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DEEP EUTECTIC SOLVENTS IN THE EXTRACTION OF BIOACTIVE COMPOUNDS FROM TWO BROWN MACROALGAE *Padina pavonica* AND *Cystoseira compressa*

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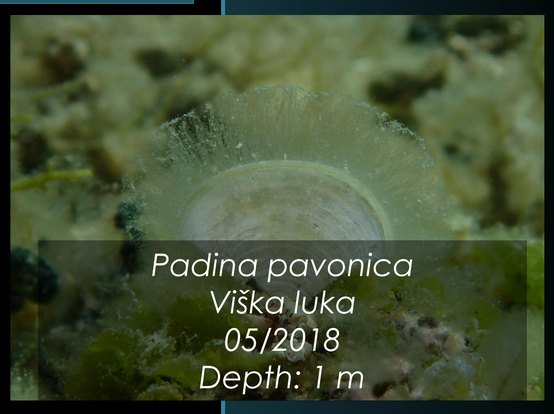
INTRODUCTION

Marine algae are known for their numerous benefits when consumed as a food and used for their pharmacological effect. Brown algae belong to the group of macroalgae and Mediterranean Sea encompasses more than 280 species of brown algae. Macroalgae of the genus *Cystoseira* are often found along shallow rocky reefs, where *C. compressa* often is found in the midlittoral zone lower level. This genus has been proved as a source of many bioactive compounds, terpenoids, meroterpenoids, lipids, phlorotannins, phenolic compounds, which are then responsible for its numerous biological activities. *Padina pavonica*, a brown calcifying algae from Dichtyophyceae family, is a warm temperate to tropical algae, common in Mediterranean sea. It has a fan-shaped thallus divided into fronds and the holdfast constituted of rhizoids. *P. pavonica* is an excellent source of bioactive compounds and is known for its antioxidant, antimicrobial, antidiabetic, anti-inflammatory activity, enzyme inhibiting, anticancer activity. Furthermore, brown algae are rich in phlorotannins, a phloroglucinol based polyphenols, biosynthesized by the acetate malonate pathway. Phlorotannins can be present in the epidermal cortex of brown algae, as well bound in the cell wall and usually serve as a plant defense system. They play an important role in algae defense system against different adverse conditions and their content in dry algae can reach 20%. They are often associated with different biological activities of brown algae extracts, such as antifungal against *Candida* sp., antibacterial, anti-inflammatory, antioxidant, antitumor, neuroprotective and proosteogenic activity. Phlorotannins possess numerous -OH groups in their structure, making them a highly hydrophilic against molecules. Therefore, it is advisable to extract those using polar solvents, such as deep eutectic solvents (DESs).

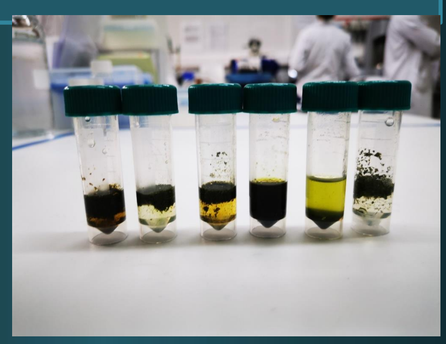
MARINE MACROALGAE SAMPLES

Macroalge were collected from different locations at different time and from certain depths. All of the samples were collected with surrounding seawater and placed in an airtight plastic bags and immediately transported to the laboratory.
For the extraction of phlorotannins, fresh macroalgae samples were freeze-dried. Before the freeze-drying, the samples were washed five times in water and twice in deionized water, then they were cut in slices (5–10 mm) and frozen at -60°C for 24 h in an ultra-low freezer. Five trays of frozen samples were placed in a laboratory freeze dryer (CoolSafe PRO, Labogene, Denmark). The freeze-drying was performed under a high vacuum (0.13–0.55 hPa) for 24 h with -30°C and 20°C as the primary and secondary drying temperatures.



DESs EXTRACTION OF PHLOROTANNINES

Ground dried algae (50 mg) were mixed with 1 mL of the selected solvent which is a mixture of DESs and ultrapure H_2O (Millipore Simplicity 185, Darmstadt, Germany) in a ratio of 80:20 (*v/v*) to reduce the viscosity of the solvent and facilitate mass transfer during the extraction process. The samples were mixed on a magnetic stirrer at 1500 rpm in an aluminum block (Stuart SHB) at 50 °C for 60 min. After extraction, the mixture was centrifuged for 15 min and then decanted. The supernatant was diluted with methanol and filtered through a PTFE 0.45 µm filter before analysis.



