CYSTOSEIRA ABIES-MARINA LIFE CYCLE: COMPARATIVE STUDY OF ITS POLAR PROFILE BY RP-HPLC-DAD-MS

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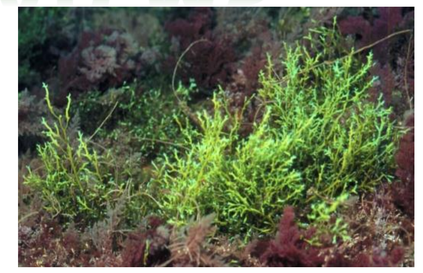


Introduction:

- Being naturally enriched in key nutrients and in various health-promoting compounds, seaweeds are largely used in food, pharmaceutical and cosmetic industries [1]. *Cystoseira abies-marina* (S.G. Gmelin) C. Agardh is a brown macroalgae (Phaeophyta) which belongs to *Cystoseira* genus and is distributed in the Mediterranean, Macaronesian Region and in the coast of Africa [2]. Phytochemical studies of this species reveled the presence of meronorditerpenoids with cytotoxic and antioxidant activities [3]. *Cystoseira abies-marina* grows abundantly in the Azorean intertidal zone [4], where it is exposed to extreme stress conditions (temperature, UV, water level, herbivory, fouling) and consequently can develop protective mechanisms and biosynthesize distinct metabolites [5].
- In this research was studied the antioxidant activity and the chemical composition of the ethyl acetate fraction of *C. abies-marina* methanolic extract, collected in two different phases of its life cycle (juvenile phase and mature phase).

Results and discussion:

- The free radical scavenging, reducing and chelating activities of the ethyl acetate fractions from the juvenile and mature *Cystoseira abies-marina* methanolic extracts were evaluated and the results shown in Table 1.

Table 1. EC_{50} values obtained in the DPPH, reducing power and chelating assays for the juvenile and mature phase and reference compounds

Assays	Samples /Standard	EC_{50} Mean ± SD (μg/mL)
Scavenging power (DPPH)	Juvenile phase	**184 ± 7.44**
	Mature phase	**27.6 ± 1.27**
	Quercetin	**3.46 ± 0.0535**
Reducing power (Fe^{3+}/Fe^{2+})	Juvenile phase	**1350 ± 65.0**
	Mature phase	**248 ± 9.76**
	BHT	**84.7 ± 3.32**
	Quercetin	**48.6 ± 1.58**

The results of table 1, allowed the following considerations:

- Mature phase clearly exhibits 7 folds higher antioxidant activity by radical scavenging mechanism than juvenile phase, although not as active as quercetin, a natural antioxidant used as reference.
- The reducing power exhibited by mature phase is also higher than to juvenile phase active.
- No metal chelation was detected in both mature and juvenile phases at the maximun concentrations tested (1.5 mg/mL)
- The main antioxidant mechanism for samples and standard is clearly through free radical scavenging effect.

- The polar profile of both fractions were evaluated by RP-HPLC-DAD-ESI/MS^n (Figure 1).

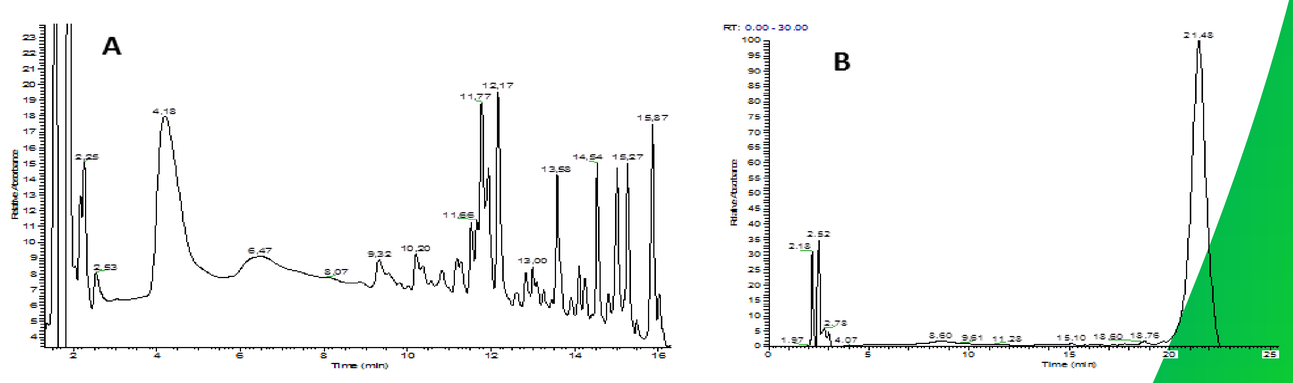
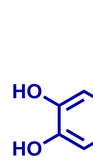


