Gilles Bedoux*, Kevin Hardouin, Christel Marty, Laure Taupin, Laurent Vandanjon and Nathalie Bourgougnon

Chemical characterization and photoprotective activity measurement of extracts from the red macroalga *Solieria chordalis*

Abstract: The photoadaptive responses of macroalgal communities to abiotic stresses have been studied, and a number of UV-absorbing molecules have been identified. Among these compounds, photoprotective compounds such as mycosporine-like amino acids and carotenoids have been isolated from various red macroalgal species. However, several substances still need to be characterized. We describe the preparation of photoprotective extracts obtained from *Solieria chordalis*. Two solvents, 2-octyl dodecanol and octyldodecyl ester of L-pyrrolidone carboxylic acid, were selected based on their cosmetic functions for performing an ultrasound-assisted extraction. The efficiency of extraction was monitored by spectrophotometry and *in vitro* photoprotective activity measurements. 2-Octyl dodecanol and octyldodecyl ester of L-pyrrolidone carboxylic acid extracts showed maximum absorption wavelengths ranging from 280 to 340 nm and 270 to 350 nm, respectively. The anti UV-B capacity for protecting a synthetic chlorophyll solution was assessed by measuring its pseudo first-order degradation kinetics at room temperature. Under irradiation at 312 nm, chlorophyll introduced in the 2-octyl dodecanol *S. chordalis* extract showed the slowest degradation kinetics with a half-life $t_{1/2}$ of 121.0 min. Several compounds were detected in the seaweed extract by high-performance liquid chromatography. Among them, the mycosporine-like amino acid, palythenic acid, was detected in the algal extract.

Keywords: mycosporine-like amino acid; palythenic acid; photoprotective activity; *Solieria chordalis*.

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***Corresponding author: Gilles Bedoux,** EA 3884, Laboratoire de Biotechnologie et Chimie Marines (LBCM), IUEM, Université de Bretagne-Sud (UBS), Campus de Tohannic, BP 573, 56017 Vannes Cedex, France, e-mail: gilles.bedoux@univ-ubs.fr
Kevin Hardouin, Christel Marty and Nathalie Bourgougnon: EA 3884, Laboratoire de Biotechnologie et Chimie Marines (LBCM), IUEM, Université de Bretagne-Sud (UBS), Campus de Tohannic, BP 573, 56017 Vannes Cedex, France
Laure Taupin: Laboratoire de Biotechnologie et Chimie Marines (LBCM), Université de Bretagne-Sud (UBS), Centre de Recherche, Rue de Saint Maudé, 56321 Lorient cedex, France
Laurent Vandanjon: EA 3884, Laboratoire de Biotechnologie et Chimie Marines (LBCM), IUEM, Université de Bretagne-Sud (UBS), Campus de Tohannic, BP 573, 56017 Vannes Cedex, France; and Laboratoire GEPEA (UMR CNRS n°6144), IUML FR-CNRS n°3473, Université de Nantes, Ifremer, France

Introduction

Solieria chordalis (Rhodophyceae, Gigartinales) is established in the subtidal zone, commonly in sheltered areas where salinities range between 26 and 35, generally either on the surface of maerl beds or on gravels (Floc'h et al. 1987). *Solieria chordalis* is found in the Mediterranean Sea (Demasi and Gargiulo 1982) and in the Atlantic Ocean from Morocco to Southern England (Gabrielson and Hommersand 1982) and is commonly encountered on French coasts (Ar Gall et al. 2008, Mineur et al. 2012). *Solieria* sp. has been observed in the Gulf of Morbihan (France) since 2005 and in the Sarzeau peninsula (Morbihan, France) where strandings have become more abundant between July and October. Wet *S. chordalis* strandings on the beaches of Sarzeau peninsula weigh between 4000 and 6000 tons in 2008 and 2009 (Tavennec 2009).

Solar radiation is crucial for aquatic photoautotrophs and enables photosynthesis to occur. In solar radiation, the harmful effects of UV rays composed of UV-B (280–315 nm) and UV-A (315–400 nm; IUPAC Recommendations 2006) are extensively documented (references in Talarico and Maranzana 2000, Rastogi et al. 2010, Pessoa 2012). Numerous organisms have developed mechanisms counteracting the damaging effects of UV-B by the production of carotenoids and xanthophyll cycle pigments and/or the UV-absorbing mycosporine-like amino acids (MAAs); (Figueroa et al. 2003, Roleda et al. 2010). During the last decade, and even

