CONSTITUENTS FROM TAIWANESE *SARCOPYRAMIS NEPALENSIS*

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Six flavonoids, quercetin (**1**), quercetin-3-*O*-α-L-rhamnoside (**2**), quercetin-3-*O*-β-D-glucoside (**3**), isorhamnetin-3-*O*-β-D-glucoside (**4**), kaempferol-3-*O*-β-D-glucoside (**5**), and rutoside (**6**), one triterpene, (+)-α-onocerin (**7**), three pheophorbides, pheophorbide A methyl ester (**8**), methyl (10*S*)-hydroxypheophorbide A (**9**), and (10*S*)-hydroxypheophorbide A (**10**), two steroids, β-sitosterol (**11**), stigmasterol (**12**), two benzenes, *p*-coumaric acid (**13**) and caffeic acid (**14**), along with three fatty acids, myristic acid (**15**), palmitic acid (**16**), and linolenic acid (**17**) have been isolated from the aerial of *Sarcopyramis nepalensis* Wall. Structures of **1-17** were elucidated by spectroscopic methods, including NMR, UV, IR, and MS techniques. Biological evaluation revealed that compounds **8-10** possessed significant cytotoxicity against KB (ED_{50} = 3.8-5.2 μg/mL) and Hela (ED_{50} = 3.4-4.2 μg/mL) human tumor cells. In addition, compounds **1**, **2**, **4**, and **14** were proved to have potent anti-oxidative activity (ED_{50} = 5.92-10.49 μM) by DPPH scavenging test.

Key words: *Sarcopyramis nepalensis*, flavonoid, pheophorbide, cytotoxicity, anti-oxidative activity

Introduction

A large family of Melastomataceae containing about 240 genera and 4000 species are widely distributed in tropical and subtropical areas, such as China, Taiwan, India, and Philippines etc[1]. Some Melastomataceae plants have been demonstrated to possess biological activities such as anti-oxidative[2], antihepatits[3], antitumor[2], and antinociceptive[4] effects, and to possess main constituents including flavonoids[2,5-6], benzoquinones[7], tannoids[8], and triterpenoids[5]. One genera of the Melastomataceae, *Sarcopyramis*, was widely used for folk medicine in China[6]. The title plant, *Sarcopyramis nepalensis* Wall, distributed through

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the Taiwan Island at altitudes from 300-2800 m high. Pervious phytochemical studies on *S. nepalensis* have demonstrated the presence of phenolic acids, fatty acids, sterols, flavonols, and its glycosides[9-11]. In our continuing search for bioactive constituents from Taiwanese plants, we found that ethanol extract of the title plant showed marginal cytotoxic activities against KB and Hela cell lines (ED_{50} = 18.51 and 23.68 μg/mL). We report herein bioassay-guided fractionations that resulted in the isolation and characterization of seventeen compounds (**1-17**), including six flavonoids (**1-6**), one triterpenoid (**7**), three pheophytins (**8-10**), two sterols (**11-12**), two benzenes (**13-14**), and three fatty acids (**15-17**) from the whole plant of Taiwanese *S. nepalensis*. The structures of the above isolated compounds were identified by extensive spectroscopic methods including 1D- and/or 2D-NMR experiments. All the isolated compounds (**1-17**) were evaluated for cytotoxic activities. In addition, compounds **1-6**, and **13-14** were further tested anti-oxidative activities by DPPH radical assay

Materials and Methods

I. General Experimental Procedure

Optical rotations were measured with JASCO P-2000 polarimeter. Infrared (IR) spectra were measured on a Nicolet AVATAR 320 FT-IR spectrophotometer using a KBr matrix. UV spectra were measured a Hitachi U-3310 spectrophotometer. ESIMS data were performed on the Waters Quattro Ultima mass spectrometer. 1D and 2D NMR spectra were performed on a Bruker NMR spectrometer (Unity Plus 400 MHz) using CD_3OD and $CDCl_3$ as solvent for measurement. Liquid chromatography was done on a Waters 2690 separations module micromass, with a Cosmosil 5SL-II column, and Waters 996 photodiode array detector. Sephadex LH-20 and silica gel (Merck 70-230 mesh and 230-400 mesh) were used for column chromatography, and precoated silica gel (Merck 60 F-254) plates were used for TLC. The spots on TLC were detected by spraying with 10% H_2SO_4 and then heating on a hot plate. HPLC separations were performed on a Shimadzu LC-6AD series apparatus with a RID-10A Refractive Index, equipped with a 250 × 20 mm preparative Cosmosil 5SL-II column.

