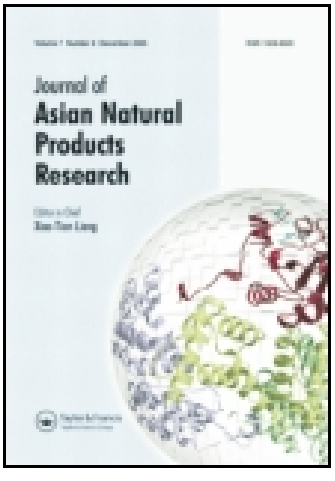


Journal of Asian Natural Products Research
Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/ganp20

Two new dendrocandins with neurite outgrowth-promoting activity from Dendrobium officinale

Liu Yang[ab], Shou-Jin Liu[b], Huai-Rong Luo[a], Juan Cui[ab], Jun Zhou[a], Xuan-Jun Wang[c], Jun Sheng[c] & Jiang-Miao Hu[a]

[a] State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming650201, China

[b] College of Pharmacy, Anhui University of Traditional Chinese Medicine, Hefei230038, China

[c] Key Laboratory of Puer Tea Science, Yunnan Agricultural University, Ministry of Education, Kunming650201, China

Published online: 07 Oct 2014.

To cite this article: Liu Yang, Shou-Jin Liu, Huai-Rong Luo, Juan Cui, Jun Zhou, Xuan-Jun Wang, Jun Sheng & Jiang-Miao Hu (2015) Two new dendrocandins with neurite outgrowth-promoting activity from Dendrobium officinale, Journal of Asian Natural Products Research, 17:2, 125-131, DOI: 10.1080/10286020.2014.942294

To link to this article: http://dx.doi.org/10.1080/10286020.2014.942294

Journal of Asian Natural Products Research, 2015
Vol. 17, No. 2, 125–131, http://dx.doi.org/10.1080/10286020.2014.942294

Two new dendrocandins with neurite outgrowth-promoting activity from *Dendrobium officinale*

Liu Yang[a,b], Shou-Jin Liu[b], Huai-Rong Luo[a], Juan Cui[a,b], Jun Zhou[a], Xuan-Jun Wang[c], Jun Sheng[c]* and Jiang-Miao Hu[a]*

[a]*State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming 650201, China;* [b]*College of Pharmacy, Anhui University of Traditional Chinese Medicine, Hefei 230038, China;* [c]*Key Laboratory of Puer Tea Science, Yunnan Agricultural University, Ministry of Education, Kunming 650201, China*

(*Received 26 March 2014; final version received 3 July 2014*)

Two new bibenzyl derivatives, dendrocandin T (**1**) and dendrocandin U (**2**), together with eight known bibenzyls, were isolated from the stems of *Dendrobium officinale*. Those compounds were sent for the first time for central nervous system-related bioassay and the results indicated that compounds **3**, **4**, and **5** have a certain degree of neurite outgrowth-promoting activity, and compounds **1**, **2**, **6**, and **7** also have weak activity. The results indicated that *D. officinale* used as health food and traditional Chinese medicine "Tiepi Shihu" has a health function of neurotrophic effects.

Keywords: *Dendrobium officinale*; bibenzyl; dendrocandin T; dendrocandin U; neurite outgrowth-promoting activity

1. Introduction

Dendrobium officinale Kimura et Migo is a perennial epiphytic herb of Orchidaceae [1]. The stem of this plant is one of the most popular and expensive biological resource for "Tiepi Shihu" that is widely used as health food and supplements and traditional Chinese medicine [2] for antipyretic, eyes-benefiting, and tonic purposes. Stimulated by the demand of market, the price of this plant becomes very high in recent years and the cultivation of this plant becomes very popular in many provinces of China, such as Yunnan, Zhejiang, Guangxi, Guangdong, and Anhui. In the past few years, pharmacological studies of *D. officinale* were mainly focused on anti-tumor [3], antioxidant [4], enhancing immune ability [5], hypoglycemic [6], and promoting glandular secretion [7]. Neurotrophic factors such as nerve growth factor (NGF) promote a wide variety of responses, including differentiation, survival, plasticity, and repair in neurons [8]. Furthermore, NGF can cause the proliferation and differentiation of PC12 cell lines. To endow a scientific base for the traditional applications of this herbal medicine, chemical research of the species was developed and 10 bibenzyls were isolated and then sent for neurite outgrowth-promoting activity assay.

