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HPLC-UV analysis of phenolic compounds and biological activities of *Padina pavonica* and *Zanardinia typus* marine macroalgae species

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Abstract: The marine macroalgae species are recognized as the food of the future with excellent bioactive properties. This study aimed to investigate phenolic compounds; cytotoxic, antibacterial, and antioxidant activities; total phenolic (TPC) and flavonoid (TFC) contents of the methanol, ethanol, and water extracts of *Padina pavonica* (PP) and *Zanardinia typus* (ZT). To the best of our knowledge, this is the first report in which ZT was evaluated in terms of phenolic content, antioxidant, antibacterial, and cytotoxic activities. The HPLC analysis allowed the identification of five phenolic compounds containing rutin (0.31 ± 0.04 – 3.00 ± 0.21 ppm) in all extracts and *trans*-*p*-coumaric acid (0.15 ± 0.02 – 3.95 ± 0.02 ppm) in MPP, EPP, MZT, EZT, WZT as the major compounds. TPC and TFC of the extracts were calculated as 11.78 ± 0.75 – 76.78 ± 0.54 µg GAEs/mg extract and 6.78 ± 0.17 – 29.50 ± 2.23 µg QEs/mg extract, respectively. The highest cytotoxicity was observed in EZT (CC_{50}: 132.3 ± 22.4 µg/mL) against MCF-7 and MZT (CC_{50}: 91.4 ± 20.9 µg/mL) against MIA PaCa-2. Among the studied extracts, EPP showed the best antibacterial activity against all test pathogens. Also, EPP indicated superior antibacterial activity against *Plesiomonas shigelloides* (MIC: 1.25 mg/mL) and *Staphylococcus aureus* (MIC: 1.25 mg/mL). EZT displayed the highest antioxidant activity in $DPPH^{\bullet}$ (IC_{50}: 49.03 ± 0.28 µg/mL), CUPRAC ($A_{0.50}$: 15.20 ± 0.14 µg/mL), and $ABTS^{\bullet+}$ (IC_{50}: 18.86 ± 0.74 µg/mL) assays. The results approved that *P. pavonica* and *Z. typus* marine macroalgae species could be valued as natural sources of bioactive agents for food and pharmacology applications.

Key words: *Padina pavonica, Zanardinia typus,* phenolic compounds, cytotoxic activity, antibacterial activity, antioxidant activity

1. Introduction

Research on marine life, which makes up more than 70% of the earth's surface with its versatile diversity of life and accompanying biodiversity, remains limited. Marine is accepted as a rich source of new metabolites with a variety of applications including nutraceuticals, cosmeceuticals, pharmaceuticals, agrochemicals, and other industrially related chemicals. With the latest studies, it has been revealed that the focus has increased on the discovery of drugs from marine sources and that the various biometabolites obtained are in the clinical phase (Malve, 2016).

A wealth of nutrients and bioactive compounds, marine macroalgae have been part of the Eastern people's traditional diet for centuries. Still, they are growing in popularity among the people of the Western world. Marine macroalgae, which have a wide application area in the food, pharmaceutical, and cosmetic industries, contain several times more minerals and many bioactive compounds that contribute to their pharmacological activities compared to terrestrial plants (Vlaisavljević et al., 2021). Also, macroalgae have low calorie, high vitamin, and fiber content, and because of that, they become an attractive material for researchers (Solak and Éva, 2011). The marine macroalgae are classified into three major phyla: Phaeophyceae (brown algae, brown color due to its fucoxanthin content), Chlorophyceae (green algae, green color due to its chlorophyll a and b contents), and

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Rhodophyceae (red algae, red color due to its phycocyanin and phycoerythrin contents) (Suganya et al., 2016). Although there are more than 1500 brown, 900 green, and 4000 red macroalgae species worldwide, the research of algae taxonomists on finding new and interesting species is ongoing. Especially brown algae have the highest biological activities and are considered as important sources of bioactive natural substances (Domínguez, 2013). The pharmacological activities (antidiabetic, antitumor, antioxidant, anticancer, immunomodulatory, antiinfluenza, antibacterial, antihypertensive) of marine macroalgae have been reported to be related to their high contents of diverse biologically active compounds such as phlorotannins, polysaccharides, protein hydrolysates, peptides, alkaloids, halogenated terpenoids, phenolic compounds, and pigments (Rengasamy et al., 2020).

The phenolic compounds in marine macroalgae include a hydroxylated aromatic ring consisting of various chemical structures ranging from simple fragments to high molecular weight polymers (Vázquez-Delfín et al., 2014). The phenolic compounds are regarded as secondary metabolites because they have protective effects against various stimuli and contribute to the defense mechanisms against UV radiation and marine macroalgae herbivores (Audibert et al., 2010). Marine macroalgae are recognized as a source of valuable polyphenolic compounds such as flavonoids, mycosporine-like amino acids, bromophenols, phenolic terpenoids, and phlorotannins. Highly purified extracts of phenolic compounds, which are hard to obtain quantitatively in industrial terms, are among the most used techniques (Generalić Mekinić et al., 2019). Most of the phenolic compounds have a wide and versalite range of biological activities such as antiallergic, antiinflammatory, antidiabetic, antiviral, antimicrobial, antiphotoaging, antioxidant, neuroprotective, antipruritic, anticancer, and hepatoprotective, and these unique properties of the phenolic compounds are explained by their interactions with proteins such as cellular receptors and enzymes (Cotas et al., 2020).

Padina pavonica (L.) Thivy species is a member of the Phaeophyceae class and Dictyotaceae family. *Zanardinia typus* (Nardo) P.C. Silva belongs to the Phaeophyceae class and Cutleriaceae family (Table 1). Both these brown macroalgae species are the largest brown algae, with a length of up to 100 m. Brown macroalgae species were reported to play a fundamental role in the functioning of coastal marine ecosystems (Mineur et al., 2015). *P. pavonica* has been reported to be a rich source of secondary metabolites with various bioactivities such as antimicrobial, insecticidal, antioxidants, antibiotics, antiinflammatory, hypoallergenic, hepatoprotective, and antidiabetic (Ansari et al., 2019). The only study in the literature on *Z. typus* investigated the fatty acid, carbohydrate, and mineral profiles (Yucetepe et al., 2023).

