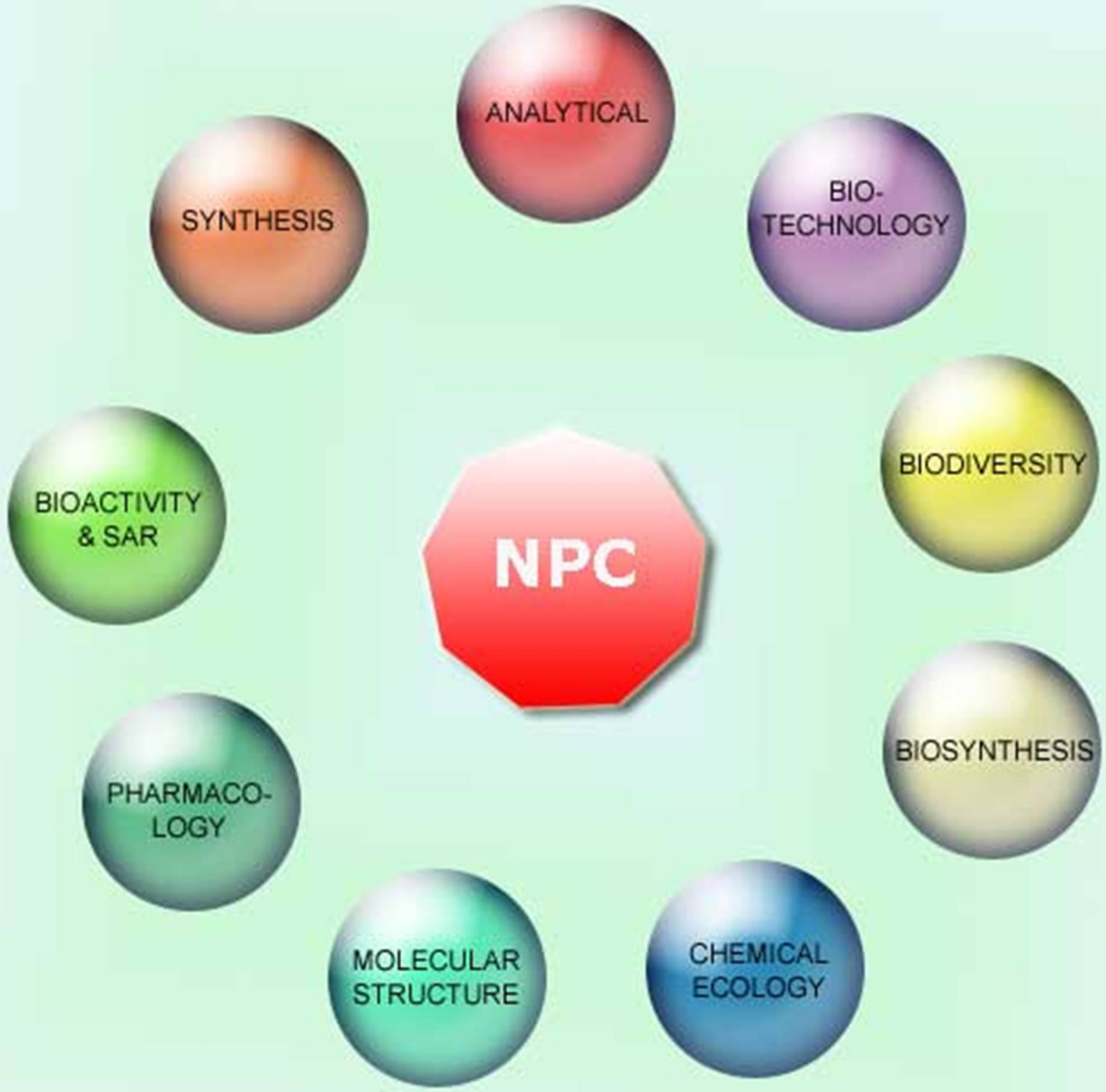
NATURAL PRODUCT COMMUNICATIONS

An International Journal for Communications and Reviews Covering all Aspects of Natural Products Research



Volume 7. Issue 1. Pages 1-142. 2012
ISSN 1934-578X (printed); ISSN 1555-9475 (online)
www.naturalproduct.us

NPC Natural Product Communications

INFORMATION FOR AUTHORS

Full details of how to submit a manuscript for publication in Natural Product Communications are given in Information for Authors on our Web site http://www.naturalproduct.us.

