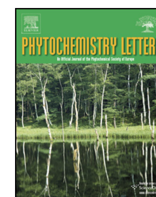
Phytochemistry Letters 20 (2017) 406–409

Contents lists available at ScienceDirect

Phytochemistry Letters

journal homepage: www.elsevier.com/locate/phytol



Caulerpenyne from *Caulerpa taxifolia*: A comparative study between CPC and classical chromatographic techniques

Estelle Sfecci [a], Céline Le Quemener [b], Thierry Lacour [c], Lionel Massi [a], Philippe Amade [a], Grégoire Audo [b], Mohamed Mehiri [a,*]

[a] Université Nice Côte d'Azur, CNRS, Institut de Chimie de Nice, UMR 7272, Produits Naturels Marins, Faculté des Sciences, Parc Valrose, 06108 Nice Cedex 02, France
[b] ARMEN Instrument, ZI Kermelin, 16 rue Ampère, 56890 Saint-Avé, France
[c] BioPreserv, 4 Traverse Dupont, Grasse, France

ARTICLE INFO

Article history:
Received 8 November 2016
Received in revised form 15 January 2017
Accepted 28 January 2017
Available online 13 February 2017

Keywords:
Caulerpa taxifolia
Caulerpenyne
Isolation
Centrifugal partition chromatography
Efficient process

ABSTRACT

Caulerpenyne (Cyn) is a cytotoxic compound firstly isolated in 1978 from *Caulerpa prolifera*. This metabolite, constituted by a highly reactive diacetoxybutadiene moiety, exhibited a wide range of biological properties with mainly antibacterial properties and antitumoral activities. Few structure–activity relationships (SAR) are available to design more potent bioactive derivatives by pharmacomodulation. Cyn can be produced by total synthesis or extracted from natural sources in particular the green alga *Caulerpa taxifolia*. Since conventional chromatographic procedures to isolate Cyn from *C. taxifolia* are time- and solvent-consuming, it was crucial to find a more efficient process to obtain pure Cyn. In our study, Cyn has been purified from *C. taxifolia* with two different techniques: Centrifugal partition chromatography (CPC) and a classical chromatographic process. The comparative study showed that CPC constitutes a very simple and efficient process to access Cyn.
© 2017 Phytochemical Society of Europe. Published by Elsevier Ltd. All rights reserved.

1. Introduction

Several seaweeds, in particular green algae, represent a rich source of molecules (Andrade et al., 2013). They contain a high concentration of marine salts, pigments such as chlorophyll or carotenoids (Dambeck and Sandmann, 2014), lipids, sugars (Athukorala and Yuan, 2013), and in some extent mid-polar to apolar bioactive secondary metabolites.

The green alga *Caulerpa taxifolia* (Vahl) C. Agardh has been of a great research interest since its accidental introduction in the Mediterranean Sea in 1984 (Meinesz and Hesse, 1991). The rapid and important proliferation of *C. taxifolia* was attributed to its high temperature tolerance, asexual reproduction, and chemical defenses (Meinesz and Hesse, 1991). Its efficient mechanism of chemical defense relies on the production of several terpenoids (Guerriero et al., 1995) in particular caulerpenyne (Cyn) (Fig. 1). Cyn, a sesquiterpenoid constituted by a reactive diacetoxybutadiene moiety, was shown to be the most abundant metabolite of *C. taxifolia* (Amico et al., 1978), acting as a grazer repulsive (Paul and Fenical, 1986) but also as a wound closure in case of cell disruption (Adolph et al., 2005).

Cyn exhibit a wide range of biological properties with mainly antitumoral activities by inhibiting the growth of several human cancer cell lines (Fischel et al., 1995) and antibacterial properties (Hodgson, 1984). To date, few structure–activity relationships (SAR) studies were performed on Cyn to yield by pharmacomodulation more potent bioactive derivatives for therapeutic purposes. In 2006, Commeiras and co-workers have shown on few Cyn derivatives a tied link between the acetoxybutadiene moiety and their ability to inhibit tubulin assembly (Commeiras et al., 2006). In 2014, Richter and co-workers have shown on Cyn and related *bis*-enols esters that the butadiene moiety was essential for the inhibition of human 5-lipoxygenase (Richter et al., 2014). Further SAR studies are still needed to better understand Cyn and Cyn derivatives biological mode of action which imply an easy access to Cyn in enough amounts.

