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SHORT COMMUNICATION

Flavonoids from the halophyte *Apocynum venetum* and their antifouling activities against marine biofilm-derived bacteria

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Eleven flavonoids were isolated from the leaves of the halophyte *Apocynum venetum*. Among them, the isolation of plumbocatechin A (**1**), 8-*O*-methylretusin (**2**) and kaempferol 3-*O*-(6″-*O*-acetyl)-β-D-galactopyranoside (**7**) was reported for the first time from this plant. Their structures were identified by using spectral methods, including 2D NMR experiments, and confirmed by comparing with the literature data. In addition, the antifouling activities of these compounds against the marine fouling bacteria, *Bacillus thuringiensis*, *Pseudoalteromonas elyakovii* and *Pseudomonas aeruginosa*, have been evaluated in this article.

Keywords: *Apocynum venetum*; flavonoids; halophyte; antifouling; antimicrobial activity

1. Introduction

Marine biofouling is recognised as the most difficult to control when humans engage in marine activities or explore marine resources. In order to resolve this problem, scientists are searching for alternative solutions to control marine biofouling, including the use of biocides as well as marine-derived natural products or secondary metabolites (Qian et al. 2010; Zhou et al. 2011). So far, a large number of natural product antifoulants have been isolated from marine organisms (Fusetani 2004, 2011). In recent years, it is reported that the active compounds from herbal plants have the potential to disrupt the settlement process of marine fouling organisms, and the medicinal plants as a valuable source of natural antifoulants may have the potential for the development of antifouling technology (Feng et al. 2009).

Apocynum venetum L. is one of the medicinal halophytes, which can grow in the salt marshes in the Yellow River delta of China (Duan et al. 2007). Its leaves have been used in traditional Chinese medicine for the treatment of neurasthenia, hypertension, nephritis and heart diseases (State Pharmacopoeia Commission of People's Republic of China 2010). Previous phytochemical investigations demonstrated flavonoids as the major active constituents in the *A. venetum* leaves exhibiting various pharmacological effects, such as antihypertensive, cardiotonic, hepatoprotective, antioxidant, antidepressant and antianxiety (Xie et al. 2012). However, the antifouling activities against the biofilm-derived bacteria of these active flavonoids have not been reported. Due to our interest on bioactive constituents of the halophytic plants (Fang et al. 2013a, 2013b), the *A. venetum* leaves, which were collected from the Yellow River delta, have been investigated. Eleven flavonoids (**1**–**11**) were isolated (Figure 1), and their

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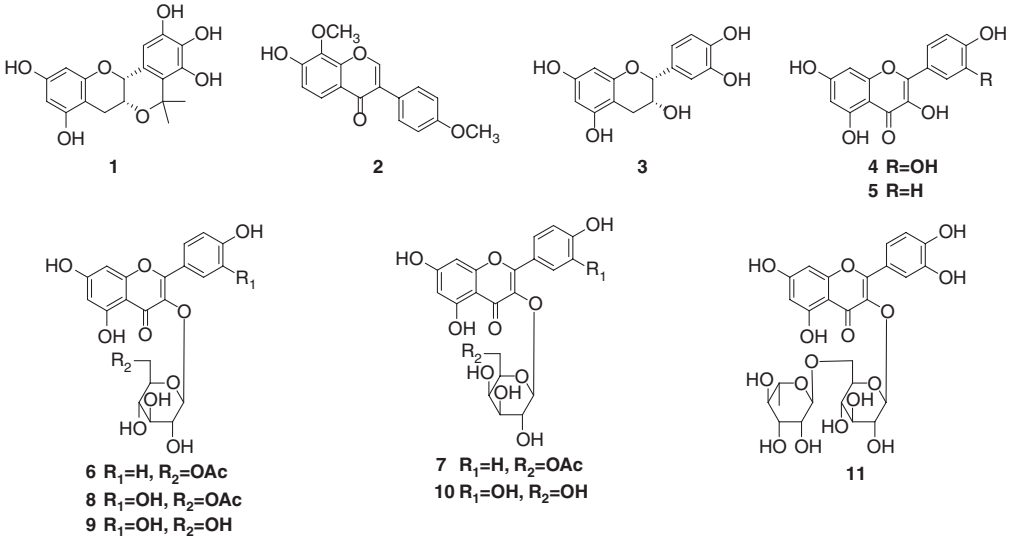

Figure 1. Structures of compounds **1**–**11**.

antifouling activities against three marine fouling bacteria, *Bacillus thuringiensis*, *Pseudoalteromonas elyakovii* and *Pseudomonas aeruginosa*, have been evaluated in this article.