II. Plant Material

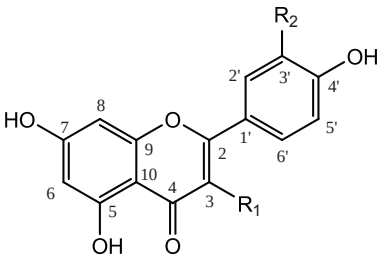
The dried aerial of *S. nepalensis* were collected in Taipei County in January 2004 and identified by Muh-Tsuen Kao. A voucher specimen (NRICM-92-003) was deposited in the National Institute of Chinese Medicine, Taipei, Taiwan.

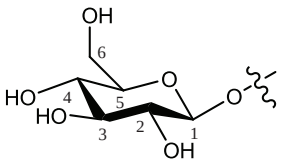
III. Extraction and Isolation

The dried aerial of *S. nepalensis* (600 g) were extracted with 95% EtOH for three times. The extracts were combined and concentrated in vacuum to dryness (101.3 g). Then the dry extract was respectively partitioned with *n*-hexane, EtOAc, *n*-BuOH, and H_2O. The *n*-hexane layer (26.9 g) was subjected to open chromatography on Silica gel (9 × 50 cm), using *n*-hexane/EtOAc solvent system (stepwise, from 100:0 to 0:100, each 600 mL), acetone, and finally MeOH to afford 34 fractions (Fr. 1~34). The Fr. 10 fraction (770.0 mg) of *n*-hexane layer was purified by repeated column chromatography on silica gel (3 × 60 cm) eluting with *n*-hexane/EtOAc in order of lower increasing polarity to afford β-sitosterol (**11**, 11.0 mg) and stigmasterol (**12**, 7.8 mg). The Fr. 13 fraction (810.0 mg) was chromatographed by silica

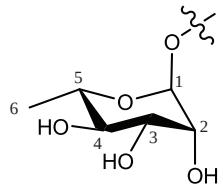
gel column (3 × 60 cm), developed with *n*-hexane/EtOAc (stepwise, 15:1 to 1:1, each 200 mL) to obtain a mixture (112.0 mg) of myristic acid (**15**), palmitatic acid (**16**), and linolenic acid (**17**). The Fr. 16 (393.4 mg) of *n*-hexane layer was purified by repeated column chromatography (3 × 40 cm) on silica gel eluting with *n*-hexane/EtOAc (stepwise, from 20:1 to 1:1, each 150 mL) to yield pheophorbide A methyl ester (**8**, 5.3 mg). The Fr. 21 (132.2 mg) was further separated by chromatography on a silica gel column (2 × 50 cm) eluting with *n*-hexane/EtOAc (from 12:1 to 0:1, each 150 mL) to yield methyl (10*S*)-hydroxypheophorbide A (**9**) and (10*S*)-hydroxypheophorbide A (**10**).

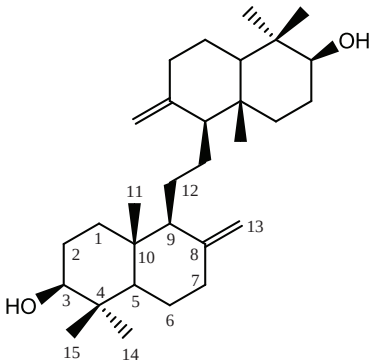
The EtOAc layer (21.3 g) was subjected to open chromatography on silica gel, using CH_2Cl_2/MeOH solvent system (stepwise, 20:1 to 0:1, each 450 mL) to afford 5 fractions (Fr. A-E). The Fr. C (1.86 g) was subjected to column chromatography by Sephadex LH-20 with MeOH to yield 7 fractions (Fr. C1-C7). The Fr. C4 (650.5 mg) was further separated by chromatography on silica gel column eluting with CH_2Cl_2/MeOH (from 10:1 to 3:1, each 500 mL) with to yield 4



Glu = 

1 R_1 = OH, R_2 = OH
2 R_1 = Rha, R_2 = OH
3 R_1 = Glu, R_2 = OH
4 R_1 = Glu, R_2 = OCH_3
5 R_1 = Glu, R_2 = H
6 R_1 = Glu_6-Rha, R_2 = OH