quite recently, marine photoprotective compounds have been the target of several studies designed to determine their structure and function (Rastogi et al. 2010, Carreto and Carignan 2011). Besides the occurrence of MAAs, carotenoids, and phenolic compounds in macroalgae, other UV-absorbing compounds still need to be characterized. Carotenoids are colored compounds involved in the photosynthetic apparatus and in the process of photoprotection in that they allow for the dissipation of excess heat energy and serve as direct quenchers of singlet oxygen or other reactive oxygen species (Deming-Adams 1990, Schubert et al. 2006, Rastogi et al. 2010). Commonly characterized as stress-induced compounds, phenolic compounds are involved in chemical protection mechanisms against biotic factors such as contamination due to bacteria and abiotic factors such as UV radiation and metal contamination (Heo et al. 2009, Rodríguez-Bernaldo de Quirós et al. 2010, Gruber et al. 2011, Holzinger et al. 2011, Stengel et al. 2011). MAAs have been detected in diverse organisms (Rosic 2012) and in macroalgae (Karentz et al. 1991, Karsten et al. 1998, Karsten and Wiencke 1999, Franklin et al. 2001; Whitehead and Hedges 2002, Peinado et al. 2004, Yuan et al. 2009, Rastogi et al. 2010, Carreto and Carignan 2011, Roleda et al. 2012, Bedoux and Bourgougnon 2014). Table 1 shows some of the most widespread MAAs in algae. MAAs are a family (32 different molecules) of secondary metabolites that absorb and protect organisms exposed to solar UV radiation (Figueroa et al. 2003). Their production is directly or indirectly related to the absorption of solar energy and they are considered as photoprotective metabolites because they have maximum UV absorption between 310 and 360 nm, high molar extinction factors, and are photostable (Conde et al. 2000, Gröniger and Häder 2000, Sinha et al. 2000, Whitehead and Hedges 2005). In addition to the photoprotective and antioxidant activities, MAA accumulation in the cells contributes to the maintenance of osmotic pressure (Oren and Gunde-Cimerman 2007). There are reports that some macroalgae only synthesize specific MAAs (Hoyer et al. 2001, Pessoa 2012) and these compounds are much more abundant in red algae, e.g., *Porphyra* sp. (Conde et al. 2000, Peinado et al. 2004), *Gelidium* sp. (Sinha et al. 1998), *Palmaria* sp. (Karsten and Wiencke 1999, Yuan et al. 2009), *Asparagopsis* sp. (Karsten et al. 1998, Sinha et al. 1998) than in brown and green algae (except *Ulva* sp.) (Pessoa 2012). Red algae can contain up to 8 mg of MAAs per gram of dried matter of *Curdiea racovitzae* (Hoyer et al. 2001) or *Bostrychia radicans* (Karsten et al. 1998). MAA content is higher in summer and at a moderate depth (0–1 m) (Reef et al. 2009). The small intracellular compounds (<400 Da) of MAAs consist of a cyclohexenone or amino-cyclohexenimine ring linked to an amino acid, amino alcohol, or amino group, and they are characterized by an absorption maximum between 320 and 360 nm. Generally, MAAs contain a glycine group on the C3 carbon and a second amino acid such as threonine, serine, taurine, and glutamate linked to the C1 carbon (Yuan et al. 2009, Carreto and Carignan 2011).

UV-B rays are more detrimental and dangerous to the human body due to their energetic properties. UV radiation induces diverse effects such as the damaging of human skin (sunburn). It can also lead to the proliferation of oncogenes, which can mutate and cause cancer (Matsumura and Ananthaswamy 2004). Various natural substances extracted from plants could have potential properties to protect from UV rays. UV-absorbing compounds are bioactive compounds that can protect the human fibroblast cells from UV-induced cell death and suppress UV-induced aging in human skin. MAAs are potentially used in cosmetics and toiletries as UV protectors and activators of cell proliferation. Moreover, a product called Helioguard® 365 that contains MAAs has been commercialized (references in Bourgougnon et al. 2011, Richa et al. 2011, Bourgougnon and Stiger-Pouvreau 2012).

Cosmetic products have to comply with strict rules concerning the use of chemical substances (EU cosmetic regulation No 1223/2009) but also satisfy those consumers who want the incorporation of natural ingredients. Moreover, industrialists have to adapt their processes to the pressing necessity of reducing their contribution to nonsustainable development (Anastas and Warner 1998). The conventional extraction technologies are very often considered to cause pollution; hence, they need to change to enable a more environmentally safe process (Afonso and Crespo 2005). Thus, the present study is focused on the extraction of natural photoprotective molecules from the red alga *S. chordalis* through an eco-designed strategy. The ability of *S. chordalis* extracts to absorb UV radiation and limit the degradation of chlorophyll was monitored for the evaluation of the photoprotective activity of these extracts.

Materials and methods

Algal material

The samples of the red alga, *Solieria chordalis* (C. Agardh) J. Agardh (Rhodophyta), used in this study were collected at low tide in October and November 2012 from the coast of Rhuys Peninsula (Saint-Gildas-de-Rhuys,

Table 1 Description of the most widespread mycosporine-like amino acids (MAAs) in macroalgae.

