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Biological evaluation of secondary metabolites from the roots of *Myrica adenophora*

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ABSTRACT

Bioassay-guided fractionation of the roots of *Myrica adenophora* led to isolation of 24 known compounds and hitherto unknown compounds, including three A-type proanthocyanidins [adenodimerins A–C], two esters of sucrose [myricadenins A and B], and the phenolic glycoside 6′-*O*-galloyl orbicularin. Spectroscopic analyses were used to determine their structures. Adenodimerin A, myricananin C, and myricetin showed strong 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activities, with SC_{50} values of 7.9, 16.3, and 15.9 μM, respectively. Adenodimerin A, myricanone, myricananin C, (−)-myricanol, myricanol 11-*O*-*β*-D-glucopyranoside, and myricetin showed stronger 2,2′-azino-bis-3-ethylbenzthiazoline-6-sulphonic acid (ABTS) radical scavenging activities than the positive control, with SC_{50} values of 7.5, 19.6, 12.0, 22.3, 19.6, and 15.6 μM, respectively. 5-Deoxymyricanone, porson, 12-hydroxymyricanone (−)-myricanol, and (+)-galeon exhibited anti-tubercular activity against *Mycobacterium tuberculosis* H37Rv in vitro and MICs values of 25.8, 40.0, 35.8, 30.0, and 15.0 μg/mL, respectively. Myricadenin A, myricanone, myricananin C, and (−)-myricanol exhibited anti-inflammatory activities in the iNOS assay with EC_{50} values of 18.1, 1.00, 13.0, and 7.5 μM, respectively.

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1. Introduction

Myrica adenophora Hance (Myricaceae) is a small tree that is distributed in China and Taiwan (Yang and Lu, 1996). The roots and bark are used as folk medicine in Taiwan for the treatment of bleeding, diarrhea, and stomach ache (Li, 2006), and the fruits of the tree are also edible. Previously, several diarylheptanoids, flavonoids, triterpenoids, tannins, monoterpenoids, and benzenoids were isolated from Myrica plants (Matsuda et al., 2002; Nhiem et al., 2010; Nonaka et al., 1983). In addition, more than 1500 species of Formosan plants have been screened for anti-tubercular, anti-oxidant, and anti-inflammatory activities. The methanolic extract of the roots of these plants showed potent bioactivities in these three assay platforms. The aims of this study were thus to isolate the chemical constituents from the roots of this species and to describe their biological activities. Bioassay-guided fractionation of the active ethyl acetate-soluble layer from the roots of this plant led to the isolation of six new compounds (**1**–**6**) and 24 known compounds (Fig. 1). Their structures of these isolates were determined by spectroscopic analysis, and assessments of their in vitro anti-tubercular, anti-oxidant, and anti-inflammatory activities are also described.

2. Results and discussion

Dried roots of *M. adenophora* were extracted to yield a MeOH extract, which was partitioned in EtOAc–H_2O to produce an EtOAc-soluble fraction and an H_2O-soluble fraction. The EtOAc-soluble fraction showed significant ABTS cation radical scavenging activity, DPPH radical scavenging activity, anti-tubercular activity, and anti-iNOS activity. Investigation of the active EtOAc-soluble fraction by column chromatography and preparative TLC led to the isolation of six new compounds (**1**–**6**) and 24 known compounds.

Compound **1** was obtained as a yellowish solid with $[\alpha]_D^{25}$ +195 (*c* 0.06, MeOH). Its HRESIMS spectrum showed a quasi-molecular $[M+Na]^+$ ion peak at *m*/*z* 789.1273, which corresponded to the molecular formula $C_{36}H_{30}O_{19}$ with 22 degrees of unsaturation. The UV spectrum of **1** showed absorptions at 206, 267, 301 sh,