Rha = 



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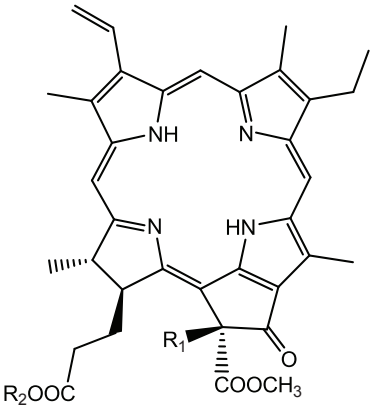
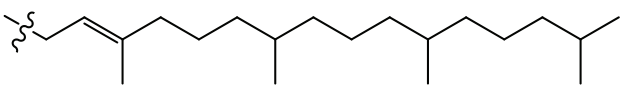


8 R_1 = H, R_2 = CH_3
9 R_1 = OH, R_2 = CH_3
10 R_1 = OH, R_2 = phytyl

phytyl = 

fractions (Fr. C4A-C4D). Fr. C4B (133.5 mg) was chromatographed on RP-HPLC (ODS, 250 × 10 mm, 20% aq. acetonitrile) to give *p*-coumaric acid (**13**, 5.6 mg), caffeic acid (**14**, 29.6 mg). The Fr. C6 (156.0 mg) was further purified by RP-HPLC (ODS, 250 × 10 mm, 50% aq. MeOH) to afford quercetin (**1**, 5.8 mg). The Fr. D (3.89 g) was subjected to column chromatography by Sephadex LH-20 with MeOH to yield 11 fractions (Fr. D1-D11). Fr. D4 (164.4 mg) was chromatographed on RP-18 SPE column (H_2O/MeOH, 3:1, 1:1, 0:1) and RP-HPLC (ODS, 250 × 10 mm, 16% aq. acetonitrile) to give rutoside (**6**, 6.3 mg). The Fr. D5 was chromatographed on RP-HPLC (ODS, 250 × 10 mm, 20% aq. acetonitrile) to afford quercetin-3-*O*-β-L-rhamnoside (**2**, 4.8 mg), kaempferol-3-*O*-β-D-glucoside (**5**, 12.3 mg) and isorhamnetin-3-*O*-β-D-glucoside (**4**, 18.5 mg). The Fr. D6 was chromatographed on repeated RP-18 SPE column eluting with MeOH/H_2O (1:2 and 1:0) to afford 2 fractions (Fr. D6A-D6B). Fr. D6A was further purified by RP-HPLC (ODS, 250 × 10.0 mm, 50% aq. MeOH) to afford quercetin-3-*O*-β-D-glucoside (**3**, 12.3 mg)

(1) Quercetin (1)

Yellow amorphous powder from CH_2Cl_2/MeOH; $[\alpha]^{23}_D$ +188.7° (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 211 (4.40), 256 (3.83), 374 (3.79) nm; IR (neat) ν_{max} 3416, 1653, 1614, 1519, 1373, 1317, 1266, 1198, 1168 cm^{-1}; ^{1}H-NMR ($CDCl_3$, 400 MHz) and ^{13}C-NMR (CD_3OD, 100 MHz), see Tables 1 and 2, respectively; Positive

Table 1. ^{1}H-NMR data of compounds 1-6 (δ, in CD_3OD).[a]

