CHEMICAL CONSTITUENTS FROM THE CLIMBERS OF *Fissistigma rubiginosum*

Napatsawan Khwanchuen*, Abdul-Wahab Salae, Uma Prawat, and Pittaya Tuntiwachwuttikul
Laboratory of Natural Products Chemistry, Faculty of Science and Technology, Phuket Rajabhat University, Muang, Phuket 83000, Thailand
*e-mail: saw0937474@gmail.com

Abstract: Chemical investigation of climbers of *Fissistigma rubiginosum* resulted in the isolation of five known compounds, aristololactam B II (**1**), goniopedaline (**2**), spathulenol (**3**), *β*-caryophyllene-9*R*,8*R*-oxide (**4**), and stigmast-4-en-3-one (**5**). The structures of the known compounds were elucidated on the basis of extensive spectroscopic analyses including, IR, UV, 1D and 2D NMR as well as comparison with reported value.

Introduction: *Fissistigma rubiginosum* (synonym: *Uvaria rubiginosum*; *Melodorum rubiginosum*), named 'Yan lueat' in Suratthani in Southern Thailand, is a large woody climber about 10 m in tall and is native India, Bangladesh, Myanmar, Thailand, Kampuchea, Malaysia, Indonesia.[1] *F. rubiginosum* is a member of genus *Fissistigma* consists about 80 species which are mainly distributed in Asia and Australia. Previous phytochemical investigations have been so far carried out on various species of *Fissistigma* genus and resulted in the isolation of many alkaloids,[2-11] cyclopentenones,[12-13] flavonoids,[14-22] and sesquiterpenoids.[23] Many biological activities have been reported for this genus, such as anti-inflammatory,[10] antimicrobial,[9] and cytotoxic.[9] As part of our ongoing search for new bioactive constituents from plants growing in Thai, either wild or cultivated, our continued interest in discovering novel bioactive metabolites of the climbers of *F. rubiginosum* resulted in the isolation and identification of five knowns (**1**–**5**) (Fig. 1). In this letter, the isolation and structure of the known compounds are elucidated using spectroscopic methods, including IR, UV, 1D and 2D NMR as well as comparison with reported value.

Methodology:

General Experimental Procedures

UV spectra were recorded in methanol using SPECORD® 210 PLUS from analytic Jena spectrophotometer. IR spectra were obtained on a Shimadzu FTIR-8900 spectrophotometer. NMR spectra were acquired on a Bruker AVANCE400 spectrometer (at 400 MHz for ^{1}H and 100 MHz for ^{13}C) using $CDCl_3$ with TMS as the internal standard. Vacuum liquid chromatography (VLC) was carried out on silica gel 60H (Merck, 5-40 μm) and RP-18 (Merck, 15-25 μm). Fractions were monitored by TLC using Merck pre-coated silica gel $60F_{254}$ and RP-18 F_{254} sheets and spots were visualized by using fluorescence (254 and 386 nm) and by heating silica gel plates sprayed with 1% $Ce(SO_4)_2$ in 10% aq. H_2SO_4 solution.

Plant material

The climbers of *F. rubiginosum* were collected from Krabi Province, Thailand, in February 2016. The identification of the plant material was authenticated by Dr. Piya Chalermglin, Thailand Institute of Scientific and Technological Research, Thailand. A voucher specimen (PKRU2016001) was deposited at the Laboratory of Natural Products Chemistry, Faculty of Science and Technology, Phuket Rajabhat University, Phuket, Thailand.