Marine macroalgae, which are in the class of functional foods because they have healing properties on health and reduce chronic diseases, are also considered as the 'food of the future' by the most researchers (Ferrara, 2020). Recently, studies on discovering bioactive compounds from marine macroalgae have become more important in relation to their functional and therapeutic properties. This study aimed to determine cytotoxic, antibacterial, and antioxidant activities of the extracts of *P. pavonica* (PP) and *Z. typus* (ZT) with total phenolic and flavonoid contents. Also, bioactivity-related phenolic compounds of the extracts were identified by using HPLC-UV.

2. Materials and methods

2.1. Marine macroalgae sampling procedures

Samples of *P. pavonica* (PP) and *Z. typus* (ZT) marine macroalgae species were collected from a sampling as deep as 0–1 m, station in Yapıldakaltı, Çanakkale, Turkey (40°14'27.03"N-26°32'29.74"E) in 2019 at the end of summer when algae formed intense populations. Possible macroalgae samples were obtained from a variety of organisms by scuba diving. PP and ZT belong to the phylum Ochrophyta. Table 1 (AlgaeBase) represented the systematic classification of the PP and ZT species and Figure 1 presented the morphological properties of PP and ZT species (Aysel et al., 2005; Fırat and Erduğan, 2022).

Foreign materials were removed from macroalgae species collected using ambient water, and the samples were transported to the laboratory by cold chain after being placed in sterile polyethylene bags. Subsequently,

Table 1. The systematic classification of PP (*Padina pavonica* (L.) Thivy) and ZT (*Zanardinia typus* (Nardo) P.C. Silva) species (AlgaeBase).

Species	Kingdom	Phylum	Class	Subclass	Order	Family	Genus
Padina pavonica (L.) Thivy	Chromista	Ochrophyta	Phaeophyceae	Dictyotophycidae	Dictyotales	Dictyotaceae	*Padina*
Zanardinia typus (Nardo) P.C. Silva	Chromista	Ochrophyta	Phaeophyceae	Fucophycidae	Tilopteridales	Cutleriaceae	*Zanardinia*

Figure 1. Morphological properties of PP and ZT. a-1, b-1) underwater view of PP and ZT, a-2, b-2) air-dried form of PP and ZT, a-3, b-3) dust form of PP and ZT.

the samples were washed with distilled water in the hydrobiology laboratory to remove epiphytic organisms and necrotic particles. In order to quickly dry the macroalgae and prevent damage to the phytochemical compounds, the samples were predried in an oven programmed at 40 °C and left for 17 h. After drying was completed, the samples were ground by using a hand homogenizer after rinsing and the chamber cells were kept airtight until extraction.

2.2. Preparation of marine macroalgae extracts

The soxhlet extraction was carried out to obtain the extracts and both macroalgae samples were extracted with different solvents according to their increasing polarity: methanol, ethanol, and water for 6 h. A vacuum by an

evaporator was used to get the methanol and ethanol extracts and a freeze-drier for the water extracts. All extracts were stored at +4 °C until analysis.

2.3. Phenolic compounds

The phenolic compounds of the marine macroalgae extracts were identified by the Shimadzu LC-20AD HPLC system (Kyoto, Japan). INERTSIL ODS-3V C_{18} column (5 µm; 4.6 × 250 mm i.d.) thermostatted at 30 °C was used to separate phenolic compounds. Mobile phase A was water comprising 0.05% glacial acetic acid, and mobile phase B was acetonitrile. The gradient conditions were as follows: 8% B (0–0.10 min); 8%–10% B (0.10–2 min); 10%–30% B (2–27 min); 30%–56% B (27–37 min); 56%–8% B (37–45 min). The condition was as follows: the solvent flow rate: 1.0 mL/min; the injection volume: 20 µL; the detector: 20A UV-Vis detector at 280 nm wavelength (Seal, 2016). The phenolic compounds were identified based on their retention times and were compared with authentic standards. The analyses were repeated three times. Calibration curves were drawn by the injection of predetermined concentrations of different standards compounds (catechin, caffeic acid, rutin, *trans-p*-coumaric acid, and *trans*-resveratrol) for the quantitative analysis of phenolic compounds. The results were stated as ppm (parts per million).

2.4. Total phenolic (TPC) and total flavonoid (TFC) contents

The Folin Ciocalteu method was applied for TPC of the marine macroalgae extracts and the aluminum nitrate method was for TFC of the marine macroalgae extracts (Slinkard and Singleton, 1977; Park et al., 1997).

2.5. Cytotoxic activity

In vitro cytotoxicity of the marine macroalgae extracts on MIA PaCa-2 (human pancreatic carcinoma), HEK 293 (human embryonic kidney 293), and MCF-7 (human breast adenocarcinoma) was studied using a modified Alamar blue experiment (Yilmaz, 2022). The sigmoidal plot of the inhibition rate (%) versus the log concentration was used to calculate the CC_{50} values of the marine macroalgae extracts. The percent viability was stated as a percentage of control in the presence of tested extracts.

2.6. Antibacterial activity

Antibacterial activities of the marine macroalgae extracts were determined by the broth microdilution method reported by Alsenani et al. (2020). The antibacterial test was performed by determining minimum inhibitory concentration values of different marine macroalgae extracts (0.1–10 mg/mL) against *Streptococcus pyogenes, Plesiomonas shigelloides, Streptococcus mutans, Enterococcus faecalis, Escherichia coli,* and *Staphylococcus aureus* bacterial strains. In parallel with this study, negative growth control contained only organic solvents (ethanol, methanol, and water), and positive growth control contained chloramphenicol. The lowest concentration values for bacterial inhibition were calculated and reported as the minimum inhibitory concentration (MIC).

2.7. Antioxidant activity

Antioxidant activities of the marine macroalgae extracts were tested using DPPH$^{\bullet}$ radical scavenging, $ABTS^{\bullet+}$ radical scavenging, and CUPRAC activity assays (Çayan et al., 2019). Ascorbic acid was used as a standard. The 50% inhibition concentration was calculated and reported as IC_{50} for radical scavenging activities, 0.50 absorbance concentration was calculated and reported as $A_{0.50}$ for CUPRAC activity.

