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Araştırma Makalesi / Research Article

Determination of Trace Elements, Heavy Metals Content, and Free Radical Scavenging Activity of the Pigments Extracted of *Donax trunculus* shells Gathered from Giresun Coast

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Abstract

The study aimed to determine free radical scavenging activity, trace elements, and the heavy metals contents of the pigments extracted from *Donax trunculus* shells obtained at the Giresun the Black Sea coast. Free radical scavenging activity was determined by spectrophotometric DPPH and ABTS methods. Heavy metal and trace elements of the pigments were measured by ICP-MS. Pigments scavenged DPPH and ABTS radicals. On the other hand, it determined that the pigments had rich trace element concentrations (Fe 90.78± 0.01 mg/kg; Cr 71.63±0.01; mg/kg, Mn 40.46±0.01 mg/kg; Zn 5.13±0.01 mg/kg; Cu 3.69±0.01 mg/kg; Se 1.22±0.01 mg/kg). Heavy metal content was within acceptable limits (Ni 4.28±0.03 mg/kg; Co 0.67±0.02 mg/kg; Pb 0.69±0.01 mg/kg; Cd 0.388±0.01 mg/kg; As 0.284±0.02 mg/kg). These results suggested that *D. trunculus* shells, considered food waste, would be a new bio-resource for obtaining natural antioxidants and micronutrients.

Keywords: Seashell, heavy metal, trace elements, free radical scavenging activity.

Giresun Sahilinden Toplanan *Donax trunculus* Kabuklarından Elde Edilen Pigmentlerin İz Element, Ağır Metal İçeriği ve Serbest Radikal Giderme Aktivitelerinin Belirlenmesi

Öz

Bu çalışma, Giresun'un Karadeniz kıyılarından toplanmış *Donax trunculus* kabuklarından elde edilen pigmentlerin serbest radikal temizleme aktivitesini, eser element ve ağır metal içeriklerini belirlemeyi amaçladı. Serbest radikal temizleme aktivitesi, spektrofotometrik DPPH ve ABTS yöntemleri ile belirlendi. Pigmentlerin ağır metal ve eser element içerikleri ICP-MS ile ölçüldü. Pigmentler DPPH ve ABTS radikallerini temizledi. Öte yandan pigmentlerin zengin eser element konsantrasyonlarına sahip olduğu belirlendi (Fe 90.78± 0.01 mg/kg; Cr 71.63±0.01; mg/kg, Mn 40.46±0.01 mg/kg; Zn 5.13±0.01 mg/kg; Cu 3.69±0.01 mg/kg; Se 1.22±0.01 mg/kg). Ağır metal içeriği kabul edilebilir sınırlar içindeydi (Ni 4.28±0.03 mg/kg; Co 0.67±0.02 mg/kg; Pb 0.69±0.01 mg/kg; Cd 0.388±0.01 mg/kg; As 0.284±0.02 mg/kg). Bu sonuçlar, gıda atığı olarak kabul edilen *D. trunculus* kabuklarının, doğal antioksidanlar ve mikro besinler elde etmek için yeni bir biyolojik kaynak olacağını sergilemiştir.

Anahtar Kelimeler: Deniz kabuğu, ağır metal, eser elementler, serbest radikal giderme aktivitesi.

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1. Introduction

The existence of polluting agents in aquatic environments has been incremented in recent years. Studies on aquatic organisms gain importance in the assessment of aquatic pollution together with traditional water analysis (Zamani-Ahmadmahmoodi et al., 2020). Marine bivalves are known to be filter feeders that bioaccumulate various pollutants in the aquatic environment. Because of this, marine bivalves are considered an important bioindicator in pollution studies (Abdel-Latif et al., 2020). Within bivalves, the clam *Donax trunculus* is among the most gathered in species in many countries, including in Turkey. *D. trunculus* spreads out an Atlantic–Mediterranean warm-temperate exposed sandy beaches (Künili et al., 2021). The studies on *D. trunculus* gain importance due to consume as food. The shell of *D. trunculus* has a smooth surface, and its color is either yellowish or light brown. Inside the shell, there is the coloration of blue-purple or brownish and orange depending on the territory and its pigments (Aydın et al., 2020). The marine bivalves accumulate certain substances in various body parts, especially by filtering the water in their habitat and ingesting some organic molecules. The marine bivalves have easily absorbed heavy metals dissolved in aquatic environments in ion or compound forms. After absorption, the toxic metals could have bound to the essential macromolecules of the cells and affect cell functions (Strehse and Maser, 2020). Free radicals are destructive molecules that break down the cell in the organism and cause undesirable effects. Free radicals can occur when living things are exposed to heavy metals. Antioxidants are molecules that eliminate free radicals formed as a result of metabolic reactions in living things. Antioxidant systems in aquatic organisms are low molecular weight ones such as glutathione (GSH), ascorbic acid, and carotenoids (Lauritano and Ianora, 2020). Deficiency or excess of some trace elements such as selenium, copper, zinc affects antioxidant systems (Le Saux et al., 2020).