Extraction and Isolation

Fresh climbers of *F. rubiginosum* (8.2 kg) were exhaustively extracted with MeOH (3 × 30 L) at room temperature, After filtration, the solvent was removed by rotary evaporation to give crude extract (624.40 g). The residue was dissolved in water and then partitioned successively with EtOAc and *n*-BuOH. The EtOAc extract (87.13 g) was adsorbed onto 175 g of silica gel 60H and fractionated by vacuum liquid chromatography (VLC) over a sintered glass filter column of silica gel 60H (1.2 kg, diameter × height: 17.5 × 10.0 cm) using an increasing amount of EtOAc in hexane (1% EtOAc to 100% EtOAc) and 100% MeOH to yield 11 fractions. Fraction 1 (0.503 g) was separated by VLC using silica gel 60H (6.28 g, 2.0 × 4.0 cm) using an increasing amount of CH_2Cl_2 in hexane (1% CH_2Cl_2 to 100% CH_2Cl_2) to provide 12 subfractions (1.1–1.12). Subfraction 1.8 was identified as **1** (1.5 mg). Fraction 3 (1.25 g) was isolated by VLC using silica gel 60H (19.3 g, 3.5 × 4.0 cm using an increasing amount of CH_2Cl_2 in hexane (1% CH_2Cl_2 to 100% CH_2Cl_2) to give 5 subfractions (3.1–3.5). Subfraction 3.1 was identified as **2** (8.0 mg). Subfraction 3.4 (0.144 g) was further purified by VLC on silica gel RP-18 (3.5 g, 1.5 × 4.0 cm), eluted with 100% MeOH to afford 9 subfractions (3.4.1–3.4.9). Subfractions 3.4.2, 3.4.5 and 3.4.6 were identified as **3** (11.7 mg), **4** (1.7mg) and **5** (7.7 mg), respectively.

aristololactam B II (**1**): Yellow solid; UV (MeOH) λ_{max} (log ε): 266 (2.87), 276 (2.25) 320 (3.13) 385 (3.13) nm; IR IR (ATR) ν_{max}: 3151, 2844, 1703, 1647, 1442, 1371 cm^{-1}; ^{1}H NMR and ^{13}C NMR (Table 1).

goniopedaline (**2**): Yellow solid; UV (MeOH) λ_{max} (log ε): 260 (2.77), 280 (2.35) 325 (3.34) 379 (3.21) nm; IR (ATR) ν_{max}: 3341, 2831, 1712, 1632, 1440, 1223 cm^{-1}; ^{1}H NMR and ^{13}C NMR (Table 1).

spathulenol (**3**): Yellow viscous oil; $[\alpha]_D^{25}$ +12° (*c* 0.1, CH_3OH); IR (ATR) ν_{max}: 3393, 2924, 1635, 914, 887 cm^{-1}; ^{1}H NMR and ^{13}C NMR (Table 2).

β-caryophyllene-9*R*,8*R*-oxide (**4**): colorless oil; $[\alpha]_D^{25}$ -54.7° (*c* 0.6, $CHCl_3$); IR (ATR) ν_{max}: 1717, 1462, 1215 cm^{-1}; ^{1}H NMR and ^{13}C NMR (Table 2).

stigmast-4-en-3-one (**5**): White solid; UV (MeOH) λ_{max} (log ε): 214 (3.15) nm; IR (ATR) ν_{max}: 1674, 1373 cm^{-1}; ^{1}H NMR and ^{13}C NMR (Table 3).

Results and Discussion:

The climbers of *F. rubiginosum* were successively extracted with MeOH at room temperature and the methanolic extract was fractionated into H_2O, EtOAc and *n*-BuOH fractions. Compounds **1**-**5** were isolated from the EtOAc extract (Fig. 1) after extensive silica gel60H vacuum liquid chromatography column. The known compounds **1**-**5** were identified by spectroscopic methods and comparison of their ^{1}H and ^{13}C-NMR spectra with the reported data.