2.8. Statistical analysis

The analysis of the obtained data was performed using SPSS 22.0 for Windows (Statistical Package for Social Sciences). Descriptive analyses were made for continuous variables and the arithmetic mean ± standard deviation (SD) values of the variables were given. The error was kept at the 0.05 level in the interpretation of the analysis results, and the results were reflected at the 95% confidence level.

3. Results

3.1. Phenolic compounds

The phenolic compounds of the extracts of PP and ZT were identified by HPLC-UV and the results are given in Table 2. The HPLC chromatograms of the standards, PP, and ZT extracts are presented in Figures 2–4. Five phenolic compounds were analyzed in the methanol, ethanol, and water extracts of PP and ZT. Rutin was identified as the main

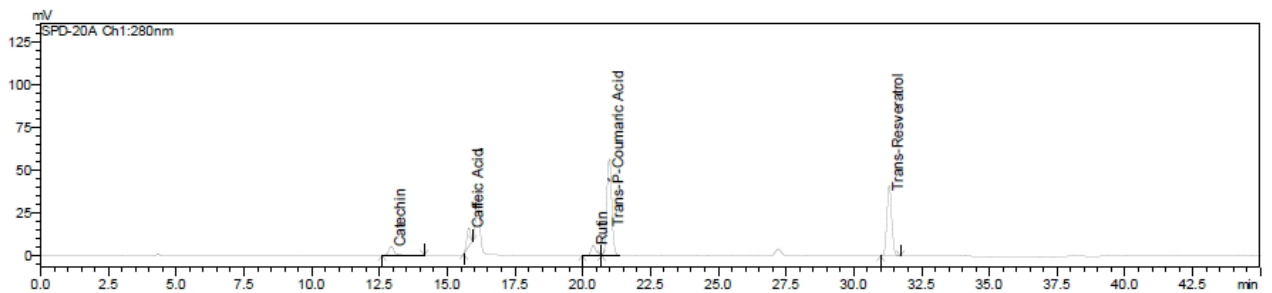

Figure 2. HPLC chromatogram of the standards

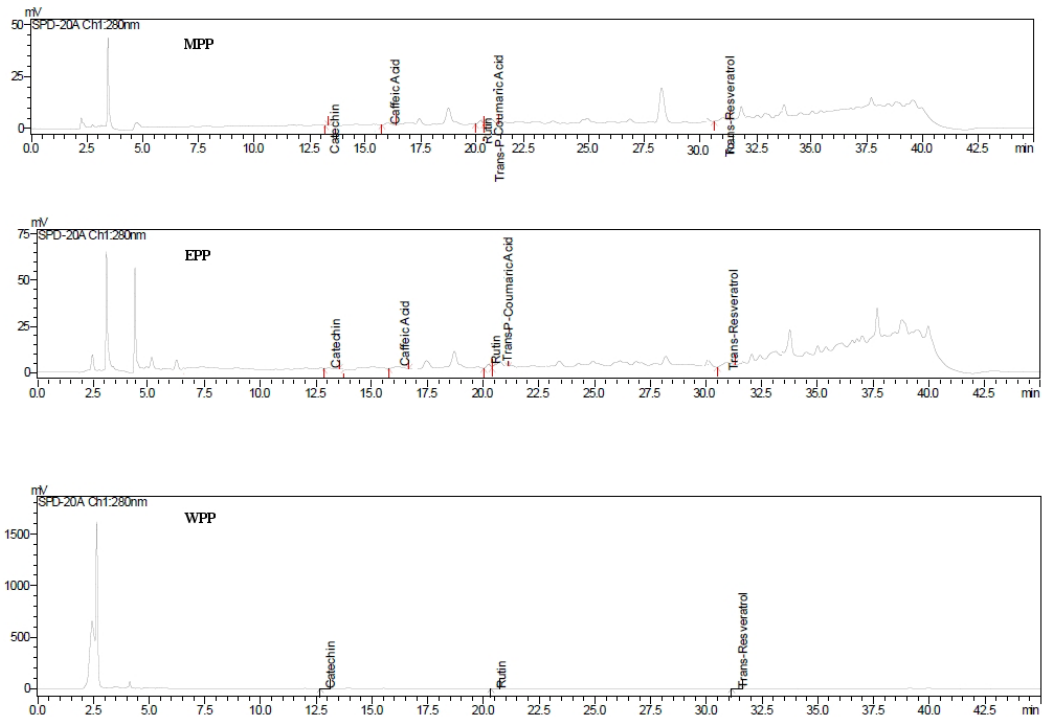

Figure 3. HPLC chromatogram of PP extracts. MPP: PP methanol extract, EPP: PP ethanol extract, WPP: PP water extract.

