

The Effect of Different Solvents on Antioxidant Properties of *Gongolaria barbata* (Phaeophyceae)

Melis Yılmaz¹, Gülen Türker², İlknur Ak^{3*}

¹Çanakkale Onsekiz Mart University, School of Graduate Studies, Department of Fisheries Basic Sciences, Terzioğlu Campus, 17100, Çanakkale/Turkey

²Department of Food Technology, Faculty of Çanakkale Applied Sciences, Çanakkale Onsekiz Mart University, Terzioğlu Campus, 17100 Çanakkale, Turkey

^{3*}Çanakkale Onsekiz Mart University, Faculty of Marine Sciences and Technology, Department of Aquaculture, Terzioğlu Campus, 17100, Çanakkale/Turkey

Correspondent: ilknurak@comu.edu.tr

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Melis Yılmaz: Orcid 0000-0002-8776-2117

Gülen Türker: Orcid 0000-0002-7554-1544

İlknur Ak: Orcid 0000-0002-0233-0025

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Abstract: In this study, the antioxidant activities, total polyphenolic contents of algal extracts from *Gongolaria barbata* were examined by using ethyl acetate (AcOEt), water(W), and phosphate buffer (PH) as solvents. According to the results, EtOH, W, and PB showed medium antioxidant potential with low IC₅₀ values. The highest total flavonoid content was detected in W (6.91±0.09 mg/g Ext.), and PB showed the maximum phenolic content (2.29±0.01 mg GAE/g ext.). The chlorophyll *a* and total carotenoid contents were detected as 40.5±1.20 and 29.13±1.12 (mg/g DW), respectively. Phycoerythrin was 0.002±0.001 (mg/g DW), while phycocyanin content was found as 0.04 ±0.01 (mg/g DW). Our results show that PB could be used as an extraction solvent for determining the antioxidant properties of *G. barbata* instead of ethanol.

Key words: Antioxidant Properties, Brown Seaweed, Flavonoids, *Gongolaria barbata*, Phenolics

Farklı Çözücülerin *Gongolaria barbata*'nın (Phaeophyceae) Antioksidan Özellikleri Üzerine Etkisi

Özet: Bu çalışmada, *Gongolaria barbata*'dan elde edilen alg ekstraktlarının antioksidan aktiviteleri, toplam fenolik ve flavonoid içerikleri sırasıyla farklı çözücüler kullanılarak incelenmiştir: etil asetat (AcOEt), su(W) ve fosfat tamponu (PH). Sonuçlara göre EtOH, W ve PB çözücülerini, sırasıyla test edilen iki ticari antioksidan olan BHT ve Vitamin C'den önemli ölçüde daha yüksek olduğu ve düşük IC₅₀ değerleri ile orta düzeyde antioksidan potansiyeli gösterdiği kaydedildi. En yüksek toplam flavonoid içeriği W (6.91±0,09 mg/g Ext.) olarak tespit edildi ve PB en yüksek fenolik içeriği (2.29±0.01 mg GAE/g ext.) gösterdi. Klorofil *a* ve toplam karotenoid değerleri sırasıyla 40,5±1,20 ve 29,13±1,12 (mg/g DW) olarak ölçüldü. Fikoeritrin 0.002±0.001 (mg/g DW) olarak ölçülürken, fikosiyenin içeriği 0.04 ±0.01 (mg/g DW) olarak bulundu. Çalışmamızın sonucuna göre, *Gongolaria barbata*'nın antioksidan içerikleri, toplam fenolik ve flavonoid içerikleri incelendiğinde, fosfat tamponunun etanolün yerine kullanılabilir bir çözücü olduğu belirlenmiştir.

Anahtar kelimeler: Antioksidan Özellikleri, Fenolik, Flavonoid, *Gongolaria barbata*, Kahverengi Alg

Introduction

Marine macroalgae, also known as seaweed, are used in many industries, including cosmetics and pharmaceuticals, and they are also good candidates as

raw materials for biofuel production due to high productivity (Ktari *et al.*, 2021). Seaweeds are photosynthetic organisms with rich pigments and

offer an excellent opportunity to the increasing global market demand for food coloring (Bizzaro *et al.*, 2022). Besides their commercial use by the food industry, seaweeds are also rich in other biochemicals such as pigments, fatty acids, and polyphenols, which possess antioxidant, antibacterial, antitumor properties (Ślusarczyk *et al.*, 2021). Especially, seaweeds from the Phaeophyceae family gained importance due to their high bioactive compounds in recent years. *Gongolaria* species are distributed in tropical and temperate waters (Guiry and Guiry, 2021). Perennial macroalgae attract the attention of researchers due to their phenol and polysaccharide contents. This seaweed contains alginic acid salts, polyunsaturated fatty acids, and minerals (Öztaşkent and Ak, 2021). The biochemical composition of *G. barbata* is affected by environmental factors such as temperature, salinity, and nutrient salts (Cirik *et al.*, 2010).