Natural environment of southern Yunnan was warm, damp, and very suitable for the cultivation of Orchidaceae; thus, the production of Shihu in Yunnan approached 60% of total Chinese national output in 2011. To ascertain the chemical composition of this species from Yunnan Province and the health care function of *D. officinale* especially for the good of elders, chemical research and assessment of neurite outgrowth-promoting activity were developed for the first time. After repeated column chromatography (CC) of

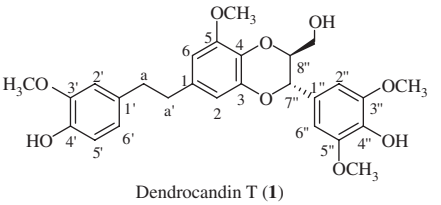
*Corresponding authors. Email: 623157535@qq.com; hujiangmiao@mail.kib.ac.cn

the ethyl acetate-soluble portion of the ethanol extract from the stems of *D. officinale* on silica gel and Sephadex LH-20, two new compounds (**1** and **2**) and eight known compounds (**3**–**10**, Figure 1) were isolated. This manuscript describes the isolation and elucidation process and the bioassay results of isolated compounds from the stems of *D. officinale* collected from Yunnan Province.

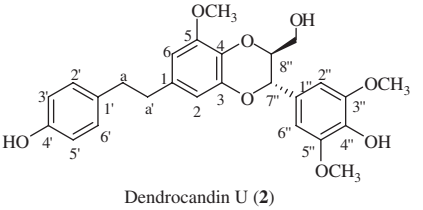
2. Results and discussion

The dried stems of *D. officinale* were labeled as powder and extracted with EtOH (90%) under reflux for three times to give EtOH extract, followed by successive extraction with EtOAc and *n*-BuOH. Further purification of the EtOAc fraction was carried out with silica gel (200–300 mesh), MCI-gel CHP-20P, Sephadex LH-20, and RP-8 and RP-18 CC. This let us to obtain two new compounds, dendrocandins T (**1**) and U (**2**), together with eight known compounds such as dendrocandin B (**3**) [9], 4-(3,5-dimethoxyphenethyl)phenol (**4**) [10], 3-(4-hydroxyphenethyl)-5-methoxyphenol (**5**) [11], 3-(3-hydroxyphenethyl)-5-methoxyphenol (**6**) [12], 4-(4-hydroxyphenethyl)-2,6-dimethoxyphenol (**7**) [13], 4-(4-hydroxy-3-methoxyphenethyl)-2,6-dimethoxyphenol (**8**) [14], gigantol (**9**) [15], and 3-*O*-methylgigantol (**10**) [16] (Figure 1). Structures of these compounds were elucidated by ^{1}H, ^{13}C NMR, and MS data analyses and comparing with those reported in the literature.

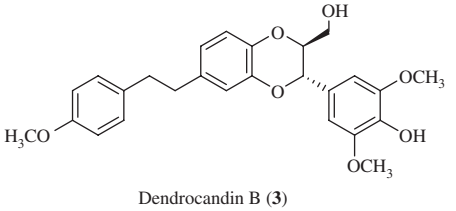
Dendrocandin T (**1**) was obtained as oil, and its molecular formula $C_{27}H_{30}O_9$ was analyzed by HR-EI-MS at *m/z* 498.1853 $[M]^+$. The IR spectrum showed absorption bands at 3440, 1615, 1512, and 1463 cm^{-1} ascribable to hydroxyl and aromatic functional groups. The UV spectrum exhibited the absorption maxima at 277 and 208 nm. The ^{1}H and ^{13}C NMR spectra indicated the presence of four methoxyl groups [δ_H 3.86 (6H, s) and 3.91 (6H, s); δ_C 55.8, 56.0, 56.3, and 56.3], three methylene groups [δ_H 2.80–2.82 (4H, m), 3.53 (1H, dd, $J = 12.0$, 3.0 Hz), and 3.90–3.91 (1H, m, oxygenated); δ_C 37.5, 38.0, and 61.5], and two oxygenated methine groups [δ_H 3.97–3.99 (1H, m)



