Biochemical Systematics and Ecology 38 (2010) 686–690

Contents lists available at ScienceDirect

Biochemical Systematics and Ecology

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

Chemical analysis of flavonoid constituents of the seagrass *Halophila stipulacea*: First finding of malonylated derivatives in marine phanerogams

Fatma Bitam [1], Maria Letizia Ciavatta*, Marianna Carbone, Emiliano Manzo, Ernesto Mollo, Margherita Gavagnin

Istituto di Chimica Biomolecolare (CNR),Via Campi Flegrei 34, I-80078 Pozzuoli (Na), Italy

ARTICLE INFO

Article history:
Received 26 January 2010
Accepted 23 April 2010

Keywords:
Seagrass
Halophila stipulacea
Chemical constituents
Malonylglucosyl flavones

ABSTRACT

The flavonoid fraction from the butanol extract of a Mediterranean sample of the seagrass *Halophila stipulacea* was chemically analyzed. A new malonylated flavone glucoside, genkwanin-4′-*O*-(6“-malonyl-glucopyranoside) (**3**), was isolated together with known flavone glucosides **4**-**9**, previously reported only from terrestrial sources. The structure of **3** was established by means of spectroscopic techniques, mainly NMR methods.

1. Introduction

The ecological importance of the marine flowering plants, the so-called seagrasses, is not only due to their extraordinarily high rate of primary production, but also to their ability to serve as nurseries, providing a habitat and protection from predators for many diverse benthic organisms. Consequently, several ecological studies on these species have been extensively carried out to determine the environmental “health” of coastal and estuary ecosystems (Dawes, 1998). Despite this, analysis and chemical elucidation of the secondary metabolite products from seagrasses has only recently been undertaken, highlighting antifouling, antibacterial, antiviral, anti-inflammatory and cytotoxic bioactivities (Kong et al., 2008; Kumar et al., 2008; Rowley et al., 2002; Qi et al., 2008).

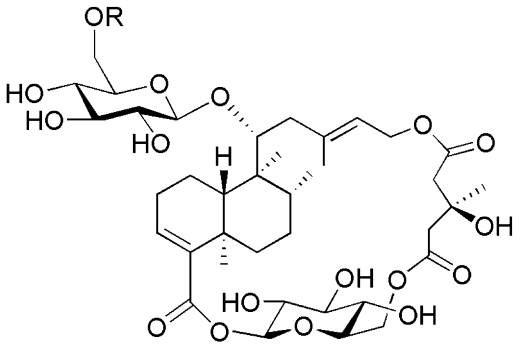
Seagrasses belonging to the genus *Halophila* are widely distributed along the western coasts of the Indian Ocean, Red Sea and South-eastern Florida coasts. This genus has been the subject of many ecological studies whereas few phytochemical investigations have been conducted to date. The latter reported the presence of unidentified sulphated phenolic compounds from nine different species of *Halophila* including *Halophila stipulacea* (McMillan et al., 1980), unidentified sulphated and non-sulphated flavones from *Halophila ovalis-H. minor* complex (McMillan, 1986), flavones and flavone glycosides from *Halophila johnsonii* (Meng et al., 2008). Antibacterial activity against a series of microorganisms has been described for methanolic and ethyl acetate extracts of *H. ovalis* from the South Indian Sea (Kumar et al., 2008).

H. stipulacea is an Indo-Pacific seagrass introduced through the Suez Canal to the Mediterranean Sea where it was recorded for the first time in 1895 (Lipkin, 1975). It is one of the nine macrophyte species that are considered as invasive playing a significant role in the recipient ecosystems, taking the place of keystone species and being economically harmful

* Corresponding author. Tel.: +39 081 8675243; fax: +39 081 8041770.
E-mail address: lciavatta@icb.cnr.it (M.L. Ciavatta).
[1] On leave from Faculté des Sciences, Département de Chimie, Université de Batna, 05000 Batna, Algeria.

