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Raman Spectroscopic Study of Antioxidant Pigments from Cup Corals *Tubastraea* spp.

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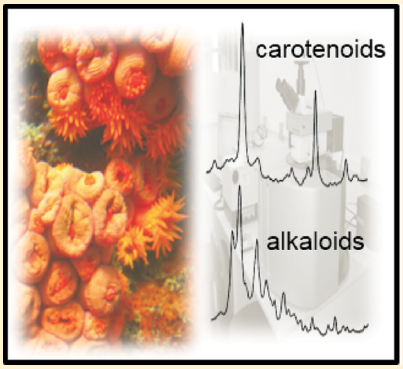
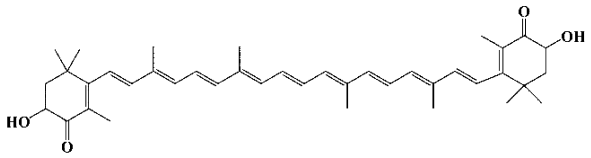
Supporting Information

ABSTRACT: Chemical investigation of nonindigenous *Tubastraea coccinea* and *T. tagusensis* by Raman spectroscopy resulted in the identification of carotenoids and indolic alkaloids. Comparison of Raman data obtained for the in situ and crude extracts has shown the potential of the technique for characterizing samples which are metabolic fingerprints, by means of band analysis. Raman bands at ca. 1520, 1160, and 1005 cm^{-1} assigned to ν_1(C═C), ν_2(C—C), and ρ_3(C—CH_3) modes were attributed to astaxanthin, and the band at 1665 cm^{-1} could be assigned to the ν(C—N), ν(C—O), and ν(C—C) coupled mode of the iminoimidazolinone from aplysinopsin. The antioxidant activity of the crude extracts has also been demonstrated, suggesting a possible role of these classes of compounds in the studied corals.



INTRODUCTION

Scleractinians *Tubastraea coccinea* and *T. tagusensis* are ahermatypic corals nonindigenous to the South Atlantic[1] and are known to contain metabolites employed in a chemical defense role.[2,3] The introduced species have shown competitive advantages over native organisms, causing a negative impact in the receptor communities.[2−5] Species of genus *Tubastraea* are sources of fatty acids, sterols,[5] polyoxazole macrolides,[6] anthraquinone derivatives,[7] and alkaloids.[5,7−10] The orange *T. coccinea* and the yellow *T. tagusensis* collected on the Southwest Atlantic Coast (Angra dos Reis, RJ, Brazil) were investigated by Near Infrared Raman Spectroscopy (NIR-RS), which is a technique of choice for the identification of different types of molecules with a conjugated system of π electrons as reported for these corals. Raman spectra from in situ analysis showed several bands that could be attributed to two classes of compounds. Fingerprint bands from the Raman analysis indicated the presence of carotenoids and alkaloids containing an indolic group together with an iminoimidazolinone system characterized as aplysinopsins (Figure 1). This is the first report of the carotenoid astaxanthin (1) in tissues of the *Tubastraea* genus and the first characterization of indolic alkaloids by Raman spectroscopy. Aplysinopsin (2) and derivatives were



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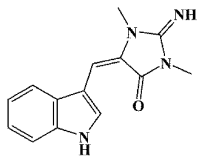

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Figure 1. Metabolites identified from cup corals *T. coccinea* and *T. tagusensis* by Raman spectroscopy: (1) astaxanthin and (2) aplysinopsin.

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Table 1. Main Observed Raman Bands with Laser Excitation at 1064 nm (cm^{-1}) and Tentative Vibrational Assignments from Metabolites of Cup Corals *Tubastraea* spp., Pure Astaxanthin and Calculated Vibrational Wavenumbers of Aplysinopsin[a]