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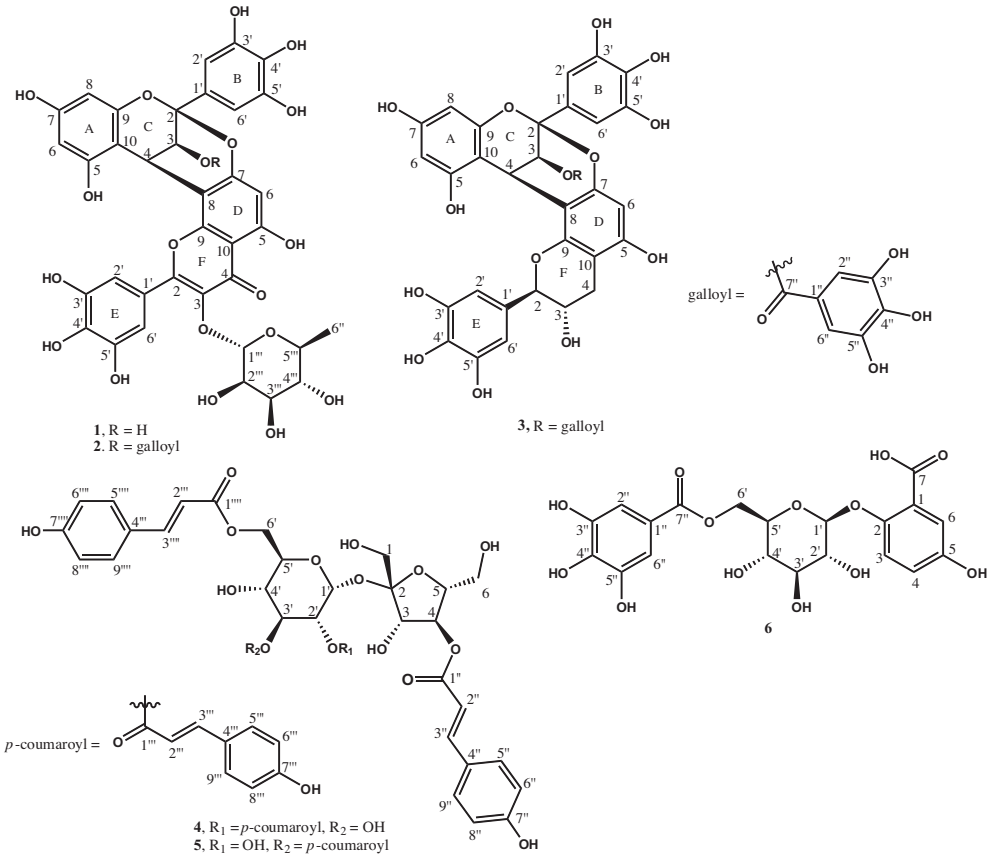

Fig. 1. Structures of compounds **1–6**.

and 357 nm. The IR spectrum displayed absorption of hydroxy groups (3363 cm^{-1}) and a carbonyl group (1655 cm^{-1}). The ^1H NMR spectrum of **1** (Table 1) gave two aromatic substitution patterns, including a gallocatechin moiety (Santos and Waterman, 2000) [δ 6.02 and 6.06 (each H, d, J = 2.4 Hz, H-6, 8 of ring A) and 6.78 (2H, s, H-2′, 6′ of ring B)] and a myricitrin moiety (Zhong et al., 1997) [δ 6.29 (1H, s, H-6 of ring D) and 7.41 (2H, s, H-2′, 6′ of ring E). A rhamnosyl moiety with an anomeric proton at δ 5.48 (1H, d, J = 1.2 Hz, H-1‴), a methyl group as a doublet at δ 0.96 (3H, d, J = 6.0 Hz, H-6‴), four oxymethine protons at δ 3.39 (1H, t, J = 9.3 Hz, H-4‴), 3.67 (1H, m, H-5‴), 3.79 (1H, dd, J = 9.3, 3.6 Hz, H-3‴), and 4.22 (1H, dd, J = 3.6, 1.2 Hz, H-2‴) were also observed.

Table 1
^1H NMR spectroscopic data for compounds **1–3**.[a]

Ring	Position	δ_H (J in Hz)		
		1	**2**	**3**
C	3	4.30, d (3.0)	5.59, d (3.6)	5.40, d (3.6)
	4	4.94, d (3.0)	5.11, d (3.6)	4.50, d (3.6)
A	6	6.02, d (2.4)	5.91, d (2.1)	5.96, d (2.4)
	8	6.06, d (2.4)	6.10, d (2.1)	6.14, d (2.4)
B	2′, 6′	6.78, s	6.72, s	6.730, s
F	2			4.74, d (7.2)
	3			4.12, ddd (7.8, 7.2, 5.4)
	4a			2.60, dd (16.5, 7.8)
	b			2.90, dd (16.5, 5.4)
D	6	6.29, s	6.40, s	6.13, s
E	2′, 6′	7.41, s	7.28, s	6.730, s
	2″		6.83, s	6.725, s
	6″		6.83, s	6.725, s
	1‴	5.48, d (1.2)	5.41, d (1.8)	
	2‴	4.22, dd (3.6, 1.2)	4.25, dd (3.6, 1.8)	
	3‴	3.79, dd (9.3, 3.6)	3.84, dd (9.6, 3.0)	
	4‴	3.37, t (9.3)	3.36, t (9.6)	
	5‴	3.67, qd (9.3, 6.0)	3.63, dd (9.6, 6.6)	
	6‴	0.96, d (6.0)	1.00, d (6.6)	
	OH	4.53, br s (3H)		
	OH	8.09, br s (8H)		
	OH-5	12.81, s		

[a] ^1H NMR data (δ) were measured in acetone-d_6 at 400 MHz for **1**, and in CD_3OD at 600 MHz for **2** and **3**.

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