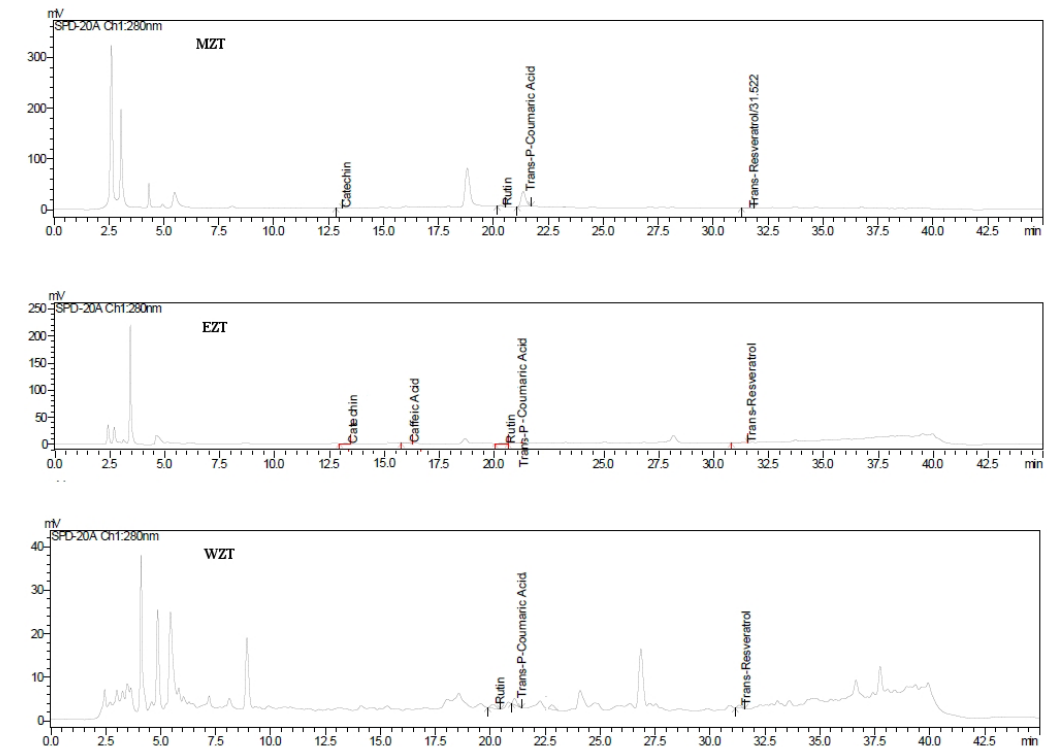

Figure 4. HPLC chromatogram of ZT extracts. MZT: ZT methanol extract, EZT: ZT ethanol extract, WZT: ZT water extract.

phenolic compound in MPP (2.28 ± 0.02 ppm), EPP (3.00 ± 0.21 ppm), and WPP (0.31 ± 0.04 ppm). When *trans-p*-coumaric acid (3.95 ± 0.02 ppm) was detected as the primary phenolic compound in the MZT, rutin was found as the primary phenolic compound in EZT (1.89 ± 0.02 ppm) and WZT (1.27 ± 0.05 ppm).

3.2. Total phenolic (TPC) and total flavonoid (TFC) contents

The findings of TPC and TFC of the methanol, ethanol, and water extracts of PP and ZT are shown in Table 3 and Figure 5. TPC of the extracts were calculated between 11.78 ± 0.75 and 76.78 ± 0.54 µg GAEs/mg extract. EZT (76.78 ± 0.54 µg GAEs/mg extract) and MZT (43.13 ± 0.99 µg GAEs/mg extract) were recorded as the richest in terms of TPC**.** TFC of the extracts were calculated between 6.78 ± 0.17 and 29.50 ± 2.23 µg QEs/mg extract. EZT (18.74 ± 1.52 µg QEs/mg extract) and EPP (29.50 ± 2.23 µg QEs/mg extract) were recorded as the richest in terms of TFC.

3.3. Cytotoxicity activity

In this study, cytotoxic activities of the methanol, ethanol, and water extracts of PP and ZT against HEK 293 (human embryonic kidney 293), MIA PaCa-2 (human pancreatic carcinoma), and MCF-7 (human breast adenocarcinoma) were tested by using Alamar Blue assay. The viability of MIA PaCa-2, MCF-7 and HEK 293 cells treated to different macroalgae extracts concentrations for 18 h is represented in Figures 6 and 7. Estimated CC_{50} values of the extracts are given in Table 4. When marine macroalgae extracts were applied at 500 µg/mL concentration, MPP and MZT were found as the most cytotoxic extracts for MIA PaCa-2 with 38% and 43% cell viability values, respectively, and the ethanol extracts of PP and ZT for MCF-7 with 52% and 46% cell viability values, respectively. As seen in Table 3, the lowest CC_{50} value was calculated in EZT (CC_{50}: 132.3 ± 22.4 µg/mL) against MCF-7 and MZT against MIA PaCa-2 (CC_{50}: 91.4 ± 20.9 µg/mL). Besides, all extracts did not

Table 2. Phenolic compounds (ppm) of PP and ZT extracts[a]

		Extracts					
Phenolic compounds	RT (min)	MPP	EPP	WPP	MZT	EZT	WZT
Catechin	12.931	0.11 ± 0.01	1.17 ± 0.03	0.20 ± 0.02	0.14 ± 0.01	0.42 ± 0.02	nd[b]
Caffeic acid	15.793	0.54 ± 0.02	0.93 ± 0.05	nd[b]	nd[b]	0.46 ± 0.02	nd[b]
Rutin	20.378	2.28 ± 0.02	3.00 ± 0.21	0.31 ± 0.04	1.68 ± 0.01	1.89 ± 0.02	1.27 ± 0.05
Trans-p-Coumaric acid	20.972	0.68 ± 0.00	1.15 ± 0.01	nd[b]	3.95 ± 0.02	0.22 ± 0.00	0.15 ± 0.02
Trans-Resveratrol	31.303	0.54 ± 0.02	1.06 ± 0.06	0.08 ± 0.00	0.13 ± 0.02	0.27 ± 0.01	0.12 ± 0.01

[a]: The results are given as a mean ±SD of three parallel measurements.
[b]: not detected.
MPP: PP methanol extract, EPP: PP ethanol extract, WPP: PP water extract, MZT: ZT methanol extract, EZT: ZT ethanol extract, WZT: ZT water extract.

Table 3. Total phenolic (TPC) and flavonoid (TFC) contents of PP and ZT extracts[a]

Extracts	Total phenolic content (TPC)	Total flavonoid content (TFC)
	(µg GAEs/mg extract)	(µg QEs/mg extract)
EPP	24.00 ± 1.03	29.50 ± 2.23
MPP	11.78 ± 0.75	14.37 ± 0.71
WPP	14.48 ± 0.54	13.36 ± 0.17
EZT	76.78 ± 0.54	18.74 ± 1.52
MZT	43.13 ± 0.99	11.72 ± 0.17
WZT	19.57 ± 0.40	6.78 ± 0.17

[a]: The results are given as a mean ±SD of three parallel measurements.
MPP: PP methanol extract, EPP: PP ethanol extract, WPP: PP water extract, MZT: ZT methanol extract, EZT: ZT ethanol extract, WZT: ZT water extract.

show a significant reduction in the cell viability of HEK 293. The methanol and ethanol extracts showed higher cytotoxic activity than the water extracts for both species.

