Interspecific variation in total phenolic content in temperate brown algae

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Abstract

Marine algae synthesize secondary metabolites such as polyphenols that function as defense and protection mechanisms. Among brown algae, Fucales and Dictyotales (Phaeophyceae) contain the highest levels of phenolic compounds, mainly phlorotannins, that play multiple roles. Four temperate brown algae (*Cystoseira amentacea*, *Cystoseira compressa*, *Dictyopteris polypodioides* and *Padina pavonica*) were studied for total phenolic contents. Total phenolic content was determined colorimetrically with the Folin-Ciocalteu reagent. Significant differences in total phenolic content were observed between leathery and sheet-like algae and also within each morphological group. Among the four species, the sheet-like alga *D. polypodioides*, living in the upper infralittoral zone, showed the highest concentration of phenolic compounds. These results are in agreement with the hypothesis that total phenolic content in temperate brown algae is influenced by a combination of several factors, such as growth form, depth, and exposition to solar radiation.

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Introduction

Environmental variables together with biotic factors strongly drive growth, reproduction and survival of macroalgae.[1,2] In response to variations of abiotic and biotic factors and different environmental stresses, seaweeds are able to produce and accumulate secondary metabolites that function as defence compounds.[3,4] Among secondary metabolites, polyphenols are commonly found in macroalgae.[5] Brown algae (Phaeophyceae) present a high content of phenols, mainly phlorotannins, that are halogenated oligomers and polymers of phloroglucinol (1,3,5-trihydroxybenzene).[5] The majority of these molecules are stored in special vesicles (physodes) within the cells and some are constituents of cell walls.[6,7] Among Phaeophyceae, Dictyotales and Fucales are particularly rich in these compounds that may constitute up to 30% of the dry weight (DW) of these algae.[8]

These molecules, that certainly represent a cost for the algae in terms of increased metabolic demand and decreased growth rates,[9] may function as defence against grazers, epiphytes and fouling, and as protection against solar radiation, especially UV radiation.[6,10] Moreover, they have various biological activities together with therapeutic, antioxidant, cytotoxic and antimicrobial properties.[4] Recently, it has been also hypothesized they may have a role in the success of invasive species.[11]

Phlorotannin concentration can vary within and among species, being affected by seaweed size, age, thallus structure and morphology, ontogenetic stages and environmental factors (*e.g.* bathymetric gradients, sea surface temperature, photosynthetically active radiation levels, UV radiation and salinity), but also in relation to geography and reef morphology.[6,10,12,13]

In the Mediterranean Sea, essentially a tideless sea, seaweeds inhabiting the infralittoral fringe, characterized by regular emersion as a result of wave movements, experience harsher environmental stress conditions (*e.g.* partial desiccation and higher irradiances) than those living in the infralittoral zone, constantly submerged. To cope with these environmentally stressful conditions, algae have developed efficient mechanisms of protection such as the production of phlorotannins. These compounds can also vary throughout the year and their seasonal variations are species specific.[14-16]

The aim of this study was to estimate total phenolic content in four Mediterranean brown algae. In particular, we analysed two leathery algae inhabiting the infralittoral fringe, *Cystoseira amentacea* (C. Agardh) Bory and *C. compressa* (Esper) Gerloff & Nizamuddin, and two sheet-like algae living in the upper infralittoral zone, *Dictyopteris polypodioides* (A.P. De Candolle) J.V. Lamouroux and *Padina pavonica* (Linnaeus) Thivy.

Materials and Methods

Study area and sampling

Samples were collected in the Marine Protected Area (MPA) Capo Gallo-Isola delle Femmine, located along the north-western coast of Sicily (NW Mediterranean Sea; N38°12'35''; E13°17'06''). Thalli (n=5) of each species were collected from exposed rocky shores (Barcarello Point) once in summer. For each species, undamaged thalli of the same length and therefore presumably of the same age were collected with a hammer and chisel, at sunrise.

After collection, thalli were placed in plastic bags filled with seawater, for transportation within 1 h to the wet laboratory at the University of Palermo. In the laboratory, thalli were gently washed with demineralised water to remove detritus and epiphytes and identified with the aid of a stereo microscope and analytical keys.[17]

Preparation of algal extracts

Approximately 30 g of algal samples were removed from the middle portion of each thallus (n=5), washed with demineralised water and oven-dried at 50 °C for 48 h. Dry samples were pulverised with mortar and pestle and 2 g (dry weight, DW) of each sample were extracted with 25 mL of 96% (v/v) ethanol in centrifuge tubes in a 37 °C water bath for 2.5 h. The mixture was centrifuged at 2200 g for 10 min and the supernatant was collected for further analyses.

