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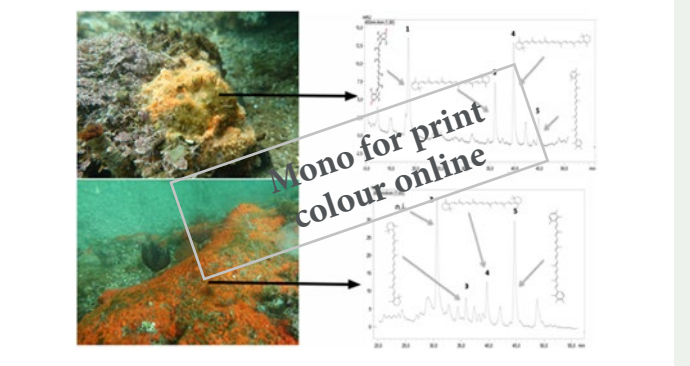
Determination and quantification of carotenoids in sea sponges *Raspaciona aculeata* and *Dictyonella marsilii* present in the Ganzirri Lake (Messina), Italy.

Andrea Salvo, Daniele Giuffrida, Archimede Rotondo, Paolo De Pasquale, Giovanna Loredana La Torre and Giacomo Dugo

Department of Biomedical and Dental Sciences and Morphofunctional Imaging, University of Messina, Messina, Italy

ABSTRACT

Considering the crucial role of carotenoids exploitable both as nutraceuticals and also as dyes in food industry, there are many efforts in seeking for new sources of these pigments, especially in the marine world. In this study, for the first time, we extracted carotenoids from sea sponges *Raspaciona aculeata* and *Dictyonella marsilii* taken from Ganzirri Lake Messina (Italy). The determination and quantification of carotenoids was made by UPLC-PDA-MS. Remarkable results concern renieratene content in *R. aculeate* found to be over 2570 ppm.



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1. Introduction

Sponges are marine animals belonging to the group of *Phylum Porifera* whose latin derivation means 'bearing holes'. These metazoans are multicellular animals with a quite simple body structure without specific tissues but rather endowed with single cell able to perform specific tasks as, for instance, the skeleton buildup or nutrition and reproduction (John et al. 2002). Sponges are typically benthic animals living on the seabeds; few species live also in fresh-water (Monaco & Quinlan 2014; Matsuno 2001). They are vertebrate suspension feeders, as

CONTACT Andrea Salvo asalvo@unime.it

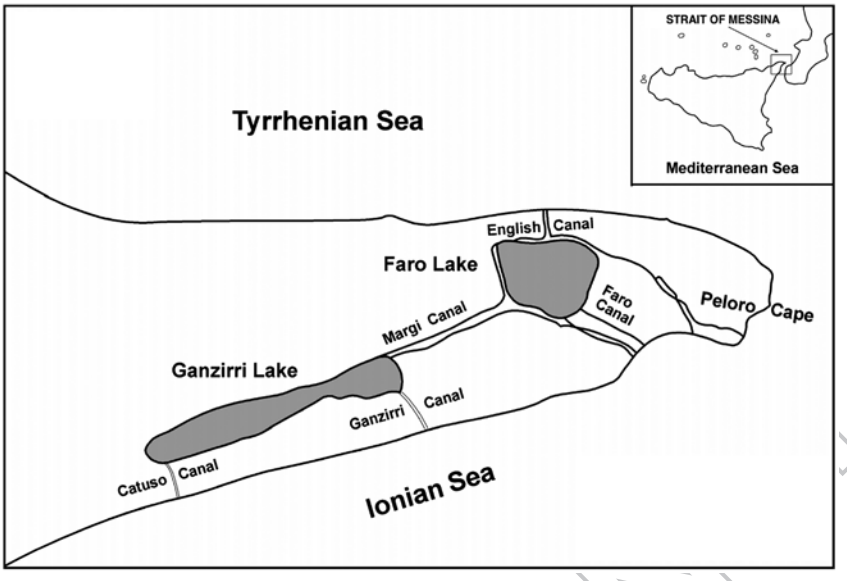

Figure 1. Area of Ganzirri and Faro Lake, with the connecting channels that flow on the Ionian and Tyrrhenian Sea.