To Subscribe: Natural Product Communications is a journal published monthly. 2012 subscription price: US$1,995 (Print, ISSN# 1934-578X); US$1,995 (Web edition, ISSN# 1555-9475); US$2,495 (Print + single site online); US$595 (Personal online). Orders should be addressed to Subscription Department, Natural Product Communications, Natural Product Inc., 7963 Anderson Park Lane, Westerville, Ohio 43081, USA. Subscriptions are renewed on an annual basis. Claims for nonreceipt of issues will be honored if made within three months of publication of the issue. All issues are dispatched by airmail throughout the world, excluding the USA and Canada.

NPC Natural Product Communications

2012
Vol. 7
No. 1
47 - 50

Phytochemical Analysis and Antioxidant Capacity of BM-21, a Bioactive Extract Rich in Polyphenolic Metabolites from the Sea Grass *Thalassia testudinum*

Erik L. Regalado[a,*], Roberto Menendez[b], Olga Valdés[b], Ruth A. Morales[b], Abilio Laguna[b], Olivier P. Thomas[a], Yasnay Hernandez[b], Clara Nogueiras[c] and Anake Kijjoa[d]

[a]*Université de Nice-Sophia Antipolis, Laboratoire de Chimie des Molécules Bioactives et des Arômes, UMR 6001 CNRS, Institut de Chimie de Nice, Faculté des Science, Parc Valrose, 06108 Nice Cedex 02, France*

[b]*Center of Marine Bioproducts (CEBIMAR), Loma y 37, Alturas del Vedado, Havana, Cuba*

[c]*Center of Natural Products, Faculty of Chemistry, University of Havana, San Lázaro y L, Havana, Cuba*

[d]*ICBAS-Instituto de Ciências Biomédicas de Abel Salazar and CIIMAR, Universidade do Porto, 4099-003 Porto, Portugal*

erikluis18@gmail.com

Received: July 14th, 2011; Accepted: December 25th, 2011

The aqueous ethanol extract of *Thalassia testudinum* leaves (BM-21) is now being developed in Cuba as an herbal medicine due to its promising pharmacological properties. Although some interesting biological activities of BM-21 have already been reported, its chemical composition remains mostly unknown. Thus, we now describe the qualitative and quantitative analyzes of BM-21 using standard phytochemical screening techniques, including colorimetric quantification, TLC and HPLC analyses. Phytochemical investigation of BM-21 resulted in the isolation and identification of a new phenolic sulfate ester (**1**), along with ten previously described phenolic derivatives (**2-11**), seven of which have never been previously reported from the genus *Thalassia*. The structures of these compounds were established by analysis of their spectroscopic (1D and 2D NMR) and spectrometric (HRMS) data, as well as by comparison of these with those reported in the literature. Furthermore, BM-21 was found to exhibit strong antioxidant activity in four different free radical scavenging assays ($HO^{\bullet}$, $RO_2^{\bullet}$, $O_2^{-\bullet}$ and $DPPH^{\bullet}$). Consequently, this is the first study which highlights the phytochemical composition of BM-21 and demonstrates that this product is a rich source of natural antioxidants with potential applications in pharmaceutical, cosmetic and food industries.

Keywords: *Thalassia*, Hydrocharitaceae, BM-21, Phytochemical analysis, Phenolic compounds, Antioxidant, Nutraceuticals.

Sea grasses are a rich source of secondary metabolites, particularly simple, conjugated, and polymeric phenolic metabolites [1]. Phenolic compounds from sea grasses include sulfated flavonoids, a group of conjugated metabolites for which the sulfate component is believed to represent a marine adaptation. A new family of sulfated flavone glycosides, named thalassiolins A-C, was discovered from a *T. testudinum* specimen collected in the Bahamas Islands [2a,2b]. These compounds were proven to be inhibitors of HIV cDNA integrase [2b], and thalassiolin A, the most active of these molecules had been previously reported as a chemical defense for *T. testudinum* against fouling microorganisms [2a]. A high content of sulfated metabolites was recently detected in *T. testudinum* volatile oil, where ethyl (Z)-1-propenyl disulfide was the major component (31%) [3a]. More recently, a lipophilic fraction rich in benzene derivatives was found to exhibit strong *in vitro* scavenging activity against six free radicals; and its topical application strikingly reduced skin damage on mice exposed to acute UVB radiation and significantly attenuated the lipid peroxidation *in vivo* after acute exposure to UVB irradiation [3b].

The product of the aqueous ethanolic extract of *T. testudinum* leaves, named BM-21 [3c], has been found to exhibit significant pharmacological properties, such as: *in vitro* hepatoprotective effects against hepatotoxicity induced by *t*-butyl-hydroperoxide, ethanol and LPS in primary cultured rat hepatocytes [3d], anti-inflammatory properties [3e], and also promotes the recovery of irradiation damaged dermis and the normal properties of the epidermis. The bioassay guided fractionation of BM-21 resulted in the isolation of thalassiolin B, which showed antioxidant activity and markedly reduced the skin UVB-induced damage [3f]. More recently, BM-21 and thalassiolin B were found to exhibit an antinociceptive effect mediated by the inhibition of acid-sensing ionic channels (ASIC). Thalassiolin B was the first ASIC inhibitor of phenolic nature [3g]. However, the phytochemical composition of BM-21 has never been reported so far.