Cyn was obtained by synthesis or directly from *C. taxifolia*. A few total syntheses of Cyn have been reported (Commeiras et al., 2001, 2003). Unfortunately, they are costly effective as several steps are

* Corresponding author.
E-mail address: mehiri@unice.fr (M. Mehiri).

http://dx.doi.org/10.1016/j.phytol.2017.01.014
1874-3900/© 2017 Phytochemical Society of Europe. Published by Elsevier Ltd. All rights reserved.

necessary and they lack enantioselectivity. Since *C. taxifolia* is a cultivable green alga which is not an endangered species in the Mediterranean Sea, the best way to obtain enantiopure Cyn remains the direct purification from the species.

In the literature, Cyn was extracted and isolated in one or several steps by using different approaches based mainly on a silica gel (Valls et al., 1994; Guerriero and D'Ambrosio, 1999; Cengiz et al., 2011; McConnell et al., 1982) and size exclusion chromatographies (Amade et al., 2001). These conventional chromatographic methods exhibited several disadvantages such as large solvent volumes and time-consuming procedures. Furthermore, the alga is also composed by several polymers (cellulose, lignin, proteins, and starch), water-soluble monosaccharides (Rabemanolontsoa and Saka, 2013), fatty acids (Ivesa et al., 2004), and pigments such as chlorophyll (Aguilar-Santos, 1970) which often stain irreversibly on the chromatographic support. Moreover, *C. taxifolia* produces in a less extent few others bioactive secondary metabolites such as caulerpin (Aguilar-Santos, 1970) which exhibit a close polarity with Cyn. Chlorophyll and caulerpin often interfere with the isolation of Cyn in a pure state. Finally, Cyn is also a sensitive secondary metabolite which can be easily and rapidly degraded in presence of water (Amade et al., 2001). All these considerations prompted us to develop a more efficient procedure for extraction and isolation of Cyn from natural raw materials.

Centrifugal partition chromatography (CPC) represents a very good alternative to conventional processes despite the instrumental higher costs. CPC is a separation process that relies on a two-phase solvent system made from a pair of immiscible solvents, one used as the stationary phase and the other as the mobile phase (Marchal et al., 2003). This technique is a liquid-liquid partition chromatography that uses the centrifugal force for the retention of the stationary phase (Chollet et al., 2015). CPC exhibits several advantages, compared to classical liquid chromatography techniques using solid supports, such as a higher sample loading capacity and the possibility to inject a relatively crude extracts, a very low solvent consumption, the absence of irreversible adsorption, and a total recovery of the sample without any deterioration. Furthermore, CPC can easily be scaled-up from analytical to preparative scale in rapid and convenient way (Yuan et al., 2008). Several studies have shown that CPC represents a suitable method to obtain pure natural products from terrestrial and marine complex extracts such as essential oils (Wei et al., 2012), lichens (Roullier et al., 2009), and algae (Hwang et al., 2012; Becerra et al., 2015; Lee et al., 2016).

In this paper, we describe a simple, efficient, and scalable CPC method to isolate pure Cyn from *C. taxifolia*.

2. Materials and methods

2.1. Reagents

Cyclohexane (*c*-Hex), *n*-heptane (Hept), ethyl acetate (EtOAc), methanol (MeOH), and dichloromethane (DCM) used for extraction and CPC were purchased from VWR (Rectapur grade). Acetonitrile (MeCN) and water (H_2O) used for high performance liquid chromatography (HPLC) analyses were analytical grade and purchased from VWR (Chromanorm grade). Sephadex LH-20 (GE Healthcare) and Diol-functionalised silica gel (75–200 μm) used for open column chromatography were purchased from Sigma–Aldrich. Thin-layer chromatography (TLC) analyses were performed with silica gel 60 plates (Merck, Silica gel F254).

Fresh *C. taxifolia* were collected from Villefranche-sur-Mer (France) in September 2013 and kept frozen until extraction.

2.2. Instruments

HPLC analyses were performed with a Waters Alliance 2695 HPLC system (Waters Corporation, Milford, MA) coupled with a Waters 996 photodiode array (PDA) detector. Analyses were performed with a bifunctional Macherey-Nagel NUCLEODUR® Sphinx RP column (5 μm, 250 mm × 4.6 mm) consisting of a balanced ratio of propylphenyl and C18 ligands, at room temperature.