DETERMINATION OF PHLOROTANNINS AND ANTIRADICAL ACTIVITY

Phlorotannins were determined according to the method of Stern et al., 1996. Phloroglucinol in the concentration range 0.01-1 mg/mL was used for the calibration curve. 10 µL of 50 mg/mL extract was prepared to which 10 µL of DMF and 700 µL of 16% HCl were added. At 1 minute intervals, 700 µL of DMBA reagent was added to each sample after which the samples were stirred and heated in a water bath at 30 °C for 60 min. After 60 min, at 1 min intervals, the absorbance of the samples was measured at 510 nm.

The DPPH method described in the paper was used to determine the antiradical activity . The methanol solution of DPPH (0.03 mM) was prepared daily and stored in the dark until analysis. 0.5 mL of DPPH solution was added to 1.2 mL of the extract, and the prepared samples were stored in the dark for 30 minutes. After 30 min, absorbance was measured using a spectrophotometer at 517 nm. All measurements were performed in triplicate, and the percentage inhibition of DPPH radicals was calculated according to the following Equation (1):

$$DPPH\ activity\ (\%) = \frac{(A_{DPPH} + A_S) - A_S}{A_{DPPH}} \times 100$$

RESULTS

Table 1. DPPH scavenging activity and phlorotannin content of the brown algae extracts.

		Padina pavonica		*Cystoseira compressa*	
No.	Solvent	%DPPH scavenging*	µg PGE/50 mg dw*	% DPPH scavenging*	µg PGE/50 mg dw*
1.	Choline chloride: lactic acid (1:2)	35.9±3.0	57.6±0.9	95.8±0.3	132.7±8.8
2.	Choline chloride: xylitol (1:1)	7.8±1.1	25.0±4.2	36.3±4.2	28.3±5.9
3.	Choline chloride: malonic acid (1:1)	47.8±1.3	31.1±0.1	58.4±6.4	63.5±1.8
4.	Choline chloride: sorbitol (1:1)	9.8±2.0	18.0±1.1	93.8±1.9	43.2±1.0
5.	Choline chloride: oxalic acid (1:1)	87.4±0.6	42.5±0.6	94.8±0.4	79.5±3.2
6.	Choline chloride: fructose (1:1)	12.9±1.0	19.1±0.2	95.2±0.4	36.3±3.0
7.	Choline chloride: levulinic acid (1:2)	34.0±4.5	19.1±0.7	97.7±0.0	100.2±5.3
8.	Choline chloride: butane-1,4-diol (1:2)	26.8±1.2	27.7±1.0	95.2±0.4	35.6±2.1
9.	Choline chloride: N-methyl urea (1:3)	30.1±4.6	49.1±5.0	61.7±0.7	29.5±3.1
10.	Choline chloride: malic acid (1:1)	25.1±6.7	51.3±1.3	92.9±2.7	98.1±0.8
11.	Choline chloride: glucose (1:1)	32.1±2.2	53.0±2.5	83.0±3.4	51.7±5.7
12.	Choline chloride: thiourea (1:2)	96.3±0.4	9.1±0.0	87.5±0.6	33.4±1.1
13.	Choline chloride: urea (1:2)	26.9±1.4	70.6±5.4	58.1±3.1	99.0±4.4
14.	Choline chloride: acetamide (1:2)	34.1±2.0	59.8±1.0	64.0±2.2	81.3±8.3
15.	Choline chloride: ethane-1,2-diol (1:2)	31.4±0.5	42.9±5.9	92.5±1.7	63.0±1.6

*average area of 3 replicates expressed in percentage (%) with standard deviation (SD)

CONCLUSION

Phlorotannins content was much higher in most of the *C. compressa* extracts compared to *P. pavonica*. For *C. compressa*, choline chloride:lactic acid (1:2) showed the best results, considering both phlorotannin yield and DPPH scavenging activity. In the case of *P. pavonica* extracts, choline chloride:urea (1:2) was the most efficient for the extraction of phlorotannins and the best DPPH scavenging activity was obtained with choline chloride:thiourea (1:2). Nevertheless, some general conclusions about the optimal DES can not be observed, since high phlorotannin content in *C. compressa* extract was achieved with choline chloride:urea as well, which is basic, more viscous and less polar than chloride:lactic acid DES. Obviously, for both algae, it is not that simple to assume which solvent is the best for the extraction, and optimization in the way described here, is necessary.

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