0305-1978/$ – see front matter © 2010 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bse.2010.04.007

(Boudouresque and Verlaque, 2002). In a previous study on a sample of this plant collected in the Gulf of Corinth, Greece, and analyzed together with the anaspidean mollusc *Syphonota geographica* feeding on the seagrass, we reported the isolation and the characterization of an unusual glycoterpenoid, syphonoside **1** (Gavagnin et al., 2007). In a subsequent work, the acetyl derivative of syphonoside, compound **2**, was also isolated from the phanerogam (Carbone et al., 2008). Furthermore, chemo-ecological implications of the introduction of both exotic species, the mollusc *S. geographica* and the seagrass *H. stipulacea*, in the Mediterranean Sea were discussed by Mollo et al. (2008).



1: R =H
2: R=Ac

Here, we report the investigation of additional chemical constituents of *H. stipulacea* with regard to the flavonoid fraction that led to the characterization of a new malonylated glucopyranosyl flavone (**3**), isolated along with known flavonoids such as malonylated glucopyranosylapigenin (**4**) and five related glucopyranosyl flavones (**5**-**9**).

2. Material and methods

2.1. Plant material

H. stipulacea (Forsskål) Aschers. was collected off Porto Germeno coasts (Gulf of Corinth, Greece) at 5–10 m depth by SCUBA diving during December 2003. The sample was stored at −20 °C until chemical analysis. A voucher specimen (Halo-71) has been deposited at the Institute of Biomolecular Chemistry (ICB), National Council of Research, Italy.

2.2. Chemical procedure

The butanolic soluble portion (1.31 g) of the acetone extract was obtained as already described (Gavagnin et al., 2007) from *H. stipulacea* frozen sample (103 g dry weight), and analyzed by TLC. Some UV polar components which gave a yellow coloration by reaction with cerium sulphate were detected at R*f* 0.15 and R*f* 0.70–0.60 (chloroform/methanol, 7:3) together with the previously reported syphonoside (**1**) (Gavagnin et al., 2007) and its acetyl derivative **2** (Carbone et al., 2008).

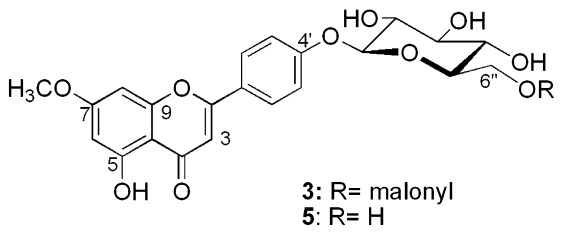
An aliquot of butanolic extract (380 mg) was subjected to Sephadex LH-20 chromatography eluting with a mixture of chloroform/methanol in 1:1 ratio. The collected fractions were analyzed by both TLC chromatography and NMR, and then combined to obtain three main flavonoid glycoside-containing fractions: A (100 mg), B (14.1 mg), and C (71.5 mg). A portion of fraction A (33.7 mg), which was also characterized by the presence of syphonosides **1** and **2**, was first submitted to preparative TLC (silica gel, 0.5 mm plates; chloroform/methanol, 7:3) to obtain a flavonoid glycoside mixture (9.4 mg) corresponding to the UV components at R*f* 0.15 (chloroform/methanol, 7:3). The latter was further purified by RP-HPLC (Synergi-HydroRP C18, 250 × 4.6 mm; 40 min gradient from 50% to 100% CH_3OH in H_2O, flow 1 mL/min, UV detector) to give the new genkwanin-4′-*O*-β-(6″-*O*-malonyl-glucopyranoside) (**3**, 1.2 mg) and the known apigenin-7-*O*-β-(6″-*O*-malonyl-glucopyranoside) (**4**, 4.0 mg). Purification of fraction B on preparative TLC (silica gel, 0.5 mm plates; chloroform/methanol, 7:3) afforded a mixture (4.1 mg) of less polar flavonoid glycosides (R*f* 0.70, chloroform/methanol, 7:3), which was further purified by RP-HPLC (Phenomenex: Kromasil 5 μ C18, 250 × 10 mm; CH_3OH/H_2O, 60:40; flow 2 mL/min, UV detector) to obtain the known genkwanin-4′-*O*-β-glucopyranoside (**5**, 1.7 mg) and chrysoeriol-7-*O*-β-glucopyranoside (**6**, 0.9 mg).