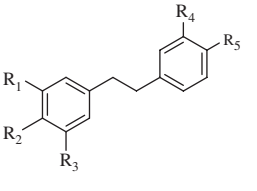
Dendrocandin T (**1**)



Dendrocandin U (**2**)



Dendrocandin B (**3**)



R_1=R_3=OCH_3,R_2=R_4=H,R_5=OH (**4**)
R_1=R_5=OH,R_2=R_4=H,R_3=OCH_3 (**5**)
R_1=R_4=OH,R_2=R_5=H,R_3=OCH_3 (**6**)
R_1=R_3=OCH_3,R_2=OH,R_4=H,R_5=OH (**7**)
R_1=R_3=R_4=OCH_3,R_2=R_5=OH (**8**)
R_1=R_5=OH,R_2=H,R_3=R_4=OCH_3 (**9**)
R_1=OH,R_2=H,R_3=R_4=R_5=OCH_3 (**10**)

Figure 1. Structures of compounds **1**–**10**.

Table 1. ^{1}H and ^{13}C NMR spectral data of compounds **1** and **2**.

No.	**1**		**2**	
Position	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C
1		134.4		134.4
2	6.51 (1H, d, $J = 1.6$)	109.5	6.50 (1H, d, $J = 1.5$)	109.5
3		144.0		144.0
4		130.8		130.8
5		148.4		148.2
6	6.33 (1H, d, $J = 1.6$)	104.6	6.30 (1H, d, $J = 1.5$)	104.7
1′		133.5		133.7
2′	6.67 (1H, br s)	110.9	7.04 (1H, dd, $J = 8.2, 1.5$)	129.5
3′		146.2	6.76 (1H, dd, $J = 8.2, 1.5$)	115.1
4′		143.6		153.7
5′	6.82 (1H, d, $J = 7.8$)	114.1	6.76 (1H, dd, $J = 8.2, 1.5$)	115.1
6′	6.69 (1H, dd, $J = 7.8, 1.6$)	120.9	7.04 (1H, dd, $J = 8.2, 1.5$)	129.5
α	2.80-2.82 (2H, m)	38.0	2.83-2.84 (2H, m)	37.9
α′	2.80-2.82 (2H, m)	37.5	2.83-2.84 (2H, m)	36.9
1″		127.2		127.2
2″	6.67 (1H, br s)	103.9	6.66 (1H, br s)	104.0
3″		147.2		147.2
4″		135.1		135.1
5″		147.2		147.2
6″	6.67 (1H, br s)	103.9	6.66 (1H, br s)	104.0
7″	4.94 (1H, d, $J = 8.2$)	76.4	4.94 (1H, d, $J = 8.2$)	76.4
8″	3.97-3.99 (1H, m)	78.2	3.99-4.00 (1H, m)	78.2
9″	3.53 (1H, dd, $J = 12.0, 3.0$)	61.5	3.56 (1H, dd, $J = 12.0$ 3.0)	61.5
	3.90-3.91 (1H, m)		3.84-3.85 (1H, m)	
5-OMe	3.86 (3H, s)	55.8	3.85 (3H, s)	56.3
3′-OMe	3.86 (3H, s)	56.0		
3″-OMe	3.91 (3H, s)	56.3	3.91 (3H, s)	56.0
5″-OMe	3.91 (3H, s)	56.3	3.91 (3H, s)	56.0