they feed by filtration from water of micro algae (Salvo et al. 2014, 2016), bacteria and protozoa. Their shape is usually asymmetric and depends on species and on environment; some are adhering over the rocks, some others show tubular shape and their figure is compared with pillows, vases or breadcrumb. They also show several colours and size being yellow, blue, purple, magenta and ranging from few millimetres to 2 m of length (Johnston 1842 book; John et al. 2002). Sponges are symbiotic and usually associated to some algae and bacteria (Britton et al. 1998). AQ1

Ganzirri lake is a hydrologic formation belonging (together with Faro Lake) to the costal reserve of Peloro Cape within the city area of Messina. As Figure 1 shows, two channels allow the exchange with the sea water; these are open or shuttered in order to maintain oxygenation and prevent eutrophication. A third channel connects Ganzirri lake to Faro lake, which in turn is connected to the Tyrrhenian and Ionian Sea. This geographic arrangement allows a constant sea water circulation so that both the water and the fishes are not contaminated by inorganic and organic substances (Dugo et al. 2010; Lo Turco et al. 2013; Di Bella et al. 2015; Naccari et al. 2015; Maisano et al. 2016; Saija et al. 2016). Moreover, this channel system allowed the formation of a micro unique ecosystem that consents to certain plant and animal species to live here only (Crescenti et al. 2010; Coppari et al. 2016).

Specifically, in this paper we report the analysis of carotenoids found in two sponge species living in Ganzirri lake: *Raspaciona aculeata* (Johnston 1797–1855) and *Dictyonella marsilii* (Crescenti et al. 2010; MaoKa 2011). The former looks like a deep red, rugged, honeycomb-like surface whose thickness ranges from 2 mm to 2 cm and its surface goes up to 6 × 2 cm, grows over rocks and shells. The latter is a yellow orange sponge occupying few square decimetres over the seabed rocks, presents a regularly puckered surface with sharp tips.

Carotenoids were not typically considered of pharmacological interest; nonetheless they have conveyed attention as important nutraceuticals because of their significant antioxidant and anti-cancer activities (Nishino et al. 2002; Baby & Sujatha 2011). Nutraceuticals are dietary

or supplemental molecules relaying a physiological benefit, or providing biological protection against chronic conditions or diseases (Ebada et al. 2010). Carotenoids have been also shown to provide both photoprotective and reproductive benefits in a variety of marine organisms (Takashi 2011), and marine sponges are a potential source of these compounds, AQ2 which are potential nutraceuticals and natural dyes (Gordon & Bauernfeind 1982). Carotenoids play their vital photoprotective and antioxidant roles via high-light energy dissipation, and free radical detoxification.

Peculiar carotenoids in sponges are aryl derivates like isorenieratene, renieratene and renierapurpurin (Matsuno & Hirao 1989; Britton et al. 1998). More than 20 aromatic carotenoid species where found in sponges (Britton et al. 2004), and beyond sponges this class of compounds is found just in green sulphur bacteria (Britton et al. 1998; Crescenti et al. 2010). The chance to identify novel interesting carotenoid molecules urged us to drive a complete pigment analysis of these sponges which have not been previously analysed.

2. Results and discussion

The HPLC chromatograms (at 450 nm) obtained for the carotenoids determination in the *R. aculeata* and *D. marsilii* sponges are reported in Figures 2–4, respectively. Five peculiar peaks are highlighted and the simultaneous use of HPLC with mass spectrometer also allowed the easy identification of four of these peaks that were characterised as reported in Table 1. The identified compounds were also confirmed by comparing the spectral data of authentic standards. Compounds α-carotene, β-carotene and renieratene were readily characterised in both of the sponge samples and therefore also the specific quantification was possible (Table 1). The obtained data show that the two examined sponges have shared some common characteristic since the concentration of two compounds resulted comparable. In particular, β-carotene showed a concentration of 650.1 ppm in *R. aculeata* and 672.9 ppm in *D. marsilii*, respectively. Also the α-carotene amount resulted similar and showed the highest amount in *D. marsilii* (393.9 ppm). Although renieratene was found in the two species of sea sponges, however it was prevalent in *R. aculeata* (2570 ppm) while it resulted the compound detected in lower amount in *D. marsilii* (277.1 ppm).