Purifications by CPC were performed on a SCPC-250 CPC system (Armen instrument, France) fitted with one rotor containing 800 twin-cells of 300 μL (total volume: 250 mL). The CPC was connected to a preparative HPLC system (Spot prep 2, Armen Instrument France) equipped with a quaternary gradient pump 50 mL/min, an automated loop injection and backflush valves, a UV/Vis PDA detector and a fraction collector, all controlled by the Armen Glider CPC software. The CPC system configuration allows to purify from 0.05 g up to 6 g.

NMR (^1H and ^{13}C) analyses were performed in deuterated MeOH (CD_3OD) on a Bruker Avance 500 MHz spectrometer.

2.3. Extraction of *C. taxifolia* and Cyn quantification

C. taxifolia was freeze-dried before extraction. The dried fronds and stolons (8 g) were crushed and then extracted six times with 200 mL of MeOH–DCM (1:1, v/v). The combined fractions were evaporated under reduced pressure to yield the crude organic extract (1.7 g).

2.4. Cyn isolation by conventional chromatography

The crude extract (1.7 g) was suspended in MeOH and extracted several times with *c*-Hex. The *c*-Hex fractions were evaporated under reduced pressure to yield 128 mg of the Cyn fraction which was subjected to a Sephadex LH-20 size exclusion chromatography (ID 1.6 cm; *h* 32 cm) in MeOH to afford 6 fractions (fr. 1–6). Fraction 4 was further chromatographed on Diol-functionalised silica gel with a gradient of AcOEt–MeOH from 100:0 to 0:100 to give 40 sub-fractions (subfr. 1–40). Subfr. 8 yielded pure caulerpenyne (0.4 mg). The remaining sub-fractions were discarded as Cyn purity by HPLC was less than 50%.

2.5. Cyn isolation by CPC

2.5.1. Selection of the two-phase solvent system

Shake flask experiments were performed to determine the partition coefficient K_{Cyn} of caulerpenyne (Cyn) between the two-phase solvent systems tested to determine a well-suited ($K_{Cyn} \sim 1$) biphasic solvent mixture for separation by CPC. The solvent systems from the Arizona family, in particular Arizona A, N, U, and Z, were screened. They were prepared by mixing at room temperature heptane, water, ethyl acetate, and methanol in different proportions (Foucault and Chevolot, 1998; Berthod et al., 2005). K_{Cyn} was determined by dissolving a suitable amount of the crude extract in 2 mL of the upper (or lower) phase. The solution was shaken with an equal volume of lower (or upper) phase until complete dissolution of the extract. After decantation, the two phases were then separated and the Cyn concentration in each phase was first estimated by TLC and then accurately quantified by HPLC. For each system, K_{Cyn} was calculated by using the following equation:

$$K_{Cyn} = \frac{[C_{Cyn}]_{stat.}}{[C_{Cyn}]_{mob.}} = \frac{A_{stat.}}{A_{mob.}}$$

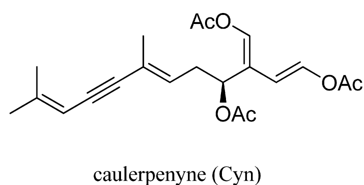


Fig. 1. Structure of caulerpenyne (Cyn) ($C_{21}H_{26}O_6$; CAS Registry Number: 70000-22-5).

Table 1
CPC conditions.

Crude extract sample	1.7 g dissolved in 5 mL of upper phase + 5 mL of lower phase
Biphasic solvent system	Arizona U
Pumping mode	Ascending
Flow	12 mL/min
Stationary phase retention	79.2%
Elution step duration	40 min
Extrusion step duration	20 min
Collection volume	12 mL during elution 24 mL during extrusion

Table 3
CPC vs classical chromatography for Cyn purification.

Technique	Crude extract weight	Isolated Cyn weight	Yield	Total volume of solvent	Time
CPC	1.7 g	8.3 mg	0.5%	~1 L	1 h
Classical	1.7 g	0.4 mg	0.03%	2–3 L	1–2 days

where $[C_{Cyn}]_{stat.}$ and $[C_{Cyn}]_{mob.}$ are the Cyn concentration in the stationary and mobile phases, respectively; $A_{stat.}$ and $A_{mob.}$ are the HPLC Cyn peak area in the stationary and mobile phases, respectively.