An aliquot of fraction C (19 mg) was subject to a preparative TLC purification (silica gel, 0.5 mm plates; chloroform/methanol, 7:3) to obtain a mixture of the flavonoid glycosides at R*f* 0.65–0.60 (chloroform/methanol, 7:3). The subsequent purification of this fraction by RP-HPLC (Phenomenex: Kromasil 5 μ C18, 250 × 10 mm; CH_3OH/H_2O, 1:1; flow 2 mL/min, UV detector) yielded the known apigenin-7-*O*-β-glucopyranoside (**7,** 6.9 mg), apigenin-4′-*O*-β-glucopyranoside (**8,** 2.2 mg), and chrysoeriol-4′-*O*-β-glucopyranoside (**9,** 1.8 mg).

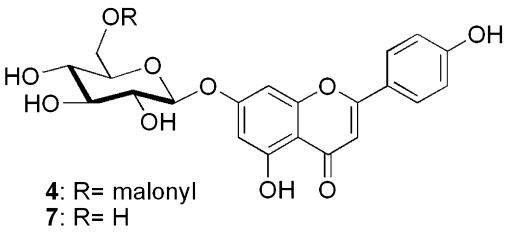
3. Results and discussion

3.1. Flavonoid characterization

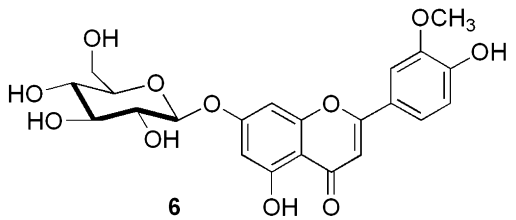
The flavonoid components of the butanol extract of the seagrass *H. stipulacea* were purified as described in Material and Methods to obtain seven pure glucosyl flavones (**3**-**9**). In particular, two malonyl derivatives, genkwanin-4′-*O*-β-(6″-*O*-malonyl)-glucopyranoside (**3**) (1% of the butanol extract) and apigenin-7-*O*-β-(6″-*O*-malonyl)-glucopyranoside) (**4**) (Takeda et al., 1993; Švehlíková et al., 2004) (3%), were isolated from the polar fraction of the flavone mixture whereas five non-esterified glucosyl flavones, genkwanin-4′-*O*-β-glucopyranoside (**5**, 0.4% of the butanol extract) (Veit et al., 1990), chrysoeriol-7-*O*-β-glucopyranoside (**6**, 0.2%) (Harput et al., 2006), apigenin-7-*O*-β-glucopyranoside (Takeda et al., 1993) (**7**, 7%), apigenin-4′-*O*-β-glucopyranoside (Nawar et al., 1994) (**8**, 2%), and chrysoeriol-4′-*O*-β-glucopyranoside (**9**, 2%) (Fukunaga et al., 1989) were isolated from the less polar portion of the extract. Malonyl derivatives **3** and **4** were unstable and a rapid degradation was observed to occur under the purification conditions used. This made the isolation of the pure compounds quite difficult. However, we were able to obtain pure **3** in an amount sufficient for a full spectroscopic analysis.



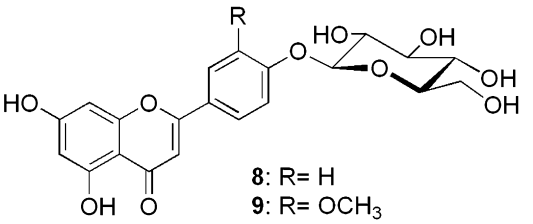
3: R= malonyl
5: R= H



4: R= malonyl
7: R= H



6



8: R= H
9: R= OCH_3

The main flavonoid metabolites of *H. stipulacea* were apigenin-7-*O*-β-glucopyranoside (**7**), a very common terrestrial flavonoid, and the corresponding malonyl ester **4**, previously described only from the blue flowers of lupin (Takeda et al., 1993) and from chamomile (Švehlíková et al., 2004). The minor co-occurring malonyl derivative of genkwanin-4′-*O*-glucoside (**3**) was an unprecedented compound. The known molecules **4**-**9** were identified by comparing NMR and mass data with those reported in the literature whereas the structure of **3** was defined as follows.