Unlike these carotenoids, the first main peak in the chromatogram of *D. marsilii* (labelled as 1 in Figure 3) is not detected in the *R. aculeata* samples; this peak was identified as alloxanthine and then quantified as 711.4 ppm within the *D. marsilii* sample. On the other hand, the first main peak of the *R. aculeata* chromatogram (marked as 2 in Figure 2) is an unidentified compound which certainly is not present in *D. marsilii.* Albeit it is not possible the specific quantification (as it is an unknown species), in *R. aculeata*, this compound is clearly very well represented within the pigments of this sponge.

3. Materials and methods

3.1. Samples and extraction process

All reactants and solvents were purchased from Sigma-Aldrich (Milan, Italy). Sponge samples were grinded and homogenised and later cooled at −80°C overnight. Afterword samples were poured into glass beakers and lyophilised by the Alpha 2–4 LD plus CHRIST for two days until the total water loss. To 0.5 g of lyophilised sample, 10 mL n-hexane is added and undergone to five cycles lasting ten minutes each at the controlled temperature of 25°C.

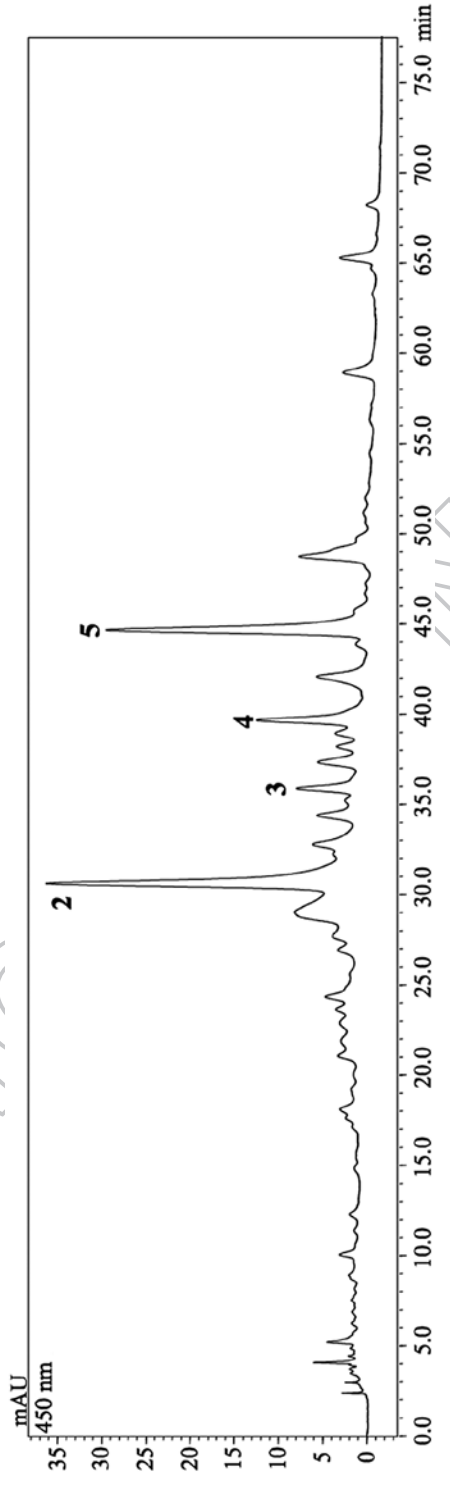

Figure 2. HPLC profile of the carotenoids detected in *Raspaciona aculeata*. For peaks identification see Figure 4.