T. coc. in situ	*T. coc.* hexane	*T. coc.* MeOH	*T. tag.* in situ	*T. tag.* DCM	*T. tag.* MeOH	astax stand.	aplysin calcd	tentative assignments
1662w	1660w	1666s	1664m	1658m	1658m		1675	ν(C═N), ν(C═O), ν(C═C)[b]
1619w	1596w	1620s	1618m	1623w	1621s		1632	ν(C═O), ν(C═N), ν(C—N),[b] ν(C═C)[c]
1573w	1573w	1573w	1573w	1575w	1573w	1591w	1615	ν(C—C), δ_s(CH), δ_s(NH),[c] ν(C═C)[d]
1519s	1521s	1510s	1519s	1521s	1521s	1512s	1546	ν(C—C), δ_s(CH), $\delta_s(CH)_3$,[c] ν(C═C)[d]
1442w	1444m	1442w		1442m	1397w	1443w	1489	ν(C—C), δ_s(CH), $\delta_s(CH)_3$,[b] ν(C—C)[d]
	1391w	1394w			1400w		1440	ν(C—C), δ_s(NH), δ_s(CH)[c]
		1336m	1341w		1336m		1376	ν(C—C), δ_s(CH)[c]
		1307w		1300w	1307w		1344	ν(C—C), δ_s(CH)[c]
1274w	1276w		1276w	1276m	1274w	1276m	1301	$\delta_s(CH_3)$, ν(C—N),[b] δ_s(C—H)[d]
		1230w			1230w		1278	δ_s(C—H), ν(C—N), δ_s(NH)[c]
1193m	1193m	1199w	1193w	1193m		1192m	1252	δ_{as}(C—H), ν(C—N), $\delta_s(CH_3)$,[b] ν(C—CH_3)[d]
1085s			1085s					$\nu(CO_3^{2-})$
1158s	1157s	1158m	1157s	1157s	1158s	1156s	1171	ν(N—CH_3), δ_s(N—H), ν(C—N),[b] ν(C—C)[d]
			1052w		1048w		1102	δ_s(CH)
1006m	1006m	1014w	1006m	1008m	1008w	1006m	1043	ν(C—N), δ_s(NH), $\delta_s(CH_3)$,[b] δ(C—CH_3)[d]
	958w		960w	961w	958w	967w	966	ν(C—N), δ_s(NH), δ_s(N—CH_3),[b] δ(C—H)[d]
705s			705w					$\nu(CO_3^{2-})$
					781w		778	ω_s(CH)[c]
							733	ring breathing
		601w			603w		661	ring breathing

[a]Raman bands intensities: s, strong; m, medium; w, weak. [b]Iminoidazolinone. [c]Indole. [d]Carotenoid.[45]

previously reported for *T. coccinea* from Indo-Pacific reefs (Oahu, Hawaii)[11] which corroborated our findings (Figure 1). As commented above, Raman spectroscopy has been used successfully to characterize conjugated polyenes, as for instance carotenoids,[12] in different living systems such as plants,[13−15] algae,[16] lichens,[17,18] microorganisms,[19] corals,[20] crustaceans,[21] fishes,[22] and birds.[23] The carotenoid astaxanthin from *T. coccinea* hexane extract and indolic alkaloids from a methanol extract were unambiguously identified on the basis of theoretical calculations and experimental analysis performed in situ and with the crude extracts. Both compounds are known to possess antioxidant activity. One of the biological functions of astaxanthin in marine animals is the quenching of the oxygen singlet state and a scavenging of free radicals.[24] The antioxidant properties of the carotenoids have been largely studied in vitro due to the complexity of studying in vivo systems. The methods used to determine the antioxidant capacity of carotenoids generally adopt a homogeneous system and measure the capacity of the carotenoids to scavenge either peroxyl radicals ($ROO^{\bullet}$)[25−27] or nonbiological radicals, such as ABTS[27,28] and to quench singlet oxygen ($^1O_2$).[24,29−35] Carotenoids have been recognized to be efficient singlet oxygen quenchers.

In this work we demonstrate by two different methods that the hexane extract containing astaxanthin and the methanol extract, which is rich in indole alkaloids, present in *T. coccinea* tissues are antioxidants. Measurement of the quenching rates of singlet oxygen using several carotenoids and hexane and methanol extracts in solution has been performed using a NIR $^1O_2$ emission method. In addition, we have also evaluated the oxidation of the methanol extract by hydroxyl radicals in a Fenton-like reaction using V_2O_5/TiO_2.

It is worth mentioning that Raman spectroscopy, using excitation near the electronic transition of the chemical system, is a suitable technique to characterize the presence of single and double carbon−carbon bonds in the delocalized π electronic system, where the C—C and C═C stretching vibrations are the ones that appear enhanced in the Raman spectra, thus permitting the chemical characterization of very small amounts of compounds.[36] Our results demonstrate the power of the Raman spectroscopic technique for the identification of natural products containing carotenoids and alkaloids without the need to isolate the specific compounds, and the technique has been successfully applied for nondestructive in situ analysis of a wide range of samples[13] as well as for crude extracts and purified compounds.[13,37−41]

EXPERIMENTAL SECTION

Materials and Experimental Methods. Raman spectroscopic measurements performed in situ and on the crude extracts from *Tubastraea* spp. were carried out using a Bruker RFS 100 instrument and a Nd^{3+}/YAG laser operating at 1064 nm with a 4 cm^{-1} spectral resolution, equipped with a Ge detector cooled with liquid nitrogen. In situ analysis of samples excited using 532 and 785 nm laser lines were recorded on a Horiba XploRA instrument equipped with an air-cooled charge-coupled detector (CCD) with the incident laser beam focused on the sample using a confocal microscope with a 50× objective. The laser output at the source was between 1 and 10 mW for the in situ measurements for the laser line at 532 nm and a higher laser power of 100 mW at the source was used for 785 nm excitation. The operating spectral resolution was 2 cm^{-1} at both 532 and 785 nm wavelengths. In situ analysis of samples excited using 514 and 632.8 nm was performed on a Horiba Jobin Yvon LabRAM HR system, with a power of ca. 2 mW. HPLC analysis was performed on a Agilent Infinity 1200 Series HPLC that consisted of a quaternary pump1260 VL, Model-G1312C, standard autosampler 1260ALS, Model-G1329B, with UV−vis dectector 1260 VWD VL+, model-G1314C, and a Waters YMC carotenoid column (250 mm × 4.6 mm i.d. × 5 μm) equipped with C_{30} reversed phase material. The mobile phase setups consisted of three solvents (solvent A, acetonitrile; solvent B, methanol; solvent C,