3.4. Antibacterial activity

Antibacterial activities of the extracts of PP and ZT were tested according to the microdilution method, and results are presented in Table 5. All the investigated marine macroalgae extracts showed antibacterial activity on all test pathogens at different concentrations. Among the studied extracts, EPP showed the best antibacterial activity against all test pathogens and indicated superior antibacterial activity against *P. shigelloides* (MIC: 1.25 mg/mL) and *S. aureus* (MIC: 1.25 mg/mL).

3. 5. Antioxidant activity

Antioxidant activities of PP and ZT extracts were investigated by using DPPH$^{\bullet}$ radical scavenging, ABTS$^{\bullet+}$ radical scavenging, and CUPRAC activity assays. The results are given in Table 6. EZT showed the highest activity in DPPH$^{\bullet}$ (IC_{50}: 49.03 ± 0.28 µg/mL), ABTS$^{\bullet+}$ (IC_{50}: 18.86 ± 0.74 µg/mL), and CUPRAC ($A_{0.50}$: 15.20 ± 0.14 µg/mL) assays among ZT extracts. The best activity was noted in EPP in ABTS$^{\bullet+}$ (IC_{50}: 75.16 ± 0.42 µg/mL) and CUPRAC ($A_{0.50}$: 119.44 ± 0.06 µg/mL) assays and WPP in DPPH$^{\bullet}$ (25.51 ± 0.67% inhibition at 400 µg/mL concentration) assay among PP extracts.

4. Discussion

As a result of the increase in the tendency to produce with natural ingredients, scientific studies have been turned in this direction. Thus, the fact that the phenolic compounds in algae are related to many bioactive properties makes the identification of phenolic compounds valuable (Cotas et al., 2020). The phenolic compounds of the extracts of PP and ZT were identified by HPLC-UV and rutin was identified as the main phenolic compound in all studied extracts except MZT. There is an inadequate number of studies in the literature on the identification of phenolic compounds of PP. HPLC was used to detect phenolic compounds of PP ethanol extract and kaempferol, ellagic acid, delphinidin-3-*O*-glucoside, naringenin, and ferulic acid were identified (Sudha and Balasundaram 2018). The previous studies revealed that marine macroalgae species contain many phenolic compounds such as ferulic, gallic, gentisic, *p*-coumaric acids, and catechin, in addition to phlorotannins that can only be synthesized by macroalgae species in nature (Jimenez-Lopez et al., 2021). Rutin was reported as an important phenolic compound of the flavonol class with broad-spectrum activities such as anticonvulsant, antioxidant, anti-Alzheimer, anticancer, antidepressant, analgesic, antidiabetic, antifungal, antibacterial, antimicrobial, antiulcer, antiviral, and antimalarial (Ganeshpurkar and Saluja, 2017). *Trans-p*-coumaric acid, a phenolic acid of the hydroxycinnamic class, has been recorded to have antioxidant, antimicrobial, antiviral, anticancer, antidiabetic, and anxiolytic activities. There are some differences and similarities between our results and the literature. These differences may be caused by the collection localities of samples, extraction, purification, quantification, and characterization methods of the phenolic compounds (Freile-Pelegrín and Robledo, 2013). EPP and EZT were found as the richest extracts in terms of TFC and TPC. In a previous study, TPC of the acetone and water extracts of PP were reported as 90.61 and 57.34 mg catechin/g extract, respectively (Leão et al., 2017). TPC of PP extracts

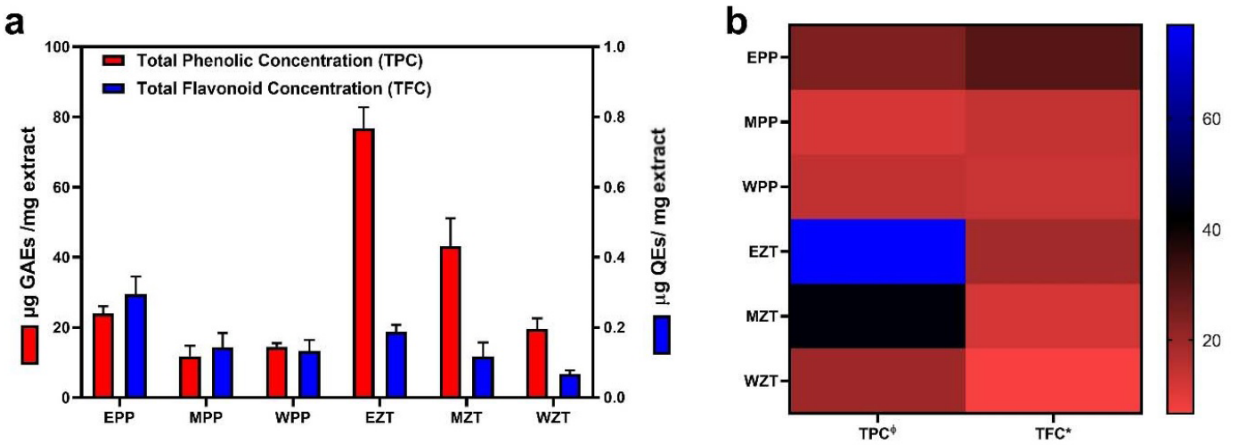

Figure 5. a. TPC and TFC of the extracts of PP and ZT. **b.** Heat Map analysis of TPC and TFC of the extracts of PP and ZT. Φ: µg GAEs/mg extract, *: µg QEs/mg extract. MPP: PP methanol extract, EPP: PP ethanol extract, WPP: PP water extract, MZT: ZT methanol extract, EZT: ZT ethanol extract, WZT: ZT water extract. The error bars signify the means ±SD of three parallel measurements.