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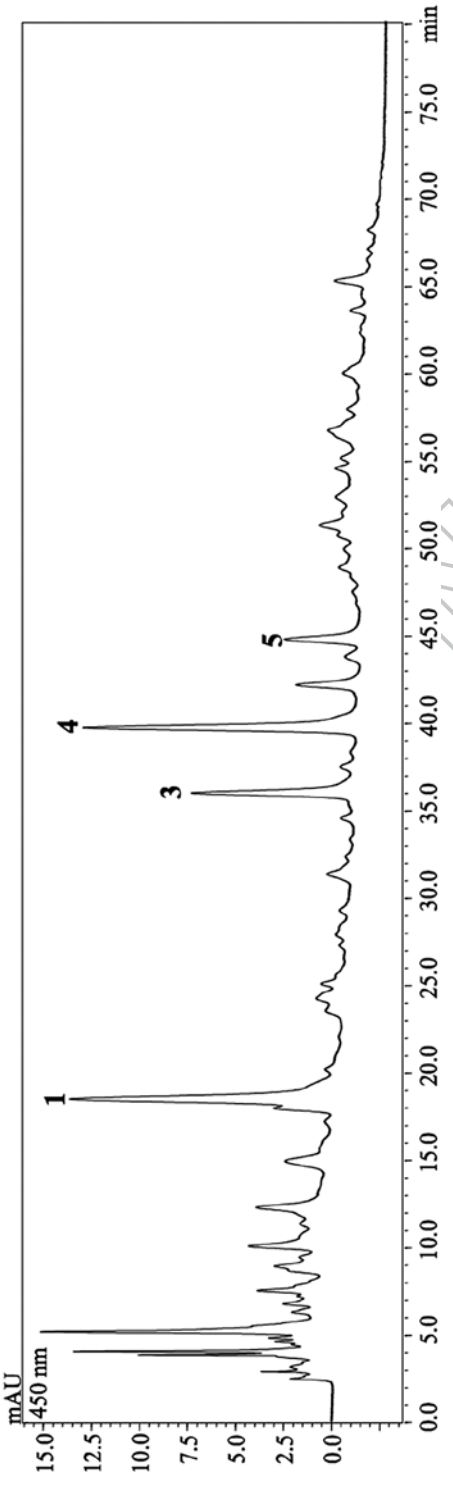

Figure 3. HPLC profile of the carotenoids detected in *Dictyonella marsilii*. For peaks identification see Figure 4.

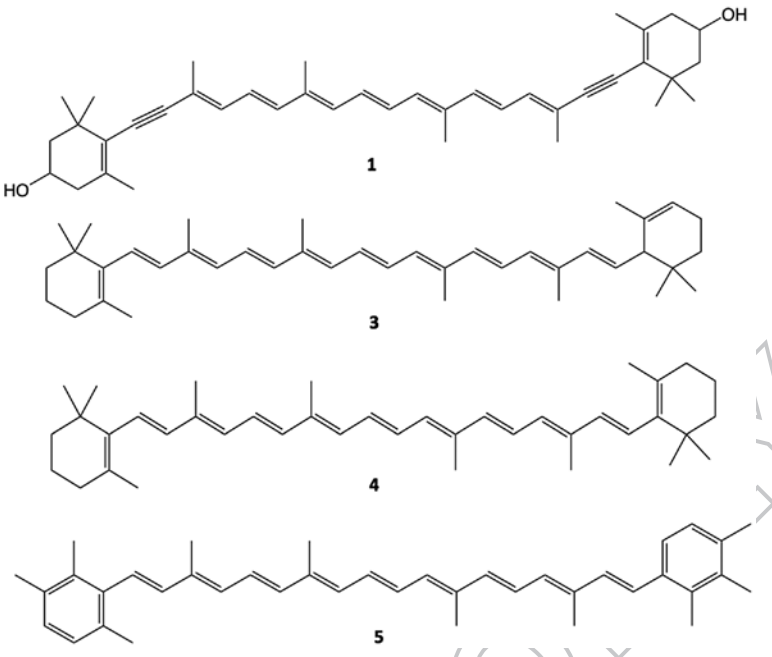

Figure 4. The chemical structures of the identified carotenoids: 1. Alloxanthin; 3. α-Carotene; 4. β-Carotene; 5. Renieratene.

Table 1. Compounds identified in marine sponges *Raspaciona aculeata* and *Dictyonella marsilii*, with corresponding retention times, UV–vis spectra data and APCI(-)/MS data, and relative amounts (dry weight).

N.	Compound	Rt (min)	PDA λ max	APCI(-)/MS (*m/z*)	R.A.[a] (ppm)	D. M.[b] (ppm)
1	Alloxanthin	18.51	423, 447, 479	564	n.d.[d]	711.4
2	n.i.[c]	30.63	422, 450, 476	596	n.d.	n.d.
3	α-Carotene	36.04	422, 444, 473	536	304.5	393.9
4	β-Carotene	39.79	425, 451, 478	536	650.1	672.9
5	Renieratene	44.75	440, 472, 506	528	2570	277.1

[a]R. A. = *Raspaciona aculeata*
[b]D. M. = *Dictyonella marsilii*
[c]n.i. = not identified
[d]n.d.= not determined.

