

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

EDITOR-IN-CHIEF**DR. PAWAN K AGRAWAL**

Natural Product Inc.
7963, Anderson Park Lane,
Westerville, Ohio 43081, USA
agrawal@naturalproduct.us

EDITORS**PROFESSOR ALEJANDRO F. BARRERO**

Department of Organic Chemistry,
University of Granada,
Campus de Fuente Nueva, s/n, 18071, Granada, Spain
afbarre@ugr.es

PROFESSOR ALESSANDRA BRACA

Dipartimento di Chimica Bioorganica e Biofarmacia,
Universita di Pisa,
via Bonanno 33, 56126 Pisa, Italy
braca@farm.unipi.it

PROFESSOR DEAN GUO

State Key Laboratory of Natural and Biomimetic Drugs,
School of Pharmaceutical Sciences,
Peking University,
Beijing 100083, China
gda5958@163.com

PROFESSOR YOSHIHIRO MIMAKI

School of Pharmacy,
Tokyo University of Pharmacy and Life Sciences,
Horinouchi 1432-1, Hachioji, Tokyo 192-0392, Japan
mimaki@ps.toyaku.ac.jp

PROFESSOR STEPHEN G. PYNE

Department of Chemistry
University of Wollongong
Wollongong, New South Wales, 2522, Australia
spyne@uow.edu.au

PROFESSOR MANFRED G. REINECKE

Department of Chemistry,
Texas Christian University,
Forts Worth, TX 76129, USA
m.reinecke@tcu.edu

PROFESSOR WILLIAM N. SETZER

Department of Chemistry
The University of Alabama in Huntsville
Huntsville, AL 35809, USA
wssetzer@chemistry.uah.edu

PROFESSOR YASUHIRO TEZUKA

Institute of Natural Medicine
Institute of Natural Medicine, University of Toyama,
2630-Sugitani, Toyama 930-0194, Japan
tezuka@inm.u-toyama.ac.jp

PROFESSOR DAVID E. THURSTON

Department of Pharmaceutical and Biological Chemistry,
The School of Pharmacy,
University of London, 29-39 Brunswick Square,
London WC1N 1AX, UK
david.thurston@pharmacy.ac.uk

HONORARY EDITOR**PROFESSOR GERALD BLUNDEN**

The School of Pharmacy & Biomedical Sciences,
University of Portsmouth,
Portsmouth, PO1 2DT U.K.
axuf64@dsl.pipex.com

ADVISORY BOARD

Prof. Berhanu M. Abegaz
Gaborone, Botswana

Prof. Viqar Uddin Ahmad
Karachi, Pakistan

Prof. Øyvind M. Andersen
Bergen, Norway

Prof. Giovanni Appendino
Novara, Italy

Prof. Yoshinori Asakawa
Tokushima, Japan

Prof. Lee Banting
Portsmouth, U.K.