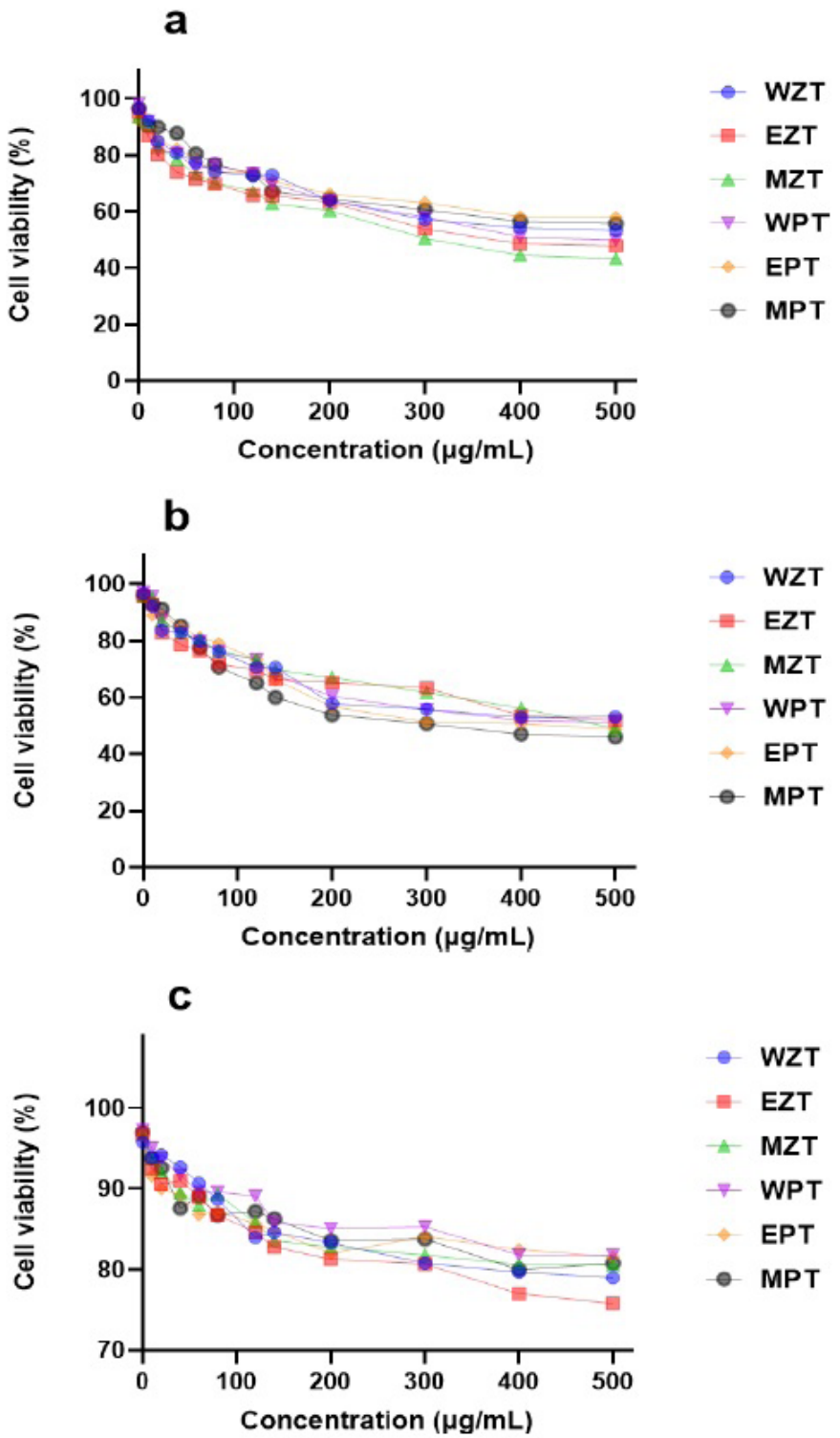

Figure 6. Viability of **a.** MCF-7, **b.** MIA PaCa-2, and **c.** HEK 293 cells exposed to 10-500 µg/mL extracts for 18 h. The results are given as a mean ±SD of three parallel measurements. MPP: PP methanol extract, EPP: PP ethanol extract, WPP: PP water extract, MZT: ZT methanol extract, EZT: ZT ethanol extract, WZT: ZT water extract.