2. Results and discussion

Eleven flavonoids were isolated from the EtOAc fraction of the methanol extract of the leaves of the halophyte *A. venetum* using extensive column chromatography over silica gel, reverse-phase ODS gel and Sephadex LH-20. Their structures were identified as plumbocatechin A (**1**) (Yue et al. 1998), 8-*O*-methylretusin (**2**) (Yang et al. 2012), epicatechin (**3**) (Yoon et al. 2007), quercetin (**4**), kaempferol (**5**) (Wu et al. 2012), kaempferol 3-*O*-(6″-*O*-acetyl)-β-D-glucopyranoside (**6**) (Li & Yuan 2006), kaempferol 3-*O*-(6″-*O*-acetyl)-β-D-galactopyranoside (**7**) (Foo et al. 2000), quercetin 3-*O*-(6″-*O*-acetyl)-β-D-glucopyranoside (**8**) (Li & Yuan 2006), isoquercitrin (**9**), hyperoside (**10**) (Cheng et al. 2007) and rutin (**11**) (Zhao et al. 2012) by using the spectral methods and confirmed by comparing with the literature data. Among them, compounds **1**, **2** and **7** were obtained from *A. venetum* for the first time.

Compound **1** was isolated as a white powder and was identified as an unnormal flavonoid named plumbocatechin A on the basis of the spectral data including ^{1}H and ^{13}C NMR (Supplementary Table S1), 2D NMR, MS, and by comparing with the literature data (Yue et al. 1998). This compound had been isolated for the first time from the whole plant of *Ceratostigma minus* (Yue et al. 1998), and it had also been synthesised previously by the reaction of epigallocatechin with acetone, and expressing much higher radical-scavenging activity than epigallocatechin (van der Westhuizen et al. 1990; Imai et al. 2011).

Compound **2** was obtained as a white powder and identified as 8-*O*-methylretusin by using the spectroscopic methods (Supplementary Table S2). In previous investigations, 8-*O*-methylretusin had been reported to be isolated from several plants such as *Wistaria brachybotrys* (Konoshima et al. 1997), *Dipteryx alata* (Puebla et al. 2010), *Sophora tonkinensis* (Yang et al. 2012) and so on. Some researches also suggested that it had exhibited antitumour-promoting (Konoshima et al. 1997) and antifouling activities (Zhou et al. 2009).

Coupounds **6** and **7** were isomers existing as a mixture (Supplementary Figure S1), which were obtained as a yellow amorphous powder. In our study, we found that they could be separated by

reverse-phase HPLC with a water–methanol system, but not using water–acetonitrile as mobile phase (Supplementary Figure S2). Finally, this mixture was separated by recycling with semi-preparative HPLC. On careful analysis of the NMR spectra of these two compounds and by comparing the ^{1}H and ^{13}C NMR spectral data with reference spectra, compound **6** was identified as kaempferol 3-*O*-(6″-*O*-acetyl)-β-D-glucopyranoside (Li & Yuan 2006). Although the NMR spectra of **7** were very similar to those of **6** (Supplementary Table S3 and Figure S4), a typical anomeric proton signal at δ_H 5.26 (1H, d, $J = 7.8$ Hz, H-1″) and six carbon signals at δ_C 102.9 (C-1″), 73.4 (C-5″), 73.2 (C-3″), 71.5 (C-2″), 68.6 (C-4″) and 63.7 (C-6″), belonging to the galactosyl group (Li & Yuan 2006), along with an additional acetyl group (δ_H 1.75, 3H, s; δ_C 170.4, 20.7) were observed individually. The downfield shift of C-6″ to 63.7 ppm indicated that the acetyl group was linked to the C-6″ position of the galactosyl residue. Therefore, compound **7** was determined to be kaempferol 3-*O*-(6″-*O*-acetyl)-β-D-galactopyranoside, which was also confirmed by the literature reported (Foo et al. 2000). To the best of our knowledge, this is the first report on the detection and isolation of this compound from the leaves of *A. venetum*.

In our study, the petroleum ether, EtOAc and *n*-BuOH parts of the MeOH extract of *A. venetum* leaves were assayed for antibacterial activities against three fouling bacteria, *B. thuringiensis*, *P. elyakovii* and *P. aeruginosa*. Only the EtOAc extract exhibited potent activity on these three strains of bacteria, which might be due to the high content of flavonoids. In addition, the antimicrobial activities of the flavonoids from the EtOAc-soluble extract were also tested (Supplementary Table S4). Compound **2** expressed weak antibacterial activities towards *B. thuringiensis* and *P. aeruginosa* but no activity against *P. elyakovii*. Compounds **3** and **4** displayed weak antibacterial activities against the three tested bacteria, while compound **5** exhibited moderate antibacterial activities towards *B. thuringiensis and P. aeruginosa.* Compounds **1** and **6**–**11** exhibited no activities against these three strains.

3. Conclusion

In conclusion, this study describes the isolation and structural elucidation of 11 flavonoids from halophyte *A. venetum* and their antifouling activities against marine biofilm-derived bacteria. The isolation of plumbocatechin A (**1**), 8-*O*-methylretusin (**2**) and kaempferol 3-*O*-(6″-*O*-acetyl)-β-D-galactopyranoside (**7**) was reported for the first time from this plant. The EtOAc extract displayed potent activities on these three strains of bacteria, while compound **5** showed moderate antibacterial activities towards *B. thuringiensis* and *P. aeruginosa*. These results suggest that halophyte *A. venetum* is a valuable source of potent antifouling agent.