All the fractions were analysed by TLC and HPLC. For TLC, the mobile phase, composed by a mixture of *c*-Hex-EtOAc (80:20, v/v), was adapted from Amade and co-workers (Amade et al., 2001). Spots were visualised under UV light (254 nm) and by spraying a 5% solution of vanillin in ethanolic sulfuric acid followed by heating. For HPLC analyses, the mobile phase was a linear gradient (v/v) of water with 0.1% of formic acid (A) and acetonitrile with 0.1% of formic acid (B) as follows: 90:10 for 5 min, then 90:10–0:100 in 30 min, and finally 0:100 for 5 min. The flow rate was set to 1.0 mL/min and the effluent was continuously monitored at 254 nm.

2.5.2. CPC process

According to the K_{Cyn} values, Arizona U (Hept, EtOAc, MeOH, H_2O; 4/1/4/1; v/v/v/v) was selected as the two-phase solvent system for Cyn purification by CPC. Hept, EtOAc, MeOH and H_2O were mixed in a separatory funnel, vigorously shaken, and thoroughly equilibrated at room temperature. The two phases were separated to yield an upper phase and a lower phase. The CPC column, rotated at 500 rpm, was fully filled in ascending mode with the lower phase, then the rotation speed was set at 2200 rpm and the upper mobile phase was eluted at 12 mL/min for the equilibration step. To perform a comparison with the conventional chromatography method, an identical amount of the crude extract (1.7 g), suspended in 10 mL of Arizona U, was injected through a 10 mL stainless steel loop. During separation, the effluent was continuously monitored at 254 nm and 12 mL fractions were collected in 25 mL tubes, examined by TLC and combined if necessary. After 40 min of elution, the CPC column was rinsed to recover all the compounds injected with the stationary phase. This process, called extrusion, was performed at 50 mL/min. The different parameters for CPC are summarized in Table 1.

Table 2
K_{Cyn} (partition coefficient) values of Cyn in the solvent systems.

Solvent system	Solvent ratio (v/v/v/v)				K_{Cyn}
	Heptane	Ethyl acetate	Methanol	Water	
Arizona A (Az A)	0	1	0	1	$>10^5$
Arizona N (Az N)	1	1	1	1	20
Arizona U (Az U)	4	1	4	1	1.2
Arizona Z (Az Z)	1	0	1	0	0.18

2.6. ^1H and ^{13}C NMR data for Cyn

^1H NMR (200 MHz, CD_3OD), δ (ppm): 7.64 (d, ^3J = 14.0 Hz, 1H, CH), 7.26 (s, 1H, CH), 5.92 (d, ^3J = 14.0 Hz, 1H, CH), 5.86 (t, ^3J = 14.0 Hz, 1H, CH), 5.66 (t, ^3J = 6.0 Hz, 1H, CH), 5.35 (s, 1H, CH), 2.61 (m, 2H, CH_2), 2.18 (s, 3H, CH_3), 2.14 (s, 3H, CH_3), 2.06 (s, 3H, CH_3), 1.87 (s, 3H, CH_3), 1.82 (s, 3H, CH_3).

^{13}C NMR (50.3 MHz, CD_3OD), δ (ppm): 171.4 (C), 169.4 (C), 168.4 (C), 148.6 (C), 138.1 (CH), 135.6 (CH), 130.6 (CH), 122.8 (C), 120.1 (C), 110.2 (C), 106.2 (CH), 94.8 (C), 86.0 (C), 70.2 (CH), 32.7 (CH_2), 24.6 (CH_3), 20.8 (CH_3), 20.6 (CH_3), 20.3 (2 CH_3), 17.7 (CH_3).

3. Results and discussion

To compare CPC to a conventional chromatographic process to isolate Cyn, we first extracted *C. taxifolia* to yield a crude organic extract. The proportion of Cyn in the crude extract was quantified by HPLC. We noticed a low abundance of Cyn (0.6%, crude extract dry weight) compared to previous studies (Valls et al., 1995). This trend was also observed by Dumay et al. (2002).