Free radicals have a crucial role in affecting both human and animal health and cause many illnesses. In recent years, antioxidants have proven their applicability in preventing various diseases in which free radicals play a role (Devi *et al.*, 2021). Many scientists have observed numerous antioxidants from unique elements of various plant species consisting of oilseeds, cereal plants, greens and spices (Banwo *et al.*, 2021). Both terrestrial plants and seaweeds are rich resources of phytochemicals possessing essential properties, including antioxidants. Because seaweeds expose various abiotic and biotic stresses, they possess robust defence systems and produce critical secondary metabolites with pharmaceutical importance (Jégou *et al.*, 2021). These metabolites are used in cancer, allergy, hypertension, oxidative stress and inflammation treatments, showing antimicrobial, antibacterial, and other pharmacological properties (Ślusarczyk *et al.*, 2021).

The phenolic compounds increase the antioxidant capacity of photosynthetic organisms (Mildenberger *et al.*, 2022). Seaweeds and plants contain phenolic compounds such as tannic acids, phenolic acids etc., which have many benefits for human health (Jégou *et al.*, 2021; Bizzaro *et al.*, 2022). Flavonoids are polyphenolic compounds and are known as safe and non-toxic antioxidants. Seaweeds' phenolic and flavonoid compounds change according to species, environmental conditions, and extraction solvents (Ak and Türker, 2019; Koru *et al.*, 2021).

Besides the pharmaceutical and biomedical potentials of seaweed pigments in the last few years, they can be used as replacements for dyes/colorants (Wang *et al.*, 2017). There has been a growing interest in natural pigments, especially in seaweeds. The primary pigments of seaweeds are chlorophylls, carotenoids and phycobiliproteins. In addition to chlorophyll *a* pigment, brown algae also have chlorophyll *c* pigment (Büchel, 2020). The carotenoids are found in the thylakoids of seaweeds,

and they have a protective role against high irradiances. The light-harvesting complexes in these seaweeds are phycobiliproteins, which are mainly two components, phycoerythrin (red) and phycocyanin (blue) (Osório *et al.*, 2020).

This study aimed to determine the antioxidant properties of algal extracts from *G. barbata* by using different solvents: water, ethyl acetate (AcOEt) and ethanol (EtOH) phosphate buffer extracts.

Material and Methods

Seaweed collection

The thallus of *Gongolaria barbata* were collected, on March 2021, at low tide along the coast of Kilitbahir, Çanakkale, Turkey (40° 9'15"N; 26°22'38"E) (Figure 1). The samples were transferred to the laboratory in ÇOMU. The samples were cleaned from epiphytes and other contaminants. Samples were dried at 30–35°C and stored for analysis.

Extraction of the seaweed

1 gr dried thallus of seaweed extracted with 10 ml of 4 different solvents (water (W), ethyl acetate (AcOEt) and ethanol (EtOH) and phosphate buffer (PB) extracts) samples were kept in solvents for 24 hours. The samples were then filtered on filter paper.

Determination of pigment contents

The chlorophyll *a* (chl *a*), chlorophyll *c* (chl *c*) and total carotenoid (TCar), phycoerythrin (PE) and phycocyanin (PC) contents of samples were determined spectrophotometrically according to Jeffrey and Humphrey (1975) and Beer and Eshel (1985).

DPPH radical scavenging activity

The DPPH radical scavenging activity was determined according to Brand-Williams *et al.* (1995). Vitamin C and Butylated hydroxytoluene (BHT) were used as the control. The methanol and DPPH solutions were added to the extracts. The mixtures were left 30 min for the reaction, and then the absorbance value was determined spectrophotometrically. Nonlinear regression was used to calculate IC₅₀ inhibition values.