The resulting mixture was centrifuged for 10 minutes at 4000 rpm and at 25°C. The surnatant was taken and dried by a Büchi Rotavapor R-215. The dry matter was dissolved in 10 mL of ethyl acetate to run the same procedure described for the n-hexane again. After the second evaporation, samples were dissolved into 2 mL of methanol and methyl-terz-buthyl-ether in 1:1 volumetric ratio (MeOH/MTBE 1:1) used as the mobile phase.

3.2. ULPC/PDA-MS analysis

Pigments in the sample were identified by comparison with available standards, elution order, UV/vis spectra, their APCI-MS spectra, recorded both in positive and negative ionization modes, and where available, by literature information. Quantification by UPLC was performed with the external standard method, for available standards; standard curves were

calculated by the linear regression analysis and any quantification was estimated as mean value of three repeated measurements.

The analyses were carried out using an HPLC system (Shimadzu, Milan, Italy) equipped with a CBM-20A controller, two LC-20AD pumps, a DGU-20A3 degaser, a SIL-20AC autosampler and a SPD-M20A photo-diode array detector. The data were processed with the software Lab solution ver. 5.10.153 (Shimadzu). For MS, analyses was used a mass spectrometer detector (LCMS-2020, Shimadzu), equipped with an APCI interface, both in positive and negative ionization mode.

Separations were performed on a YMC C30 column (250 × 4.6 mm; 5 µm) and the injection volume was 20 µL. The mobile phase consisted of a binary gradient of methanol/methyl-tert-butyl ether/water (MeOH/MTBE/H2O) (90:8:2; v/v/v) (A), and MeOH/MTBE/H2O (8:90:2; v/v/v) (B), starting with 0% B toward a linear gradient increase of 30% of B in 20 min, to 80% B at 35 min, to 100% B at 65 min and to 100% B at 75 min, then re-equilibrating the column to initial B concentration at a flow rate of 1 mL/min. The UV–Vis spectra were acquired in the range of 250–600 nm, while the chromatograms were extracted at 450 nm (sampling frequency: 1,5625 Hz; time constant: 0.64 s). The MS APCI+, APCI- parameters were set as follows: Acquisition Mode, Scan; Interface Temperature, 350°C; Interface Voltage: 4.5 kV; Heat Block, 300°C; CDL Voltage: 0 V; CDL temperature, 300°C; m/z range, 300–1200; Nebulizing gas flow (N2): 4 mL min; Event Time: 1 sec; Detector Voltage: 0.8 kV; Q-array: 0.0 V; RF: 90 V; Sampling: 2 Hz. AQ3

4. Conclusions

This is the first identification and characterisation of carotenoids in sponges; it was performed on the species *R. aculeata* e *D. marilii* living in the Ganzirri lake. Chromatograms extracted at 450 nm (Figures 2 and 3), show total five peculiar peaks. AQ4

We have identified five peculiar peaks associated to specific carotenoids; four of these were characterised and just three of them were present in both sponge species, whereas the alloxanthine was well represented just in the *D. marilii* samples. Interestingly, the *R. aculeata,* unlike *D. marilii*, bears a peculiar pigment which is uncharacterised so far.

This paper christen the fascinating science to find pigments in unusual living matrices leading to the selection of nutraceutical sources, moreover it paves the way to the discovery of new pigments with unprecedented molecular structure. This study presents an overview about the presence of some carotenoids in sea sponges; in order to improve the knowledge and to understand the difference and similarity, it would be of great help to process many other samples and to conduct structural analysis for the definitive chemical characterisation.

Disclosure statement

No potential conflict of interest was reported by the authors. AQ5

ORCID

Andrea Salvo http://orcid.org/0000-0002-0519-5084
Archimede Rotondo http://orcid.org/0000-0001-6228-7228
Giovanna Loredana La Torre http://orcid.org/0000-0001-6304-6899
Giacomo Dugo http://orcid.org/0000-0003-0185-6591

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