MAAs	Monoisotopic mass (Da)	λ_{max} (nm)	Organisms	References
Palythine		320	*Chondrus crispus*	Franklin et al. 2001
			Palmaria palmata	Yuan et al. 2009
	244.2455			Karsten and Wiencke 1999
	($C_{10}H_{16}N_2O_5$)		*Grateloupia lanceola*	Huovinen et al. 2006
			Porphyra columbina	Peinado et al. 2004
			Curdiea racovitzae	Hoyer et al. 2001
Palythene		360	*C. crispus*	Franklin et al. 2001
	284.3097			Kräbs et al. 2002
	($C_{13}H_{20}N_2O_5$)		*Desmarestia menziesii*	Karentz et al. 1991
			P. palmata	Karsten and Wiencke 1999
			Palmaria decipiens	Hoyer et al. 2001
Porphyra 334		334	*Porphyra* sp.	Conde et al. 2000
			P. columbina	Peinado et al. 2004
			Curdiea racovitzae	Hoyer et al. 2001
	346.3346		*Palmaria decipiens*	
	($C_{14}H_{22}N_2O_8$)		*Palmaria palmata*	Karsten and Wiencke 1999
				Yuan et al. 2009
			Grateloupia lanceola	Huovinen et al. 2006
			Gracilaria cornea	Sinha et al. 2000
			Desmarestia menziesii	Karentz et al. 1991
Asterina 330	288.2983	330	*Palmaria palmata*	Yuan et al. 2009
	($C_{12}H_{20}N_2O_6$)			Karsten and Wiencke 1999
			Porphyra columbina	Peinado et al. 2004
			Chondrus crispus	Franklin et al. 2001
				Kräbs et al. 2002
			Curdiea racovitzae	Hoyer et al. 2001
Palythinol	302.3250	332	*Palmaria palmata*	Yuan et al. 2009
	($C_{13}H_{22}N_2O_6$)			Karsten and Wiencke 1999
Shinorine		334	*Chondrus yendoi*	Tsujino et al. 1980
	332.3080		*Chondrus crispus*	Franklin et al. 2001
	($C_{13}H_{20}N_2O_8$)			Kräbs et al. 2002
			Palmaria palmata	Yuan et al. 2009
				Karsten and Wiencke 1999
			Palmaria decipiens	Hoyer et al. 2001
			Gracilaria cornea	Sinha et al. 2000
			Porphyra columbina	Peinado et al. 2004
			Curdiea racovitzae	Hoyer et al. 2001
			Phyllophora appendiculata	Sinha et al. 1998
Mycosporine-glycine		335	*Palmaria palmata*	Karsten and Wiencke 1999
	245.2303			Yuan et al. 2009
	($C_{10}H_{15}NO_6$)		*Grateloupia lanceola*	Huovinen et al. 2006
			Porphyra columbina	Peinado et al. 2004
			Curdiea racovitzae	Hoyer et al. 2001
			Desmarestia menziesii	Karentz et al. 1991

Latitude/Longitude 47°30′0″ North/2°49′60″ West, Morbihan, Southern Brittany, France) when the algae were still immersed. This species is a rapidly growing alga that constitutes a variable but abundant and renewable resource (Mineur et al. 2012). The alga is found in the form of a pink-red thallus up to 15–20 cm high and 0.5–2 mm in diameter. The thallus develops from a fibrous base consisting of entangled filaments. The haptera form studs surrounding the anchor point of the algae. After removal of the epiphytes, the thalli were rinsed in water and then freeze-dried.

Preparation of the eco-designed algal extracts

Ethyl acetate was purchased from Fisher Scientific (Illkirch, France). Octyldodecyl ester of L-pyrrolidone

carboxylic acid (OE L-PCA) (cosmetic emollient and moisturizer) was obtained from the Solabia Group (Pantin, France), and 2-octyldodecanol (2-OD) was purchased from Sasol (Paris, France). Six algal extracts were prepared from *Solieria chordalis* samples collected in October and November 2012. The extractions were repeated three times. Prior to extraction, frozen *S. chordalis* was ground using a Jupiter T8 grinder equipped with a 3-mm hole grinder plate. Freeze-dried samples (100 g) were soaked for 1 h at 20°C in 200 ml of solvent prior to sonication for 30 min at 40°C (Elmasonic S40 H sonicator, 37 kHz, Fisher Scientific, Illkirch, France). Ultrasound-assisted extraction led to cell breaking resulting in a reduction of the extraction time and the quantity of the solvent. Samples were kept overnight at 4°C. Following centrifugation (4°C, 10 min, 5000 *g*), the supernatants were collected and kept at 4°C in the dark. Table 2 lists the extracts used for the anti-UV activity. Three different extracts in 2-OD were prepared and labeled E1, E2, and E3. E1 was used fresh, while E2 and E3 were used after storage in the dark at 4°C for 6 weeks and 5 months, respectively. Two extracts E4 and E5 were obtained with OE L-PCA and used fresh or after storage in the dark at 4°C for 5 months. An extract (E6) of the alga in ethyl acetate was studied for spectral and structural analysis (HPLC and LC/MS analyses). The extracts were prepared in triplicate. The 2-OD and OE L-PCA extracts were used for the anti-UV activity measurements described below.

Spectrophotometry

All absorbance and spectrum measurements were established with a UV-SHIMADZU-1800 scanning spectrophotometer (Marne La Vallée, France). The spectra of ethyl acetate, 2-OD, and OE L-PCA algal extracts were recorded between 250 and 400 nm. For each spectrum, the blank was the solvent used for the preparation of the extracts.

Table 2 Evaluation of the photoprotective activity of *Solieria chordalis* extracts (E) and solvents (S).