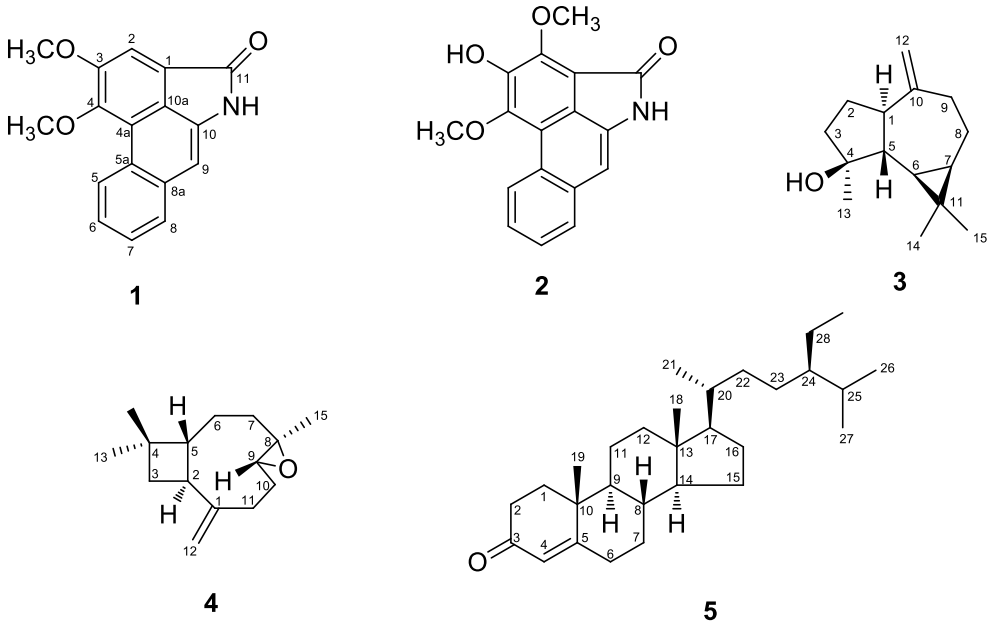

Figure 1. Structures of compounds **1-5**.

Compound **1** was obtained as yellow solid, showing a positive reaction with dragendorff reagent, indicating its alkaloid nature. The molecular formula $C_{17}H_{13}NO_3$ as determined by ^{1}H and ^{13}C NMR spectroscopic data, indicating 12 degrees of unsaturation. The UV absorption in methanol showed maxima at 266, 276, 320 and 385 nm, which corresponds to the phenenthrene chromophore[14] which was further confirmed by IR spectrum showing peaks at 1703 cm^{-1} (C=O) with a shoulder at 1647 cm^{-1} (C=C stretching of aromatic ring). The ^{13}C NMR spectrum of **1** displayed 17 carbon signals (Table 1), when analyzed with the help of its DEPT spectrum, showed the presence of 2 methyls, 6 olefinic methines, 8 olefinic quaternarys and a carbonyl carbon, including two methoxy, one carbonyl group and three benzene rings. The ^{1}H NMR spectrum data (Table 1) indicates six aromatic proton signals and two methoxyl protons at δ 3.91 (3H, s) and 4.12 (3H, s). The aromatic proton signal at δ 9.50 (1H, m) assigned to H-5, and the signals at δ 7.63 (2H, dd, *J* = 6.0, 3.6 Hz) and 7.96 (1H, dd, *J* = 6.0, 3.6 Hz) assigned to the remaining protons of ring C suggested four adjacent aromatic protons at C-5 (δ 127.6), C-6 (δ 127.7), C-7 (δ 127.7) and C-8 (δ 129.1), respectively. The singlet signal at δ 12.22 (1H, s) was attributed to NH group. This proton showed long range correlations with the signals at δ_C 124.7 (C-10a), 136.3(C-10), and 169.7 (C-11). The methoxyl groups at δ 3.91 (3H, s) and 4.12 (3H, s) were assigned at C-3 and C-4 according to the HMBC and NOESY correlations (**Fig**. **2**). Examination of NOESY spectra also confirmed the positions of methoxyl groups at C-3 and C-4, showing NOE correlation between methoxyl signal at δ 3.91 and the singlet at δ 8.07 (assigned to H-2) and between the methoxyl signal at δ 4.12 and a doublet of doublet at δ 9.50 (H-5). The HMBC correlations (**Fig**. **2**) between δ 8.07 (1H, s) and C-1 (δ 121.0), C-3 (δ 154.7), C-4 (δ 151.2), C-10a (δ 124.7), C-11 (δ 1 69.7) and between δ 7.33 (1H, s) and C-5a, (δ 127.0), C-8 (δ 129.1), C-8a (δ 135.7), C-10 (δ 136.3) and C-10a (δ 124.7) confirmed that the two singlet aromatic protons were assigned to H-2 and H-9, respectively. The other HMBC correlations between the aromatic protons at δ 7.63 (H-6, H-7) and C-5 (δ 127.6), C-5a (δ 127.0), C-8 (δ 129.1) and C-8a (δ 135.7), between the aromatic proton at δ 9.50 (H-5) and C-4a (δ 122.7) and C-7 (δ 127.7), and between the aromatic proton at δ 7.96 (H-8) and C-6 (δ 125.5) and C-9 (δ 104.9) confirmed the connectivity of the complete structure of **1**. This is in great agreement with the aristolactam skeleton. Thus, on the basis of