Total phenolic (TPC) and flavonoid contents (TFC)

Total phenolic and total flavonoid contents of *G. barbata* were found spectrophotometrically according to Djeridane *et al.* (2006) and Quettier-Deleu *et al.* (2000).

Statistical analyses

One-way analysis of variance (ANOVA) was used to determine the significance of differences between groups. Moreover, the correlations between the means were found with Pearson's correlation.

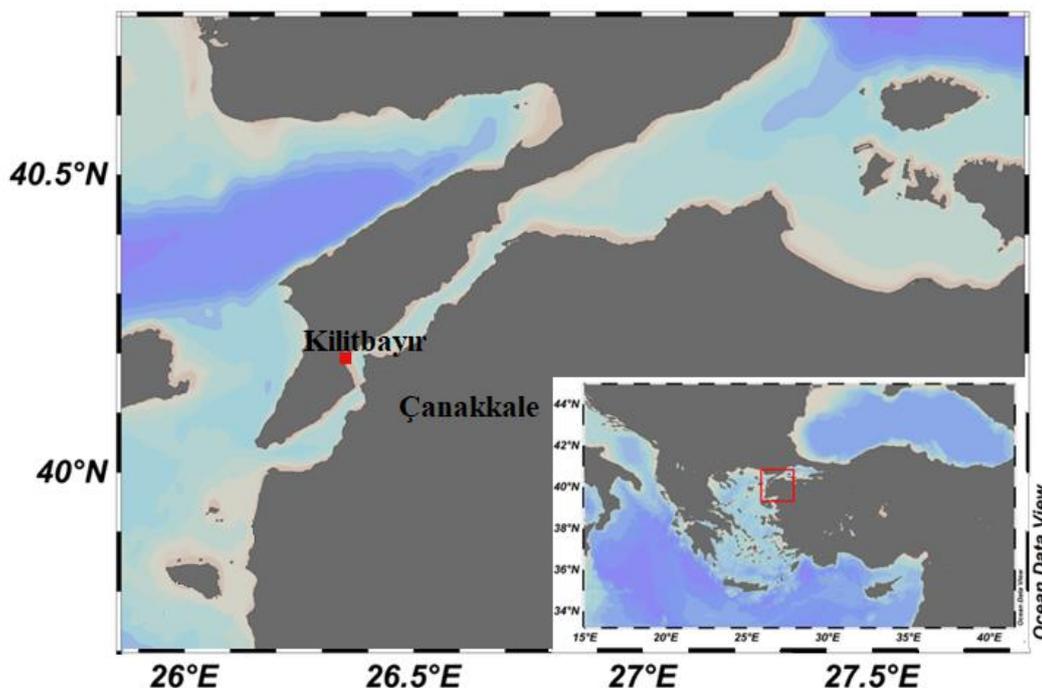


Figure 1. Sampling area

Results and Discussion

In this study, the antioxidant properties of four different seaweed extracts were evaluated (Table 1). AcOEt solvents showed the lowest antioxidant potential with a high IC₅₀ (9.56±0.09 mg/g Ext.). No significant differences were found between W, PB and EtOH (p>0.05), and these values are significantly higher than BHT and Vitamin C, respectively. Ak and Türker (2019) reported that IC₅₀ inhibition values of *Eucheuma* sp. and *Laminaria* sp. were found at 2.26±0.03 to 2.63±0.03, respectively. According to our results, EtOH, W and PB solvents were determined as the most suitable scavengers.

TPC and TFC contents of *G. barbata* extracts are shown in Table 2. It was found that all the groups were significantly different (p<0.05), and PB showed the highest phenolic potential (2.29±0.01 mg GAE/g ext.). The lowest value (0.41±0.01 mg GAE/g ext.) was found in the extract of AcOEt. Phenolics are one of the effective antioxidant sources, and they are dominant in seaweeds (Mildenberger et al., 2022). Mildenberger et al. (2022) and Zhu et al. (2022) reported that brown seaweed extracts changed phenolic content according to species.

The highest TFC was determined in W (6.91±0.09 mg/g Ext.) (Table 2). The flavonoid contents of brown seaweeds varied according to species and environmental conditions (Mildenberger et al., 2022). Flavonoids play an essential role in seaweeds' defence systems, and they are associated with preventing vascular diseases and diabetes (Jégou et al., 2021). Also, they show antioxidant activity by chelation of metals (Shubina et al., 2021).