Prof. Julie Banerji
Kolkata, India

Prof. Anna R. Bilia
Florence, Italy

Prof. Maurizio Bruno
Palermo, Italy

Prof. César A. N. Catalán
Tucumán, Argentina

Prof. Josep Coll
Barcelona, Spain

Prof. Geoffrey Cordell
Chicago, IL, USA

Prof. Ana Cristina Figueiredo
Lisbon, Portugal

Prof. Cristina Gracia-Viguera
Murcia, Spain

Prof. Duvvuru Gunasekar
Tirupati, India

Prof. Kurt Hostettmann
Lausanne, Switzerland

Prof. Martin A. Iglesias Arteaga
Mexico, D. F., Mexico

Prof. Jerzy Jaroszewski
Copenhagen, Denmark

Prof. Leopold Jirovetz
Vienna, Austria

Prof. Karsten Krohn
Paderborn, Germany

Prof. Hartmut Laatsch
Gottingen, Germany

Prof. Marie Lacaille-Dubois
Dijon, France

Prof. Shoei-Sheng Lee
Taipei, Taiwan

Prof. Francisco Macias
Cadiz, Spain

Prof. Imre Mathe
Szeged, Hungary

Prof. Joseph Michael
Johannesburg, South Africa

Prof. Ermino Murano
Trieste, Italy

Prof. M. Soledade C. Pedras
Saskatoon, Canada

Prof. Luc Pieters
Antwerp, Belgium

Prof. Peter Proksch
Düsseldorf, Germany

Prof. Phila Raharivelomanana
Tahiti, French Polynesia

Prof. Luca Rastrelli
Fisciano, Italy

Prof. Monique Simmonds
Richmond, UK

Prof. John L. Sorensen
Manitoba, Canada

Prof. Valentin Stonik
Vladivostok, Russia

Prof. Winston F. Tinto
Barbados, West Indies

Prof. Sylvia Urban
Melbourne, Australia

Prof. Karen Valant-Vetschera
Vienna, Austria

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>.

Authors may reproduce/republish portions of their published contribution without seeking permission from NPC, provided that any such republication is accompanied by an acknowledgment (original citation)-Reproduced by permission of Natural Product Communications. Any unauthorized reproduction, transmission or storage may result in either civil or criminal liability.

The publication of each of the articles contained herein is protected by copyright. Except as allowed under national "fair use" laws, copying is not permitted by any means or for any purpose, such as for distribution to any third party (whether by sale, loan, gift, or otherwise); as agent (express or implied) of any third party; for purposes of advertising or promotion; or to create collective or derivative works. Such permission requests, or other inquiries, should be addressed to the Natural Product Inc. (NPI). A photocopy license is available from the NPI for institutional subscribers that need to make multiple copies of single articles for internal study or research purposes.

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.

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

^aUniversité 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

^bCenter of Marine Bioproducts (CEBIMAR), Loma y 37, Alturas del Vedado, Havana, Cuba

^cCenter of Natural Products, Faculty of Chemistry, University of Havana, San Lázaro y L, Havana, Cuba

^dICBAS-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[•], RO₂[•], O₂[•] and DPPH[•]). 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 $21.0 \pm 2.3\%$, respectively) in this extract. Also, the proanthocyanidins (condensed tannins) content in this extract was found to be higher than those previously 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	(% \pm 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 chlorophylls ($\mu\text{g/mL}$), and all data are means \pm SD (n=3).

Fractionation of BM-21 by RP-C₁₈ 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.

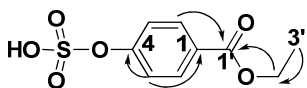


Figure 1: Key HMBC (¹H \rightarrow ¹³C) correlations for compound **1**.

Compound **1** was isolated as a white amorphous solid with a molecular formula of C₉H₁₀O₆S, 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 ¹³C 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-C₁₈ 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 $5.8 \pm 0.3\%$ (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 ($R_f = 0.35$) 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 IC₅₀ and values represent average of three determinations with \pm standard deviation (S.D.). DMSO (HO[•]), quercetin (RO₂[•] and O₂[•]) and ascorbic acid (DPPH[•]) were used as standards.

Radical specie	Concentration range ($\mu\text{g/mL}$)	IC ₅₀ ($\mu\text{g/mL}$)	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
RO ₂ [•]	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 (IC₅₀ = 171.0 ± 2.8 $\mu\text{g/mL}$) 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 RO₂[•] radical (IC₅₀ = 131.0 ± 3.2 $\mu\text{g/mL}$), up to a concentration of 1 mg/mL, where 99.4 \pm 0.4% scavenging was detected, and no further increase of the effect was observed at higher concentrations. The extract also scavenged the O₂[•] radical in a dose dependent manner (IC₅₀ value of 154 $\mu\text{g/mL}$). Maximum scavenging capacity (81.4 \pm 2.7%) occurred at 1 mg/mL

since no additional effect was observed at higher concentrations. Besides, BM-21 also scavenged DPPH• dose-dependently with a maximal effect of $75.8 \pm 2.4\%$ and an IC_{50} value of 161.0 ± 3.7 $\mu\text{g/mL}$, 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 $\mu\text{g/mL}$.