and 4.94 (1H, d, $J = 8.2$ Hz); δ_C 78.2 and 76.4] (Table 1). Furthermore, the signals of seven aromatic protons were observed in the ^{1}H NMR spectrum, and the signals of 18 aromatic carbons (7 protonated carbons and 11 quaternary carbons) were shown in the ^{13}C NMR spectrum. Also, comparison of the ^{1}H and ^{13}C NMR spectra for **1** with those of dendrocandin B indicated that compound **1** also contains three aromatic rings [9]. The cross-peaks of H-α, α′ at δ_H 2.80−2.82 (4H, m) with C-1 at δ_C 134.4, C-1′ at δ_C 133.5, C-2 at δ_C 109.5, C-2′; at δ_C 110.9, C-6 at δ_C 104.6, and C-6′; at δ_C 120.9 in the HMBC spectrum (Figure 2) showed the presence of a bibenzyl unit. In addition, The HMBC correlation peaks between H-2″/C-1″, C-3″, C-4″, and C-7″, and between H-7″/C-1″, C-2″, C-6″, C-8″, and C-9″ deduced the presence of a phenylpropane unit. By comparing the ^{1}H and ^{13}C NMR spectra of **1** with those of dendrocandin B [9], compound **1** was deduced to have one more methoxy and hydroxyl residues. The methoxy (δ_H 3.86; δ_C 55.8) was linked to C-5 (δ_C 148.4) by referring to the HMBC correlation, while the hydroxyl was linked to the remaining quaternary carbon (C-4′ at δ_C 143.6). The cross-peaks of δ_H 3.86 (5-OCH_3) with δ_H 6.33 (H-6), δ_H 3.86 (3′-OCH_3) with δ_H 6.67 (H-2′) in the ROESY spectrum (Figure 2) confirmed this connection further. The coupling constant ($J_{7'',8''} = 8.2$ Hz) between H-7″ and H-8″ indicated the *threo* configuration of the

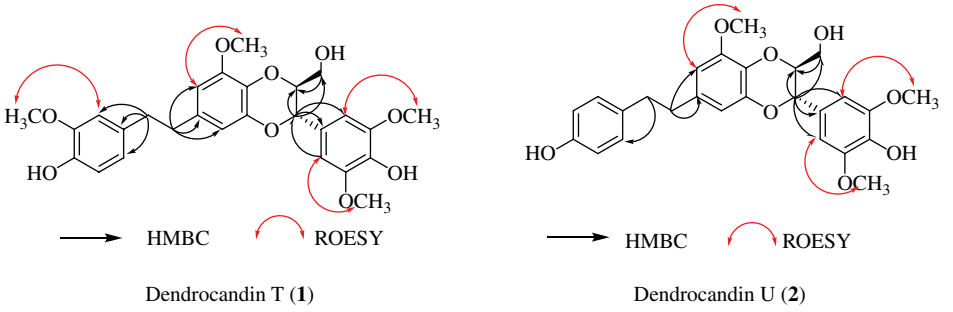


Figure 2. Key HMBC and ROESY correlations of compounds **1** and **2**.

chiral centers of the dioxane ring [17–19]. The optical rotation of compound **1** (−4.3) was similar to that of dendrocandin B (−4.6), indicating that both compounds may have the same trans-configurations at C-7″ and C-8″. Therefore, compound **1** was elucidated as 1′-[α′-[(7″*S*,8″*S*)-7″-(4″-hydroxy-3″, 5″-dimeth oxyphenyl)-8″-hydroxy-methyl-5-meth-oxy-7″,8″-dihydrobenzo[3a,4a]dioxin-1-yl] ethyl]-4′-hydroxy-3′-methoxylbenzene, named dendrocandin T.

Dendrocandin U (**2**) was obtained as a white powder and its molecular formula $C_{26}H_{28}O_8$ was analyzed by HR-EI-MS at *m/z* 468.1784 $[M]^+$. The IR spectrum showed absorption bands at 3441, 1614, 1512, and 1462 cm^{-1} ascribable to hydroxyl and aromatic functional groups. The UV spectrum exhibited the absorption maxima at 275 and 209 nm. The ^1H and ^{13}C NMR data of **2** indicated the presence of three methoxy groups [δ_H 3.85 (3H, s) and 3.91 (6H, s); δ_C 56.3, 56.0, and 56.0], three CH_2 groups [δ_H 2.83–2.84 (4H, m), 3.56 (1H, dd, J = 12.0, 3.0 Hz), and 3.84–3.85 (1H, m, oxygenated); δ_C 37.9, 36.9, and 61.5], and two oxygenated CH groups [δ_H 3.99–4.00 (1H, m) and 4.94 (1H, d, J = 8.2 Hz); δ_C 78.2 and 76.4] (Table 1). In addition, the signals of eight aromatic protons were observed and distributed to three aromatic rings on the basis of the coupling constants in the ^1H NMR spectrum. Comparing ^1H, ^{13}C NMR, and HMBC spectra (Figure 2) with those of dendrocandin T (**1**), the methoxy at C-3′ of dendrocandin T was substituted by an aromatic proton. The coupling constant ($J_{7'',8''}$ = 8.2 Hz) between H-7″ and H-8″ indicated the *threo* configuration of the chiral centers of the dioxane ring [17–19], same as that of compound **1**. The optical rotation of compound **2** (−4.7) was similar with that of dendrocandin B (−4.6) indicating that two compounds may all have the trans-configurations at C-7″ and C-8″. Therefore, compound **2** was elucidated as 1′-[α′-[(7″*S*,8″*S*)-7″-(4″-hydroxy-3″,5″-dimethoxyphenyl)-8″-hydroxymethyl-5-methoxy-7″,8″-dihydro-benzo[3a,4a]dioxin-1-yl]ethyl]-4′-hydro-xybenzene, named as dendrocandin U.