its spectroscopic data and comparison of the ^{1}H and ^{13}C NMR spectral data with the previous report[23], **1** was identified as aristolactam BII.

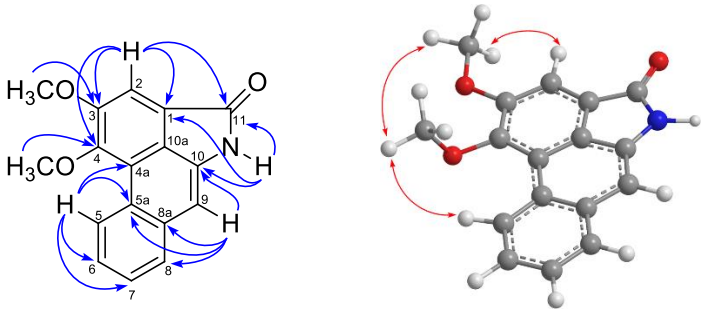

Figure 2. Selected HMBC and NOESY correlations of **1**.

Compound **2** was isolated as yellow solid, showing a positive reaction with dragendorff reagent, indicating its alkaloid. the molecular formula $C_{17}H_{13}NO_4$ as determined by ^{1}H and ^{13}C NMR spectroscopic data. Its UV and IR spectra were similar to those compound **1**. The ^{1}H and ^{13}C NMR spectra (**Table 2**) were closely related to **1**. The appearance of an oxyquaternary olefinic carbon in the ^{13}C NMR spectrum of **1** at δ_C 142.3 and the absence of aromatic methine proton (δ_H 8.07 (1H, s) and δ_C 110.1), suggested an additional a hydroxyl group. Substitution of a hydroxyl group at C-3 was supported by HMBC correlations. Detailed analysis of the HMBC correlations (**Figure 3**) confirmed that the other parts of **2** were the same as those of **1**. Thus, on the basis of its spectroscopic data and comparison of the ^{1}H and ^{13}C NMR spectral data with the previous report[24], **2** was concluded to be goniopedaline.

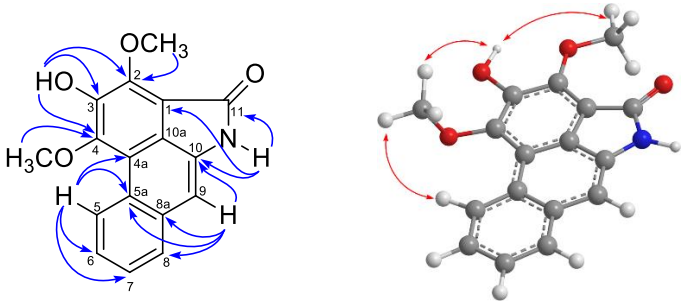

Figure 3. Selected HMBC and NOESY correlations of **2**.