Then, we selected a classical chromatographic process to purify Cyn. We combined the most efficient conventional processes to isolate Cyn. We thus proceed in 3 steps: (i) a liquid–liquid extraction to remove polar analytes from the crude extract, (ii) a size exclusion chromatography on the organic fraction, and finally (iii) a diol column chromatography to offer Cyn with a yield of 0.03% (*C. taxifolia* crude extract dry weight, 5% recovery) for the overall process.

Subsequently, CPC was investigated as an alternative process to obtain pure Cyn directly from the crude extract which implies a careful search for a suitable two-phase solvent system that would provide an ideal partition coefficient for an optimal separation of Cyn. The partition coefficient (*K*), defined as the ratio of a solute distributed in two mutually equilibrated solvent phases, should be in a range between 0.5 and 2 to obtain the best resolution. A smaller *K*-value elutes the solute closer to the solvent front with lower resolution while a larger *K*-value tends to give better resolution, but broader, more dilute peaks due to a longer elution time (Ito, 2005). Hence, the partition coefficient K_{Cyn} of Cyn was investigated to determine the optimal two-phase solvent systems for CPC. We selected the Arizona family as it allows a rapid screening (Berthod et al., 2005). The K_{Cyn}-values determined for the Arizona A, Arizona N, Arizona U, and Arizona Z solvent systems are reported in Table 2.

Arizona A and Arizona N solvent systems were investigated and gave large K_{Cyn}-values ($K_{Cyn(Az\ N)} = 20$) implying a too long separation time ($Vr_{(Az\ N)} = 334$ min). For the next trial, Arizona Z solvent system gave a too small K_{Cyn}-value ($K_{Cyn(Az\ Z)} = 0.18$), implying a very short separation time ($Vr_{(Az\ Z)} = 7$ min) and poor peak resolution. These solvent systems were therefore not suitable for the separation of Cyn by CPC. Finally, Arizona U was tested and

gave a well-suited K_{Cyn}-value of 1.2 ($Vr_{(Az\ U)}$ = 24 min). Therefore, Arizona U was selected to isolate Cyn. For this purpose, the crude extract (1.7 g), suspended in 10 mL of Arizona U, was injected through the CPC system. Sixty-four fractions of 12 mL were collected based on the UV detection (254 nm) and 2 of them were pooled after TLC analyses to yield at the end of the process 8.3 mg of pure Cyn (0.5%, *C. taxifolia* crude extract dry weight, 83% recovery). The structure and purity of Cyn was further confirmed by NMR spectroscopy.

The different parameters (crude extract weight, amount of Cyn isolated, yields, total volume of solvent used, time) for CPC and the classical chromatographic process are summarized in Table 3. Hence, CPC constitutes a short, simple, and efficient one step process to isolate pure Cyn with a higher yield and a lower solvent consumption compared to chromatographic techniques traditionally used.

4. Conclusion

Caulerpenyne (Cyn) is a sesquiterpenoid which exhibited a wide range of biological activities. This secondary metabolite can be produced by total synthesis or extracted from natural sources and in particular from the green alga *C. taxifolia*. Traditional chromatographic techniques used imply at least three steps: (i) a liquid–liquid partition on the crude extract, (ii) a size exclusion chromatography, and finally (iii) a diol column chromatography to give Cyn with a yield of 0.03% (dry weight). In the present study, we have shown that CPC represents an excellent alternative process as pure Cyn is isolated with a higher yield, 0.5% (dry weight), in only one step with a lower solvent consumption in a shorter time. To the best of our knowledge, this is the first time that CPC is used to isolate pure Cyn from a natural matrix. This work may serve as a model for further scale-up and industrial development of Cyn with a larger volume CPC system.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgements

E. Sfecci is the recipient of a thesis grant from the "Conseil Régional Provence Alpes Côte d'azur". M. Mehiri research is supported by the ANR/Investissements d'Avenir program via the OCEANOMICs project (grant #ANR-11-BTBR-0008), and the H2020 European program via the EMBRIC project.