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chloroform). The detection of carotenoids (UV/vis detector) was accomplished at a wavelength of 430 nm. Data acquisition and processing were achieved with Agilent ChemiStation LC Systems software. The identification of the characteristic peaks was based on the relative retention times, namely, standard astaxanthin purchased from Sigma-Aldrich Co. (19.189 min), *T. coccinea* extracts (t_R = 19.742 min) and cochromatographed astaxanthin standard and *T. coccinea* extracts 19.282 min.

Pure Astaxanthin (1). UV (MeOH) $_{\lambda max}$ 476 nm, UV ($CHCl_3$) λ_{max} 491 nm. Raman bands see Table 1.

Extract in Hexane from T. coccinea. Orange gum; UV (MeOH) λ_{max} 402, 476 nm, UV ($CHCl_3$) λ_{max} 387, 473 nm. Raman bands see Table 1.

Extract in CH_2Cl_2 from T. coccinea. Dark orange gum; UV (MeOH) λ_{max} 384 nm. Raman bands see Table 1.

Extract in MeOH from T. coccinea. Yellow-brownish solid; UV (MeOH) λ_{max} 283, 385 nm. Raman bands see Table 1.

Extract in CH_2Cl_2 from T. tagusensis. Yellow-brownish solid; UV (MeOH) λ_{max} 283, 393 nm. Raman bands see Table 1.

Extract in MeOH from T. tagusensis. Yellow-brownish solid; UV (MeOH) λ_{max} 283, 385 nm. Raman bands see Table 1.

Calculations. The structure of aplysinopsin was fully optimized in the gas phase at the B3LYP[42,43] level using a 6-311++G(d,p)[44] triple-quality basis set with inclusion of polarization functions for both the heavy and hydrogen atoms (hereafter abbreviated as B3LYP/6-311G(d,p). The geometry was considered as neutral species. The final geometry was characterized as minima on the potential energy surface through harmonic frequency calculations (all frequencies were found to be real). The Raman intensities were also calculated and the band spectra were simulated by fitting a Lorentzian type function[45] with parameters set to 10 cm^{-1} for the average width of the peaks at half height and 2×10^{-6} mol cm^{-3} for sample concentration. The spectra for all species were then assigned according to the normal-mode analysis. Frequency scaling was not needed once the predicted values and theoretical spectrum profiles were in satisfactory agreement with the experimentally observed spectra, allowing unambiguous band assignments. All calculations were carried out with Gaussian 09 program as installed in the computers of the Núcleo de Estudos em Química Computacional (NEQC-UFJF).[46]

Catalytic Assays. The hydrogen peroxide (Synth) decomposition study was carried out with 0.1 mL solution of H_2O_2 (0.32 mol L^{-1}) with 5 mg of catalyst V_2O_5/TiO_2 (6% of vanadium oxide) by measuring the formation of gaseous O_2 in a glass vial. The catalytic activity of the composite was evaluated by measuring the degradation rate of aqueous methylene blue (2.5 mL), monitored at a fixed wavelength of 633 nm in a UV/vis spectrophotometer, Shimadzu model UV1800. The oxidative reaction promotes a discoloration of the dye with time. Assays using methylene blue as a probe were performed in both the presence and absence of the MeOH extract (2.0 mg in 0.4 mL) in triplicate. The hexane extracts were not totally soluble in the reaction medium.

Time-resolved NIR emission of singlet molecular oxygen generated by photosensitizer. The third harmonic of a CryLas Nd:YAG HP 355-50 laser (pulse width of 1.0 ns and energy <150 mJ) was used as the excitation source for an aerated perinaphthenone (photosensitizer, A_{355} = 0.36) solution in $CHCl_3$. In experiments using hexane extracts, samples were solubilized in $CHCl_3$ and in experiments using methanol extracts samples were solubilized in EtOH.

The singlet oxygen emission at 1270 nm was measured with a spectrofluorometer (FS920 Edinburgh Instruments TMS300 monochromator). The detection system was equipped with a NIR Hamamatsu model H1033-45 photomultiplier. Slit 10 nm, T = 25.6 °C.