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 F₂₅₄, 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 $(\text{CD}_3)_2\text{SO}$. 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:H₂O (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 diphenylboryloxyethylamine and 5% ethanolic polyethyleneglycol 4000). Typical intense fluorescence in UV light at $\lambda = 365$ 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/H₂O (50:50) (3 x 10 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-C₁₈ flash chromatography {elution with H₂O (100%), H₂O/MeOH (80:20) and MeOH (100%)}. The 80:20 H₂O/MeOH fraction (12 g) was subjected to DIOL CC and eluted with *n*-butanol/H₂O/acetic acid (90:7:1). The final fractions (1.5 g) were combined and further separated by RP-C₁₈ 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 H₂O/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-C₁₈ analytical HPLC-DAD (Phenomenex Luna C₁₈, 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

¹H 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').

¹³C 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 C₉H₁₀NaO₆S, 269.0084, Δ 0.86 ppm).

Antioxidant activity (more details in the supplementary data)

Assay of hydroxyl radical (HO•) 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 peroxy radical (RO₂•) 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 (O₂•⁻) scavenging activity:** This test was assessed according to [14e]. **Assay of 2,2-diphenyl-2-picrylhydrazyl (DPPH•) 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 HNO₃ 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. ^1H , ^{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 zoospore 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, Fernández X, Menéndez R, Hernández I, Morales RA, Fernández MD, Thomas OP, Pino JA, Concepción AA, 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 AA. (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 AA, 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, Biología y Producción Vegetal, Buenos Aires, Argentina*, **VI**; (b) Schabra SC, Ulso M, Mshin EN. (1984) Phytochemical screening of Tanzanian medicinal 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- β 25-35-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 thyriforme*. *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 autoxidation 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.

Ternary Liquid-Liquid Equilibria Measurement for Epoxidized Soybean Oil + Acetic Acid + Water Shuang-Fei Cai, Li-Sheng Wang, Guo-Qing Yan, Yi Li, Yun-Xia Feng and Rong-Gang Linghu	75
Chemical Constituents of the Essential Oil from Aerial Parts and Fruit of <i>Anisosciadium orientale</i> Vahid Rowshan, Ahmad Hatami, Atefeh Bahmanzadegan and Mahnaz Yazdani	79
GC-MS Analysis of <i>Ziziphora clinopodioides</i> Essential Oil from North Xinjiang, China Xiaoying Zhou, Qian Yu, Haiyan Gong and Shuge Tian	81
Analysis of the Essential Oil of <i>Teucrium polium</i> ssp. <i>capitatum</i> from the Balkan Peninsula Violeta Mitić, Olga Jovanović, Vesna Stankov-Jovanović, Bojan Zlatkovic and Gordana Stojanovic	83