Determination of total phenolic content

Total phenolic content (TPC) was determined colorimetrically with the Folin-Ciocalteu reagent (Sigma-Aldrich Chemie, Steinheim, Germany), using phloroglucinol as the reference standard.[18,19] The Folin-Ciocalteu method was chosen because it provides more consistent results and is less affected by interfering non-phenolic compounds than other colorimetric methods.[20,21]

Briefly, 0.4 mL of the ethanol extract was transferred into a test tube containing 0.8 mL of the 10% Folin-Ciocalteu-phenol reagent. After 3 min, 1.6 mL of a 10% sodium carbonate solution was added. The contents of the tube were mixed thoroughly using a glass rod and left to stand at room temperature for 1 h. After the reaction, the absorbance of the samples was measured with a spectrophotometer (DU 800, Beckman Coulter Inc., Fullerton, CA, USA) at 750 nm. TPC was expressed as phloroglucinol equivalents in percentage of DW (% DW).

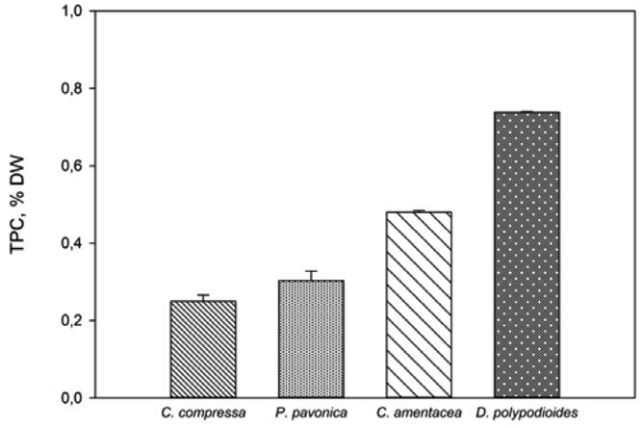

Figure 1. Variations of total phenolic content in the four examined species. Bars represent mean±standard deviation (n=5).

Statistical analysis

All analyses were run in duplicate, and values are expressed as mean±standard deviation (SD). Statistical analysis was carried out using SigmaPlot 12 software (Systat Software, Inc., San Jose, CA, USA). The significance of differences in TPC among the four species was tested with a one-way ANOVA. To satisfy the criteria of normality and variance homogeneity, data were square-transformed prior to performing ANOVA. Fisher's LSD post hoc tests were performed when data showed significant differences ($P<0.05$).

Results

TPC differed significantly among the four analysed species (Figure 1; Table 1). *D. polypodioides* showed significantly higher phenolic content (0.74±0.001% of DW) than the other species whereas *C. compressa* displayed the lowest value (0.25±0.016% of DW). Differences in TPC between *D. polypodioides* and each of the other seaweeds, *C. amentacea* ($P<0.05$), *C. compressa* ($P<0.001$) and *P. pavonica* ($P<0.001$), resulted also significant.

TPC differed significantly between the two leathery algae ($P<0.05$), *C. amentacea* (0.48±0.06% of DW) and *C. compressa* (0.25±0.02% of DW), and between the two sheet-like algae ($P<0.001$), *D. polypodioides* (0.74±0.001% of DW) and *P. pavonica* (0.30±0.02% of DW).

Discussion

The mean TPC values of the analysed species, ranging from 0.25±0.02% to 0.74±0.001% of DW, are consistent with those reported in previous studies for brown algae.[12,21]

Variations in reported TPC among brown algae are partly attributed to differences in the methods used both in sample preparation and phenol extraction, since different solvents vary in their extraction efficiency.[20,22,23] As we used the same method in this interspecific analysis of TPC, differences can be attributed to other factors such as thallus morphology, life cycle, bathymetric level and grazing pressure.

Thallus morphology significantly affects the algal palatability so sheet-like algae, as a consequence of their high palatability,[24] produce high amounts of phenols. This was the case for the sheet-like alga *D. polypodioides*, in which we found a higher phenolic content than in the leathery *Cystoseira* species. On the contrary, we found a low TPC in the sheet-like alga *P. pavonica*. However, this species presents consistent blade calcification, making *P. pavonica* a hardly palatable species and thus possibly not requiring greater investment in phenols.