Genkwanin-4′-*O*-β-(6″-*O*-malonyl)-glucopyranoside (**3**): $C_{25}H_{24}O_{13}$, obtained as a yellow amorphous powder; UV (CH_3OH) λ_{max} (log ϵ): 206 (4.32), 269 (4.66) nm; MALDI/MS, *m/z* 533 $[M + H]^+$, 447 $[M - 86$ (malonyl) $+ H]^+$; HR-MALDI/MS *m/z* 533.1269 (533.1295 calculated for $C_{25}H_{24}O_{13} + H$). The molecular formula of **3** was consistent with both the MS and ^{13}C NMR data. Analysis of the ^{13}C NMR and DEPT spectra immediately indicated that compound **3** had the same flavonoid nature as the co-occurring genkwanin-4′-*O*-β-glucopyranoside (**5**) (Veit et al., 1990). In fact, analogously with **5**, the aglycone moiety was identified as genkwanin as well as the sugar residue was determined to be glucose (see Table 1).

The additional mass fragment $C_3H_3O_3$ required by the molecular formula of **3** was attributed to an acyl residue attached to an hydroxyl group of the molecule. Comparison of the ^1H NMR spectrum of **3** recorded in DMSO-d_6 with **5** (Veit et al., 1990) clearly revealed that in **3** the hydroxyl esterified was at C-6 position of the glucose unit. In fact, H_2-6″ protons resonated at δ_H 4.08 and 4.25, which were downfield shifted with respect to the corresponding proton values (δ_H 3.11–3.79) observed for the non-esterified compound (Veit et al., 1990). According to the expected acylation chemical shift difference, C-6″ resonated at δ_C 64.5 in **3**, compared with δ_C 60.7 in **5** (Veit et al., 1990). Finally, the acyl residue was identified as a malonyl unit by the diagnostic signals observed in the ^1H and ^{13}C NMR spectra of **3** (in DMSO-d_6) [δ_C 169.5 (C-3‴), 168.2 (C-1″), 45.7 (C-2‴); δ_H 2.94 (2H, s, H_2-2‴)] (Table 1).

A detailed analysis of 2D-NMR experiments (^1H-^1H COSY, HSQC and HMBC) carried out in CD_3OD and DMSO-d_6 (Table 1) allowed the full assignment of proton and carbon values of **3** and also confirmed the proposed structure including the sites of malonylation and glucosylation at C-6″ and C-4′, respectively. Accordingly, diagnostic long-range correlations were observed in the HMBC spectrum of **3** (in CD_3OD) between C-4′ and H-1a″ as well as between C-1‴ and H-1′ (Table 1).

The stability of compound **3** was very low. In fact it was observed that **3** underwent a rapid deacylation reaction during work-up to give the corresponding non-esterified genkwanin-4′-*O*-β-glucopyranoside (**5**). An analogous chemical behaviour

Table 1
NMR data of genkwanin-4'-*O*-β-(6"-*O*-malonyl-glucopyranoside) (**3**)