In this context, our present work has focused on the phytochemical study of BM-21, which includes its metabolomic qualitative and quantitative analysis by phytochemical screening, colorimetric quantification, TLC and HPLC analysis, as well as quantification of its heavy metals content by flame ionization detection. Using a combination of chromatographic techniques (flash chromatography, HPLC), 11 compounds were isolated from BM-21 and their structures were established by spectroscopic and spectrometric techniques (ESIMS, 1D and 2D NMR). Furthermore, the antioxidant capacity of BM-21 was also assessed by four well established *in vitro* free radical scavenging models.

Qualitative phytochemical analysis conducted on BM-21 using a combination of two standard phytochemical screening tests [4a,4b] revealed the presence of triterpene-steroids, tannins, phenols, flavonoids, proanthocyanins, saponins and reducing sugars. Among these, phenolic compounds were found to be the most abundant components. Curiously, the results from phytochemical quantifications (Table 1) of this extract showed it to have a higher total phenolic content (29.5 ± 1.2%) than that of the previous extract (18 ± 1.5%) obtained from a specimen of *T. testudinum* collected at "La Concha" beach (22° 05' 45'' N, 82° 27' 15'' W)

[3f]. Furthermore, metabolites of phenolic nature (flavonoids and proanthocyanidins) were detected in significant concentrations (4.6 ± 0.2 and 21.0 ± 2.3%, respectively) in this extract. Also, the proanthocyanidins (condensed tannins) content in this extract was found to be higher than those previous reported, which ranged from 10 to 150 mg tannin per g of tissue, dry mass (DM), in leaves and up to 250 mg tannin per g of DM in roots and rhizomes [1,5]. Additionally, other primary metabolites were quantified and results are shown in Table 1.

Table 1: Quantification of components of BM-21.

Metabolites	(% ± S.D.)*	Standard used
Total polyphenols	29.5 ± 1.2	Pyrogallol
Flavonoids	4.6 ± 0.2	Quercetin
Proanthocyanidins	21 ± 2.3	Malvidine glucoside
Polysaccharides	5.8 ± 1.6	β-D-Glucose
Lipids	0.59 ± 0.01	-
Soluble proteins	16.2 ± 0.7	BSA
Chlorophylls a and b	3.43 and 1.44	-

*Values are expressed as g per 100 g of the dry extract (%, w/w) except for cholorophylls (µg/mL), and all data are means±SD (n=3).

Fractionation of BM-21 by RP-C_{18} flash chromatography furnished a brown syrupy fraction, which was further purified by column chromatography. Subsequent purification by HPLC-DAD resulted in the isolation of eleven pure metabolites (**1-11**) and, after structure elucidation and literature search, compound **1** was found to be a new metabolite.

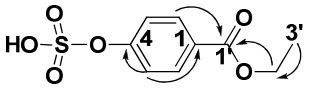


Figure 1: Key HMBC ($^1H \rightarrow ^{13}C$) correlations for compound **1**.

Compound **1** was isolated as a white amorphous solid with a molecular formula of $C_9H_{10}O_6S$, which was established by the HRESIMS (*m/z* 269.0096 $[M + Na]^+$). The ^1H NMR spectrum of **1** showed signals at δ 7.80 (2H, d, *J*=8.8 Hz, H-2) and 6.84 (2H, d, *J*=8.8 Hz, H-3), characteristic of a *para* disubstituted aromatic ring, as well as two signals at δ 4.24 (2H, q, *J*=7.2 Hz, H-2') and 1.29 (3H, t, *J*=7.1 Hz, H-3'), corresponding to an ethyl ester group. In the ^{13}C NMR spectrum, resonances of the disubstituted benzene ring at δ 161.9 (s), 131.3 (d), 120.5 (s), and 115.3 (d) were observed, as well as an ester group at δ 165.7 (s), and an ethyl ester at δ 60.0 (t) and 14.3 (q). HMBC (Figure 1) and HSQC correlations further led to the construction of the structure of **1**, which was consistent with a phenol sulfate ester derivative and fitted with the molecular formula. NMR data comparison with other analogues from marine sources confirmed this assumption [6].