Solvent (S)/ Extract (E)	Solvent	Month algae collected	Extract age	Half life $t_{1/2}$ (min)
S1	Ethyl acetate	–	–	66.0±5.8[a]
S2	OE L-PCA	–	–	3.0±0.2[b]
S3	2-OD	–	–	44.0±9.5[c]
E1	2-OD	Nov	Fresh	39.0±12.7[c]
E2	2-OD	Oct	6 weeks	121.0±6.0[d]
E3	2-OD	Oct	5 months	27.0±1.3[e]
E4	OE L-PCA	Nov	Fresh	61.0±0.6[a]
E5	OE L-PCA	Oct	5 months	27.0±0.5[e]
E6	Ethyl acetate	Oct	Fresh	–

$t_{1/2}$ values correspond to the half-life of chlorophyll.
Values of $t_{1/2}$ are means±SD (n=3). Values with different superscript letters are significantly different ($p<0.05$).

Anti-UV activity of *Solieria chordalis* extracts

The experimental device (Vilbert Lourmat, Fisher Scientific, Illkirch, France) was composed of an irradiation chamber (300×280×240 mm) supplied with UV-B 312 nm lamps (6 W, intensity of the source 680 μW cm^{-2}). The lamps are supplied by Vilbert Lourmat (Marne La Vallée, France), and their emission curve reaches a maximum at 312 nm. The distance between the samples and the lamp was 20 cm. Synthetic chlorophyll (composed of 90% Chl *a* and 10% Chl *b*) was purchased from Fisher Scientific (Illkirch, France). The synthetic chlorophyll (designated Chl in the following) was used for the preparation of the solutions and introduced in the extracts for the anti-UV activity measurements. Chl was chosen as a sensitive indicator of photodegradation and was added to the solvents (S) and extracts (E) described in Table 2 at a final concentration of 10 mg l^{-1}. The effect of UV-B radiation (312 nm) on Chl photodegradation was determined as decrease in absorbance at 662 nm measured every 10 min. Each solvent (S) and extract (E) was irradiated for 120 min in order to determine the half-life $t_{1/2}$ of Chl. Ethylhexylmethoxycinnamate (EHM; Uvinul MC 80, BASF, Mississauga, Canada) was chosen as a photoprotective and photostable synthetic filter. EHM is currently approved worldwide and used in skin and sun care products (EU Cosmetics regulation; U.S. OTC Drugs regulation). EHM was introduced at a final concentration of 0.1 g l^{-1} as a standard UV filter in a solution containing Chl. Solvents (S) were also tested alone with Chl (10 mg l^{-1}). As a control, photostability of the 2-OD and OE L-PCA extracts was monitored at 310 nm (maximum wavelength), while the extracts were irradiated at 312 nm.

Kinetics study – apparent first-order degradation

The photodegradation of Chl followed apparent first-order kinetics and is described by the equation:

$$\ln A_{(t)} = \ln A_{(t=0)} - kt$$

where $A_{(t)}$ and $A_{(t=0)}$ refer to the absorbance of Chl at t and t=0, k is the apparent degradation rate constant, and t is

the irradiation time (min). Absorbance of Chl, $A_{(t)}$, is linear with the concentration of Chl, $[Chl]_{(t)}$, in the Beer-Lambert-Bouguer law:

$$A_{(t)} = \varepsilon.1.[chl]_{(t)}.$$

To evaluate the protective effects of the algal extracts (Table 2), the half-life $t_{1/2}$ of Chl, which is the time necessary for a 50% decrease of initial concentration, could be calculated as:

$$t_{1/2} = \ln 2 / k$$

High-pressure liquid chromatography – UV detection (UV DAD-HPLC)

The ethyl acetate algal extract was analyzed by HPLC equipped with a diode array detector. HPLC-grade solvents (methanol, ethyl acetate, and acetonitrile) were purchased from Fisher Scientific (Illkirch, France). Qualitative analyses of the ethyl acetate extract were achieved by the HPLC system (Thermo Scientific Dionex, Voisins Le Bretonneux, France) after concentration under vacuum and dilution in methanol at 10 mg l^{-1}. The HPLC device consisted of a P680 gradient pump, an ASI100 automated sampler injector connected to a separating column in inverse phase C18 (Nucleodur C18 250×4.6, 5 µm, Macherey-Nagel), preceded by a precolumn and a photodiode array detector (UVD 340U, 200–595 nm, Thermo Scientific Dionex, Voisins Le Bretonneux, France). HPLC data were collected using Chromeleon software (Thermo Scientific Dionex, Voisins Le Bretonneux, France). Solvent A consisted of Milli-Q water (13%)/methanol (51%)/acetonitrile (36%)/ammonium acetate (300 mM); solvent B was acetonitrile (30%)/ethyl acetate (70%). A linear gradient (solvent A 100% 0–26 min, solvent B 100% 26–35 min) was used at a flow rate of 1.4 ml min^{-1}. Twenty microliters was injected three times, and total run time was 35 min.

