A comparative evaluation of biological activities and bioactive compounds of the seagrasses *Zostera marina* and *Z. noltei* from Southern Portugal

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Abstract

This work assessed the antioxidant potential, acetylcholinesterase (AChE) inhibition and the *in vitro* cytotoxic activity of extracts of the seagrasses *Zostera marina* and *Z. noltei* collected from southern Portugal. The total phenolic contents (TPC), the rosmarinic acid (RA) concentration (HPLC/DAD) and the fatty acid profile (GC/MS) is also described. *Z. marina* had the highest TPC, radical scavenging activity against DPPH radicals, and copper chelating activity. *Z. noltei* had metal chelation capacity to copper and iron ions. None of the species was able to inhibit AChE. Both seagrasses had high levels of polyunsaturated fatty acids. *Z. marina* significantly and selectively reduced the viability of tumorous neuronal cells. *Z. noltei* was highly toxic for the three cell lines tested and was selective against hepatocarcinoma cells at the concentration of 100 µg/mL. RA was the main compound identified in *Z. marina*, but not in *Z. noltei*.

Keywords: Cytotoxicity; oxidative stress; PUFA; rosmarinic acid, seagrasses

1. Introduction

There are four species of seagrasses native to Europe, namely *Zostera marina*, *Z. noltei*, *Cymodocea nodosa* and *Posidonia oceanica*. Water and methanol extracts of *Z. marina* and *Z. noltei* collected from the French Mediterranean coast displayed algicidal effects on *Alexandrium catenella*, a dinoflagellate responsible for the occurrence of harmful algal blooms (Laabir et al., 2013). *Z. marina* leaf extracts have antioxidant and antibiotic activities (Kim et al., 2004, Kolenchenko et al., 2005; Choi et al., 2009), while organic extracts of *Z. noltei* collected in the Central Mediterranean area had strong antifungal activity against *Candida albicans* and *Aspergillus niger* (Ballesteros et al., 1992). Some bioactive compounds have been isolated from *Z. marina*, such as apigenin-7-O-β-D-glucoside and chrysoeriol (Kim et al., 2004). Rosmarinic (RA) and zosteric acids have been detected in both *Zostera* species (Kolenchenko et al., 2005; Achamlale et al., 2009; Grignon-Dubois et al., 2012). The *Zostera* genus exhibits a high chemical plasticity and displays different phenolic profiles for different geographic areas (Grignon-Dubois et al., 2012). However, nothing has been reported so far about the biological activities and / or chemical components of *Z. marina* and *Z. noltei* from southern Portugal. Therefore, evaluated the antioxidant, anti-acetylcholinesterase (AChE) and the *in vitro* cytotoxic activities of extracts of both species, collected in Southern Portugal (Algarve). It also describes some biocompounds present in both species, including total phenolics content, rosmarinic acid (RA) levels and fatty acids (FA) profile.

2. Results and discussion

The extraction yields of the crude methanol extracts were 18.3% for *Z. marina* and 9.3% for *Z. noltei*, while the extraction yields of the ethyl acetate fractions were similar for both species: 0.16 and 0.14% for *Z. marina* and *Z. noltei*, respectively (Table 1). *Z. marina* had a higher TPC than *Z. noltei* (Table 1). RA was found in the ethyl acetate extracts for the two species and was the main component in *Z. marina*, but not in Z. *noltei* (Fig S1 and Fig. S2). The different results about RA composition between the Achamlale et al. (2009) report and our study can be ascribed to the chemical plasticity and different patterns of phenolic composition depending on the collection site, and / or to the extraction procedure (Grignon-Dubois and Rezzonico, 2012; Santos-Buelga et al., 2012).

The fatty acids (FA) profiles of both species were mainly composed of palmitic, linoleic (LA) and α-linolenic (ALA) acids, which represent more than 70% of the total FA detected (Table 2). All

results were consistent with those previously reported by other authors (Coelho et al., 2011). The proportion of polyunsaturated fatty acids (PUFA) in both species was higher than the proportion of saturated FA and monounsaturated FA combined (Table 2). Moreover, the PUFA found in these species were mainly from the n-3 series, and although in less amounts, the hexadecatrienoic, eicosapentaenoic (EPA) and docosahexaenoic (DHA) acids were also detected. The proportion of n-3 PUFA detected in *Z. marina* and *Z. noltei* were approximately 40% of the total FA; therefore, taking into account their FA profile, both seagrasses can be considered to be a promising source of n-3 PUFA with valuable nutritional, pharmaceutical and biomedical applications.

Z. marina had the highest RSA (Table 1), which was lower than the RSA value previously reported for the ethyl acetate fraction of a crude methanol extract for the same species collected in Korea (Choi et al., 2009). *Z. marina* also had a good capacity to chelate copper, although not iron (Table 1). The antioxidant activity detected on the ethyl acetate fraction of *Z. marina* could be ascribed to RA, which is the most abundant compound in this fraction, with recognized antioxidant activity (Lu and Foo, 2002). *Z. noltei* had capacity to chelate both copper and iron ions (Table 1), but none of the extracts exhibited capacity to inhibit AChE (data not shown). Nonetheless, they could help to alleviate AD symptoms by the inhibition of oxidative stress processes and deposition of β-amyloid plaques through the chelation of copper and iron ions (Weinreb et al., 2011).