Composition of the Essential Oil of <i>Pogostemon travancoricus</i> var. <i>travancoricus</i> Ramar Murugan and Gopal Rao Mallavarapu	87
Liquid CO₂ Extraction of <i>Jasminum grandiflorum</i> and Comparison with Conventional Processes Om Prakash, Deeptanjali Sahoo and Prasant Kumar Rout	89
Chemical Composition of Volatile Oils from the Pericarps of Indian Sandalwood (<i>Santalum album</i>) by Different Extraction Methods Xin Hua Zhang, Jaime A. Teixeira da Silva, Yong Xia Jia, Jie Tang Zhao and Guo Hua Ma	93
Fast Quality Assessment of German Chamomile (<i>Matricaria chamomilla</i> L.) by Headspace Solid-Phase Microextraction: Influence of Flower Development Stage Mohammad Rafieiohossaini, An Adams, Hamid Sodaeizadeh, Patrick Van Damme and Norbert De Kimpe	97
Floral Scent Composition of <i>Plumeria tuberculata</i> Analyzed by HS-SPME Disnelys Báez, Jorge A. Pino and Diego Morales	101
Identification and Quantification of the Antimicrobial Components of a Citrus Essential Oil Vapor Carol A. Phillips, Konstantinos Gkatzionis, Katie Laird, Jodie Score, Avinash Kant and Mark D. Fielder	103
Composition, Antioxidant and Antimicrobial Activities of the Leaf Essential Oil of <i>Machilus japonica</i> from Taiwan Chen-Lung Ho and Yu-Chang Su	109
Chemical Composition, Antimicrobial, Antiradical and Anticholinesterase activity of the Essential Oil of <i>Pulicaria stephanocarpa</i> from Soqatra Nasser A. Awadh Ali, Rebecca A. Crouch, Mohamed A. Al-Fatimi, Norbert Arnold, Axel Teichert, William N. Setzer and Ludger Wessjohann	113
Composition of the Essential Oils and Antibacterial Activities of <i>Hymenocrater yazdianus</i>, <i>Stachys obtusirena</i> and <i>Nepeta asterotricha</i> Three Labiatae Herbs Growing Wild in Iran Shiva Masoudi, Abdolhossein Rustaiyan, Raziieh Mohebat and Mohammad Hossein Mosslemin	117
Antibacterial Activities of Essential Oils Extracted from Leaves of <i>Murraya koenigii</i> by Solvent-Free Microwave Extraction and Hydro-Distillation Naciye Erkan, Zhou Tao, H. P. Vasantha Rupasinghe, Burcu Uysal and Birsen S. Oksal	121
Chemical Composition, Antifungal and Herbicidal Effects of Essential oil Isolated from <i>Chersodoma argentina</i> (Asteraceae) Rosana Alarcón, Soledad Ocampos, Adriana Pacciaroni and Virginia Sosa	125
Essential Oil Composition and Acaricidal Activity of <i>Schinus terebinthifolius</i> from Atlantic Forest of Pernambuco, Brazil against <i>Tetranychus urticae</i> Aline Fonseca do Nascimento, Claudio Augusto Gomes da Camara, Marcilio Martins de Moraes and Clécio Souza Ramos	129
Evaluation of the Anti-<i>Leishmania major</i> Activity of <i>Satureja bahhtiarica</i> Essential Oil in vitro Ghasem Mohammadpour, Eisa Tahmasbpour Marzony and Mahin Farahmand	133
<i>Spartium junceum</i> Aromatic Water: Chemical Composition and Antitumor activity Teresa Cerchiara, Serafina V. Straface, Giuseppe Chidichimo, Emilia L. Belsito, Angelo Liguori, Barbara Luppi, Federica Bigucci and Vittorio Zecchi	137