The marine bivalves have been evaluated as a good bioindicator in determining heavy metals, trace elements, antioxidants and toxic substances concentration in many parts of the world due to various studies. In previous studies generally carried out on shells and the soft tissues of *D. trunculus*. However, there is no study on the pigments extracted from the shells found in the literature. This study evaluated the availability of dumped and unused *D. trunculus* shells. We studied the antioxidant property of pigments extracted from shells and the toxic and trace elements contents of these pigments in the present research.

2. Materials and Methods

2.1. Extraction of pigments of Seashells

D. trunculus shells were washed with a stream of cold water and air- dried at 4 °C for 2 days in the dark. The dried shells were grounded and then dissolved by gradually adding 6 M HCl and then the pigments were extracted with diethyl ether. The ether layer was washed with 5% NaCl until the acid was almost removed. The ether solution including the pigments was dried over anhydrous sodium sulfate and the solvent was evaporated under reduced pressure. The extract including the pigments was again dissolved in ethanol and stored at - 30°C in the dark. Then, the pigment solution was ready to be used (Kuwahara et al., 2009).

2.2. DPPH (1,1-Diphenyl 2-Picryl Hydrazil) Free Radical Scavenger Activity Measurement

DPPH Radical Scavenger Activity measurement was done according to the method of Brand–Williams et al. (1995). 1 mL of 10-3 M DPPH ethanol solution was taken, 3 mL of extract solution was added and shaken by the vortex. It was kept in the dark for 30 minutes, and absorbance was read at 517 nm. BHT and Trolox were used as standard antioxidants. The DPPH radical scavenging activity, expressed as percent inhibition of the reaction, was calculated using the following formula.

Inhibition % = [Ac - As / Ac] x 100

Ac: Absorbance of control (without antioxidant)

As: Absorbance of sample (with antioxidant)

Antioxidant concentrations (IC50), which caused 50% inhibition, were read from the graph drawn with the % Inhibition values calculated against the antioxidant concentrations.

2.3. Determination of Total Antioxidant Status

Total antioxidant status (TAS) was evaluated based on the conversion of the dark blue-green coloured 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) radical cation (ABTS*+) into the colourless reduced ABTS form of the antioxidants in the sample (Re et al., 1999). The TAS level of the sample is related to the change in absorbance at 660 nm. For calibration, (±)-6-Hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (Trolox Equivalent), a vitamin E analogue, was used. Results are expressed as mmol Trolox Equiv/L.

Two reagents were prepared for the measurement.

0.4 M pH:5.8 acetate buffer was prepared for Reagent 1.

For Reagent 2, acetate buffer with 30 mM pH:3.6 was first prepared. Then, using this buffer, a 2mmol/L H2O2 solution was prepared. Reagent 2 with a final ABTS concentration of 10 mmol/L was prepared using the last prepared solution. The previously described method for TAS was applied to the ARCHITECT c8000 (Abbott, Abbott Park, Illinois, U.S.A.) clinical chemistry auto-analyzer as shown below:

Mode: Endpoint (descending)

Reagent 1 volume: 160 µL

Reagent 2 volume: 25 µL

Sample volume: 10 µL

Primary wavelength (λ): 660 nm

Secondary wavelength (λ): 476 nm

Reading points: 31-33

Standard (Trolox Equivalent) concentration value: 1.0 mmol/L Trolox

2.4. Heavy Metal and Trace Elements Measurements

To determine the samples' toxic and trace elements contents, a microwave digestion procedure and solubilization of the samples were chosen and performed with nitric acid in GRUMLAB. The samples were then allowed to cool and made ready for ICP-MS (Bruker 820-MS) by filtration with distilled water. The toxic and trace elements were determined with ICP-MS in GRUMLAB, concentrations were calculated based on the standards.

3. Results

Figure 1 shows the DPPH radical scavenging activity of pigments of *D. trunculus* shells. The pigments exhibited DPPH scavenging activity (%), and its potency increased almost linearly with an increasing amount of pigments. Figure 2 shows the total antioxidant status (TOS, %) of pigments. TOS capacity increased with increasing the added amounts of pigments.