References

Adolph, S., Jung, V., Rattke, J., Pohnert, G., 2005. Wound closure in the invasive green alga *Caulerpa taxifolia* by enzymatic activation of a protein cross-linker. Angew. Chem. Int. Ed. 44, 2806–2808.
Aguilar-Santos, G., 1970. Caulerpin, a new red pigment from green algae of the genus *Caulerpa*. J. Chem. Soc. C 6, 1842–1843.
Amade, P., Joncheray, L., Loru, F., Pesando, D., 2001. Caulerpenyne behaviour in seawater: by-product investigations. In: Gravez, V., Ruitton, S., Boudouresque, C.F., Le Direac'h, L., Meinesz, A., Scabbia, G., Verlaque, M. (Eds.), Fourth International Workshop on *Caulerpa taxifolia*. GIS Posidonie Publ., France, pp. 158–167.
Amico, V., Oriente, G., Piattelli, M., Tringali, C., Fattorusso, E., Magno, S., Mayol, L., 1978. Caulerpenyne, an unusual sesquiterpenoid from the green alga *Caulerpa prolifera*. Tetrahedron Lett. 38, 3593–3596.
Andrade, P.B., Barbosa, M., Matos, R.P., Lopes, G., Vinholes, J., Mouga, T., Valentão, P., 2013. Valuable compounds in macroalgae extracts. Food Chem. 138, 1819–1828.
Athukorala, Y., Yuan, Y.V., 2013. Fucoidans from marine brown macroalgae: isolation, identification, and potential biological activities. In: Kim, S.-K. (Ed.), Marine Nutraceuticals: Prospects and Perspectives. CRC Press, ISBN: 978-1-4665-1351-8, pp. 393–435.
Becerra, M., Boutefnouchet, S., Córdoba, O., Pinto Vitorino, G., Brehu, L., Lamour, I., Laimay, F., Efstathiou, A., Smirlis, D., Michel, S., Kritsanida, M., Flores, M.L., Grougnet, R., 2015. Antileishmanial activity of fucosterol recovered from *Lessonia vadosa* Searles (Lessoniaceae) by SFE, PSE and CPC. Phytochem. Lett. 11, 418–423.
Berthod, A., Hassoun, M., Ruiz-Angel, M.J., 2005. Alkane effect in the Arizona liquid systems used in countercurrent chromatography. Anal. Bioanal. Chem. 383, 327–340.
Cengiz, S., Cavas, L., Yurdakoc, K., Pohnert, G., 2011. The sesquiterpene caulerpenyne from *Caulerpa* spp. is a lipoxygenase inhibitor. Mar. Biotechnol. 13, 321–326.
Chollet, S., Marchal, L., Meucci, J., Renault, J.-H., Legrand, J., Foucault, A., 2015. Methodology for optimally sized centrifugal partition chromatography columns. J. Chromatogr. A 1388, 174–183.
Commeiras, L., Santelli, M., Parrain, J.-L., 2001. First total synthesis of (±)-taxifolial A and (±)-iso-caulerpenyne. Org. Lett. 3, 1713–1715.
Commeiras, L., Santelli, M., Parrain, J.-L., 2003. On the construction of 2-substituted 1,4-diacetoxybutadiene moiety: application to the synthesis of (±)-caulerpenyne. Tetrahedron Lett. 44, 2311–2314.
Commeiras, L., Bourdron, J., Douillard, S., Barbier, P., Vanthuyne, N., Peyrot, V., Parrain, J.-L., 2006. Total synthesis of terpenoids isolated from caulerpale algae and their inhibition of tubulin assembly. Synthesis 1, 166–181.
Dambeck, M., Sandmann, G., 2014. Antioxidative activities of algal keto carotenoids acting as antioxidative protectants in the chloroplast. Photochem. Photobiol. 90, 814–819.
Dumay, L., Pergent, G., Pergent-Martini, C., Amade, P., 2002. Variations in caulerpenyne contents in *Caulerpa taxifolia* and *Caulerpa racemosa*. J. Chem. Ecol. 28, 343–351.
Fischel, J.L., Lemee, R., Formento, P., Caldani, C., Moll, J.L., Pesando, D., Meinesz, A., Grelier, P., Pietra, P., Guerriero, A., 1995. Cell-growth-inhibitory effects of caulerpenyne, a sesquiterpenoid from the marine alga *Caulerpa taxifolia*. Anticancer Res. 15, 2155–2160.
Foucault, A.P., Chevolot, L., 1998. Counter-current chromatography: instrumentation, solvent selection and some recent applications to natural product purification. J. Chromatogr. A 808, 3–22.