C	DMSO-d_6					CD_3OD				
	δ ^{13}C[a]	m[b]	δ ^{1}H[c]	m *J* (Hz)	HMBC[d]	δ ^{13}C[a]	m[b]	^{1}H[c]	m *J* (Hz)	HMBC[d]
2	163.4	s	–			165.6	s	–		
3	104.1	d	6.96	s	C-2, C-4	105.2	d	6.75	s	C-l′, C-2, C-4
4	182.8	s	–			184.6	s	–		
5	160.3	s	–			164.2	s	–		
6	98.0	d	6.40	d (2.4)	C-5, C-7	98.9	d	6.39	d (2.4)	C-5, C-7, C-8, C-10
7	165.2	s	–			167.4	s	–		
8	92.8	d	6.80	d (2.1)	C-9, C-10	93.6	d	6.71	d (2.1)	C-6, C-9
9	157.3	s	–			159.3	s	–		
10	104.7	s	–			106.6	s	–		
1'	123.8	s	–			126.1	s	–		
2',6'	128.2	d	8.05	d (8.9)	C-2	129.6	d	8.05	d (8.9)	C-2, C-4'
3',5'	116.5	d	7.15	d (8.9)	C-l'	118.2	d	7.26	d (8.9)	C-4', C-2′, C-6'
4'	160.3	s	–	s		161.8	s	–		
1"	99.8	d	5.05	d (6.1)	C-4'	101.5	d	5.05	d (7.2)	C-4'
2"	73.6	d	3.28	m		74.7	d	3.54	m	
3"	75.9	d	3.30	m		77.8	d	3.52	m	
4"	69.6	d	3.20	m		715	d	3.43	m	
5"	73.8	d	3.64	m		75.7	d	3.78	m	
6"a	64.5	t	4.08	d (11.8)		64.7	t	4.33	d (11.8)	C-1""
6"b			4.25	dd (11.8, 6.1)				4.54	dd (11.8, 6.1)	
$-OCH_3$	56.0	q	3.82	s		56.4	q	3.95	s	C-7
1""	168.2	s	–			170.5		–		
2""	45.7	t	2.94	s	C-l"", C-3""	45.4	t	2.95	s	
3""	169.5	s	–			nd		–		

[a] Bruker 300 MHz
[b] Multiplicity deduced by DEPT
[c] Bruker 600 MHz
[d] Long-range coupling constants optimized for $J = 10$ and 6 Hz)

was observed for apigenin-7-*O*-β-(6″-*O*-malonyl)-glucopyranoside) (**4**) which degraded under silica–gel chromatographic conditions to form the corresponding non-esterified derivative **7**.

3.2. Previous studies and chemo-ecological significance

Following our previous study on the chemical constituents of the invasive seagrass *H. stipulacea* (Gavagnin et al., 2007; Carbone et al., 2008) that resulted in the isolation of the glucoterpenoid macrocycles, syphonoside (**1**) and its acetyl derivative **2**, we have now investigated the flavonoid content of the plant and identified the components. This study indicated that the flavonoid profile of *H. stipulacea* was dominated by apigenin-7-*O*-β-glucopyranoside (**7**) co-occurring with other minor flavone glucosides including two malonyl derivatives, the new compound **3** and apigenin-7-*O*-β-(6″-*O*-malonyl)-glucopyranoside) (**4**). It is noteworthy that this is the first finding of malonylated flavone glycosides in the marine environment. However, to the best of our knowledge, with the exception of a malonyl amide alkaloid that has been recently found in a marine bacterium associated with a zoanthid (Kita et al., 2007), no other malonyl-containing metabolites have been reported from marine organisms. On the contrary, malonyl flavone glucosides derivatives have been reported from many terrestrial sources (Veit et al., 1990; Takeda et al., 1993; Švehlíková et al., 2004; Montoro et al., 2005; Kim et al., 2009).

The instability of malonylated flavone glycosides that is due to the known reactivity of the malonyl ester linkage *in vitro*, has been studied for different acetyl and malonyl derivatives of apigenin glucosides from chamomile by Švehlíková et al. (2004). In this paper, it was reported that apigenin-7-*O*-β-(6″-*O*-malonyl)-glucopyranoside) (**4**) undergoes rapid decarboxylation at room temperature forming apigenin-7-(6″acetyl-glucopyranoside) and, subsequently, under different solvent conditions, deacetylates completely giving apigenin-7-*O*-β-glucopyranoside. This was in agreement with the observations in the present work. The deacylation reaction of compounds **3** and **4** occurring during the work-up suggested that malonylated flavone glycosides were most likely more abundant in the plant than chemical analysis indicates and, consequently, the corresponding non-esterified derivatives **5** and **7** should be considered as artefacts.