Liquid chromatography-mass spectrometry (LC-MS)

LC-MS analyses were performed on a LCMS-Q-TOF (Dionex, Ultimate 3000, Bruker, micrOTOF Q) system (Bruker Daltonik GmbH, Bremen, Germany). Liquid chromatography separation was achieved with injection of 20 µl of extract on a C18 column (250×4.6 mm, 5 µm) at a flow rate of 1.4 ml min^{-1} with a total run of 45 min. The mobile phase consisted of solvent A, methanol/water/acetonitrile 51/13/36 with 0.3 M ammonium acetate, and solvent B methanol. The program corresponded to an isocratic elution of 100% A for the initial 10 min, followed by a linear gradient 0–100% B from 10 to 35 min and an isocratic elution of 100% B for 10 min. The sample detection was simultaneously performed by a UV detector at 307 nm and in the positive mode by the Q-TOF mass spectrometer detector. This hybrid triple quadrupole time-of-flight was equipped with an electrospray source (Bruker Daltonik GmbH, Bremen, Germany). The source conditions were the following: nebulizer 40 psi, dry gas 9 l min^{-1}, and temperature 200°C. The scan range was 50–1000 m/z.

Statistical analysis

Half-life $t_{1/2}$ of Chl is expressed as means±standard deviation (SD; n=3). The statistical analysis was carried out on SPSS (IBM, Armonk, NY, USA) using the one-way analysis of variance (ANOVA) followed by a Duncan test at the 5% level ($p<0.05$) to evaluate differences between samples. For each series of values, the significant differences are labeled by superscript letters.

Results

Extraction

The extraction using ethyl acetate led to a mass of 1.2–1.5 g (d.w.) from 100 g (d.w.) of algae.

Spectrophotometry of algal extracts

The spectra of ethyl acetate, 2-OD and OE L-PCA algal extracts were recorded between 250 and 400 nm (Figure 1). All the samples showed absorption in the UV-B region (290–315 nm) with absorption maxima (λ_{max}) at 292, 305, and 319 nm. The spectrum of 2-OD extract had an additional absorption peak at 346.2 nm in the UV-A region (315–400 nm). The OE L-PCA extract had lower absorption at λ_{max} 302.0 and 315.7 nm. These results suggested the presence of UVB-absorbing compounds in the extracts.

Photoprotective activity measurements

The photostability of the 2-OD and OE L-PCA extracts was monitored at 310 nm (maximum wavelength), while the

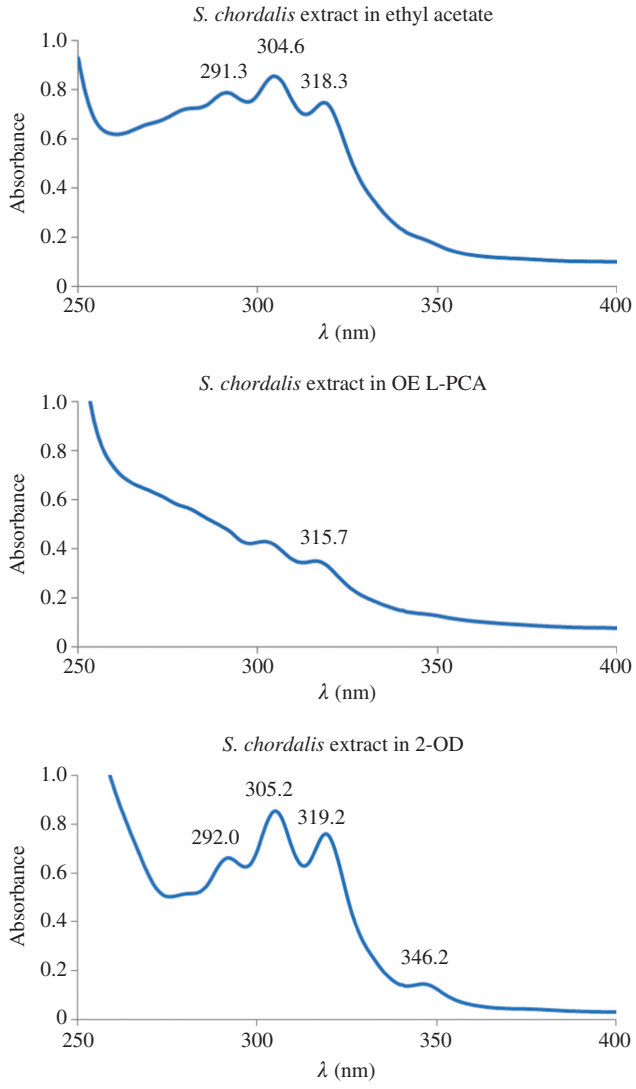

Figure 1 UV spectra of *Solieria chordalis* extracted in three solvents.

extracts were irradiated at 312 nm. For both samples, a very low decrease in absorption maximum at 310 nm was observed (data not shown).

The effect of UV-B radiation (312 nm) on Chl degradation was measured as decrease in absorbance at 662 nm. The $t_{1/2}$ (half-life) of Chl after exposure to radiation is shown in Table 2. The solution of Chl in pure OE L-PCA (S2) had the lowest $t_{1/2}$, 3.0 min, which indicated that Chl degraded at a faster rate than in the other two solvents, ethyl acetate and 2-OD with $t_{1/2}$ values of 66.0 and 44.0 min, respectively. This implies that the nature of the solvent influences the degradation behavior of analytes. 2-OD was a better UV-absorbing solvent as it had a higher $t_{1/2}$. However, the most effective solvent used was ethyl acetate, which had the highest $t_{1/2}$. 2-OD is used as an emollient in cosmetic formulae, and it was useful to study its potential for extracting anti-UV-absorbing molecules from *Solieria chordalis*.