3. Experimental

3.1 General experimental procedures

Optical rotations were determined on a JASCO model 1020 polarimeter (Horiba, Tokyo, Japan). UV spectra were measured on a Shimadzu UV-2401A spectrophotometer (Shimadzu, Kyoto, Japan). IR spectra were obtained on a Bio-Rad FTS-135 infrared spectrometer (Bio-Rad, Hercules, CA, USA) using KBr pellets. ESI-MS (including HR-ESI-MS) were recorded on an API QSTAR Pulsar i (MDS Sciqaszex, Concord, Ontario, Canada) mass spectrometer. 1D and 2D NMR spectra were recorded on a Bruker AV-400 or DRX-500 spectrometer (Bruker, Bremerhaven, Germany) with

TMS as the internal standard. Silica gel (200–300 mesh; Qingdao Marine Chemical, Inc., Qingdao, China), MCI gel CHP 20P (75–150 μm; Mitsubishi Chemical Corp., Tokyo, Japan), Sephadex LH-20 (Amersham Biosciences, Uppsala, Sweden), and LiChroprep RP-18 gel (40–63 μm, Merck, Darmstadt, Germany) were used for normal pressure CC. Fractions were monitored by TLC, and spots were visualized with UV light at 254 nm.

3.2 Plant material

The stems of *D. officinale* were collected in Yunnan Province in 2011 and identified by Prof. Kaicong Fu of the Pu'er National Institute of Traditional Medicine. A voucher specimen (No. Zsh-10) was deposited in the herbarium of Kunming Institute of Botany, Chinese Academy of Sciences.

