TLC-Densitometric Investigation of Bioactive Components from Mediterranean Algae

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Abstract

Several photosynthetic pigments from two algae, namely *Padina pavonica* and *Codium fragile*, were extracted in different solvents and subsequently analyzed through TLC. The optimization of the separation parameters led to the choosing of the optimum eluent (hexane/acetone mixture) for polar silica gel plates. The isolated compounds were evaluated through densitometric measurements and by the acquisition of their UV-Vis spectra. While xanthophylls, chlorophyll *a* and pheophytin *a* were the most typical pigments of *Padina pavonica*, chlorophyll *a* and *b*, xanthophyll and β-carotene were the most characteristic pigments for *Codium fragile*.

Introduction

The algae, omnipresent in seas and oceans, concentrate a wealth of various bioactive substances, which find numerous applications in beauty and health products. Used both internally and externally, they are today a veritable weapon against aging. While strenuous efforts have been made to separate the photosynthetic pigments of phytoplankton, the literature dedicated to those of seaweeds is limited [1–3]. Thus, the present paper aims to expand the knowledge related to the composition and biological roles of the bioactive components of various seaweeds. We selected two species for our study: *Padina pavonica*, a brown algae which is part of the Phaeophyceae class, Dictyotaceae family, and a green algae, *Codium fragile*, from the Bryopsidophyceae class, Codiaceae family. As a reliable and trustworthy analysis method, thin-layer chromatography (TLC) coupled with densitometric analysis was used, through which a multitude of data on the composition of these two algae (including UV-Vis spectra of pure components) were obtained. Some of these constitute the subject of this paper.

Experimental

Both species of seaweeds were collected from the Tunisian coast in May 2015. They were washed with tap water several times, dried and preserved on ice (-28°C) for further processing. The algae were cut in small pieces, powdered with a blender, and 30 g of dried sample was weighted for each extraction, which was performed for both species with three different solvents, namely methanol, hexane and acetone. Extractions were carried out at room temperature for 24 h under heavy stirring. The extraction mixtures were filtered and the solvent was removed using a rotary evaporator. The evaporated samples were dissolved in a methanol/acetone mixture (1:1 v/v), stirred a couple of minutes, and then placed on the TLC Silica gel $60F_{254}$ plates (10 x 10 cm) from Merck. The plates were eluted in a vertical developing chamber. The densitometric evaluation of the TLC plates was performed using the CAMAG TLC Scanner 3 which is controlled by the winCATS software.

Results and discussion

Because the dedicated literature contains a scarce amount of experimental data regarding the TLC [5,6] or HPTLC [7,11] separating conditions, in the first stage a study was conducted for determining the optimal eluent which allows the separation of a maximum number of components from these species. The tested eluting systems are shown in Table 1. The most efficient eluent which led to the highest number of separated components was determined to be hexane/acetone 70:30 (v/v).

Table 1. Eluents tested for the TLC separation on Silica gel $60F_{254}$ plates

Eluent mixture	% volumes
ethyl acetate : acetone : methanol	60:30:10
ethyl acetate : acetone : methanol	80:10:10
toluene : ethyl acetate : methanol	60:30:10
chloroform : ethyl acetate: methanol	60:30:10
hexane : acetone	65:35
hexane : acetone	70:30

At close inspection of the TLC plates for the methanol extracts (Figure 1), eight peaks are revealed in the case of *Padina pavonica* and nine peaks for *Codium fragile*. Based on the spectral (UV-Vis) characteristics [11], three pigments were identified for *Padina pavonica* (Figure 2A) and four pigments for *Codium fragile* (Figure 3A). The major photosynthetic pigments identified in the two algae, together with their R_f and the maximum absorbance values, are shown in Table 2. For the quantitative evaluation of the isolated components, the TLC plates were analyzed by densitometry using a 254 nm wavelength UV radiation. The corresponding densitograms are shown in Figures 2B and 3B. Chlorophyll *a* and *b* [4] are characterized by two absorption bands, located in the blue-violet and red region of the spectrum (green photosynthetic pigments), while carotenoids [4,8-10] generally give yellow or orange pigments.