Quenching of Singlet Oxygen. Stock solutions of the quenchers were prepared so that it was only necessary to add microliter volumes to the sample cell to obtain appropriate concentrations of the quencher. The rate constants for the quenching of singlet oxygen emission with the different quenchers employed in this work were obtained from the Stern−Volmer plots after eq 1.

$$k_{obs} = k_o + k_q[Q] \quad (1)$$

where k_o is the emission decay rate constant in the absence of quencher, k_{obs} is the emission decay rate constant in the presence of the quencher, and [Q] is the quencher concentration. Plots based on this equation were found to be linear, from which the value of k_q was determined. In a typical experiment, one adds microliter volumes of the quencher to a 3 mL solution of perinaphthenone (Table S3, Supporting Information).

Collection. Samples of *T. coccinea* and *T. tagusensis* (69.5 g) were collected at a depth of 1−4 m at Ilha dos Macacos, Angras dos Reis, RJ (S 23° 04.713′/W 42° 13.479′). After collection, the colonies were immediately frozen in dry ice and stored in a freezer.

Extraction. Frozen colonies of *T. coccinea* and *T. tagusensis* were freeze-dried prior to extraction. Soxhlet extraction of *T. coccinea* (78.8 g dry weight) with hexane followed by dichloromethane gave orange extracts (631.2 mg and 76.9 mg, respectively). Extraction with methanol furnished a brownish yellow extract (1.9 g). The Soxhlet extraction procedure with *T. tagusensis* (69.5 g dry weight) was carried out with dichloromethane and methanol giving extracts (76.9 mg and 1.91 g, respectively) with coloration patterns similar to those of *T. coccinea*.

RESULTS AND DISCUSSION

Spectroscopic Analysis. Hexane crude extracts and in situ analysis of *Tubastraea* spp. specimens investigated by Raman spectroscopy using NIR laser excitation (1064 nm) showed vibrational bands characteristic of carotenoids[13,14] at ca. 1520, 1160, and 1005 cm^{-1} assigned to ν_1(C═C), ν_2(C—C), and ρ_3(C—CH_3) modes (Figures 2 and 3). The identification of the carotenoid content performed by HPLC (Figure 4) was based on the analysis of retention times of major peaks from hexane crude extract and standard astaxanthin (Sigma-Aldrich Co). Co-chromatography of pure astaxanthin and hexane crude extract confirmed the presence of astaxanthin in *T. coccinea* tissues. Comparison of Raman bands attributed to ν_1(C═C) of pure astaxanthin[47] with and samples from *Tubastraea* spp. showed a shift to higher wavenumbers from 1512 to ca. 1521 cm^{-1}, respectively (Table 1). An explanation for this phenomena has already been proposed; the wavenumber position of the ν_1 band is dependent on the length of the polyconjugated chain and matrix effects as a consequence of chemical interactions in natural samples.[14]

Astaxanthin is a dominant carotenoid in marine animals[48] and is mostly found in fishes and crustaceans that have

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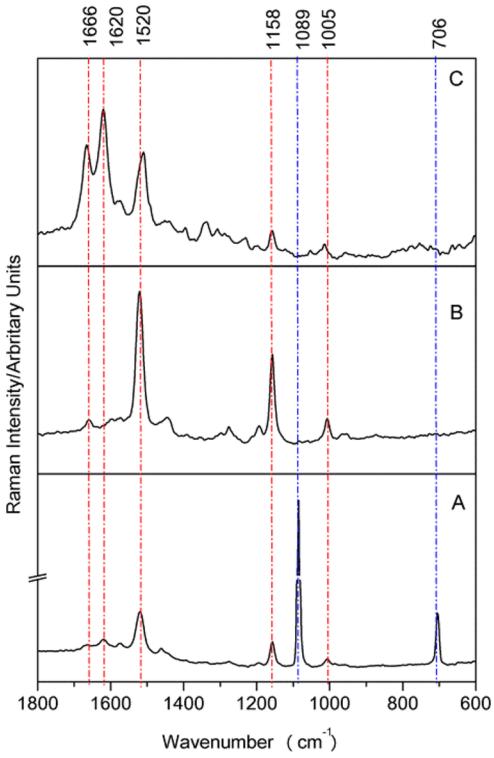

Figure 2. Raman spectra recorded at laser line 1064 nm of *T. coccinea*: A, in situ analysis; B, hexane extract; C, methanol extract.

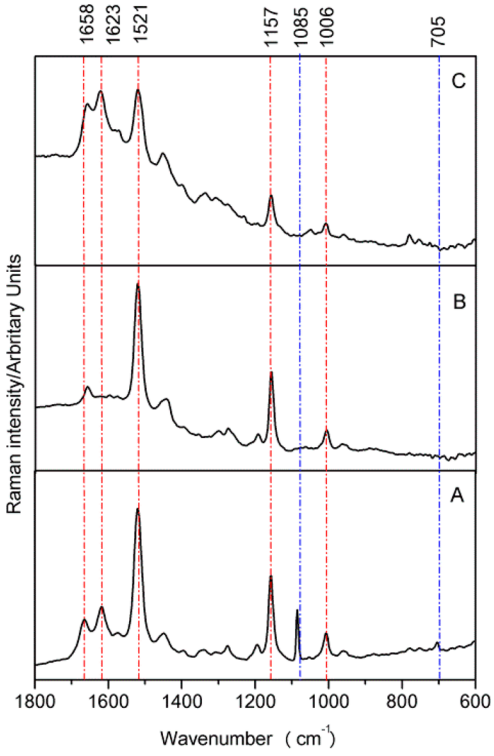

Figure 3. Raman spectra recorded at laser line 1064 nm of *T. tagusensis*: A, in situ analysis; B, hexane extract; C, methanol extract.