Table 1.
NMR (400 MHz, $CDCl_3$) data for compounds **1** and **2**.

	aristololactam B II (**1**)		goniopedaline (**2**)	
Position	δ_C, type	δ_H (*J* in Hz)	δ_C, type	δ_H (*J* in Hz)
1	121.0, C		107.6, C	
2	110.1, CH	8.07, s	147.3, C	
3	154.7, C		142.3, C	
4	151.2, C		149.1, C	
4a	122.7, C		116.5, C	

5a	127.0, C		133.4, C	
5	127.6, CH	9.50, dd (6.0, 3.2)	126.6, CH	9.21, m
6	125.5, CH	7.63, dd (6.0, 3.6)	125.6, CH	7.55, m
7	127.7, CH	7.63, dd (6.0, 3.6)	126.6, CH	7.55, m
8	129.1, CH	7.96, dd (6.0, 3.6)	128.6, CH	7.83, m
8a	135.7, C		125.6, C	
9	104.9, CH	7.33, s	105.0, CH	7.15, s
10	136.3, C		133.4, C	
10a	124.7, C		122.6, C	
11	169.7, C=O		166.9, C=O	
2-OCH_3			63.3, CH_3	4.62, s
3-OH				6.28, s
3-OCH_3	56.6, CH_3	3.91, s		
4-OCH_3	59.9, CH_3	4.12, s	60.2, CH_3	4.16, s
-NH		12.22, s		8.07, s

Compound **3** was obtained as a colorless oil, and its molecular formula was determined to be $C_{15}H_{24}O$ by ^1H and ^{13}C NMR spectroscopic data, indicating 3 degrees of unsaturation. Its IR spectrum exhibited a hydroxyl absorption band at ν_{max} 3393 cm^{-1} and a double bond absorption band at 1635 cm^{-1}. The ^1H and ^{13}C NMR, DEPT, and HMQC data of **3** supported the presence of a double bond (δ_C 106.3 and 153.5), two tetrasubstituted carbons (δ_C 81.0 and 20.3), four methines, five methylenes, and three methyl groups (**Table 3**). Elucidation of the structure of the aromadendrane skeleton of **3** was accomplished by analyses of COSY and HMBC data (**Figure 4**). Two upfield methine protons at δ_H 0.46 (H-6) and 0.70 (H-7) suggested the presence of a cyclopropane. The presence of geminal methyl groups δ_H 1.03 (H_3-14) and 1.05 (H_3-15) on the cyclopropane ring was determined by the HMBC correlations from both of the methyl protons, H_3-12 and H_3-13, to the cyclopropyl methines, C-6 and C-7, and an upfield quaternary carbon at δ_C 20.3 (C-11) in addition to the correlations from H_3-12 to C-13 and from H_3-13 to C-12. The location of a hydroxyl (C-4) was assigned based on the HMBC correlations from H_3-13 to this oxyquternary carbon. The presence of the neighboring tertiary alcohol (δ_C 81.0, C-10) was demonstrated by the HMBC correlations from H_3-13 to the oxyquaternary carbon. The relative configuration was established on the basis of the NOESY data. Thus, on the basis of its spectroscopic data and comparison of the ^1H and ^{13}C NMR spectral data with the previous report[25], **3** was identified as spathulenol.

Compound **4** was obtained as a colorless oil, and its molecular formula was determined to be $C_{15}H_{24}O$ by ^1H and ^{13}C NMR spectroscopic data, indicating a sesquiterpene with four degrees of unsaturation. The ^{13}C NMR spectrum (Table 2) displayed 15 carbons, which were assigned by HSQC, HMBC and DEPT experiments to the resonances of three methyl (δc 17.0, 21.6 and 29.9), six methylene (δc 27.2, 29.8, 39.2, 39.8 and 112.8), three methine (δc 48.7, 50.7, 63.8) and three quaternary (δc 34.0, 59.9 and 151.8) carbons. In the ^{13}C NMR spectra (Table 2) of the compound, the presence of signals at C-1 and C-12 of an olefinic bond and the presence of one oxyquaternary carbon (δ_C 59.9) and one oxymethine carbon (δ_C 63.8) were observed which suggests that caryophyllenol A was an oxide derivative. Detailed 2D NMR analysis showed that the compound is a caryophyllene type sesquiterpene which is composed of cyclobutane, cyclononene and epoxide ring cyclic systems. Thus, on the basis of its spectroscopic data and comparison of the ^1H and ^{13}C NMR spectral data with the previous report[26], **4** was determined as *β*-caryophyllene-9*R*,8*R*-oxide.