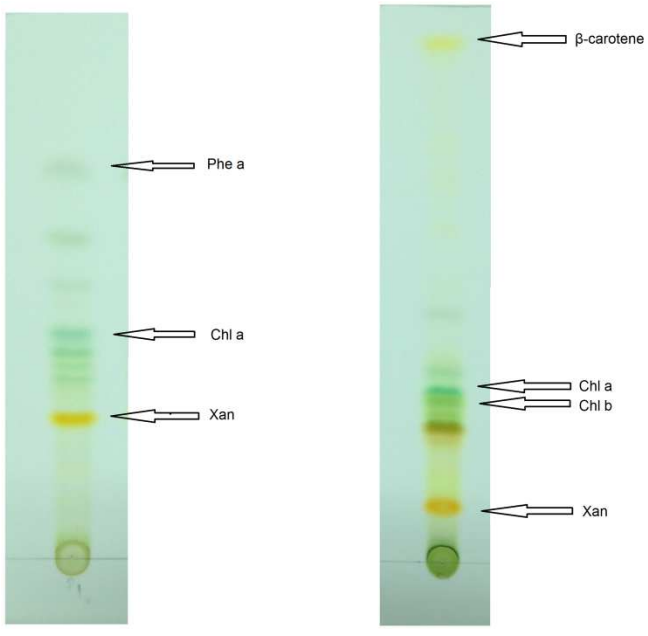
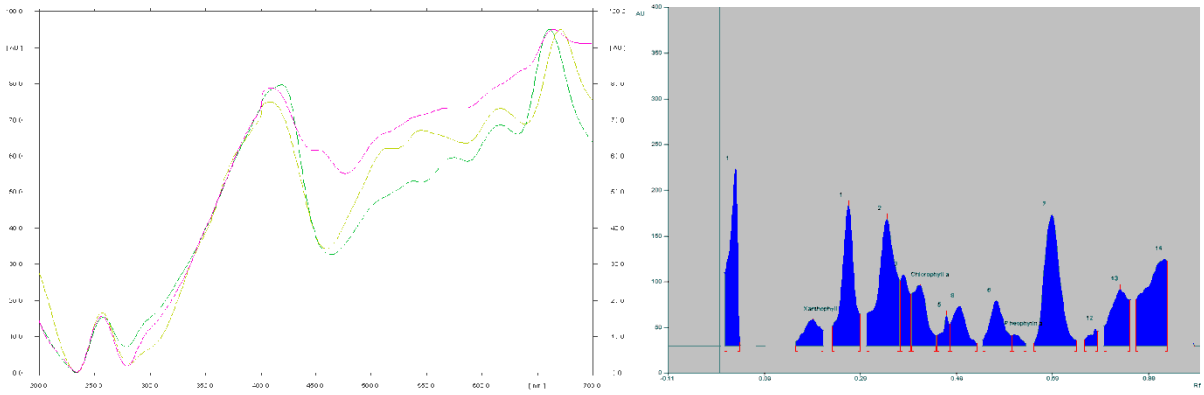


Figure 1. TLC plates after elution with hexane/acetone (70:30):
(a) *Padina pavonica*, and (b) *Codium fragile*

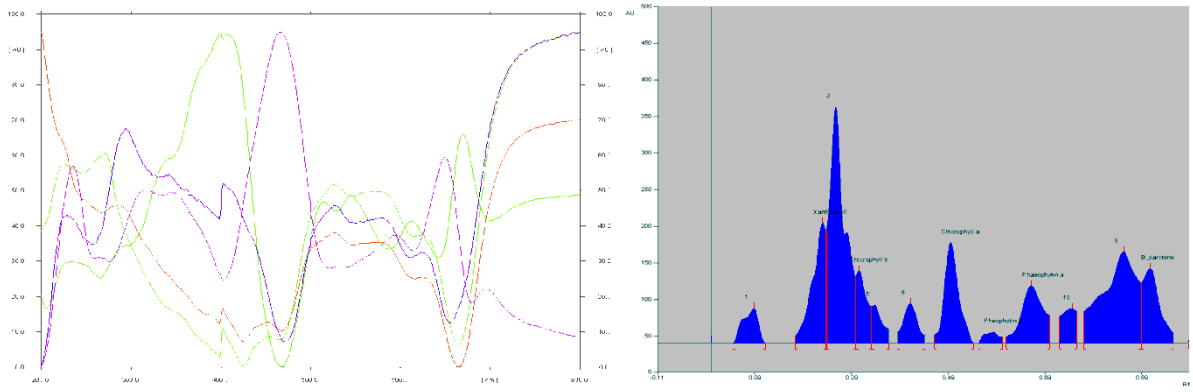
Table 2. The major photosynthetic pigments: R_f and UV-Vis maxima

Substance	R_f (literature)	R_f (determined)	Spectral data (nm)
Chlorophyll *a*	0.44 [5]	0.42	426, 662
Chlorophyll *b*	0.32 [5]	0.31	453, 643
Pheophytin *a*	0.60 [5]	0.58	425, 468, 663
Xanthophyll	0.16 [4]	0.15	421, 667
β-carotene	0.91 [4]	0.91	402, 529



(a) (b)

Figure 2. *Padina pavonica* methanol extract: (a) UV-Vis spectrum of xanthophyll, chlorophyll *a* and pheophytin, and (b) densitogram of all found peaks



(a) (b)

Figure 3. *Codium fragile* methanol extract: (a) UV-Vis spectrum of xanthophyll, chlorophyll *a* and *b*, pheophytin and β-carotene, and (b) densitogram of all found peaks

Conclusion

Several photosynthetic pigments from two algae, namely *Padina pavonica* and *Codium fragile*, were extracted in various solvents and then analyzed through TLC. The optimization of the separation conditions led to the election of the most favorable eluent (hexane/acetone 70:30) for the silica gel plates. Separated compounds were evaluated through densitometric measurements and UV-Vis spectroscopy [7]. While xanthophylls, chlorophyll *a* and pheophytin *a* were the most typical pigments of *Padina pavonica*, chlorophyll *a* and *b*, xanthophyll and β-carotene were the most characteristic pigments in *Codium fragile.*

Acknowledgements

This work was supported by the Romanian National Authority for Scientific Research (CNCS-UEFISCDI) through project PN-II-PCCA-2011-142.

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