On this basis, the structure of compound **1** was established as ethyl 4-(sulfooxy)benzoate. The biosynthesis of compound **1** could be postulated to arise directly from the sulfation and esterification of 4-hydroxybenzoic acid (**2**), a metabolite commonly found in plants and also detected in *T. testudinum.* Interestingly, 4-(sulfooxy) cinnamic acid, commonly known as zosteric acid was discovered from the seagrass *Zostera marina* and, together with other synthetic sulfate ester analogues, have offered promise for the development of environmentally benign antifouling agents [7]. Metabolites **2-11** (figure 1, supplementary data) were identified as 4-hydroxybenzoic acid (**2**), 4-hydroxybenzaldehyde (**3**), chrysoeriol-7-*O*-β-D-glucopyranosyl-2″-sulfate (thalassiolin B, **4**), apigenin 7-*O*-β-D-glucopyranosyl-2″-sulfate (thalassiolin C, **5**), chrysoeriol 7-*O*-β-D-glucopyranoside (**6**), apigenin 7-*O*-β-D-glucopyranoside (**7**), 5,7-dihydroxy-3',4'-dimethoxyflavone 7-*O*-β-D-glucopyranoside (**8**), luteolin-3'-sulfate (**9**), chrysoeriol (**10**) and apigenin (**11**) by a combination of spectroscopic methods (MS, 1D and 2D NMR) and comparison with literature data [2b,8a-8e]. Compounds **3** and **6-11** are herein reported for the first time in a *Thalassia* species. The RP-C_{18} analytical HPLC-DAD-ELSD profile of BM-21 clearly showed the abundant 21-min peak (figure 2, supplementary data), corresponding to thalassiolin B (**4**), and proved its high concentration in this product, which was consistent with the TLC analysis. LC-ESIMS analysis employing the same conditions showed the ion at m/z 541 ($[M-H]^-$) confirming the identity of this major metabolite. Previous quantification of thalassiolin B by using analytical-scale HPLC peak integration was equivalent to 5.8 ± 0.3% (w/w) of the crude extract [3g]. TLC analysis of BM-21 confirmed the results obtained from our phytochemical quantification (see details in supplementary data, figure 3). The high intensity and diameter of the orange spot corresponding to thalassiolin B (Rf = 0.35) clearly indicated that its concentration in BM-21 is relatively high when compared with other flavonoid derivatives.

Seagrasses are marine angiosperms that colonize seashore environments, and concern has arisen over increasing concentrations of metals in these systems. Sea grasses, being primary producers, may be utilized as the first level indicator for monitoring trace metal levels in the coastal marine environment. It has been established that seagrasses sequester trace metals from the marine environment via leaves, as well as root-rhizomes and these concentrations can be correlated with the water column and sediments, respectively [9a]. Concentrations of the heavy metals under study (Cd, Pb and Hg) were below the limits of acceptance established for these toxic metals [9b], meaning that BM-21 fulfills the requirements of an active product to be used in human consumption.

Table 2: Scavenging effects of BM-21 on "five" free radicals. Antioxidant effectiveness expressed as IC_{50} and values represent average of three determinations with ± standard deviation (S.D.). DMSO ($HO^•$), quercetin ($RO_2^•$ and $O_2^{-•}$) and ascorbic acid ($DPPH^•$) were used as standards.

Radical specie	Concentration range (µg/mL)	IC_{50} (µg/mL)	Maximum inhibitory effect (%)
	DMSO		
$HO^•$	20-0.2	7.5 ± 0.8	95.9 ± 1.8
	QUERCETIN		
$RO_2^•$	50-0.05	0.72 ± 0.09	92.3 ± 1,7
$O_2^{-•}$	100-0.05	2.46 ± 0.07	90.3 ± 2.5
	ASCORBIC ACID		
$DPPH^•$	100-0.5	45.0 ± 2.6	95.0 ± 2.7
	BM-21		
$HO^•$	4000-8	171.0 ± 2.8	85.6 ± 2.5
$RO2^•$	5000-15	131.0 ± 3.2	99.4 ± 0.4
$O_2^{-•}$	500-5.00	154.0 ± 3.0	81.4 ± 2.7
$DPPH^•$	850-40	161.0 ± 3.7	75.8 ± 2.4

Results concerning the antioxidant capacity of BM-21 and the standards used are shown in Table 2 and Figure 4 (supplementary data). In cell-free systems, BM-21 showed a dose-dependent scavenging effect on the $OH^•$ radical (IC_{50} = 171.0 ± 2.8 µg/mL) where the optimal activity (greater than 80%) was seen at doses of 2 mg/mL as no additional effect was observed by increasing the concentration of BM-21. It also showed a dose dependent elevation of the scavenging activity of the $RO_2^•$ radical (IC_{50} = 131.0 ± 3.2 µg/mL), up to a concentration of 1 mg/mL, where 99.4 ± 0.4% scavenging was detected, and no further increase of the effect was observed at higher concentrations. The extract also scavenged the $O_2^{-•}$ radical in a dose dependent manner (IC_{50} value of 154 µg/mL). Maximum scavenging capacity (81.4 ± 2.7%) occurred at 1 mg/mL

since no additional effect was observed at higher concentrations. Besides, BM-21 also scavenged DPPH$^{\bullet}$ dose-dependently with a maximal effect of 75.8 ± 2.4% and an IC_{50} value of 161.0 ± 3.7 µg/mL, which did not show significant difference from that previously reported for the same product obtained from *T. testudinum* collected at "La Concha" beach [3f]. On the other hand, the new compound, ethyl 4-(sulfooxy)benzoate (**1**) was assayed for DPPH radical trapping activity and was found not to scavenge this radical at concentrations >400 µg/mL.