3.3 Extraction and isolation

The dried stems of *D. officinale* (4.0 kg) were powdered and extracted three times with 90% EtOH at 70°C for 2 h to give EtOH extract of 248 g, followed by successive extraction with EtOAc and *n*-BuOH. The EtOAc extract (89 g) was initially chromatographed on MCI gel and gradiently eluted with 40% MeOH to 100% MeOH to afford seven fractions (A–G). Fraction A (5.9 g) was subjected to silica gel CC (200–300 mesh, 60 g) and eluted by petroleum ether/acetone (20:1–1:1) to give four subfractions (Fr. A-1–A-4). Fr. A-2 (0.85 g) was further separated by octadecylsilyl (ODS) column [MeOH/H_2O (65:35–95:5, v/v)], then Sephadex LH-20 column [MeOH/H_2O (95:5, v/v)] to yield compound **10** (3 mg). Fraction B (8.7 g) was subjected to repeated silica gel CC (200–300 mesh, 87 g, petroleum ether/acetone (15:1–1:1, v/v)), and then purified by CC [Sephadex LH-20, MeOH/H_2O (95:5, v/v)] to give compound **3** (2 mg). Fraction C (5.2 g) was submitted to repeated silica gel CC [petroleum ether/acetone (10:1–2:3)] to give five subfractions (Fr. C-1–C-5). Fr. C-3 (1.3 g) was chromatographed over silica gel [(petroleum ether/acetone (6:1–1:1, v/v)], then purified by Sephadex LH-20 column [MeOH/H_2O (95:5, v/v)] to yield **4** (2 mg). Fraction D (9.2 g) was submitted to repeated silica gel CC [petroleum ether/acetone (10:1–2:3)] to give five subfractions (Fr. D-1–D-5). Fr. D-3 (0.9 g) was purified by Sephadex LH-20 column [MeOH/H_2O (95:5, v/v)], then chromatographed over silica gel [petroleum ether/acetone (2:1, v/v)] to yield **1** (7 mg). Similarly, fraction D-4 (1.2 g) was further purified by Sephadex LH-20 column [MeOH/H_2O (95:5, v/v)], then chromatographed over silica gel [(petroleum ether/acetone (2:1, v/v)] to yield **2** (8 mg). Fraction E (15.6 g) was subjected to repeated silica gel CC, eluted with a gradient of petroleum ether/acetone (8:1–0:1, v/v) to give six subfractions. Fraction E-1 (3.5 g) was separated further by CC [silica gel, $CHCl_3$/MeOH (15:1, v/v)], and then passed over ODS column [MeOH/H_2O (1:1–1:0, v/v)] and purified by Sephadex LH-20 column [MeOH/H_2O (95:5, v/v)] to afford **6** (2 mg) and **7** (41 mg). Fraction E-3 (1.1 g) was treated as fraction E-1 to give **5** (7 mg). Fraction F (5.8 g) was submitted to repeated silica gel CC [petroleum ether/acetone (8:1–0:1)] to give three subfractions (Fr. C-1–C-3). Fr. F-1 (0.87 g) was chromatographed over silica gel [(petroleum ether/acetone (2:1, v/v)], then purified by Sephadex LH-20 column [MeOH/H_2O (95:5, v/v)] to yield **9** (23 mg). All purified compounds had a degree of purity $>95\%$.

3.3.1 Dendrocandin T (*1*)

Oily, $[\alpha]_D^{20}$ −4.3678 ($c = 0.261$, MeOH); UV(MeOH) λ_{max} (log ε): 277, 208 nm; IR (KBr) ν_{max}: 3440, 2925, 2851, 1615, 1601, 1512, 1463, 1363, 1339, 1219, 1115,

1033 cm^{-1}; EI-MS *m/z*: 521 $[M + Na]^+$; HR-EI-MS *m/z*: 498.1853 $[M]^+$ (calcd for $C_{27}H_{30}O_9$, 498.1890).

*3.3.2 Dendrocandin U (**2**)*

White powder, $[\alpha]_D^{20}$ −4.7489 ($c = 0.219$, MeOH); UV (MeOH) λ_{max} (log ε): 275, 209 nm; IR (KBr) ν_{max}: 3441, 2927, 2851, 1614, 1601, 1512, 1462, 1361, 1340, 1219, 1116, 1037 cm^{-1}; EI-MS: *m/z* 491 $[M + Na]^+$; HR-EI-MS: *m/z* 468.1784 $[M]^+$ (calcd for $C_{26}H_{28}O_8$, 468.1784).

3.4 Cell culture and evaluation of neurite outgrowth-promoting activity

The neurotrophic activity of the test compounds was examined according to an assay using PC12 cells [20]. Briefly, PC12 cells were maintained in F12 medium supplemented with 12.5% horse serum, and 2.5% fetal bovine serum, and incubated at 5% CO_2 and 37°C. Test compounds were dissolved in DMSO. For the neurite outgrowth-promoting activity bioassay, PC12 cells were seeded at a density of 5×10^4 cells/ml in 48-well plate coated with poly-L-lysine. After 24 h, the medium was changed to that containing 10 μM of each test compound plus 5 ng/ml NGF, or various concentrations of NGF (50 ng/ml for the positive control; 5 ng/ml for the negative control). The final concentration of DMSO was 0.05%, and the same concentration of DMSO was added into the negative control. After 72 h incubation, the neurite outgrowth was assessed under a phase-contrast microscope. Neurite processes with a length equal to or greater than the diameter of the neuron cell body were scored as neurite-bearing cells. The ratio of the neurite-bearing cells to total cells (with at least 100 cells examined/view area; 5 viewing area/well) was determined and expressed as a percentage. The data of neurite outgrowth-promoting activity were shown in Table 2.