Table 2 shows that the *S. chordalis* extract prepared with the algae collected in November (E4) had a higher $t_{1/2}$ (61.0 min) than the algal extract collected 1 month before (E5; 27.0 min). However, this latter algal sample had been stored at 4°C for 5 months and might have been degraded, whereas E4 was freshly prepared. The *S. chordalis* extracts E4 and E5 demonstrated a higher anti-UV protection activity than the pure OE L-PCA (3.0 min).

The 2-OD extract prepared with algae collected in October and measured after 6 weeks' storage (E2) had a higher $t_{1/2}$ (121.0 min) than the same extract stored for 5 months (E3; 27.0 min). The freshly prepared 2-OD extract from November (E1) showed Chl half-life of 39.0 min. Of these measurements, only E2 showed a higher anti-UV protection activity than the pure 2-OD.

HPLC-DAD

The HPLC-DAD chromatogram registered at 307 nm is shown in Figure 2A. Three peaks were detected with retention time at 3.8 min, 6.4 min, and 7.9 min. The corresponding compounds all had absorption maxima in the UV-B region. The largest peak (1) had an absorption maximum at 309.7 nm (Figure 2B), and peaks 2 (Figure 2C) and 3 (Figure 2D) had maxima at 306.4 and 321.2 nm with an additional maximum at 293.5 nm for peak 3. The three-dimensional UV-DAD spectrum registered between 255 nm and 400 nm did not reveal other absorption and/or another peak (data not shown).

LC-MS, detection of palythenic acid

The sample extracted with ethyl acetate was submitted to LC-MS analysis. The mass spectrum of the first peak separated by HPLC (Figure 3) revealed two singly charged ion peaks $[M+H]^+$ at m/z 209.09 and m/z 329.14 and the corresponding sodium adduct at m/z 231.07 and m/z 351.12, respectively. The second peak eluted by HPLC led to $[M+H]^+$ at m/z 299.1918 and $[M+Na]^+$ at 321.1736, while the third peak led to $[M+H]^+$ at m/z 301.2017 and $[M+Na]^+$ at 323.1893. Peak 1 eluted at 3.786 min showed a large absorption maximum peak including the absorption region of MAAs, and the m/z 329.1422 corresponded to the monoisotopic molecular mass of the proton adduct of palythenic acid (Figure 4). The other compounds detected in the extract (peaks 2 and 3 eluted by HPLC) are still under investigation.