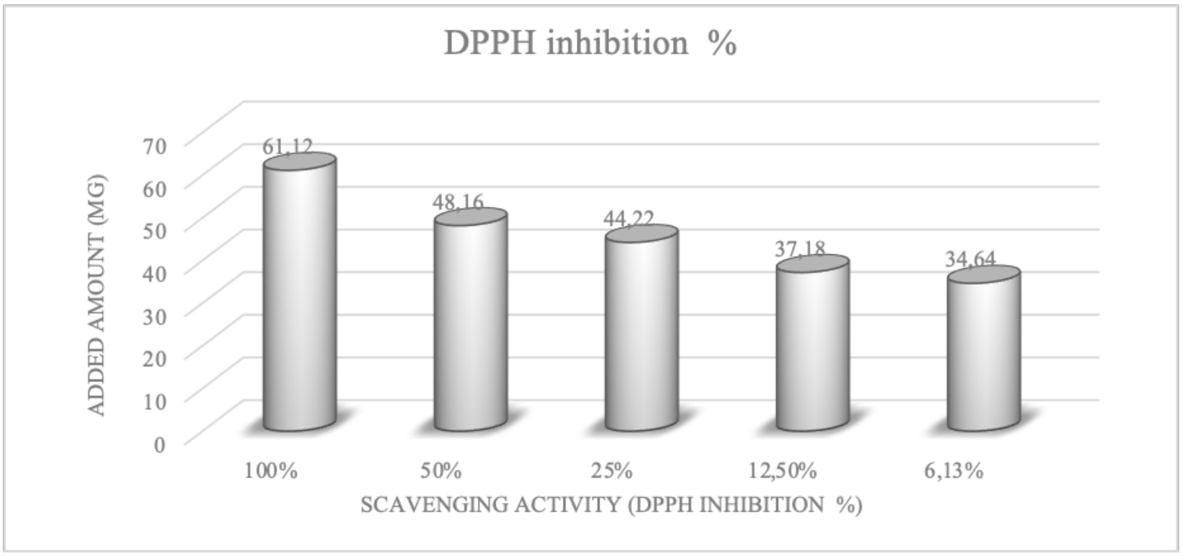

Figure 1. DPPH radical scavenging activity of pigments of *D. trunculus* shells

Table 1 shows the trace and toxic element contents measured by ICP-MS. According to the results, the trace element found in the highest amount in pigments was iron (Fe). In addition, the amounts of chromium (Cr), manganese (Mn) and aluminum (Al) elements were found to be high levels. Other trace element amounts of the pigment extracts were determined as zinc (Zn), copper (Cu), and selenium (Se), from the highest to the lowest concentration, respectively.

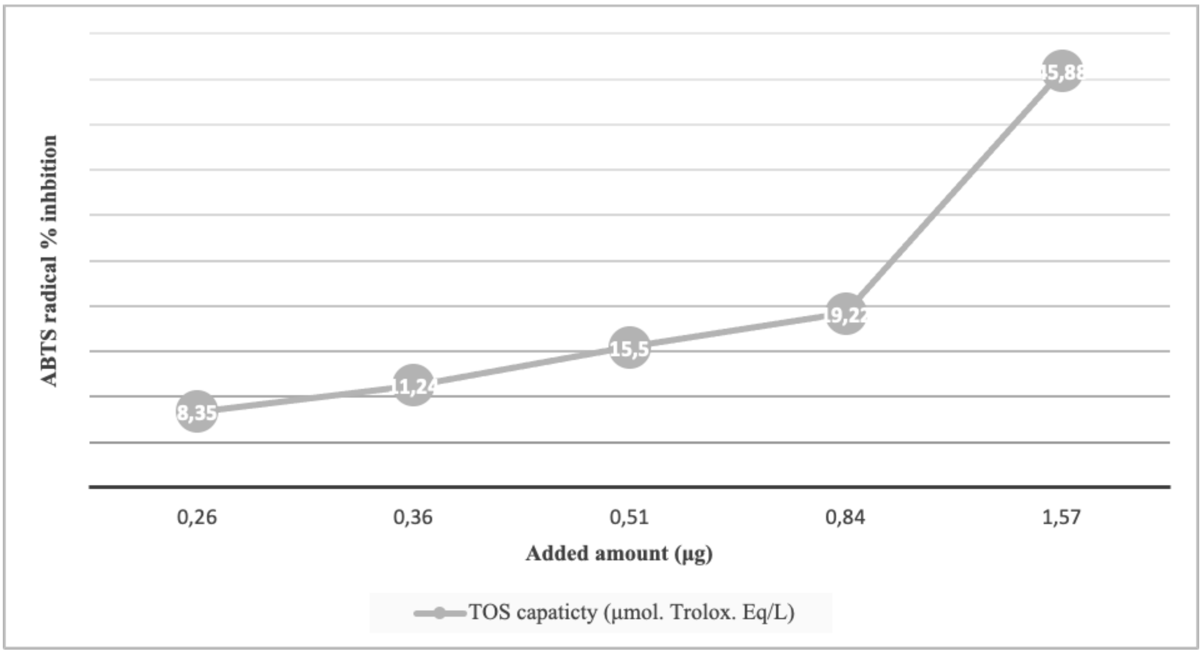

Figure 2. The total antioxidant status (TOS, %) of pigments of *D. trunculus* shells

The element with the highest concentration among toxic heavy metals was determined as nickel (Ni). On the other hand, the toxic metal contents were determined as, in order from highest to lowest, are lead (Pb), cobalt (Co), cadmium (Cd), and arsenic (As).