Table 2. The neurite outgrowth-promoting activity of compounds **1**–**10**.

Groups	72-h differentiation rate (%)
Blank	No activity
Negative 5 ng/ml NGF	6.20
Positive 50 ng/ml NGF	27.89
1	7.86
2	9.46
3	15.95
4	14.87
5	12.08
6	8.20
7	6.94
8	No activity
9	No activity
10	No activity

Acknowledgments

This work was financially supported by Pu'er Institute of Pu'er Tea and Yunnan Province (No. 2012CG001 and 2013IB021). The authors are also grateful to the members of the analytical group of the State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, for all of the spectral measurements.

References

[1] Flora Repubulicae Popularis Sinicae Editorial Committee, *Flora of China*, Vol. 19 (Science Publishers, Beijing, 2004), p. 117.
[2] China Pharmacopoeia Committee, *Chinese Pharmacopoeia*, Part I (Chemistry and Industry Press, Beijing, 2010), p. 265.
[3] G.X. Ma, G.J. Xu, and L.S. Xu, *J. China Pharm. Univ.* **25**, 188 (1994).
[4] X.Q. Zha and J.H. Wang, *Food Sci.* **28**, 90 (2007).
[5] Y.S.Y. Hsieh, C. Chien, S.K.S. Liao, S.F. Liao, W.T. Hung, W.B. Yang, C.C. Lin, T.J.R. Cheng, C.C. Chang, J.M. Fang, and C.H. Wong, *Bioorg. Med. Chem.* **16**, 6054 (2008).
[6] H.S. Wu, J.H. Xu, L.Z. Chen, and J.J. Sun, *China J. Chin. Mater. Med.* **29**, 160 (2004).
[7] J.H. Xu and L.Z. Chen, *Chin. Tradit. Herb. Drugs* **26**, 79 (1995).
[8] G.X. Zhang and Q.F. Zhu, *J. Biol. (Shengwuxue Zazhi)* **19**, 14 (2002).

[9] Y. Li, C.L. Wang, S.X. Guo, J.S. Yang, and P.G. Xiao, *Chem. Pharm. Bull.* **56**, 1477 (2008).
[10] G.R. Pettit, S.B. Singh, J.M. Schmidt, M. L. Niven, E. Hamel, and C.M. Lin, *J. Nat. Prod.* **51**, 517 (1988).
[11] M. Wang, C.F. Zhang, Z.T. Wang, and M. Zhang, *China J. Chin. Mater. Med.* **32**, 701 (2005).
[12] J.M. Hu, W.W. Fan, F.W. Dong, Z.H. Miao, and J. Zhou, *Chin. J. Chem.* **30**, 1327 (2012).
[13] E. Malan and E. Swinny, *Phytochemistry* **34**, 1139 (1993).
[14] G.N. Zhang, C.F. Zhang, Z.T. Wang, and L.S. Xu, *Chin. J. Nat. Med.* **2**, 78 (2004).
[15] Y.G. Chen, J.J. Xu, H. Yu, C. Qing, Y.L. Zhang, L.Q. Wang, Y. Liu, and J.H. Wang, *Food Chem.* **107**, 169 (2008).
[16] Y. Tezuka, H. Hirano, T. Kikuchi, and G. J. Xu, *Chem. Pharm. Bull.* **39**, 593 (1991).
[17] M.G. Banwell, A. Bezos, S. Chand, G. Dannhardt, W. Kiefer, U. Nowe, C.R. Parish, G.P. Savage, and H. Ulbrich, *Org. Biomol. Chem.* **1**, 2427 (2003).
[18] T.H. Kim, H. Ito, K. Hayashi, T. Hasegawa, T. Machiguchi, and T. Yoshida, *Chem. Pharm. Bull.* **53**, 641 (2005).
[19] C.Y. Ma, H.J. Zhang, G.T. Tan, N.V. Hung, N.M. Cuong, D.D. Soejarto, and H.H.S. Fong, *J. Nat. Prod.* **69**, 346 (2006).
[20] L.A. Greene and A.S. Tischler, *Proc. Natl Acad. Sci. USA*. **73**, 2424 (1976).