Table 2.
NMR (400 MHz, $CDCl_3$) data for compounds **3** and **4**.

	spathulenol (**3**)		*β*-caryophyllene-9*R*,8*R*-oxide (**4**)	
Position	δ_C, type	δ_H (*J* in Hz)	δ_C, type	δ_H (*J* in Hz)
1	53.4, CH	2.21, m	151.8, C	
2	26.7, CH_2	1.58, m	48.7, CH	2.62, dt (9.7, 9.2)
		1.85, m		
3	41.7, CH_2	1.55, m	39.8, CH_2	1.60, m
		1.78, m		1.70, m
4	81.0, C		34.0, C	
5	54.3, CH	1.31, m	50.7, CH	1.77, t
6	29.9, CH	0.46, dd (11.1, 9.6)	27.2, CH_2	1.45, m
				1.64, m
7	27.5, CH	0.70, m	39.2, CH_2	0.97, m
				2.11, m
8	24.8, CH_2	1.01, m	59.9, C	
		2.00, m		
9	38.9, CH_2	2.02, m	63.8, CH	2.88, dd (10.6, 4.2)
		2.42, dd (13.1, 6.0)		
10	153.5, C		30.2, CH_2	1.31, m
				2.25, m
11	20.3, C		29.8, CH_2	2.12, m
				2.35, m
12	106.3, CH_2	4.66, br s	112.8, CH_2	4.86, br s
		4.69, br s		4.99, br s
13	26.1, CH_3	1.28, s	21.6, CH_3	1.01, s
14	16.3, CH_3	1.03, s	29.9, CH_3	0.99, s
15	28.7, CH_3	1.05, s	17.0, CH_3	1.20, s

Compound **5** was isolated as a white solid. The strong IR absorption at 1722 cm^{-1} gives evidence for the presence of ketonic group and its ^{13}C NMR signal at δ 171.7 supports the presence of the carbonyl group. The ^{13}C NMR spectrum (Table 2) displayed 29 carbons, which were assigned by HSQC, HMBC and DEPT experiments to the resonances of two methyl singlets and four methyl doublets, eleven methylene, seven methine and four quaternary carbons. The ^1H NMR terminal signal at δ 0.82 and 0.83 (6H, d, *J* = 7.0 Hz) confirmed the presence of an isopropyl moiety with the methyl groups at C-26 and C-27. The unsaturation at Δ^3 was suggested by the ^1H NMR signal at δ 5.72 (s). These were supported by the ^{13}C NMR signals at δ 123.7 (C-4), δ 171.7 (C-3). The HMBC, HSQC, and DEPT-135 experiments provided further convincing evidence for the structure assignment of **5** and accordingly, it was elucidated as stigmast-4-en-3-one.[27]

Table 3.
NMR (400 MHz, $CDCl_3$) data for stigmast-4-en-3-one (**5**).

Position	δ_C, type	δ_H (*J* in Hz)	Position	δ_C, type	δ_H (*J* in Hz)
1	35.7, CH_2		16	28.2, CH_2	
2	33.9, CH_2		17	56.0, CH	
3	199.7, C		18	11.9, CH_3	0.71, s
4	123.7, CH	5.72, s	19	17.4, CH_3	1.18, s
5	171.7, C		20	36.1, CH	
6	33.0, CH_2		21	18.7, CH_3	0.92, d (6.5)
7	32.0, CH_2		22	34.0, CH_2	
8	35.6, CH		23	26.1, CH_2	
9	53.8, CH		24	45.9, CH	
10	38.6, C		25	29.1, CH	
11	21.0, CH_2		26	19.8, CH_3	0.83, d (7.0)
12	39.6, CH_2		27	19.0, CH_3	0.82, d (7.0)
13	42.4, C		28	23.0, CH_2	
14	55.9, CH		29	12.0, CH_3	0.84, s
15	24.1, CH_2				

Conclusion: Phytochemical studies of the climbers of *F. rubiginosum* afforded the isolation of five known compounds. All the compounds were isolated from *F. rubiginosum* for the first time.