accumulated the carotenoid unchanged from either dietary or metabolic modifications.[49−51] The occurrence of astaxanthin in corals is restricted to a few species of zoantharians,[52] to hydrocorals,[53−55] and to the octocorals *Sinularia flexibilis*,[56] *Leptogorgia punicea*, and *Muricea atlantica*.[41] Perhaps the most

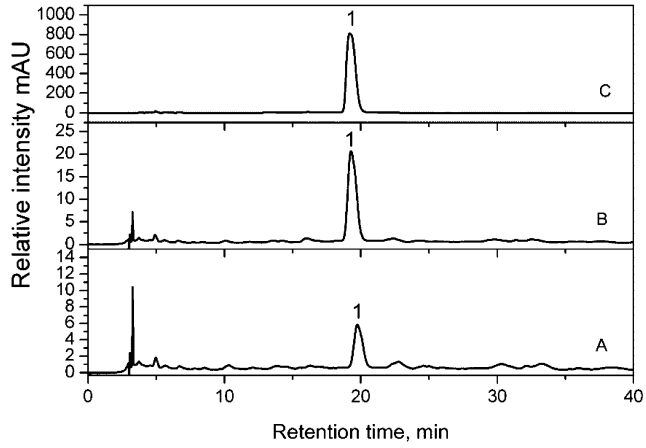

Figure 4. HPLC chromatograms of (a) *T. coccinea* hexane extract, (b) cochromatography of *T. coccinea* hexane extract with authentic astaxanthin, and (c) standard astaxanthin. The peak number refers to astaxanthin present in the analysis. Analytical conditions: waters YMC C_{30} column (250 mm × 4.6 mm i.d. × 5 μm), flow rate 1 mL/min. Mobile phase: solvent A, acetonitrile; solvent B, methanol; solvent C, chloroform. UV/vis detector set at 430 nm.

relevant bioactive role of astaxanthin in animals is its ability to either quench or scavenge active oxygen species.[24] The function and origin of astaxanthin in *Tubastraea* spp. is unknown; pigmentation based on light-absorbing chromophores may act as a defense against oxygen singlet and free radical production. However, the in situ spectrum recorded here showed that astaxanthin occurs along with indole derivatives, which are also known to be effective antioxidants.[57,58] Raman spectroscopic analysis together with literature data have identified the presence of the indole alkaloids related to aplysinopsins previously described for *T. coccinea*[11] and sponge. These bioactive molecules are composed of an indole moiety and a creatinine based skeleton described as an iminoimidazolinone system. Methanol extracts of both species showed major bands at ca. 1665, 1620, 1574, 1510, 1452, 1441, 1396, 1338, 1158, and 1014 cm^{-1}. Most of these bands were observed in the spectrum recorded in situ; however, bands at 1510, 1159, and 1014 cm^{-1} were overlapped with carotenoid bands at ca. 1519, 1159, and 1005 cm^{-1} (Figures 2 and 3). In situ spectra also showed bands at 1089 [$\nu(CO_3^{2-})$] and 706 [$\nu(CO_3^{2-})$] cm^{-1} assigned to vibrational modes of carbonate ion in aragonite[59] (Figure 2 and 3). The tentative assignment of each band attributed to alkaloids has been proposed by comparison with the Raman bands in the literature[60−62] and theoretical calculations made for aplysinopsin [(2) Table 1, Figure 5]. Key bands from both moieties have been indicated in Table 1. The strong band at 1665 cm^{-1} was attributed to [ν(C—N) + ν(C—O) + ν(C—C)] of the iminoimidazolinone. An intense band at 1619 cm^{-1} was assigned to both the ν(C—C) of the indole nucleus and the [ν(C—O) + ν(C—N)] of the iminoimidazolinone system. The bands at 1510 cm^{-1} due to [ν(C—N) + ν(C—C)] and at 1337 cm^{-1} due to [ν(C—C) + δ_s(C—H)] were assigned to the aromatic system of the indole. Bands at 1158 and 1014 cm^{-1} were assigned to [ν(N—CH_3) + δ_s(N—H) + ν(C—N) + ν(C—C)], and [ν(C—N) + δ_s(NH) + δ_s(CH_3)], respectively, of the iminoimidazolinone. Figure 6 shows a correlation between the experimental and the predicted frequencies from theoretical calculations made on aplysinosin. The experimental Raman shifts appear to have shifted linearly