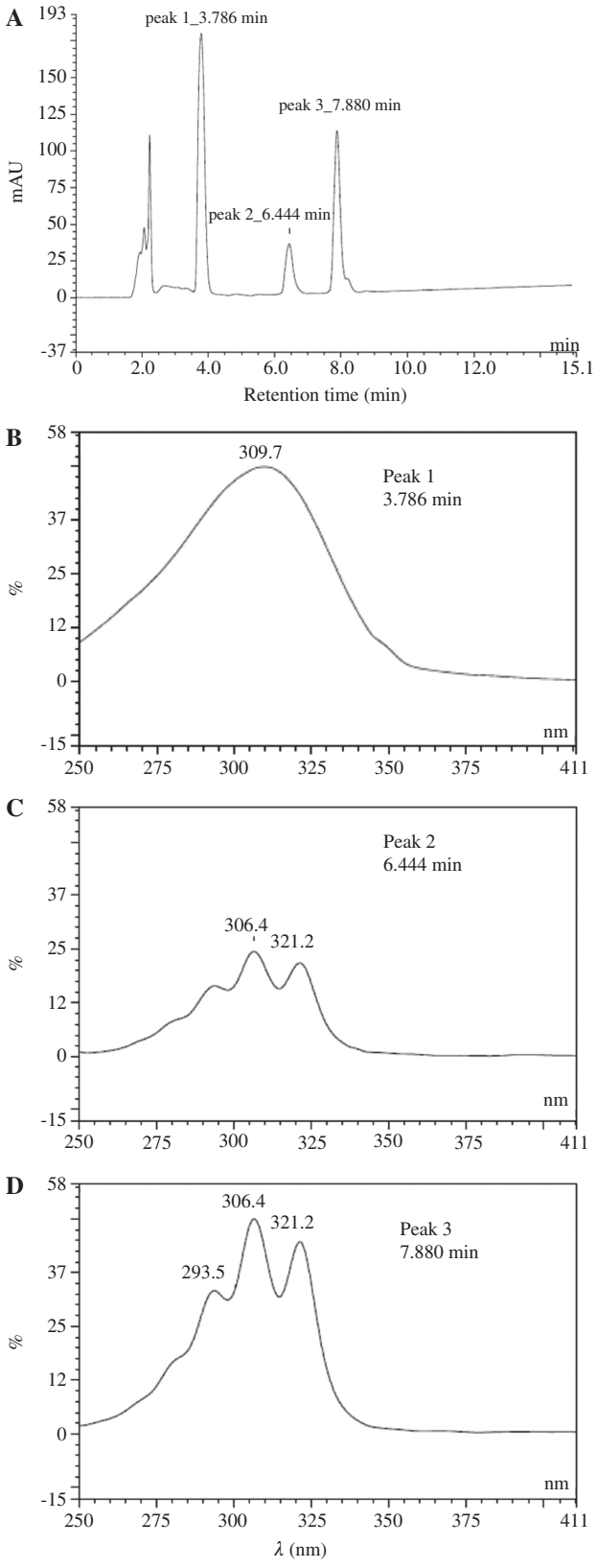

Figure 2 HPLC analysis of extract of *Solieria chordalis* in ethyl acetate. (A) HPLC chromatogram – UV detector fixed at 307 nm. (B–D) UV absorption spectra of the three peaks numbered 1, 2, and 3 with maximum wavelengths in nm.

Discussion

Macroalgae growing in the intertidal zone (6–12 m of tidal level variation in Brittany in France) have to counteract the extreme changes in solar radiation, seawater temperature, salt concentration, water column depth, and emersion stress. Hence, various secondary metabolites are produced, and these bioactive compounds could represent useful leads in the development of new functional ingredients in the pharmaceutical and cosmetic industries. UV-B radiation induces damage on human skin (Matsumura and Ananthaswamy 2004), and the use of anti-UV macroalgal extract in sunscreen composition is of high interest. In this study, the extracts prepared in OE L-PCA and in 2-OD were conceived with a view to being incorporated as a natural ingredient in photoprotective cosmetic products. It was expected to enhance the efficiency of chemical anti-UV filters while displaying the positive image of an eco-extracted ingredient.

UV-absorbing molecules in *Solieria chordalis* extracts

In this study, *Solieria chordalis* extracts prepared in the solvents ethyl acetate, 2-OD, and OE L-PCA showed four absorption maxima (λ_{max}) centered at 292, 305, 318, and 346 nm. The absorption maxima were also affected by the nature of the solvent. This was illustrated by the OE L-PCA extract, which showed a hypsochromic displacement of 3 nm with λ_{max} at 302.0 and 315.7 nm. These results suggested the presence of UVB-absorbing compounds in the extracts. The known amino-cyclohexenone MAAs are characterized by λ_{max} at 310 nm (Carreto and Carignan 2011). MAAs, which contain an amino-cyclohexenimine ring, have absorption maxima between 320 and 360 nm. Therefore, the presence of MAAs and/or substituted MAAs in addition to spectrally similar compounds (polyunsaturated compounds) can be suggested for the three extracts. Furthermore, the extracts of *S. chordalis* in 2-OD and OE L-PCA have been shown to significantly reduce the photodegradation of "synthetic Chl" by UV-B radiation.

Photoprotective activity measurements

In vitro measurement of the anti-UV capacity of macroalgal extracts has been rarely reported, and data are really scarce. The measurement of absorbance of the 2-OD and OE L-PCA extracts monitored at 310 nm confirmed the photostability previously observed for samples of MAAs

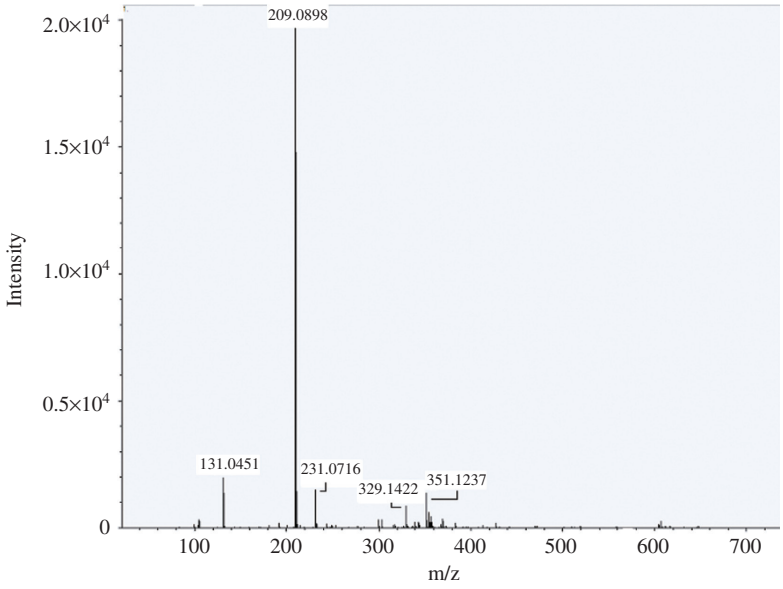

Figure 3 Liquid chromatography mass spectrum obtained in the positive ion mode for the compound of HPLC peak 1 eluted at 3.786 min (see Figure 2).