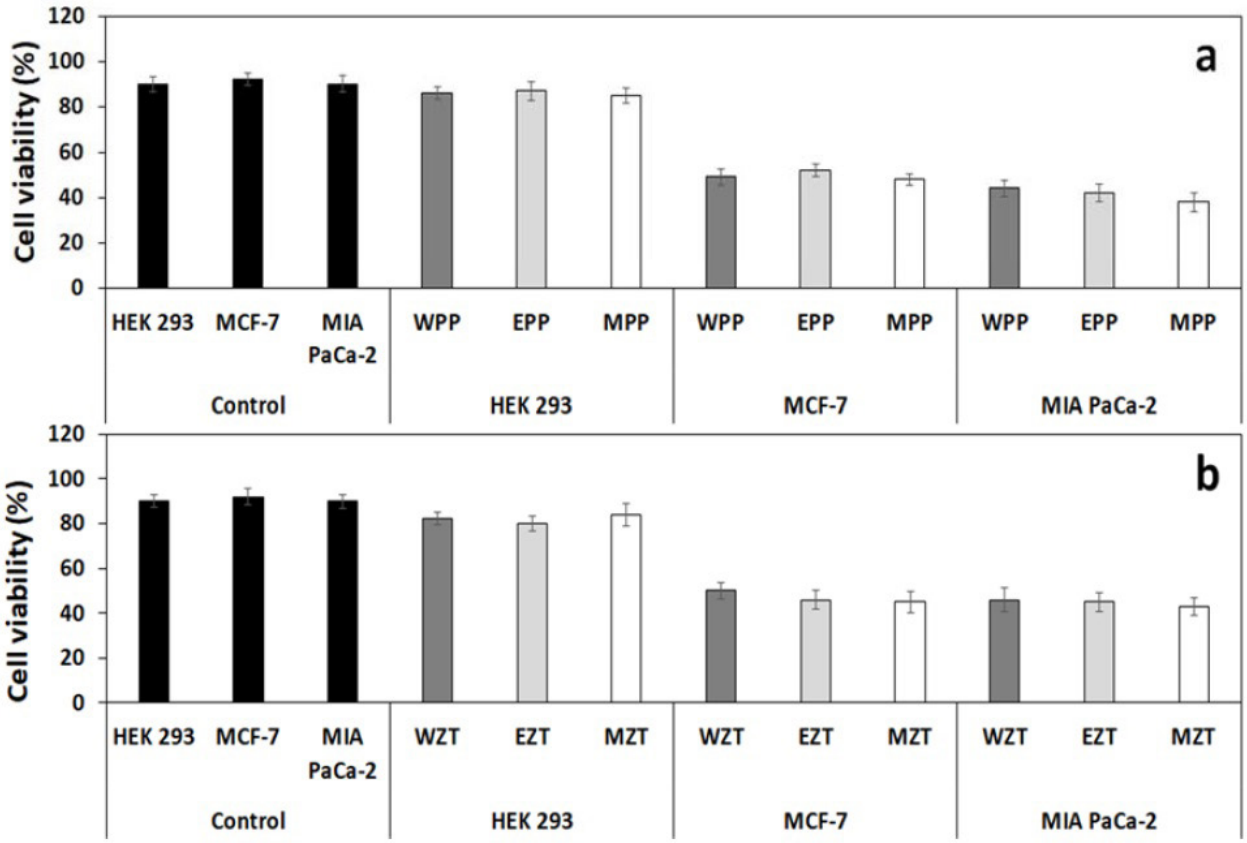

Figure 7. a. The effect of ZT extracts on the inhibition of MCF-7, MIA PaCa-2, and HEK 293 following exposure for 18 h at 500 µg/mL concentration. **b.** The effect of PP extracts on the inhibition of MCF-7, MIA PaCa-2, and HEK 293 following exposure for 18 h at 500 µg/mL concentration. MPP: PP methanol extract, EPP: PP ethanol extract, WPP: PP water extract, MZT: ZT methanol extract, EZT: ZT ethanol extract, WZT: ZT water extract. The error bars signify the means ±SD of three parallel measurements.

Table 4. The CC_{50} values of the extracts[a]

Extracts	**Cytotoxicity (CC_{50}, µg/mL)**		
	MIA PaCa-2	MCF-7	HEK 293
MPP	109.7 ± 21.4	169.3 ± 26.2	>500
EPP	127.1 ± 28.2	152.2 ± 32.3	>500
WPP	142.5 ± 32.3	196.7 ± 34.5	>500
MZT	91.4 ± 20.9	134.5 ± 24.6	>500
EZT	111.3 ± 23.5	132.3 ± 22.4	>500
WZT	137.8 ± 18.1	143.9 ± 20.9	>500

[a]: The results are given as a mean ±SD of three parallel measurements.
MPP: PP methanol extract, EPP: PP ethanol extract, WPP: PP water extract, MZT: ZT methanol extract, EZT: ZT ethanol extract, WZT: ZT water extract.

Table 5. Minimum inhibitory concentration (MIC) of PP and ZT extracts.

	MIC (mg/mL)						
	MPP	EPP	WPP	MZT	EZT	WZT	Chloramphenicol
P. shigelloides	2.50	1.25	3.75	3.75	3.75	5.00	0.15
E. faecalis	2.50	3.75	5.00	5.00	5.00	6.25	1.25
S. mutans	3.75	2.50	5.00	5.00	4.37	6.25	3.75
E. coli	5.00	3.75	6.25	6.25	5.00	7.50	5.00
S. aureus	3.75	1.25	3.75	5.00	4.37	5.00	3.75
S. pyogenes	3.75	2.50	5.00	3.75	4.37	7.50	1.25

MPP: PP methanol extract, EPP: PP ethanol extract, WPP: PP water extract, MZT: ZT methanol extract, EZT: ZT ethanol extract, WZT: ZT water extract.

Table 6. Antioxidant activity of PP and ZT extracts.

	Antioxidant activity					
	DPPH$^{\bullet}$ assay		ABTS$^{\bullet+}$ assay		CUPRAC assay	
Extracts	Inhibition (%)[a]	IC_{50} (μg/mL)[b]	Inhibition (%)[a]	IC_{50} (μg/mL)[b]	Absorbance[c]	$A_{0.50}$ (μg/mL)[d]
MPP	18.65 ± 1.09	>400	88.30 ± 0.17	178.20 ± 0.19	0.47 ± 0.01	>400
EPP	0.20 ± 0.05	>400	87.05 ± 0.66	75.16 ± 0.42	0.98 ± 0.06	119.44 ± 0.06
WPP	25.51 ± 0.67	>400	73.60 ± 1.16	195.50 ± 1.24	0.23 ± 0.00	>400
MZT	3.00 ± 0.41	>400	88.90 ± 0.13	30.15 ± 0.50	2.64 ± 0.01	44.13 ± 0.01
EZT	76.14 ± 0.20	49.03 ± 0.28	89.42 ± 0.33	18.86 ± 0.74	3.60 ± 0.01	15.20 ± 0.14
WZT	69.75 ± 0.91	78.73 ± 0.87	88.59 ± 0.12	35.47 ± 0.59	1.86 ± 0.03	85.72 ± 0.17
Standard						
Ascorbic acid	85.65 ± 0.03	6.68 ± 0.22	89.70 ± 0.04	5.24 ± 0.18	3.42 ± 0.01	44.06 ± 0.09

[a]: Inhibition values % of 400 μg/mL concentration of the extracts are given as a mean ±SD of three parallel measurements.
[b]: IC_{50} values are given as a mean ±SD of three parallel measurements.
[c]: Absorbance values of 400 μg/mL concentration of the extracts are given as a mean ±SD of three parallel measurements.
[d]: $A_{0.50}$ values are given as a mean ±SD of three parallel measurements.
MPP: PP methanol extract, EPP: PP ethanol extract, WPP: PP water extract, MZT: ZT methanol extract, EZT: ZT ethanol extract, WZT: ZT water extract.