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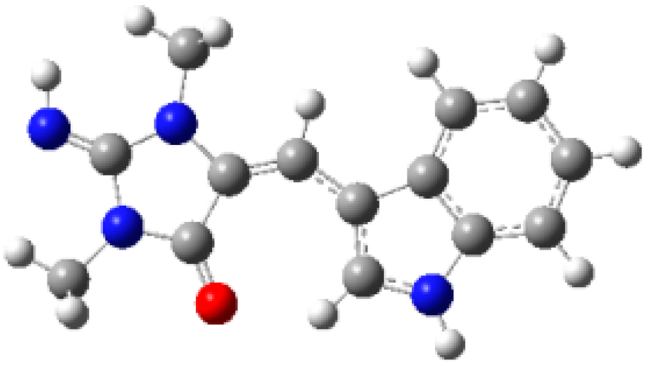

Figure 5. Optimized structure for aplysinopsin at theoretical level B3LYP/6-311++G(d,p).

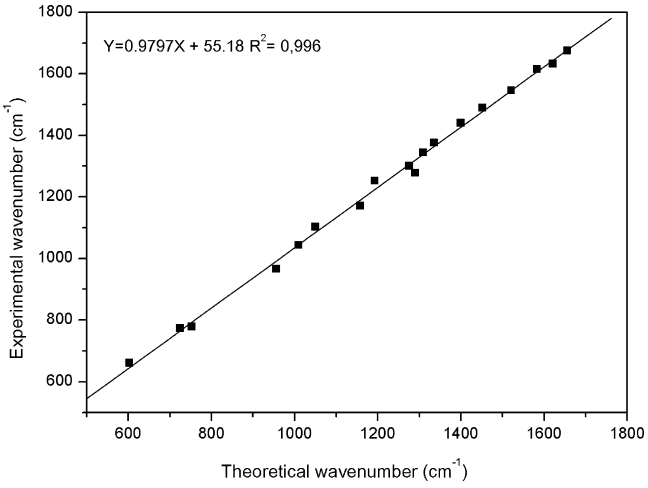

Figure 6. Correlation coefficient and scatter plot of Raman frequencies between experimental data from *T. coccinea* methanol extract and theoretical data from aplysinopsin.

and are in good agreement (R^2 = 0.996) with the theoretical data. Both pigments were identified by NIR-RS (1064 nm) in tissues of the orange *T. coccinea* and the yellow *T. tagusensis*. However, Raman measurements of *T. coccinea* tissues made with different laser lines at 514, 532, 632.8, 785, and 1064 nm showed fingerprint bands attributed to carotenoids and carbonate ion (Figure 7). It is important to note that under resonance conditions, such as the Raman spectra obtained with green light excitations, some of the vibrational modes that are involved with the electronic transition are the ones that appear with their intensity enhanced, according to the theory predict by inelastic light scattering;[63−66] in this case, the literature has demonstrated that the chromophoric species are the C—C, C═C, and C—CH_3 chemical groups present in the structure of carotenoids.[67]

Aplysinopsins are yellow pigments isolated from sponges,[69] mollusc,[10] and scleractinians[70−72] whereas astaxanthins are reddish orange pigments widely distributed in nature.[48] It is possible that either aplysinopsin or astaxanthin from *T. coccinea* and *T. tagusensis* could be involved in the coloration of their tissues and even the coloration of their predators as the nudibranch *Phestilla melanobrachia*, which feeds on *T. coccinea*, exhibit the same color pattern[11] as well as the same chemical composition.[71] Aplysinopsins are biologically active against cancer cells, are antiplasmodial and antimicrobial, and act as modulators in neurotransmission.[10] Chemical and ecological

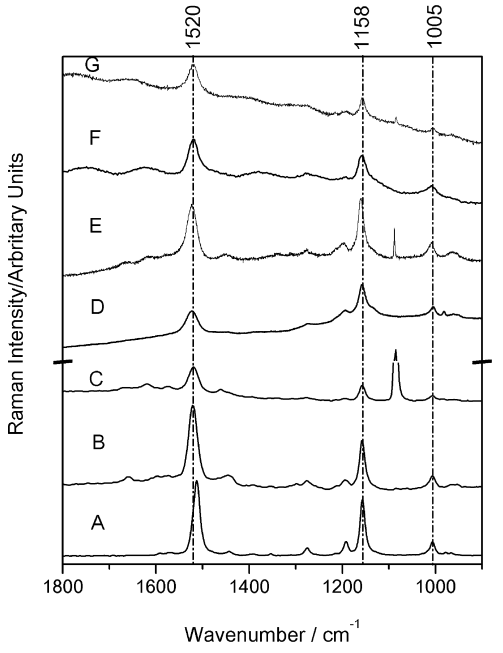

Figure 7. Raman spectra recorded at different laser lines: (A) standard astaxanthin recorded at 1064 nm. *T. coccinea* analysis: (B) hexane extract at 1064 nm; (C) in situ at 1064 nm; (D) in situ at 785 nm; (E) in situ at 632.8 nm; (F) in situ at 532 nm; (G) in situ at 514 nm.