Table 1. The trace and toxic element contents of pigments of *D. trunculus* shells

	Analyte	Amount (mg/kg)
Trace elements	Fe	90.788±0.017
	Cr	71.631±0.016
	Mn	40.469±0.017
	Al	27. 315±0.006
	Zn	5.137±0.006
	Cu	3.695±0.016
	Se	1.222±0.005
Toxic elements	Ni	4.028±0.034
	Co	0.675±0.002
	Pb	0.695±0.002
	Cd	0.388 ±0.0002
	As	0.284±0.002

4. Discussion

Exposure to free radicals is known to cause biological toxicity like lipid peroxidation as well as food deterioration. To impair these harmful effects, antioxidants have been utilized as food supplements and additives. Nowadays, for the reason that some synthetic antioxidants such as BHA are opined of owning carcinogenic effects, effective natural antioxidant substances such as carotenoids, herbal polyphenols, catechins, and alpha-tocopherol are preferred (Liu and Mabury, 2020). Trace elements, such as chromium, zinc, manganese, copper, cobalt, selenium, and iron, are critical in cell metabolism. The oxidant or antioxidant function of these metals also affects the organism (Gudioncik et al., 2014). Trace metal concentrations in aquatic organisms are highly variable according to intervals of place and time (Roussiez et al., 2013). It is known that heavy metals have toxic effects and change some physiological and biochemical processes of the cell by affecting cellular components such as the membranes. Heavy metals are regarded as the most important pattern of pollution in the aquatic environment due to their toxicity and easily accumulated by marine organisms (Pontoni et al., 2021). In the present study, we evaluated trace elements, heavy metals content, and some antioxidant properties of the pigments extracted from *D. trunculus* shells, discarded without further application.

The pigments from *D. trunculus* shells showed antioxidant activity against DPPH and ABTS radicals. We also found that the scavenging activities of the pigments increased for both DPPH and ABTS radicals as the amount of pigment added to the experimental medium increased. To our knowledge, no study has been carried out on the antioxidant status of pigments obtained from *D. trunculus* shells. On the other hand, in Kuwahara and colleagues' study, the free radical scavenging activity of pigments obtained from purple sea urchin (*Anthocidaris crassispina*) has been investigated. (Kuwahara et al., 2009). They showed that the pigments had significant activity to scavenge hydrogen peroxide and superoxide anion radicals.

Minerals with a daily requirement of less than 100 mg are called microminerals. They are also called trace elements because their concentrations in the body are very low. Insufficient intake of these elements causes dysfunction. On the other hand, elements that are not essential for the body's structure and functions are known as non-essential or toxic elements (Strain and Cashman, 2009). Exposure to toxic elements, such as lead, nickel, cadmium, and cobalt can result in several pathological conditions like toxicity, impaired reproductive function, kidney damage, hepatic dysfunction, and cancers (Buha et al., 2021). On the other hand, the metals such as iron, copper, zinc, magnesium, chromium, selenium, and manganese are essential nutrients required for various biochemical and physiological functions (Godswill et al., 2020). Insufficient intake of these trace elements results in various pathological conditions. According to the results of this study, the amounts of toxic elements in pigment extracts were within acceptable limits, and other trace elements were found in satisfactory amounts (EFSA, 2009; EFSA, 2021). Therefore, it can be said that the aquatic environment in which the *D. trunculus* lives is less polluted or that there is no significant accumulation in the shells, even if there is environmental pollution. Because of their rich antioxidants and trace elements, pigments obtained from *D. trunculus* shells could be used as a significant source of natural antioxidants and trace elements to promote their economic value.

The pigments extracted from *D. trunculus* shells were analyzed for antioxidant properties. The pigments had significant activity to scavenge DPPH and ABTS radicals. Therefore, these pigments may suppress the formation of its related active oxygen radical species. In addition, the pigments had a rich content of micronutrient metals. These results suggested that *D. trunculus* shells, considered as food waste, could be a new bio-source of natural antioxidants and trace elements.

Authors' Contributions

All authors contributed equally to the study.

Statement of Conflicts of Interest

There is no conflict of interest between the authors.

Statement of Research and Publication Ethics

The author declares that this study complies with Research and Publication Ethics.

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