Natural Product Communications

2012

Volume 7, Number 1

Contents

<u>Original Paper</u>	<u>Page</u>
New Iridoid from Aerial Parts of <i>Mussaenda roxburghii</i> Utpal Chandra De, Ranjit Ghosh, Sanjib Chowdhury and Biswanath Dinda	1
A New Megastigmane Glycoside, Phoenixoside A, from <i>Phoenix dactylifera</i> Sumbul Azmat, Aqib Zahoor, Rehana Ifzal, Viqar Uddin Ahmad and Faryal Vali Mohammed	3
New 3,4-Seco-ent-kaurene Dimers from <i>Croton micans</i> Elsa Mateu, Katuska Chavez, Ricarda Riina, Reinaldo S. Compagnone, Franco Delle Monache and Alírica I. Suárez	5
Diacarperoxide S, New Norterpene Cyclic Peroxide from the Sponge <i>Diacarnus megaspinorhabdosa</i> Sabrin R. M. Ibrahim	9
Constituents of Kenyan <i>Gardenia volkensii</i> Esther W. Kinuthia, Moses K. Langat, Elizabeth M. Mwangi and Peter K. Cheplogoi	13
Oral Administration of <i>Cimicifuga racemosa</i> Extract Attenuates Immobilization Stress-Induced Reactions Isao Nadaoka, Kazuki Watanabe, Masaaki Yasue, Manabu Sami, Yasushi Kitagawa and Yoshihiro Mimaki	15
Complete NMR Assignments of Tubulosine Venkata Siva Satyanarayana Kantamreddi and Colin W. Wright	19
Application of Mixture Analysis to Crude Materials from Natural Resources (III)^{III}: NMR Spectral Studies to Analyze Chalcones from <i>Angelica keiskei</i> Eriko Fukuda, Masaki Baba, Yoshihiro Uesawa, Osamu Kamo, Kazunori Arifuku, Koji Tsubono and Yoshihito Okada	21
A Validated Chromatographic Method for the Determination of Flavonoids in <i>Copaifera langsdorffii</i> by HPLC João Paulo B. de Sousa, Ana Paula S. Brancalion, Milton G. Júnior and Jairo K. Bastos	25
Luteolin Induces Mitochondria-dependent Apoptosis in Human Lung Adenocarcinoma Cell Qing Chen, Shengming Liu, Jinghong Chen, Qianqian Zhang, Shijie Lin, Zhiming Chen and Jianwei Jiang	29
Diuretic Activity of <i>Lophophytum leandri</i> Antonio Bracci, Anibal G. Amat, Francesco Maione, Carla Cicala, Nicola Mascolo and Vincenzo De Feo	33
Evaluation of the Hypocholesterolemic Effect and Phytochemical Screening of the Hydroethanolic Extract of <i>Crataegus aronia</i> from Jordan Entisar K. Al-Hallaq, Fatma U. Afifi and Shtaywy S. Abdalla	35
Secondary Metabolites, Cytotoxic Response by Neutral Red Retention and Protective Effect Against H₂O₂ Induced Cytotoxicity of <i>Sedum caespitosum</i> Didem Şöhretoğlu and Suna Sabuncuoğlu	39
Effect of <i>Hibiscus sabdariffa</i> and its Anthocyanins on some Reproductive Aspects in Rats Badredin H. Ali, Intisar Al-Lawati, Sumyia Beegam, Amal Ziada, Suhail Al salam, Abderrahim Nemmar and Gerald Blunden	41
New Galloyl Derivative from Winged Sumac (<i>Rhus copallinum</i>) Fruit Hang Ma, Tao Yuan, Antonio González-Sarrias, Liya Li, Maxwell E. Edmonds and Navindra P. Seeram	45
Phytochemical Analysis and Antioxidant Capacity of BM-21, a Bioactive Extract Rich in Polyphenolic Metabolites from the Sea Grass <i>Thalassia testudinum</i> Erik L. Regalado, Roberto Menendez, Olga Valdés, Ruth A. Morales, Abilio Laguna, Olivier P. Thomas, Yasnay Hernandez, Clara Nogueiras and Anake Kijjoa	47
Herbicidal Activity of Curvulinic Acid Isolated from <i>Nimbya alternantherae</i> Jun Li, Yonghao Ye, Xiaoyang Wang and Liyao Dong	51
Xanthenes with Antiproliferative Effects on Prostate Cancer Cells from the Stem Bark of <i>Garcinia xanthochymus</i> Feng Ji, Zhanlin Li, Gaofeng Liu, Shengli Niu, Nan Zhao, Xiaoqiu Liu and Huiming Hua	53
Aromatic Hydroxyl Group Plays a Critical Role in Antibacterial Activity of the Curcumin Analogues Mi Kyoung Kim, Jun Cheol Park and Youhoon Chong	57
Analysis of Danshen and Twelve Related <i>Salvia</i> Species Luyang Lu, Yuan Liu, Zhifeng Zhang and Hao Zhang	59
Meliloester, a New Melilotic Ester from <i>Melilotus alba</i> Rasheeda Khatoon, Nikhat Saba, Aqib Zahoor, Shazia Summer and Viqar Uddin Ahmad	61
A New Long-Chain Unsaturated Ester and Other Constituents of <i>Hypericum tomentosum</i> Ouassila Touafek, Zahia Kabouche, Joël Boustie and Christian Bruneau	63
Microbial Conversion of Tomato by a Plant Pathogenic Bacterium <i>Pectobacterium atrosepticum</i>: a Plant-Microbial Approach to Control Pathogenic <i>Candida</i> Species Vivek K. Bajpai, Sun Chul Kang, Soon-Gu Lee and Kwang-Hyun Baek	65
Antibacterial and Antiparasitic Effects of <i>Bothropoides lutzi</i> venom Ramon R.P.P.B. de Menezes, Alba F. C. Torres, Thiala S. J. da Silva, Daniel F. de Sousa, Danya B. Lima, Diva B. Norjosa, Nádia A. P. Nogueira, Maria F. Oliveira, Márcia R. de Oliveira, Helena S. A. Monteiro and Alice M. C. Martins	71

Continued inside backcover