With the exception of a recent investigation on *H. johnsonii* resulting in the chemical characterization of all main components of the flavone profile of this seagrass (Meng et al., 2008), previous studies on different *Halophila* species including *H. stipulacea* only reported qualitative analysis of flavone profiles and indicated the presence of unidentified flavones and flavone sulphates (McMillan et al., 1980; McMillan, 1986). In particular, these studies revealed a high variability in the flavone composition for the different *Halophila* species analyzed with regard to the complexity of the flavone mixture and to the presence of sulphate derivatives thus preventing reliable interspecific taxonomic relationships in *Halophila* seagrasses based on qualitative evaluation of flavone profiles. It is interesting to note that some polar flavonoids exhibiting a chromatographic behaviour similar – but not identical – to that of sulphate flavone derivatives found in other seagrasses were detected in the *H. ovalis-H. minor* complex (McMillan, 1986). By analogy with *H. stipulacea* and considering that flavone

malonylglycosides show chromatographic properties similar to those of sulphate derivatives, malonyl-containing structures could be tentatively suggested for these flavonoids.

In conclusion, our studies on *H. stipulacea* revealed the presence of a flavone glycoside pattern less complex and characterized by different aglycone moieties with respect to that reported for *H. johnsonii* (Meng et al., 2008). The occurrence of flavone malonylglycosides has not been previously reported in marine phanerogams even though it should be taken into consideration that flavone acetylglycosides isolated from *H. johnsonii* (Meng et al., 2008) could be derived from the corresponding malonyl derivatives by decarboxylation. Unfortunately, chemical data on the identity of flavonoid metabolites of other *Halophila* species are lacking in the literature. Additional studies in this field should be necessary to get useful information for relating flavone chemistry to taxonomy in seagrasses of genus *Halophila*, and to explore the ecological role of malonylated flavone glycosides in marine phanerogams.

Acknowledgements

This research work was financially supported by PRIN-MIUR 2007 Project "Antitumor natural products and synthetic analogues". F.B. is deeply grateful to the Ministry of Superior Education and Scientific Research, Algeria for financial support. The authors acknowledge the staff of ICB NMR Service and, in particular, Mr. A. Esposito and V. Mirra for 2D-NMR spectra. The authors are also grateful to Dr. V. Carbone of ISA-CNR for recording MALDI/MS spectra. Thanks are also due to Prof. V. Roussis, University of Athens, for the help in the collection of *H. stipulacea*.