References:

1. Sinclair J. The Gardens' Bulletin Singapore. 1976;14:149–169.
2. Lu SH, Wu YC. Heterocycles. 1983;20:813–815.
3. Wu YC, Kao SH, Huang JF, Duh CY, Lu ST. Phytochemistry. 1990;29:2387–2388.
4. Chia YC, Chang FR, Wu YC. J Nat Prod. 1998;61:1430–1432.
5. Chia YC, Chang FR, Teng CM, Wu YC. J Nat Prod. 2000;63:1160–1163.
6. Lo WL, Chang FR, Wu YC. J Chin Chem Soc. 2000;47:1251–1256.
7. Alias A, Hazni H, Jaafar FM, Awang K, Ismail NH. Molecules. 2010;15:4583–4588.
8. Sawasdee K, Chaowasku T, Likhitwitayawuid K. Molecules. 2010;15:639–648.
9. Thuy TTT, Quan TD, Anh NTH, Sung TV. Nat Pro Res. 2012;26:1296–1302.
10. Ge YW, Zhu S, Shang MY, Zang XY, Wang X, Bai YJ, Li L, Komatsu K, Cai SQ. Phytochemistry. 2013;86:201–207.
11. Zhou Q, Fu YH, Zhang YQ, Wu SY, Song XP, Cao CK, Xu W, Chen GY. Tetrahedron Lett. 2016;57:4162–4164.
12. Chia YC, Chang FR, Wu YC. Tetrahedron Lett. 1999;40:7513–7514.
13. Chia YC, Wu JB, Wu YC. Tetrahedron Lett. 2000;41:2199–2201.
14. Allas Y, Awang K, Hadi AHA. J Nat Prod. 1995;58:1160–1166.
15. Jonbunprasert V, Bavovada R, Theraratchailert P, Rungserichai R, Likhitwitayawuid K. ScienceAsia. 1999;25:31–33.
16. Porzel A, Lien TP, Schmidt J, Drosihn S, Wagner C, Merzweiler K, Sung TV, Adam G. Tetrahedron. 2000;56:865–872.
17. Lien TP, Pozel A, Schmidt J, Sung TV, Adam G. Phytochemistry. 2000;53:991–995.

18. Lan YH, Chia YC, Chang FR, Hwang TL, Liaw CC, Wu YC. Helvetica Chimica Acta. 2005;88:905–909.
19. Hongping Z, Xiaoling L, Xiaohong S, Qiangzhi X, Binghua J. Journal of medical colleges of PLA. 2010;25:226–234.
20. Lan YH, Leu YL, Peng YT, Thang TD, Lin CC, Bao BY. Planta Med. 2011;77:2019–2022.
21. Lan YH, Peng YT, Thang TD, Hwang TL, Dai DN, Leu YL, Lai WC, Wu YC. Chem Pharm Bull. 2012;60:280–282.
22. Wu YC, Sureshbabu M, Fang YC, Wu YH, Lan YH, Chang FR, Chang YW, Hwang TL. Toxicol Appl Pharmacol. 2013;266:399–407.
23. Priestap HA. Planta Med. 2017;83:217–223.
24. Sun NJ, Antoun M, Chang CJ, Casady JM. J Nat Prod. 1987;50:843–846.
25. Wu TS, Chan YY, Leu YL. Chem Pharm Bull. 2000;48:357–361.
26. Damodaram NP, Dev S. Tetrahedron. 1968;24:4113–4122.
27. Greace MD, Monaco P. J Nat Prod. 1990;53:1430–1435.

Acknowledgements: We are indebted to the Office of the National Research Council of Thailand and Phuket Rajabhat University for financial support. We thank Dr. Piya Chaleamklin (Thailand Institute of Scientific and Technological Research, Thailand) for the identification of the plant. We are grateful to Department of Natural Products Chemistry, Faculty of Science and Technology, Phuket Rajabhat University for the provision of laboratory facilities and technical assistance.