:1 n-9	12.86 ±0.14	2.86 ±0.14	4.062±0.33	1.12 ±0.23
C18:1 n-7	1.47 ±0.05	UD	2.51 ±0.07	UD
UD MUFA	2.20 ±1.08	8.68 ±0.00	10.03±0.19	9.16 ±0.43
Total MUFA	**25.97 ±1.03**	**21.31 ± 0.99**	**21.40±0.37**	**14.39 ±0.34**
C18:2 n-6	1.21 ±0.10	1.27 ±0.17	4.02 ±0.11	2.55 ±0.24
C20:4 n-6	4.39 ±0.66	UD	17.16±0.59	10.55 ±0.09
C20:5 n-3	15.43±3.85	15.43 ±3.85	10.02±0.38	7.46 ±0.81
C20:3 n-6	4.22 ±0.55	2.99 ±0.24	UD	UD
C22:6 n-3	5.10 ±0.62	5.43 ±0.74	3.16 ±0.13	13.48 ±0.29
UD PUFA	1.64 ±1.39	24.94±1.55	1.65 ±0.30	19.90 ±0.35
n-3 PUFA	20.96±4.59	20.86±2.29	13.80±0.25	20.94 ±0.55
n-6 PUFA	9.88 ±1.38	4.26 ±0.20	21.18±0.35	13.10 ±0.33
Total PUFA	**32.48±1.84**	**50.06 ±1.25**	**36.01± 076**	**53.94 0.31**

Data are expressed as mean ± SD of 6 replicates.
UD: undetermined; SFA: saturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids.

Abyssocucumis abyssorum, Oneirophanta mutabilis and *Peniagone vitrea*. They showed an abundance of SFA compared to PUFA and MUFA. A dissimilar dominance of PUFA over SFA was reported for *H. tubulosa* from the southern Adriatic and Aegean Seas (Aydin *et al*., 2011; Bilgin and Tanrikulu, 2018; Sicuro *et al*., 2012). This variation could be due to the abundance of a dietary source such as phytoplankton, which is considered a major food for sea cucumbers and is rich in SFA, especially C18:0. It might be also related to several factors like climatic conditions, habitat ecosystem and reproduction practices (Taboado *et al*., 2003).

The nutritional benefits of sea cucumber are mainly attributed to their fatty acid contents such as omega 3, which cannot be produced by the human body and mostly comes from seafood (Ridzwan *et al*., 2014). In our study, n-3 PUFA was the main compound (20.96%) as compared to n-6 PUFA (9.88%). Among n-3 PUFA, eicosapentaenoic (C20:5n-3, EPA) and docosahexaenoic (C22:6n-3, DHA) were found to be the prominent fatty acids at 15.43 and 5.53%, respectively. These PUFA are known for their interesting pharmacological and therapeutic properties (Ridzwan *et al*., 2014). They are also responsible for wound healing, involved in growth and have anti-inflammatory effects (Wu *et al*., 2014). However, n-6 PUFA was characterized by the dominance of arachidonic acid (C20:4n-6) and dihomo- γ-linolenic (C20:3n-6), which showed a similar trend at 4.39 and 4.22%, respectively. Our investigation was in agreement with other researchers, who reported the dominance of n-3 PUFA over n-6 PUFA in *H. tubulosa* tissues from Turkey (Aydin *et al*., 2011; Bilgin and Tanrikulu, 2018) and in *H. scabra* from Sri Lanka (Nishanthan *et al*., 2018). However, Ridzwan *et al*., (2014) and Sicuro *et al*., (2012) showed an abundance of n-6 PUFA compared to n-3 PUFA in *S. horrens* from Malaysia and in *H. tubulosa* from the Adriatic sea.

3.3. Nutritional value

Nutritional quality indices such as n-3 PUFA/n-6 PUFA, EPA+DHA, atherogenic (AI) and thrombogenic (TI) indices are extensively used to assess the beneficial effects of different aquatic organisms (Bejaoui *et al*., 2017). The n-3 PUFA/n-6 PUFA ratio has a great effect on the human diet because it is attributed to the prevention of many coronary complications. Our results showed a high value for the n-3 PUFA/n-6 PUFA ratio, which was around 2.1 (Table 3) and is considered as a good index which is higher than the recommended value (0.25) reported by the UK Department of Health (1994).

In addition, the intake of EPA+DHA could prevent the progression of some types of cancer and cardiovascular diseases and decrease the symptoms of rheumatoid arthritis (Shahidi, 2009). Several findings have described that EPA+DHA is the most essential index for evaluating the nutritional quality of food because it is recognized as playing a major role in biological processes in the human body (Shahidi, 2009; Bejaoui *et al*., 2017). Our results revealed higher levels of EPA+DHA (Table 3) and were more prominent that those reported by the British Nutrition Foundation (1992), which establishes that the daily consumption of at least 0.2 g of EPA and DHA is necessary for a stable and healthy intake in humans.

Other nutritional indices (AT and TI) reflected the effects of fatty acids on human health, particularly concerning the possibility of thrombus and/or atheroma development. In this context, AI revealed the relationship between pro-atherogenic and the anti-atherogenic compounds. Furthermore, this index addresses the circulation of the blood system (such as cholesterol, etc.). TI establishes a relationship between pro-thrombogenetic and anti-thrombogenetic fatty acids. Several investigations

Table 3. Indices of nutritional value of *H. tubulosa* teguments.

NQI	**Teguments**
n-3/n-6	2.118 ± 0.003
EPA+DHA	20.96 ± 0.030
AI	1.387 ± 0.002
TI	0.548 ± 0.001

Results are expressed as means ± SD (n= 6).
NQI: Nutritional quality indices; EPA: eicosapentaenoic acid; DHA: docosahexaenoic acid; AI: Atherogenicity index; TI: Thrombogenicity index.