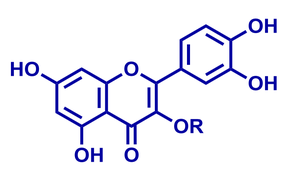
Figure 1. HPLC-DAD chromatogram obtained for ethyl acetate fractions of methanol extracts from juvenile (A) and mature (B) *Cystoseira abies-marina.*

Figure 1, clearly shows that:

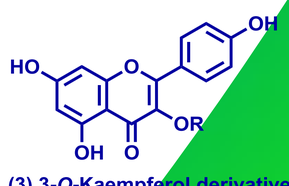
- The polar profile change significantly from juvenile to mature phase.
- The antioxidant activity is gained by different metabolite production and not from accumulation of the same metabolites.
- Interesting compounds were identified in juvenile phase, such as, cinnamic acid (1), quercetin (2) and kaempferol (3) derivatives.
- Kaempferol-*O*-rutinoside derivative (4) is the most abundant compound in the mature phase and could be the major responsible for antioxidant activity of mature phase fraction.



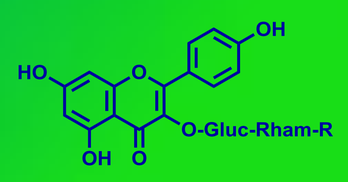
(1) Cinnamic acid



(2) 3-*O*-Quercetin derivatives



(3) 3-*O*-Kaempferol derivatives



(4) 3-*O*-Kaempferol rutinoside derivatives

Conclusion:

- *Cystoseira abies-marina* in mature phase is a source of antioxidant compounds;
- The chemical composition of this seaweed varies significantly with the life cycle phase.

Experimental procedures:

Cystoseira abies-marina was collected in Mosteiros, S. Miguel, Azores, in Winter and Spring. After grinding, fresh alga samples were separately extracted with methanol. Each MeOH extract (94.3 g and 124.1 g respectively) was evaporated to dryness, dissolved in water and then fractionated by liquid/liquid partition with hexane, chloroform and ethyl acetate yielding 2 ethyl acetate fractions one from mature phase (0.442 g) and other from juvenile phase (0.170 g).

The DPPH and reducing power assays were performed as described in Leal *et al.* [6] while chelating activity was carried out according to Chua *et al.* [7]. EC_{50} value is the effective concentration of ethyl acetate fractions capable of causing a variation of 50% in the reference value.

The RP-HPLC-DAD-ESI/MS^n analysis was performed on Dionex Ultimate 3000 apparatus equipped with an Ultimate 3000 DAD and a LTQXL detectors. Analysis was run on a Hichrom Nucleosil C18 column (250 mm x 4.6 mm i.d.; 5 μm particle diameter, end-capped) at 30 ºC. The mobile phase was composed by water/formic acid (0.1%, v/v) (A) and acetonitrile (B) at a flow rate of 1.5 mL/min. The following gradient program was used: 5% B (0 min), 30% B (12 min), 100% B (15 min), 100% B (19 min), 5% B (25 min) and 5% B (30 min).

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