experimental data have demonstrated that methanol crude extracts from *T. coccinea* and *T. tagusensis*, which are rich in alkaloids were effective against fish fouling and induced different responses in the fouling community, inhibiting or enhancing settlement, survival or the growth of different taxa.[2] Alcoholic extracts and aplysinopsin derivatives from *T. faulkneri* were toxic to larvae of potential competitors and antimicrobial against marine bacteria and cyanobacteria.[72] The antioxidant activity of 6-bromo-2′-de-*N*-methylaplysinopsin isolated from the sponge *Hyrtios* sp. has been reported previously.[58]

In this work, we have evaluated the antioxidant activity of crude extracts from *T. coccinea* against hydroxyl radical species and singlet molecular oxygen. Hydroxyl radicals were generated from hydrogen peroxide by a catalysis system using vanadium pentoxide (V_2O_5) supported on titanium dioxide (TiO_2). V_2O_5 is an important metal oxide catalyst and in combination with TiO_2 is useful for photocatalytic reactions.[73] The composite layers are biologically and chemically inert, in addition vanadium peroxide complexes are known to be strong oxidants capable of decomposing hydrogen peroxide even in biological systems.[74] The hydrogen peroxide decomposition was investigated in the presence of the catalyst with the addition of the crude extracts and using methylene blue as a probe. The oxidation of methylene blue in the presence of hydroxyl radicals was monitored by absorption at 663 nm and the discoloration plots showed a color reduction of 42.83% over the time of the experiment (Figure 8). However, a lower activity for methylene blue oxidation was observed in the presence of hydroxyl radicals and of the MeOH crude extract. Figure 8 shows a color retention of 70.61% against 42.83% in the absence of the crude extract at the end of 1 h. The inhibition of discoloration by the MeOH extract suggested that radical species reacted more efficiently with the mixture of alkaloids than with the methylene blue.

To evaluate the antioxidant activity of different reactive oxygen species, the ability of crude extracts in quenching singlet

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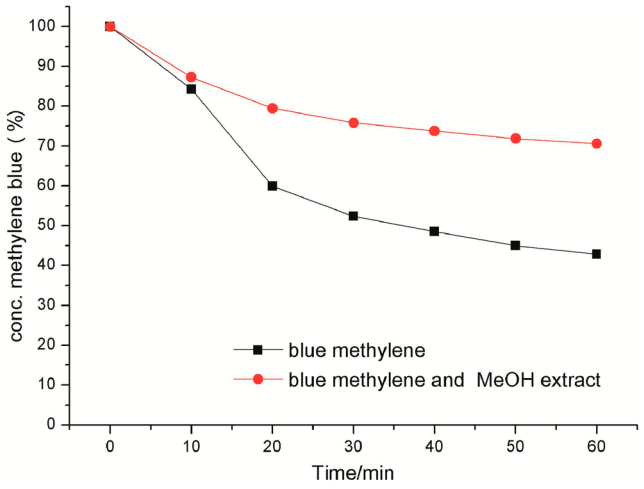

Figure 8. Discoloration plot of methylene blue oxidation in the presence of H_2O_2 and catalyst in the absence and presence of *T. coccinea* methanol crude extracts.

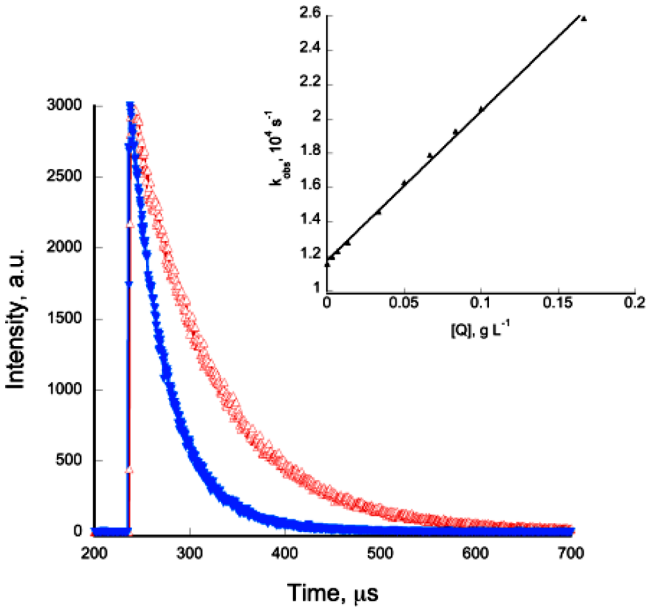

Figure 9. Singlet oxygen decays in the absence (Δ) and in the presence of 1.17 g L^{-1} (▼) of hexane extract, perinaphthenone as sensitizer, and chloroform as solvent. Inset: Stern–Volmer plot according to eq 1.