Supplementary material

Experimental details relating to this article are available online, alongside Tables S1–S4 and Figures S1–S4.

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References

Cheng XL, Zhang SQ, Li QS. 2007. Chemical constituents of flavonoids from *Apocynum venetum*. J Chin Med Mater. 30:1086–1088.

Duan DX, Liu JH, Zhang XL, Wu T, Liu XH. 2007. Study on medicinal halophytes resources in Shandong province. J Anhui Agric Sci. 35:4186–4188.

Fang ST, Liu X, Kong NN, Liu SJ, Xia CH. 2013a. A new essential oil component from *Datura stramonium*. Chin Trad Herb Drug. 44:2035–2038.

Fang ST, Liu X, Kong NN, Liu SJ, Xia CH. 2013b. Two new with anolides from the halophyte *Datura stramonium* L. Nat Prod Res. 27:1965–1970.

Feng DQ, Ke CK, Lu CY, Li SJ. 2009. Herbal plants as a promising source of natural antifoulants: evidence from barnacle settlement inhibition. Biofouling. 25:181–190.

Foo LY, Lu Y, Molan AL, Woodfield DR, McNabb WC. 2000. The phenols and prodelphinidins of white clover flowers. Phytochemistry. 54:539–548.

Fusetani N. 2004. Biofouling and antifouling. Nat Prod Rep. 21:94–104.

Fusetani N. 2011. Antifouling marine natural products. Nat Prod Rep. 28:400–410.

Imai K, Nakanishi I, Anzai K, Ozawa T, Miyata N, Urano S, Okuda H, Nakamura A, Fukuhara K. 2011. Synthesis and enhanced radical scavenging activity of a conformationally constrained epigallocatechin analogue. Chem Lett. 40:1417–1419.

Konoshima T, Takasaki M, Kozuka M, Tokuda H, Nishino H, Matsuda E, Nagai M. 1997. Anti-tumor promoting activities of isoflavonoids from *Wistaria brachybotrys*. Biol Pharm Bull. 20:865–868.

Li LH, Yuan Z. 2006. Study on flavonoids in leaves of *Apocynum venetum*. China J Chin Mater Med. 31:1337–1340.

Puebla P, Oshima-Franco Y, Franco LM, Dos Santos MG, da Silva RV, Rubem-Mauro L, Feliciano AS. 2010. Chemical constituents of the bark of *Dipteryx alata* Vogel, an active species against *Bothrops kararacussu* Venom. Molecules. 15:8193–8204.

Qian PY, Xu Y, Fusetani N. 2010. Natural products as antifouling compounds: recent progress and future perspectives. Biofouling. 26:223–234.

State Pharmacopoeia Commission of People's Republic of China. 2010. Pharmacopoeia of the People's Republic of China. Vol. 1. Beijing: China Medical Science & Technology Press.

van der Westhuizen JH, Steenkamp JA, Ferreira D. 1990. An unusual reaction of flavan-3-ols with acetone of relevance to the formation of the tetracyclic ring system in peltogynoids. Tetrahedron. 46:7849–7854.

Wu Y, Qu W, Geng D, Liang JY, Luo YL. 2012. Phenols and flavonoids from the aerial part of *Euphorbia hirta*. Chin J Nat Med. 10:40–42.

Xie W, Zhang X, Wang T, Hu J. 2012. Botany, traditional uses, phytochemistry and pharmacology of *Apocynum venetum* L. (Luobuma): a review. J Ethnopharmacol. 141:1–8.

Yang RY, Lan YS, Huang ZJ, Shao CL, Liang H, Chen ZF, Li J. 2012. Isoflavonoids from *Sophora tonkinensis*. Chem Nat Compd. 48:674–676.

Yoon KD, Jeong DG, Hwang YH, Ryu JM, Kim J. 2007. Inhibitors of osteoclast differentiation from *Cephalotaxus koreana*. J Nat Prod. 70:2029–2032.

Yue JM, Zhao Y, Zhao QS, Wu HM, Xu JF, Lin ZW, Sun HD. 1998. A novel compound from *Ceratostigma minus*. Chin Chem Lett. 9:647–649.

Zhao J, Yang WJ, Ren Y, Xu F, Xu JG. 2012. Chemical constituents of *Capparis spinosa* L. Nat Prod Res Dev. 24:52–54.

Zhou SW, Yang CY, Xia CH. 2011. The prevention of marine fouling organisms by natural antifoulants: a review. Nat Prod Res Dev. 23:186–197.

Zhou X, Zhang Z, Xu Y, Jin C, He H, Hao X, Qian PY. 2009. Flavone and isoflavone derivatives of terrestrial plants as larval settlement inhibitors of the barnacle *Balanus amphitrite*. Biofouling. 25:69–76.