Table 1. Results of the one-way ANOVA on total phenolic content of the four species collected at Barcarello Point in summer.

Source of variation	SS	df	MS	F	P
Between groups	3.825	3	1.275	10.514	***
Residual	1.940	16	0.121		
Total	5.765	19			

SS, sum of squares; df, degree of freedom; MS, mean sum of squares. ***P<0.001.

Dictyopteris polypodioides is a semi-perennant species producing mature fronds in the autumn-spring period while in summer, a period of rest, we only found the mid-rib and the remains of the frond. High TPC is often found in early vegetative phases in order to increase protection in young tissues.[21] Finding such a high TPC in summer would confirm the need for this alga to invest in defense strategies all year around, to protect both young and adult thalli.

On the contrary, *P. pavonica* is an annual alga that reaches the maximum development in summer. The low TPC found in the period of maximum development, would confirm the low palatability of adult thalli of this species.

Cystoseira amentacea and *C. compressa* are semi-perennant algae with adult thalli present in the spring-summer period. These species did not appear to invest highly in the protection of adult thalli in terms of TPC. The differences found between the two *Cystoseira* species could be related to some differences in thallus structure or in chemical defences. Indeed, we know *Cystoseira* species differ for the secondary metabolites they produce,[25] that may also act as defensive metabolites (constitutive or induced).

The bathymetric level may affect TPC differently, acting both on the herbivore pressure and on the exposure to solar radiation. Consumer pressure is generally lowest in wave exposed shores where the feeding ability of most mobile consumers is limited to calm periods.[26] Therefore, in the infralittoral fringe a greater water movement makes feeding on algae more difficult than in the constantly submerged zones. Our results are consistent with these observations. Indeed, *D. polypodioides* inhabiting the upper infralittoral zone showed a higher TPC than that found in *Cystoseira* species inhabiting the infralittoral fringe. *Cystoseira* species, even though they are leathery algae, are generally considered highly palatable,[27] therefore we expected to find a TPC similar to that of *D. polypodioides*. But since both *Cystoseira* species live in a wave exposed zone, that makes feeding on algae more difficult, they do not need to invest many resources in defensive metabolites due to a lower grazer pressure. Even though *P. pavonica* lives in the upper infralittoral zone where herbivore pressure is higher, as mentioned before, blade calcification makes it a less palatable species.

The bathymetric level also affects exposure to solar radiation. Algae such as *Cystoseira* species, inhabiting the infralittoral fringe, experience regular even if brief emersion of the apical parts of the thalli, whereas algae such as *D. polypodioides* and *P. pavonica*, living in the upper infralittoral zone, are continually submerged. The emersion of thalli, even if incomplete, implies a direct exposure to solar radiation that gradually decreases as it passes through seawater and TPC is affected by environmental factors such as light quantity and quality.[6,28,29]

Therefore, we would have expected a higher phenolic content in algae inhabiting the infralittoral fringe as compared with those growing in the constantly submerged zones, in agreement with the findings of Pavia and Brock.[29] Instead, our findings are not consistent with these observations, but partially agree with Connan *et al.*[14] who found higher TPC in brown algae growing at mid-tide level compared to that of species inhabiting either the low-tide level or the upper mid-littoral fringe. To explain that, the authors hypothesized that mechanisms of photo-protection other than phenol production exist in seaweeds. For example, carotenoids might limit effects of excessive light in plants and maybe act together with phenolic compounds in algal species.[14,30] Thus the light stressing condition of the emersion phases does not necessarily result in an increase of TPC. On the contrary, no relation between bathymetric level and TPC in Sargassaceae from Brittany (France) was observed.[6]

Conclusions

Our results seem to confirm that TPC in brown algae is a response to a combination of several factors.[7] However, due to the complexity of TPC responses in brown algae and the multiple roles of phlorotannins, for a better understanding of this process, it is still necessary to identify which types of phlorotannins are responsible for the different activities in order to clarify *who does what*.

Since these algae may produce different metabolites in response to stress conditions (grazing, pathogens), the entire range of defensive mechanisms should be studied in more detail. It is also noteworthy that these algae are a source of different bioactive substances, among which phenolic compounds, which deserve great attention for the benefits they may provide for human health.

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