oxygen was tested. Singlet oxygen can be formed through an energy transfer process from a suitable donor, usually a sensitizer in the triplet excited state (^{3}Sens*). A tiny fraction of $^1O_2$ molecules undergoes radioactive decay, thereby emitting a photon in the near-infrared (NIR). This extremely weak phosphorescence, centered at 1270 nm, provides the means for the most direct and unambiguous method for $^1O_2$ detection. In this study singlet oxygen was generated using perinaphthenone as photosensitizer. After laser excitation (355 nm), the aerated chloroform solution of perinaphthenone emits phosphorescence at 1270 nm that decays with a rate constant (k_0) of approximately 12 000 s^{-1}, corresponding to a lifetime of around 85 μs. The lifetime of state shows a strong solvent dependence. The experimental values observed here ranging from 83.80 to 88.97 μs (Table S1–4, Supporting Information) is lower than the lifetime of singlet oxygen in chloroform (literature value is τ_Δ = 207 μs).[75] Decreasing in lifetime of singlet oxygen in solution has already been reported and may be attributed, among other reasons, to subtle variations in experimental condition.[55]

Quenching of Singlet Oxygen. In homogeneous media, the singlet oxygen reaction with carotenoids can involve physical and/or chemical processes. In this study, the total (physical and chemical) quenching rate constant, k_q, for the reaction of $^1O_2$ was performed with different quenchers, such as β-carotene, astaxanthin, hexane extract in , and MeOH extract in EtOH as solvent. Quenching rates were obtained from the experimentally measured first-order decay of $^1O_2$ phosphorescence at 1270 nm in the absence and the presence of the quencher. The singlet oxygen decays obtained in chloroform with perinaphethenone in the absence and in the presence of the hexane extract is shown in Figure 9, and those of β-carotene and astaxanthin in Figures S1 and S2 (Tables S1 and S2) of the Supporting Inforamation, respectively. All decays fit first-order kinetics from which singlet oxygen lifetimes were computed. The k_q values were calculated from the slope of the Stern–Volmer plots according to eq 1 (Figure 9 and Figures S1 and S2 of the Supporting Information). Quenching of the singlet oxygen by the hexane extract occurred with a rate constant approaching that of diffusion control ($k_{diff}(CHCl_3)$ = 1.12 × 10^{10} L mol^{-1} s^{-1}),[75] which is in accord with the physical nature of the energy transfer process. The results obtained with the MeOH extract did not quench singlet oxygen showing rate constant and lifetime similar to that of the observed for the probe (Figure S3, Table S3, Supporting Information). For comparison, Table 2 shows the literature values of k_q for the carotenoids in $CHCl_3$.[24]

Table 2. Rate Constants of Quenching of Singlet Oxygen by the Investigated Carotenoids

quencher	k_q (obs)	k_q, L mol^{-1} s^{-1}
β-carotene[72]	(1.63 ± 0.10) × 10^{10} L mol^{-1} s^{-1}	1.1 × 10^{10}
astaxanthin[72]	(2.18 ± 0.09) × 10^{10} L mol^{-1} s^{-1}	1.2 × 10^{10}
hexane extract	(8.68 ± 0.15) × 10^4 L g^{-1} s^{-1}	

The systems used to assay the antioxidant activity of pigments from the cup corals showed selectivity of the reactive oxygen species tested toward the chemical nature of compounds. Scavenging of hydroxyl radical was demonstrated in experiments performed with methanol extract rich in alkaloids and quenching of singlet oxygen was observed only in assays with hexane extracts containing carotenoids.

CONCLUSIONS

The investigation by Raman spectroscopy of the pigment composition of orange tissues from *T. coccinea* and yellow tissues of *T. tagusensis* revealed that they are due to carotenoids and indolic alkaloids such as aplysinopsin derivatives. Occurrence of aplysinopsin derivative in *T. coccinea* tissues is known; however, this is the first report to *T. tagusensis*. Also new is the presence of astaxanthin in tissues of the genus *Tubastraea*, although it is a common compound in marine organisms, the identification have been neglected. In our work we used Raman spectroscopy, which is the technique of choice in studying polyconjugated system as identified in cup corals. Aplysinopsin carbon skeleton showed fingerprint bands in Raman spectra that could be used as a chemical marker, because this class of bioactive alkaloid may occur in other

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marine organisms rather than sponges, corals, and their predators. The data we presented here has also shown that pigments in *T. coccinea* and *T. tagusensis* are used for ornamental purposes and may be implied in physiological processes. The antioxidant assays provided a clue that *Tubastraea* spp. use compounds from different chemical classes for different oxidative systems. Methanol extract rich in alkaloids were effective in scavenging hydroxyl radical, whereas hexane extracts containing carotenoids were effective in quenching of singlet oxygen.

■ ASSOCIATED CONTENT

Supporting Information

Experimental conditions and measured parameters of quenching oxygen singlet by β-carotene, astaxanthin, hexane extract, and methanol extract (Tables S1−S5), singlet oxygen decays (Figures S1−S5), and complete ref 46 are available free of charge via the Internet at http://pubs.acs.org.

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Notes

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