(Carreto and Carignan 2011). The extracts prepared are active against UV-B radiation. However, the activity of the algal extracts varied with the solvent used and the length of storage. There could also be more anti-UV-absorbing molecules present in the algal sample collected in October than in the November sample. The production of MAAs has been shown to be multiparameter dependent (irradiance and spectral composition of solar radiation, water column depth) (Karsten et al. 1998, Karsten and Wiencke 1999, Rozema et al. 2002, Pessoa 2012). Seasonal and geographical variations have also been reported (Karsten et al. 1998). Furthermore, the solvent nature had a profound influence on the degradation behavior of analytes (Kundu et al. 2005). Thus, we compared the half-life of Chl introduced in the algal extracts. Among the two emollients used, it was found that the 2-OD extract revealed the highest capacity for protecting Chl. The possible reaction or interaction between solvent and anti-UV substances was not investigated in this work. This study has demonstrated that the *Solieria chordalis* extracts in 2-OD or in OE L-PCA showed a higher anti-UV protection activity than the corresponding pure solvent solution. The method used in this study appeared to be well-adapted for screening the efficiency of compounds against UV-B rays and was first applied by Hupel et al. (2011). Nevertheless, the use of "synthetic chlorophyll" as a UV-sensitive target is an original approach for *in vitro* anti-UV activity measurement. The use of sodium magnesium chlorophyllin (SMC) was reported by Hupel et al. (2011), but SMC appeared much less sensitive to UV-B radiation in our experiments. Variations of Chl and SMC absorbance under UV-B radiation were shown to be strongly correlated, whereas the loss in absorbance was twice as fast for chlorophyll as for SMC (Hupel et al. 2011). Degradation of Chl under UV-B radiation was in agreement with previous studies (Cuny et al. 1999, Santabarbara 2006, Zvezdanović et al. 2009).

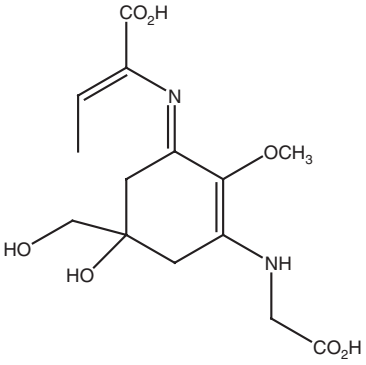

Figure 4 Structure of the mycosporine-like amino acid: palythenic acid.

Detection of palythenic acid

We have described for the first time the detection of one MAA in a *Solieria chordalis* extract along with other unknown UV-absorbing compounds. Using HPLC and LC-MS, we have shown the presence of palythenic acid in an ethyl acetate extract. The presence of palythenic

acid has been shown in microalgae, phytoplankton, and coral reef organisms (Carreto et al. 1990, Carreto et al. 2001, Whitehead et al. 2001, Sommaruga et al. 2006, Llewellyn and Airs 2010), while palythine, palythinol, and palythene were also revealed in *Palmaria palmata* (Karsten and Wiencke 1999) and *Chondrus crispus* (Franklin et al. 2001, Kräbs et al. 2002). To our knowledge, this is the first time that the form of palythenic acid has been characterized in macroalgae. Palythenic acid, palythene, and palythinol differ by the nature of the group linked to the imino moiety, and they are characterized by similar ring chromophores having different absorption maxima λ_{max} ranging between 320 and 360 nm (Carreto and Carignan 2011). The absence of a by-product from MAAs in the extract confirms its photostability. Despite the high diversity of MAAs observed in macroalgae, no other single MAA was identified in the ethyl acetate extract; this could be due to the apolar solvent character. The chemical structures of other compounds detected by HPLC and LC-MS are still under investigation.

In skin care products, synthetic UV filters are used for preventing sunburn on the assumption that commercial sunscreens will also prevent skin cancer. However, sun protecting agents still have to be complemented by other compounds to make sun protection cosmetics more efficient and to prevent skin cancer and skin photoaging. Highly effective UV-absorbing compounds are produced by marine organisms like algae that can counteract the damaging effect of UV-B rays. In this study, we report the preparation of eco-designed emollient algal extracts for protection against UV-B-induced chlorophyll degradation. The algal extract introduced at a final content of 5% (w/w) allowed significant anti UV-B activity. UV radiation induces diverse effects for human skin like sunburn, inflammation, alteration of cytokine levels in cells found in the epidermis and dermis, and causes skin aging by the reaction of free radicals and reactive oxygen species and/or formation of aggressive malignant skin cancer (Muthusamy and Piva 2013). The UV-B-induced pyrimidine dimers in DNA can lead to a form of programmed apoptosis or can cause DNA replication errors. MAAs exhibit interesting anti-UV activity allowing development of cosmetic applications for UVB and UVA-exposure protection. The mycosporine-like amino acid, palythenic acid, was characterized for the first time in *S. chordalis*. The occurrence of MAAs in red algae has been reported to provide protection as UV-B-absorbing/screening compounds. To date, no studies have been reported on the preparation of emollient algae extracts, and this study shows the potential application of algal extracts in sun care products. However, the algal extracts were acting to delay the degradation of Chl, and these results must be completed by the measurement of other *in vitro* tests. Further development could be performed by using UV-B-sensitive substances present in human beings and/or in human skin. Further developments now need to be carried out, such as the optimization of extraction conditions for increasing bioactivity, a study of Chl degradation in a dose-dependent manner, the determination of the Sun Protection Factor and of *in vivo* biological activity, and the prevention of UV-B-induced erythema (Torres et al. 2004, Hupel et al. 2011).

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