Excessive amounts of ROS formation, including $OH^{\bullet}$, $O_2^{-\bullet}$ and $RO_2^{\bullet}$, are deleterious to various physiologically important molecules [10]. Thus, the ability of BM-21 to quench these natural formed radical species may prevent initiation and propagation of lipid peroxidation thus reducing its deleterious effects on living cells. Therefore, it is possible that the cytoprotective capacities previously described for BM-21 [3d,3f] may be attributed, at least in part, to its antioxidant properties. A previous work has demonstrated that thalassiolin B (**4**), the major component isolated from BM-21, exhibited a DPPH trapping effect [3f]. Other minor flavones identified as components of BM-21, such as chrysoeriol (**10**) and apigenin (**11**), are known to exhibit a broad spectrum of pharmacological properties, including free radical scavenging effects [8a,11]. In addition, flavone glycosides: chrysoeriol 7-*O*-β-D-glucopyranoside (**6**), apigenin 7-*O*-β-D-glucopyranoside (**7**) and 5,7-dihydroxy-3',4'-dimethoxyflavone 7-*O*-β-D-glucopyranoside (**8**) also possess strong antioxidant capacities [12a-12c]. Thus, the multiple free radical capacity of BM-21 may be justified in a great part by its high content of diversified molecular scaffolds of phenolic nature.

Experimental

General: UV measurements were obtained on a Shimadzu UV-1201 spectrophotometer. Aluminum sheets (4.5×10 cm), coated with silica gel 60 F_{254}, were used for analytical TLC, and compounds were visualized under UV light with a Vilber Lourmat lamp and subsequently detected after spraying with chemical reagents and heating. HPLC analyzes were carried out on a Waters 600 system equipped with a Waters 717 plus autosampler, and a Waters 996 photodiode array detector coupled with a Sedex 55 evaporative light-scattering detector (Sedere, France). NMR experiments were performed on a Bruker Avance 500 MHz spectrometer. Chemical shifts (δ in ppm) are referenced to the carbon (δ_C 39.52) and residual proton (δ_H 2.50) signals of $(CD_3)_2SO$. Low resolution electrospray ionization (ESI) MS were recorded on a Bruker Esquire 3000 Plus spectrometer in the positive or negative mode. HRESIMS were conducted on a LTQ Orbitrap mass spectrometer (Thermo Finnigan).

Plant material: *Thalassia testudinum* Banks and Soland ex. Koenig was collected in April 2009 from "Guanabo" Beach (22° 05' 45'' N, 82° 27' 15'' W) and identified by Dr Areces J.A. (Institute of Oceanology, La Havana, Cuba). A voucher sample (No. IdO 039) has been deposited in the herbarium of the Cuban National Aquarium.

Phytochemical studies: Screening for major constituents was undertaken using two qualitative methods [4a,4b]. Quantification of metabolite families was performed by standard phytochemical reaction methods using UV detection: total polyphenols [9b], flavonoids [13a], total proanthocyanidins [13b], and total carbohydrates by phenol-sulfuric methods [13c], total lipids by solvent extraction [13d] and their quantification by colorimetric reaction with potassium dichromate in acid media [13e], chlorophylls [13f] and soluble proteins [13g]. Flavonoid screening was carried out by TLC using two mobile phases constituted of $CHCl_3$:MeOH:H_2O (70:30:2.5) (**A**) and $CHCl_3$:MeOH (70:40) (**B**). The developed chromatogram was observed under short wave UV light (254 nm) and in long wave UV light (365 nm) after spraying with NP-PEG solution (1% methanolic diphenylboryloxyethyl-amine and 5% ethanolic polyethyleneglycol 4000). Typical intense fluorescence in UV light at λ = 365 nm was produced immediately on spraying (flavonoids appeared as orange-yellow spots, whereas phenolic acids formed blue fluorescent zones) [13h]. Thalassiolin B, the major compound in BM-21, and pyrogallol were used as references. Flavonoids were identified as orange and yellow fluorescent spots.