was decreased in the order of pressurized hot water (1.17 ± 0.08 mg GAE/g dry wt.) > ethanol (1.13 ± 0.02 mg GAE/g dry wt.) > boiling water (0.80 ± 0.02 mg GAE/g dry wt.) > cold water (0.67 ± 0.01 mg GAE/g dry wt.) in the study of El-Shazoly and Fawzy (2018). The findings obtained were consistent with previous studies. Anticancer supplements of complementary and alternative medicinal compounds and extracts with fewer side effects derived from marine flora, especially marine algae, have recently attracted scientists' attention (Abd El-Hack et al., 2019). Unique bioactive active molecules obtained from algae extracts act by interacting with specific cancer-related receptors or cancer cell molecules, triggering certain mechanisms that cause cancer cell death (Wang et al., 2008). The methanol and ethanol extracts of PP and ZT showed higher cytotoxic activity than the water extracts. This high activity is thought to be related to the high phenolic contents when HPLC-UV, TPC, and TFC results were evaluated. Previous studies have proven that phenolic compounds from several subgroups have cytotoxic effects on various cancer types discussed by Jafari et al. (2018). A vast number of marine macroalgae extracts have been found to exhibit cytotoxic activity in many studies. Cytotoxic activity of PP ethanol extract on A549 (IC_{50}: 58.9 ± 0.1 μg/mL), CACO-2 (IC_{50}: 115 ± 0.9 μg/mL), HCT-116 (IC_{50}: 54.5 ± 0.3 μg/mL), Hela (IC_{50}: 59.0 ± 0.1 μg/mL), HEp-2 (IC_{50}: 101.0 ± 0.2 μg/mL), HEPG-2 (IC_{50}: 101.0 ± 0.4 μg/mL) and MCF-7 (IC_{50}: 97.6 ± 0.3 μg/mL) was reported by Al-Enazi et al. (2018). In a

different research, the cell viability of PP methanol extract on MCF-7 was observed as ~80% at 0.5 mg/mL concentration described by Pinteus et al. (2017). Çelenk et al. (2016) also reported the cell viability value of PP methanol extract on MCF-7 as around 35% at 50 µg/mL concentration. The bacterial resistance epidemic that has developed against antibiotics that are currently in use; has led to the exploration of new antibacterial agents of natural origin without side effects. In this regard, studies on the discovery of new agents from terrestrial and marine sources are gaining momentum (Shannon and Abu-Ghannam, 2016). Among the studied extracts, EPP was founded as the best antibacterial activite extract against all test pathogens and superior antibacterial activity against *P. shigelloides* and *S. aureus*. It has been deciphered that phenolic compounds contain many domains of action at the cellular level. It has been proven that these compounds exhibit antibacterial effects by causing variations in several intracellular functions induced by hydrogen bonding to enzymes or alteration of cell wall stiffness with loss of integrity owing to various interactions with the cell membrane reported by Bouarab-Chibane et al. (2019). As a result of HPLC-UV and spectrophotometric analysis, the highest amount of phenolic compounds was determined in EPP. It was observed that this extract contained a higher amount of rutin (3.00 ± 0.21 ppm) compared to other extracts. Besides, rutin was an important antibacterial phenolic compound already available in the literature of Dutta and Ray (2020). Since antioxidants have different action mechanisms, more than one method is preferred to determine the antioxidant activity rather than a single method. Antioxidant activities of PP and ZT extracts were screened using DPPH$^{\bullet}$ radical scavenging, ABTS$^{\bullet+}$ radical scavenging, and CUPRAC activity assays. EZT was obtained as the most antioxidant-active extract in all antioxidant activity assays. Antioxidant activities of various extracts of PP were described in previous studies. Scavenging activities of the water and acetone extracts of PP of DPPH$^{\bullet}$ (47.20 ± 0.02% and 72.92 ± 0.10% inhibition at 1 mg/mL concentration, respectively) and ABTS$^{\bullet+}$ (IC_{50}: 0.01 ± 0.00 mg/mL and IC_{50}: 0.01 ± 0.01 mg/mL, respectively) were reported by Fernando et al. (2016). Total antioxidant activity (69.4 ± 0.8–339.9 ± 12.5 µg/g dry wt.) and reducing power (231.4 ± 6.7–886.1 ± 54 µg/g dry wt.) of pressurized hot water, boiling water, cold water, and ethanol extracts of PP were investigated in a different study by El-Shazoly and Fawzy (2018). The IC_{50} value of the ethanol extract of PP was found as 5.59 ± 1.55 µg/mL in DPPH$^{\bullet}$ assay by Al-Enazi et al. (2018). When the previous studies were scanned, it was stated that the ethanol extracts generally had higher antioxidant activity. Herein, the antioxidant activities of the ethanol extracts of both studied macroalgae species were observed as higher when compared to other extracts. It is well-reported that phenolic compounds have strong antioxidant effects by scavenging free radicals, activating the endogenous antioxidant system, and preventing lipid peroxidation. Numerous studies have described that algae extract rich in phenolic compounds had higher antioxidant activity (Jimenez-Lopez et al. 2021). Apart from the study, Yuan et al. (2018) also reported that the highest antioxidant activity of the ethanol extracts of macroalgae species can be associated with the highest amounts of TPC and TFC.

5. Conclusion

The marine flora and fauna need exploration as they contain a diverse variety and lack scientific validation. In this direction, a new scan was made for updating and validation of this study. In this study, cytotoxic, antibacterial, and antioxidant activities of the various extracts of *P. pavonica* and *Z. typus* species were investigated with TPC and TFC. This is the first report on phenolic compounds and bioactive properties of *Z. typus* extracts. The ethanol extracts obtained from both studied marine macroalgae species with the highest TPC and TFC demonstrated close or higher antioxidant and antibacterial activities than the standards. Furthermore, all the studied extracts did not cause any toxicity in healthy cells, while they showed significant cytotoxicity on MCF-7 and MIA-PaCa-2 cancer cells. Presence of possible phenolic compounds that could be the source of bioactivities was confirmed by HPLC-UV analysis.

The findings of this study emphasized that *P. pavonica* and *Z. typus* species, which are considered the food of the future, could serve to discover promising, new and natural antioxidant, antibacterial, and anticancer agents that are critically important for a wide variety of industries such as medicine, food, and cosmetics. However, it is necessary to perform isolation studies to determine the biologically active compounds apart from phenolic compounds that may cause these bioactive properties.

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Conflicts of interest

The authors have no financial or nonfinancial interests to disclose.

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