Table 4. Estimated IC_{50} values for tegument extracts and standard BHT from four different antioxidant and free radical scavenging activity tests.

	IC_{50} (95% CI)	
Antioxidant tests	**Tegument extracts**	**Standard (BHT)**
ABTS	25.46** (20.50–30.66)	0.92 (0.62–2.62)
DPPH	23.23** (23.54–30.56)	8.03 (2.04–10.96)
FRAP	26.95 (19.37–32.45)	14.89 (10,91–20,32)
T-AOC	0.5*** (0.34–1.42)	4.22 (2,04–12,02)

IC_{50}: 50% inhibitory concentration; 95% CI: 95% confidence interval. Data are expressed as IC_{50} and its 95% CI and the geometric means of ten independent experiments are presented. Comparison among the means of IC_{50} was analyzed by the Student *t*-test.
IC_{50} of DPPH and ABTS are expressed in µg/ml; IC_{50} of FRAP and T-AOC are expressed in µM and µg EAG/mg DW, respectively.
Significant difference between tegument extracts and standard a are presented by asterisk (** $p < 0.01$; *** $p < 0.001$).

have demonstrated worldwide that the low AI and TI in seafood maintained the nutritional value of their fatty acids (Lira *et al*., 2014). Our study showed that AI and TI values were lower in the edible tegument of *H. tubulosa* (Table 3). Our results concerning AI and TI were in line with other works carried out on aquatic vertebrates and invertebrates (Lira *et al*., 2014).

3.4. *In vitro* antioxidant and free radical scavenging activities

Sea cucumber tissues have a strong natural defense system to counter and scavenge free radicals (Zhou *et al*., 2012). In the present study, the antioxidant activity of the *H. tubulosa* body wall was determined and scored as the ability to scavenge free radicals. This antioxidant capacity was estimated by the IC_{50} value, which is the concentration of *H. tubulosa* extract able to scavenge 50% of the free radicals. Thus, the higher the IC_{50} value, the greater the antioxidant activity is. DPPH & ABTS antioxidant assays are widely used to evaluate the free radical scavenging ability of different biological extracts *in vitro*. The IC_{50} values for DPPH inhibition were 26.26 and 8.03 µg/ml for the tegument extract of *H. tubulosa* and for standard BHT, respectively. Similarly, the IC_{50} values for ABTS inhibition were 25.46 and 0.92 µg/ml for the tegument extract of *H. tubulosa* and for standard BHT, respectively. Our *in vitro* assays demonstrated that the free radical (DPPH and ABTS) scavenging activities of the tegument extract of *H. tubulosa*, were important, although significantly lower than those of the BHT standard. The antioxidant activities of the *H. tubulosa* extracts were estimated by T-AOC and FRAP assays, which are used by several authors to evaluate antioxidant activity in vitro. The IC_{50} values determined by the T-AOC assay were 0.5 and 4.22 EAG/mg DW for the tegument extracts of *H. tubulosa* and for standard BHT, respectively. Moreover, the IC_{50} values for FRAP inhibition were not significantly different between the tegument extracts of *H. tubulosa* and the BHT standard (26.95 vs. 19.89 µg/ml). The tegument extracts of *H. tubulosa* revealed a strong antioxidant activity compared to those of the BHT standard, especially with in vitro T-AOC test ($p < 0.001$). The tegument extracts of *H. tubulosa* from the Bizerta lagoon had the lowest IC_{50} values compared to the others, suggesting that these extracts have a potential antioxidant power. In contrast, no significant antioxidant activity was detected in *Stichopus regalis* extracts (Santos *et al*., 2015). On the other hand, Althunibat *et al*., (2009) showed that the extracts of *H. Tubulosa* possessed the highest antioxidant activity among the extracts of the three sea cucumber species from Malaysia (2 vs. 10 mg/ml). Similarly, Santos *et al*., (2013) concluded that *Holothuria forskali* from the Peniche coast (Portugal) had potential antioxidant activity.

Zhong *et al*., (2007) showed a good correlation between the high levels of PUFA n-3 and the high ability to scavenge free radicals and these results were quite close to our findings. Also, Wu *et al*., (2014) studied the antioxidant function of EPA from the sea cucumber *Cucumaria frondosa*. Their result showed that EPA was endowed with a potential power to increase the total antioxidant capacity (T-AOC) and the superoxide dismutase (SOD) activities, which have a protective effect against oxidative damages. Furthermore, Zheng *et al*., (2012) identified the antioxidant oligopeptides of sea cucumber (*Stichopus japonicus*) guts, which might be involved, in part, in the observed antioxidant effect. In addition, it was reported (Althunibat *et al*., 2009) that sea cucumber contains phenolic compounds, which are well known to play an important role in the scavenging of free radicals. These phenols could therefore contribute to the antioxidant properties of the holothurian extracts.

4. CONCLUSIONS

In conclusion, our results revealed that the sea cucumber *H. tubulosa* from the Bizerta lagoon (southwestern border of the Mediterranean Sea) contain a good proximate composition and a rich fatty acid profile, especially in n-3 PUFA such as DHA and EPA. The edible tegument of *H. tubulosa* was considered a healthy food for consumers because it possesses important nutritional qualities such as EPA+DHA, AI, TI and the n-3PUFA/n-6PUFA ratio. Also, the studied tegument was endowed with a highly potential power to inhibit and scavenge free radicals. Finally, different parts of *H. tubulosa* might have a favorable effect on maintaining or improving antioxidant systems and could be considered as an antioxidant potential. Further works in the nutritional biochemistry field will be needed to highlight the active extracts and determine their antioxidant mechanisms and activities.

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