Extraction and isolation: Dried and ground leaves of *Thalassia testudinum* (2.3 kg) were extracted with 30 L of EtOH/H_2O (50:50) (3 x10 L) at room temperature. The combined aqueous ethanol solutions were filtered, concentrated under reduced pressure and dried by sprinkler to yield 170 g of extract (BM-21), which was fractionated by RP-C_{18} flash chromatography {elution with H_2O (100%), H_2O/MeOH (80:20) and MeOH (100%)}. The 80:20 H_2O/MeOH fraction (12 g) was subjected to DIOL CC and eluted with *n*-butanol/H_2O/acetic acid (90:7:1). The final fractions (1.5 g) were combined and further separated by RP-C_{18} semipreparative HPLC-DAD (SymmetryPrepth C18, 7.8 x 300 mm, 7 µm) equipped with a UV detector set at 254 and 280 nm, and using a linear gradient of H_2O/MeOH/TFA (flow rate: 3 mL/min from 80:20:0.1 to 0:100:0.1 in 35 min). The subsequent mixtures were finally purified by RP-C_{18} analytical HPLC-DAD (Phenomenex Luna C_{18}, 150 x 4.6 mm, 5 µm, 1.0 mL/min) to provide 11 pure metabolites: **1** (3.0 mg), **2** (8 mg), **3** (3.4 mg), **4** (150 mg), **5** (15 mg), **6** (4.8 mg), **7** (1.6 mg), **8** (3.0 mg), **9** (4.8 mg), **10** (2.7 mg) and **11** (1.2 mg). UV detections were set at 254 and 280 nm.

Ethyl 4-(sulfooxy)benzoate (**1**)
White amorphous powder
^{1}H NMR (500 MHz, DMSO-d_6): 7.80 (2H, d, *J* = 8.8 Hz, H-2), 6.84 (2H, d, *J* = 8.8 Hz, H-3), 4.24 (2H, q, *J* = 7.2 Hz, H-2'), 1.29 (3H, t, *J* = 7.1 Hz, H-3').
^{13}C NMR (125 MHz, DMSO-d_6) δ: 165.7 (C-1'), 161.9 (C-4), 131.3 (C-2), 120.5 (C-1), 115.3 (C-3), 60.0 (C-2'), 14.3 (C-3').
HRESIMS (+) *m*/*z* 269.0096 $[M + Na]^+$ (calcd for $C_9H_{10}NaO_6S$, 269.0084, Δ 0.86 ppm).

Antioxidant activity *(more details in the supplementary data)*

Assay of hydroxyl radical (HO$^{\bullet}$) scavenging activity: The experiments were performed according to the modified method of Aruoma [14a]. Deoxyribose damage was assessed by determining thiobarbituric acid reactive substances (TBARS) according to [14b]. *Assay of peroxyl radical ($RO_2^{\bullet}$) scavenging effects:* The assay was performed according to a modification of the method described by [14c]. The degree of ABAP-mediated oxidation was measured by TBARS assay and protein concentration was estimated by a modification of the Lowry procedure [14d]. *Assay of superoxide anion ($O_2^{\bullet-}$) scavenging activity:* This test was assessed according to [14e]. *Assay of 2,2-diphenyl-2-picrylhydrazyl (DPPH$^{\bullet}$) scavenging activity*: The experiments were carried out according to the method previously described [14f], with minor modifications.

Quantification of heavy metals: Metal extraction was carried out by digesting 0.5 g of BM-21 in 10 mL of concentrated HNO_3 in Teflon® bombs in a microwave oven. The extracts were made up to 25 mL with distilled water and, later on, samples were diluted 50 times more. Concentrations of Cd, Pb and Hg were determined by flame atomic absorption spectrophotometry (AAS) using a Perkin-

Elmer 2100 spectrophotometer with background deuterium correction. Quality control was carried out by parallel analysis of certified reference material.

Supplementary data: Details on the antioxidant assays, structures of **1-11**, HPLC-DAD-ELSD profile, TLC analysis of BM-21, dose-response curve for the antioxidant assays. ^{1}H, ^{13}C, HSQC and HMBC NMR spectra for compound **1** are also available.

Acknowledgments - We are grateful to the European Molecular Biology Organization (EMBO) for financial support provided by a grant to Erik L. Regalado (Ref: ASTF 50.00-2010).

References

[1] Arnold TM, Tanner CE, Rothen M, Bullington J. (**2008**) Wound-induced accumulations of condensed tannins in turtlegrass, *Thalassia testudinum*. *Aquatic Botany*, ***89***, 27-33.