Table 1. IC₅₀ inhibition values (mg/g ext) and Inhibition rates (%) of *G. barbata*

Solvents	IC ₅₀ inhibition values	Inhibition
EtOH	6.94±0.03 ^b	19.43±0.07 ^b
W	6.91±0.09 ^b	19.52±0.26 ^b
PB	6.96±0.10 ^b	19.40±0.28 ^b
AcOEt	9.56±0.09 ^a	14.20±0.13 ^c
BHT	1.40±0.01 ^c	99.00±0.01 ^a
Vitamin c	1.36±0.01 ^c	99.00±0.01 ^a

Different superscripts in a column indicates statistical differences (p<0.05).

The chl *a*, chl *c*, Tcar, PE and PC of *G. barbata* are shown in Table 3. The Chl *a* and Tcar values were 40.5±1.20 and 29.13±1.12 (mg/g DW), respectively. *G. barbata* is particularly rich in pigments and could be used as a powerful antioxidant source. Recent studies have shown that foods rich in carotenoids can reduce the risk of diseases such as cancer and heart disease (Jégou et al., 2021). and PC content are shown in Table 2. It was determined that PC content was higher than PE content.

Table 2. The total phenolics (mg GAE/g ext.) and total flavonoids (mg rutin/g ext.) of *G. barbata*.

Solvents	TFC	TFE
EtOH	1.67±0.01 ^a	1.14±0.02 ^b
W	1.69±0.01 ^a	1.01±0.01 ^c
PB	1.65±0.01 ^a	2.29±0.01 ^a
AcOEt	1.39±0.02 ^b	0.41±0.01 ^d

Different superscript letters in a column indicates statistical differences (p<0.05).

Table 3. Chl *a*, Chl *c*, TCar, phycoerythrin and phycocyanin values of *G. barbata* (mg/g DW)

Chl <i>a</i>	Chl <i>c</i>	TCar	PE	PC
40.5±1.20	8.66±0.45	29.13±1.12	0.002±0.001	0.04 ±0.01

Correlation coefficient analysis results are shown in Table 3. Our results showed a negative correlation between DPPH scavenging activities and both flavonoids and phenolic values. In previous studies, the total phenolic and flavonoid were significantly correlated to IC₅₀ inhibition values, and results show significant negative relationships (Ismail, 2017; Ak and Türker, 2019).

Table 4. Correlation coefficients (n=9) between variables.

	IC ₅₀ inhibition values	Total flavonoids
Total phenolics	-0.671*	0.609*
Total flavonoids	-0.987*	

* Significant correlation at the 0.05 level.

Conclusion

Seaweeds are rich sources of chemical compounds having antibacterial, antioxidant, antitumor and other properties. As a result, increasing attention has been paid regarding the application of algae in the pharmaceutical, cosmetic, and food industries. Most of the studies that aim to determine antioxidant activities of polyphenolic compounds of seaweeds were carried out with methanol and ethanol as extracting solvents. According to the results of this study, phosphate buffer (PB) can be used as effective as methanol and ethanol solvents for determining antioxidant capacity, total phenolic and flavonoid contents of *G. barbata*. Also, in this study, phycoerythrin and phycocyanin contents of *G. barbata* were determined for the first time. At the end of the study, we found that the antioxidant activities and total polyphenolic contents of *G. barbata* show differences based on the solvent type. Extraction solvents of seaweeds used in primarily cosmetic and pharmaceutical industries should not be harmful to human and animal health and should be cheap and

PE Besides chlorophyll *a* which is mainly responsible for photosynthesis, seaweeds also contain other pigments such as carotenoids and phycobiliproteins (such as phycoerythrin and phycocyanin) (Osório et al., 2020). The phycobiliproteins, one of the groups of water-soluble compounds, are accessory pigments for photosynthetic light collection (Ak and Yücesan, 2012). In this study, for the first time, phycocyanin and phycoerythrin determinations were carried out in dry weights of brown algae. It has been determined that *G. barbata* contains these two pigments, albeit in small amounts.

sustainable materials. From this point of view we suggest phosphate buffer for extraction solvent as well as ethanol.

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Conflict of Interest

There is no conflict of interest between the authors.

Author Contributions

M.Y., G.T. and I.A. contributed to concepts and design of experiments. M.Y. and G.T. have performed the experiments M.Y. and I.A. were carried out data analysis I.A. has responsibility for the integrity of the work and the correspondence.

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