Guerriero, A., Depentori, D., D'Ambrosio, M., Pietra, F., 1995. Caulerpenyne-amine reacting system as a model for *in vivo* interactions of ecotoxicologically relevant sesquiterpenoids of the Mediterranean-adapted tropical green seaweed *Caulerpa taxifolia*. Helv. Chim. Acta 78, 1755–1762.
Guerriero, A., D'Ambrosio, M., 1999. Epoxycaulerpenynes: reactivity, and diastereoselective and highly regioselective synthesis by dimethyldioxirane oxidation of caulerpenyne. Eur. J. Org. Chem. 1985–1990.
Hodgson, L.M., 1984. Antimicrobial and antineoplastic activity in some South Florida seaweeds. Bot. Mar. 27, 387–390.
Hwang, S.H., Jang, J.M., Lim, S.S., 2012. Isolation of fucosterol from *Pelvetia siliquosa* by high-speed countercurrent chromatography. Fish Aquat. Sci. 15, 191–195.
Ivesa, L., Blazina, M., Najdek, M., 2004. Seasonal variations in fatty acid composition of *Caulerpa taxifolia* (M. Vahl.) C. Ag. in the northern Adriatic Sea (Malinska, Croatia). Bot. Mar. 47, 209–214.
Ito, Y., 2005. Golden rules and pitfalls in selecting optimum conditions for high-speed counter-current chromatography. J. Chromatogr. A 1065, 145–168.
Lee, J.-H., Han, J.W., Ko, J.Y., Lee, W., Ahn, G., Kim, C.Y., Kim, G.H., Jeon, Y.-J., 2016. Protective effect of a freshwater alga, *Spirogyra* sp., against lipid peroxidation in vivo zebrafish and purification of antioxidative compounds using preparative centrifugal partition chromatography. J. Appl. Phycol. 28, 181–189.
Marchal, L., Legrand, J., Foucault, A., 2003. Centrifugal partition chromatography: a survey of its history, and our recent advances in the field. Chem. Rec. 3, 133–143.
McConnell, O.J., Hughes, P.A., Targett, N.M., Daley, J., 1982. Effects of secondary metabolites from marine algae on feeding by the sea urchin, *Lytechinus variegatus*. J. Chem. Ecol. 8, 1437–1453.
Meinesz, A., Hesse, B., 1991. Introduction et invasion de l'algue tropicale *Caulerpa taxifolia* en Mediterranée nord-occidentale. Oceanol. Acta 14, 415–426.
Paul, V.J., Fenical, W., 1986. Chemical defense in tropical green algae, order Caulerpales. Mar. Ecol. Prog. Ser. 34, 157–169.
Rabemanolontsoa, H., Saka, S., 2013. Comparative study on chemical composition of various biomass species. RSC Adv. 3, 3946–3956.
Richter, P., Schubert, G., Schaible, A.M., Cavas, L., Werz, O., Pohnert, G., 2014. Caulerpenyne and related *bis*-enol esters are novel-type inhibitors of human 5-lipoxygenase. Chem. Med. Chem. 9, 1655–1659.
Roullier, C., Chollet-Krugler, M., Bernard, A., Boustie, J., 2009. Multiple dual-mode centrifugal partition chromatography as an efficient method for the purification of a mycosporine from a crude methanolic extract of *Lichina pygmaea*. J. Chromatogr. B 877, 2067–2073.
Valls, R., Artaud, J., Amade, P., Vincente, N., Piovetti, L., 1994. Determination of caulerpenyne, a toxin from the green alga *Caulerpa taxifolia* (Caulerpaceae). J. Chromatogr. A 663, 114–118.
Valls, R., Lemee, R., Piovetti, L., Amade, P., Bouaïcha, N., 1995. Dosage de la caulerpényne, toxine de l'algue verte *Caulerpa taxifolia*. Acta Bot. Gallica 142, 131–135.
Wei, Y., Du, J., Lu, Y., 2012. Preparative separation of bioactive compounds from essential oil of *Flaveria bidentis* (L.) Kuntze using steam distillation extraction and one step high-speed counter-current chromatography. J. Sep. Sci. 35, 2608–2614.
Yuan, Y., Wang, B., Chen, L., Luo, H., Fisherd, D., Sutherland, I.A., Wei, Y., 2008. How to realize the linear scale-up process for rapid purification using high-performance counter-current chromatography. J. Chromatogr. A 1194, 192–198.