[2] (a) Jensen PR, Jenkins KM, Porter D, Fenical W. (**1998**) Evidence that a new antibiotic flavone glycoside chemically defends the sea grass *Thalassia testudinum* against zoosporic fungi. *Applied and Environmental Microbiology*, ***64***, 1490-1496. (b) Rowley DC, Hansen MS, Rhodes D, Sotrifer CA, Ni H, Mccammon JA, Bushman FD, Fenical W. (**2002**) Thalassiolins A-C: new marine derived inhibitors of HIV cDNA integrase. *Bioorganic and Medicinal Chemistry*, ***10***, 3619-3625.

[3] (a) Pino JA, Regalado EL. (**2010**) Volatile constituents of *Thalassia testudinum* Banks ex König leaves. *Journal of Essential Oil Research*, ***22***, 421-423; (b) Regalado EL, Rodríguez M, Fernandez X, Menéndez R, Hernández I, Morales RA, Fernández MD, Thomas OP, Pino JA, Concepción ÁA, Laguna A. (**2011**) Photoprotecting action and phytochemical analysis of a multiple radical scavenger lipophilic fraction obtained from the leaf of the seagrass *Thalassia testudinum*. *Photochemistry and Photobiology*, ***87***, 1058-1066; (c) Rodríguez M, Laguna A, Regalado EL, Menéndez R, Garateix A, Concepción ÁA. (**2010**) Procedimiento de obtención de un producto de origen marino procedente de la planta marina *Thalassia testudinum*. Cuban Patent 23607; (d) Rodeiro I, Donato MT, Martínez I, Hernández I, Garrido G, González-Lavaut JA, Menéndez R, Laguna A, Castell JV, Gómez-Lechón MJ. (**2008**) Potencial hepatoprotective effects of new Cuban natural products in rat hepatocytes culture. *Toxicology In Vitro*, ***22***, 1242-1249; (e) Nuñez R, Garateix A, Laguna A, Fernández MD, Ortiz E, LLanio M, Valdés O, Rodríguez AA, Menéndez R. (**2006**) Caribbean marine biodiversity as a source of new compounds of biomedical and others industrial applications. *Pharmacologyonline*, ***3***, 111-119; (f) Regalado EL, Rodríguez M, Menéndez R, Concepción ÁA, Nogueiras C, Laguna A, Rodríguez AA, Williams DE, Lorenzo-Luaces P, Valdés O, Hernandez Y. (**2009**) Repair of UVB-damaged skin by the antioxidant sulphated flavone glycoside thalassiolin B isolated from the marine plant *Thalassia testudinum* Banks ex König. *Marine Biotechnology*, ***11***, 74-80; (g) Garateix A, Salceda E, Menéndez R, Regalado EL, López O, García T, Morales RA, Laguna A, Thomas OP, Soto E. (**2011**) Antinociception produced by *Thalassia testudinum* extract BM-21 is mediated by the inhibition of acid-sensing ionic channels by the phenolic compound thalassiolin B. *Molecular Pain*, ***7***, 10.

[4] (a) Rondina RV, Coussio JD. (**1969**) Estudio fitoquímico de plantas medicinales argentinas. *Revista de Investigaciones Agropecuarias, INTA, Serien 2, Biologia y Producción Vegetal, Buenos Aires, Argentina*, ***VI***; (b) Schabra SC, Ulso M, Mshin EN. (**1984**) Phytochemical screening of Tanzanian medical plants. *Journal of Ethnopharmacology*, ***11***, 157-159.

[5] Arnold TM, Targett NM. (**2002**) Marine tannins: the importance of a mechanistic framework for predicting ecological roles. *Journal of Chemical Ecology*, ***28***, 1919-34.

[6] Todd JS, Zimmerman RC, Crews P, Alberte RS. (**1993**) The antifouling activity of natural and synthetic phenol acid sulphate esters. *Phytochemistry*, ***34***, 401-414.

[7] Zimmerman RC, Alberte RS, Todd JS, Crews P. (**1997**) Phenolic acid sulfate esters for prevention of marine biofouling. U.S. Patent 5,607,741.

[8] (a) Choi D-Y, Lee JY, Kim M-R, Woo E-R, Kim YG, Kang KW. (**2005**) Chrysoeriol potently inhibits the induction of nitric oxide synthase by blocking AP-1 activation. *Journal of Biomedical Science*, ***12***, 949-959; (b) Markham KR, Geiger H. (**1994**) The Flavonoids: Advances in Research Since 1986. Chapman & Hall, London; (c) Agrawal PK (**1989**) *Carbon-13 NMR of Flavonoids*. Elsevier, Amsterdam; (d) Kim JH, Cho YH, Park SM, Lee KE, Lee JJ, Lee BS, Pyo HB, Song KS, Park HD, Yun YP. (**2004**) Antioxidants and inhibitor of matrix metalloproteinase-1 expression from leaves of *Zostera marina* L. *Archives of Pharmacal Research*, ***27***, 177-183; (e) Barbosa WLR, dos Santos WRA, Pinto LN, Tavares ICC. (**2002**) Flavonóides de *Cissus verticillata* e a atividade hipoglicemiante do chá de suas folhas. *Revista Brasileira de Farmacognosia*, ***12***, 13-15.