No.	1	2	3	4	5	6
6	6.18 (d, *J* = 1.2[b])	6.20 (d, *J* = 2.0)	6.18 (d, *J* = 2.0)	6.19 (br s)	6.19 (s)	6.18 (br s)
8	6.38 (d, *J* = 1.2)	6.37 (d, *J* = 2.0)	6.35 (d, *J* = 2.0)	6.38 (br s)	6.39 (s)	6.35 (br s)
2'	7.79 (d, *J* = 2.0)	7.33 (d, *J* = 2.0)	7.69(d, *J* = 2.0)	7.91(d, *J* = 1.6)	8.04 (d, *J* = 8.8)	7.66 (br s)
3'					6.88 (d, *J* = 8.8)	
5'	6.87 (d, *J* = 8.8)	6.89 (d, *J* = 8.4)	6.85 (d, *J* = 8.4)	6.90 (d, *J*= 8.4)	6.88 (d, *J* = 8.8)	6.85 (d, *J* = 8.4)
6'	7.61 (dd, *J* = 8.8, 2.0)	7.28 (dd, *J* = 8.4, 2.0)	7.56 (dd, *J* = 8.4, 2.0)	7.58 (dd, *J* = 8.4, 1.6)	8.04 (d, *J* = 8.8)	7.60 (dd, *J* = 8.4, 2.0)
Glu-1			5.21 (d, *J* = 7.6)	5.37 (d, *J* = 7.2)	5.24 (d, *J* = 7.2)	5.09 (d, *J* = 7.6)
Glu-2			3.24 (m)	3.24 (m)	3.22 (m)	3.45 (m)
Glu-3			3.46 (m)	3.45 (m)	3.45 (m)	3.41 (m)
Glu-4			3.34 (m)	3.40 (m)	3.33 (m)	3.27 (m)
Glu-5			3.37 (m)	3.45 (m)	3.45 (m)	3.30 (m)
Glu-6			3.58 (m); 3.71 (m)	3.54 (m); 3.74 (m)	3.53 (m); 3.68 (m)	3.39 (m); 3.77 (m)
Rha-1		5.34 (s)				4.54 (s)
Rha-2		4.22 (m)				3.60 (m)
Rha-3		3.74 (m)				3.51 (m)
Rha-4		3.30 (m)				3.26 (m)
Rha-5		3.42 (m)				3.44 (m)
Rha-6		0.93 (d, *J* = 6.0)				1.10 (d, *J* = 6.4)
OCH_3				3.93 (s)		

[a]Spectra recorded at 400 MHz in CD_3OD, [b]The *J* values are in Hz in parentheses.

ESIMS *m/z* 226 $[M+Na]^+$; Negative ESIMS *m/z* 202 $[M-H]^-$ (Calcd for $C_{15}H_{10}O_7$).

(Ⅱ) Quercetin-3-*O*-α-L-rhamnoside (2)

Yellow amorphous powder from CH_2Cl_2/MeOH; $[\alpha]^{23}_D$ -71.7° (*c* 0.1, MeOH; UV (MeOH) λ_{max} (log ε) 213 (4.30), 260 (3.81), 362 (3.74) nm; IR (neat) ν_{max} 3416, 2920, 1652, 1600, 1494, 1451, 1362, 1267, 1064 cm^{-1}; ^{1}H-NMR ($CDCl_3$, 400 MHz) and ^{13}C-NMR (CD_3OD, 100 MHz), see Tables 1 and 2, respectively; Positive ESIMS *m/z* 471 $[M+Na]^+$; Negative ESIMS *m/z* 447 $[M-H]^-$ (Calcd for $C_{21}H_{20}O_{11}$).

(Ⅲ) Quercetin-3-*O*-β-D-glucoside (3)

Yellow amorphous powder from CH_2Cl_2/MeOH; $[\alpha]^{23}_D$ -7.4° (*c* 1.0, MeOH); UV (MeOH) λ_{max} (log ε) 210 (4.45), 256 (3.73), 358 (3.68) nm; IR (neat) ν_{max} 3365, 1660, 1651, 1605, 1557, 1515, 1504, 1445, 1367, 1301,

Table 2. ^{13}C-NMR data of compounds 1-6 (δ, in CD_3OD).

No.	1	2	3	4	5	6
2	148.0 s	159.1 s	158.4 s	158.5 s	158.6 s	159.1 s
3	137.2 s	136.1 s	135.6 s	135.3 s	135.4 s	135.6 s
4	177.3 s	179.9 s	179.4 s	179.3 s	179.5 s	179.3 s
5	162.5 s	163.8 s	162.9 s	163.0 s	163.1 s	163.8 s
6	99.3 d	99.9 d	99.9 d	100.0 d	100.0 d	100.2 d
7	165.6 s	165.5 s	166.2 s	166.3 s	166.4 s	166.2 s
8	94.4 d	94.7 d	94.7 d	94.8 d	94.9 d	94.5 d
9	158.2 s	158.1 s	159.0 s	158.6 s	161.6 s	158.4 s
10	104.5 s	105.6 s	105.6 s	105.7 s	105.7 s	105.5 s
1'	121.7 s	123.0 s	123.0 s	123.1 s	122.8 s	123.5 s
2'	116.0 d	116.5 d	116.0 d	114.4 d	132.3 d	117.2 d
3'	146.2 s	146.1 s	145.8 s	148.4 s	116.1 d	145.6 s
4'	148.8 s	149.6 s	149.8 s	150.8 s	159.1 s	149.8 s
5'	116.2 d	116.1 d	117.5 d	116.0 d	116.1 d	116.2 d
6'	124.6 d	122.8 d	123.2 d	123.8 d	132.3 d	123.5 d
Glu-1			104.4 d	103.7 d	104.1 d	104.5 d
Glu-2			75.7 d	75.9 d	75.7 d	75.5 d
Glu-3			78.1 d	78.1 d	78.1 d	78.1 d
Glu-4			71.2 d	71.5 d	71.4 d	71.4 d
Glu-5			78.3 d	78.5 d	78.4 d	77.8 d
Glu-6			62.5 t	62.5 t	62.6 t	68.6 t
Rha-1		103.5 d				102.5 d
Rha-2		71.9 d				72.3 d
Rha-3		72.1 d				72.4 d
Rha-4		73.2 d				73.8 d
Rha-5		72.0 d				70.1 d
Rha-6		17.7 q				17.6 q
OCH_3				56.8 q		