References

Boudouresque, C.F., Verlaque, M., 2002. Biological pollution in the Mediterranean Sea: invasive versus introduced macrophytes. Mar. Biol. Bull. 44, 32–38.
Carbone, M., Gavagnin, M., Mollo, E., Bidello, M., Roussis, V., Cimino, G., 2008. Further syphonosides from the sea hare *Syphonota geographica* and the seagrass *Halophila stipulacea*. Tetrahedron 64, 191–196.
Dawes, C.J., 1998. Marine plants and their habitats. In: Marine Botany. John Wiley & Sons, Inc., 605 3° Avenue, New York, pp. 1–7.
Fukunaga, T., Nishiya, K., Kajikawa, I., Takeya, K., Itokawa, H., 1989. Studies on the constituent of Japanese mistletoes from different host trees, and their antimicrobial and hypotensive properties. Chem. Pharm. Bull. 37, 1543–1546.
Gavagnin, M., Carbone, M., Amodeo, P., Mollo, E., Vitale, R.M., Roussis, V., Cimino, G., 2007. Structure and absolute stereochemistry of syphonoside, a unique macrocyclic glycoterpenoid from marine organisms. J. Org. Chem. 72, 5625–5630.
Harput, U.S., Calis, I., Saracoglu, I., Dönmea, A.A., Nagatsu, A., 2006. Secondary metabolites from *Phlomis syriaca* and their antioxidant activities. Turk. J. Chem. 30, 383–390.
Kim, D.H., Kim, S.K., Kim, J.H., Kim, B.G., Ahn, J.H., 2009. Molecular characterization of flavonoid malonyltransferase from *Oryza sativa*. Plant Physiol. Biochem. 47, 991–997.
Kita, M., Miwa, R., Widianti, T., Ozaki, Y., Aoyama, S., Yamada, K., Uemura, D., 2007. Revised structure of kasarin, an antibacterial pyrazinone compound from the marine microorganism *Hyphomycetes* sp. Tetrahedron Lett. 48, 8628–8631.
Kong, C.S., Kim, Y.A., Kim, M.M., Park, J.S., Kim, J.A., Kim, S.K., Lee, B.J., Namf, T.J., Seo, Y., 2008. Flavonoid glycosides isolated from *Salicornia herbacea* inhibit matrix metalloproteinase in HT1080 cells. Toxicol. Vitro 22, 1742–1748.
Kumar, C.S., Sarada, D.V.L., Gideon, T.P., Rengasamy, R., 2008. Antibacterial activity of three South Indian seagrasses, *Cymodocea serrulata, Haplophila ovalis* and *Zostera capensis*. World J. Microbiol. Biotechnol. 24, 1989–1992.
Lipkin, Y., 1975. On the male flower of *Halophila stipulacea*. Israel J. Bot. 24, 198–200.
McMillan, C., Zapata, O., Escobar, L., 1980. Sulphated phenolic compounds in seagrasses. Aquat. Bot 8, 267–278.
McMillan, C., 1986. Sulphated flavonoids and leaf morphology in the *Halophila ovalis – H. minor* complex (Hydrocharitaceae) of the Indo-Pacific Ocean. Aquat. Bot. 25, 63–72.
Meng, Y., Krzysiak, A.J., Durako, M.J., Kunzelman, J.I., Wright, J.L.C., 2008. Flavones and flavone glycosides from *Halophila johnsonii*. Phytochemistry 69, 2603–2608.
Mollo, E., Gavagnin, M., Carbone, M., Castelluccio, F., Pozone, F., Roussis, V., Templado, J., Ghiselin, M.T., Cimino, G., 2008. Factors promoting marine invasions: a chemoecological approach. PNAS 105, 4582–4586.
Montoro, P., Carbone, V., Pizza, C., 2005. Flavonoids from the leaf of Cyclanthera pedata: two new malonyl derivatives. Phytochem. Anal 16, 210–216.
Nawar, M.A.M., Hussein, S.A.M., Merfort, I., 1994. Leaf phenolics of *Punica granatum*. Phytochemistry 37, 1175–1177.
Qi, S.H., Zhang, S., Qian, P.Y., Wang, B.G., 2008. Antifeedant, antibacterial, and antilarval compounds from the South China Sea seagrass *Enhalus acoroides*. Bot. Mar. 51, 441–447.
Rowley, D.C., Hansen, M.S.T., Rhodes, D., Sotriffer, C.A., Ni, H., McCammon, J.A., Bushman, F.D., Fenical, W., 2002. Thalassiolins A–C: new marine-derived inhibitors of HIV cDNA integrase. Bioorg. Med. Chem. 10, 3619–3625.
Švehlíková, V., Bennett, R.N., Mellon, F.A., Needs, P.W., Piacente, S., Kroon, P.A., Bao, Y., 2004. Isolation, identification and stability of acylated derivatives of apigenin 7-*O*-glucoside from chamomile (*Chamomilla recutita* [L.] Rauschert. Phytochemistry 65, 2323–2332.
Takeda, K., Harborne, J.B., Waterman, P.G., 1993. Malonylated flavonoids and blue flower colour in lupin. Phytochemistry 34, 421–423.
Veit, M., Geiger, H., Czygan, F.C., Markham, K.R., 1990. Malonylated flavone 5-*O*-glucosides in the barren sprouts of *Equisetum arvense*. Phytochemistry 29, 2555–2560.