[9] (a) Prange JA, Dennison WC. (**2000**) Physiological responses of five seagrass species to trace metals marine. *Pollution Bulletin*, ***41***, 327-336; (b) British Pharmacopoeia B. (**2009**) Herbal Drugs and Herbal Drug Preparation Kelp, Volume III. The Stationary Office, London.

[10] Stadtman ER. (**1992**) Protein oxidation and aging. *Science*, ***257***, 1220-1224.

[11] Liu R, Zhang T, Yang H, Lan X, Ying J, Du G. (**2011**) The flavonoid apigenin protects brain neurovascular coupling against amyloid-&beta25-35-induced toxicity in mice. *Journal of Alzheimer's Disease*, ***24***, 85-100.

[12] (a) Mooi JLY, Lajis NH, Ali AM, Sukari MA, Rahman AA, Othman AG, Kikuzaki H, Nakatani N. (**2003**) Antioxidant and antitumor promoting activities of the flavonoids from *Hedychium thyrsiforme*. *Pharmaceutical Biology*, ***41***, 506-511; (b) Ma C, Wang W, Chen Y-Y, Liu R-N, Wang R-F, Du L-J. (**2005**) Neuroprotective and antioxidant activity of compounds from the aerial parts of *Dioscorea opposita*. *Journal of Natural Products*, ***68***, 1259-1261; (c) Nazemiyeh H, Bahadori F, Delazar A, Ay M, Topçu G, Nahar L, Majinda RR, Sarker SD. (**2008**) Antioxidant phenolic compounds from the leaves of *Erica arborea* (Ericaceae). *Natural Product Research*, ***22***, 1385-1392.

[13] (a) Aiyegoro OA, Okoh AI. (**2010**) Preliminary phytochemical screening and *in vitro* antioxidant activities of the aqueous extract of *Helichrysum longifolium* DC. *BMC Complementary and Alternative Medicine*, ***10***, 21; (b) Fuleki T, Francis FJ. (**1968**) Quantitative methods for anthocyanins. *Journal of Food Science*, ***33***, 78-83; (c) Dubois M, Gilles KA, Hamilton JK, Rebers PA, Smith F. (**1956**) Colorimetric method for determination of sugars and related substances. *Analytical Biochemistry*, ***28***, 350-356; (d) Bligh EG, Dyer WJ. (**1959**) A rapid method of total lipid extraction and purification. *Canadian Journal of Biochemistry and Physiology*, ***37***, 911-917; (e) Craigie JS, Leigh C. (**1978**) Carrageenan and agars. In *Handbook of Phycological Method.*. Por Hellebust JA, Craigie JS. (Eds) Cambridge University Press. Cambridge, pp. 109-31; (f) Wrolstad R, Acree T, Decker E, Penner Reid D, Schwartz S, Shoemaker C, Smith D, Sporns P. (**2005**) *Handbook of Food Analytical Chemistry*. John Wiley & Sons, Hoboken; (g) Bradford MM. (**1976**) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry*, ***72***, 248-54; (h) Wagner H, Bladt S. (Eds) (**1996**) Plant Drug Analysis. Springer, Berlin.

[14] (a) Aruoma OI. (**1994**) Deoxyribose assay for detecting hydroxyl radicals. *Methods in Enzymology*, ***233***, 57-66; (b) Ohkawa H, Ohishi N, Yagi K. (**1979**) Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. *Analytical Biochemistry*, ***95***, 351-358; (c) Yu-Jun C, F. J-G, Lan-Ping M, Li Y, Zhong-Li L. (**2003**) Inhibition of free radical-induced peroxidation of rat liver microsomes by resveratrol and its analogues. *Biochimica et Biophysica Acta*, ***1637***, 31-38; (d) Markwell MA, Haas SM, Beiber LL, Tolbert NE. (**1978**) A modification of the Lowry procedure to simplify protein determination in membrane lipoprotein samples. *Analytical Biochemistry*, ***87***, 206-210; (e) Marklund S, Marklund G. (**1974**) Involvement of superoxide anion radical in the antioxidation of pyrogallol and a convenient assay for superoxide dismutase. *European Journal of Biochemistry*, ***47***, 469-474; (f) Brand-Williams W, Cuvelier ME, Berset C. (**1995**) Use of a free radical method to evaluate antioxidant activity. *Food Science and Technology*, ***28***, 25-30.

Natural Product Communications
2012
Volume 7, Number 1

Contents

Continued inside backcover