The *Z. marina* extract was less toxic to HepG2 and S17 cells (IC_{50} > 100 μg mL^{-1}), than to the neuroblastoma cell line (IC_{50} = 63.2 μg mL^{-1}), and was able to selectively reduce the viability of this cell line at the concentrations of 50 and 100 μg mL^{-1} (Fig. 1). Conversely, *Z. noltei* was highly toxic for the three cell lines tested (IC_{50} < 3 μg mL^{-1}) although it was selective (SI = 2.1) against HepG2 cells at the highest concentration tested (100 μg mL^{-1}; Fig. 1). In *Z. marina*, the reduction of cell viability can be attributed to the presence of RA (Stanojković et al., 2013). In *Z. noltei* the cytotoxic activity is likely attributed to others compounds present in the extracts. Etoposide allowed IC_{50} values of 1.9 μg mL^{-1} (HepG2 cells), 38.6 μg mL^{-1} (SH-SY5Y) and 10 μg mL^{-1} (S17), and selectivity values of 5.2 for HepG2 cells and 0.2 for the SH-SY5Y cell line.

3. Conclusions

Z. marina and *Z. noltei* contain of antioxidant compounds, namely RA. *Z. noltei* could be a source of potential chemotherapeutic agents and / or valuable lead compounds for the semi-synthesis or total

synthesis of effective new drugs. The isolation and identification of the compound(s) responsible for the bioactivities of *Z. noltei* are now being pursued.

Supplementary material

Experimental details relating to this article are available online.

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Tables

Table 1. Extraction yields (%), total phenolic contents (TPC), radical scavenging (RSA) on DPPH radicals, and metal chelation activity on iron (MCAI) and copper (MCAC) of extracts *Z. marina* and *Z. noltei*, expressed as IC_{50} values (mg/mL)

Samples	Extraction yields (%)		TPC[1]	RA[2]	RSA	MCAI	MCAC
	ME	EAE					
Z. marina	18.3	0.16	0.14 ± 0.01	0.24	0.31 ± 0.01^{b}	> 10	0.61 ± 0.02^{c}
Z. noltei	9.37	0.14	0.09 ± 0.00	0.09	1.10 ± 0.15^{c}	4.99 ± 0.71^{b}	0.88 ± 0.05^{b}
BHT*					0.07 ± 0.00^{a}		
EDTA*						0.08 ± 0.00^{a}	$0.05 \pm .01^{a}$

Values are means ± SD (n = 6). [1]Expressed as gallic acid equivalents [(mg GAE/g, dry weight (DW)]; [2]RA content quantified by HPLC analysis of the ethyl acetate fractions (mg/g^{-1}, DW); *Positive control; ME: methanol extract; EAE: ethyl acetate extract.

Figures

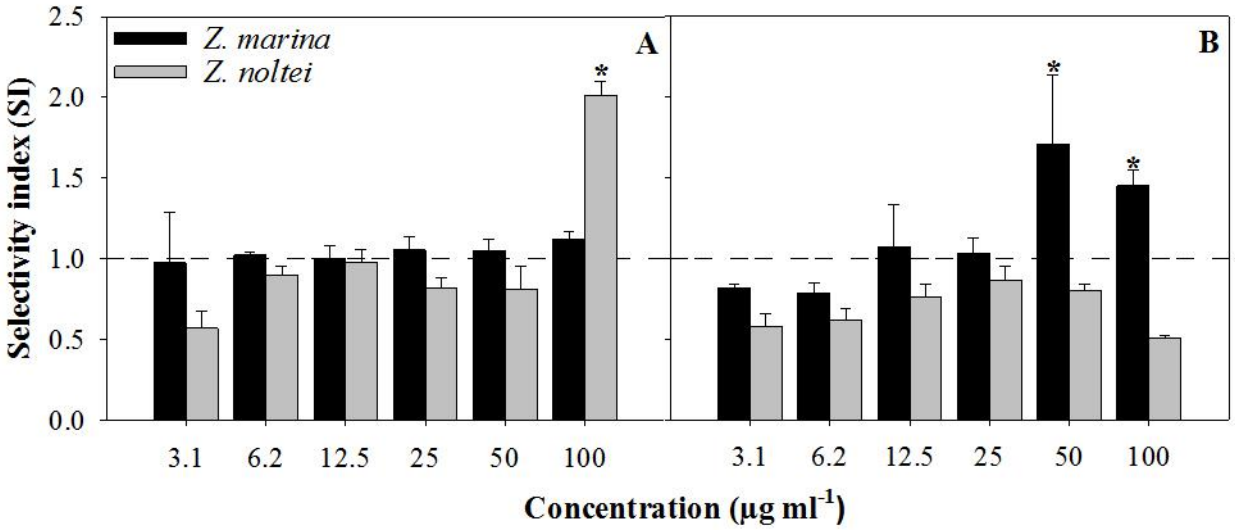

Figure 1. Selectivity index (SI) of *Z. marina* and *Z. noltei* extracts against human hepatocarcinoma (**A**, HepG2 cells) and neuroblastoma (**B**, SH-SY5Y) cell lines, calculated in relation to non-tumoural cell line (S17 cells). Solid bars and errors bars represent the average and standard error values, respectively (n = 9). *Significantly higher than 1 (dashed line) ($p < 0.05$).