[a]Recorded at 100 MHz. [b]Multiplicities and assignments made by HMQC and HMBC techniques.

1198, 1124, 1066, 1012 cm^{-1}; ^{1}H-NMR (CD_3OD, 400 MHz) and ^{13}C-NMR (CD_3OD, 100 MHz), see Tables 1 and 2, respectively; Positive ESIMS *m/z* 487 $[M+Na]^+$; Negative ESIMS *m/z* 463 $[M-H]^-$ (Calcd for $C_{21}H_{20}O_{12}$).

(Ⅳ) Isorhamnetin-3-*O*-β-D-glucoside (4)

Yellow amorphous powder from CH_2Cl_2/MeOH; $[\alpha]^{23}_D$ -68.2° (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 211 (4.34), 254 (3.82), 264 (3.76), 367 (3.75) nm; IR (neat) ν_{max} 3378, 2931, 1655, 1616, 1510, 1362, 1261, 1227, 1189, 1088, 1024 cm^{-1}; ^{1}H-NMR (CD_3OD, 400 MHz) and ^{13}C-NMR (CD_3OD, 100 MHz), see Tables 1 and 2, respectively; Positive ESIMS *m/z* 501 $[M+Na]^+$; Negative ESIMS *m/z* 477 $[M-H]^-$ (Calcd for $C_{22}H_{22}O_{12}$).

(Ⅴ) Kaempferol-3-*O*-β-D-glucoside (5)

Yellow amorphous powder from CH_2Cl_2/MeOH; $[\alpha]^{23}_D$ -10.4° (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 213 (4.41), 265 (3.78), 352 (3.59) nm; IR (neat) ν_{max} 3351, 2920, 1651, 1606, 1504, 1359, 1283, 1207, 1180, 1063 cm^{-1}; ^{1}H-NMR (CD_3OD, 400 MHz) and ^{13}C-NMR (CD_3OD, 100 MHz), see Tables 1 and 2, respectively; Positive ESIMS *m/z* 471 $[M+Na]^+$; Negative ESIMS *m/z* 447 $[M-H]^-$ (Calcd for $C_{21}H_{20}O_{11}$).

(Ⅵ) Rutoside (6)

Yellow amorphous powder from CH_2Cl_2/MeOH; $[\alpha]^{23}_D$ -30.4° (*c* 0.2, MeOH); UV (MeOH) λ_{max} (log ε) 210 (4.42), 256 (3.68), 267 (3.59), 352 (3.55) nm; IR (neat) ν_{max} 3375, 2920, 1651, 1600, 1557, 1513, 1486, 1454, 1429, 1352, 1292, 1204, 1128, 1092, 1062 cm^{-1}; ^{1}H-NMR (CD_3OD, 400 MHz) and ^{13}C-NMR (CD_3OD, 100 MHz), see Tables 1 and 2, respectively; Positive ESIMS *m/z* 633 $[M+Na]^+$; Negative ESIMS *m/z* 609 $[M-H]^-$ (Calcd for $C_{27}H_{30}O_{16}$).

(Ⅶ) (+)-α-Onocerin (7)

white amorphous powder from *n*-hexane/EtOAc; $[\alpha]^{25}_D$: +8.3° (*c* 0.1, $CHCl_3$); IR (neat) ν_{max} 3472, 2937, 2845, 1644, 1470, 1435, 1384, 1029 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$):δ 4.81 (2H, s, H-13), 4.53 (2H, s, H-13), 3.23 (2H, dd, *J* = 11.6, 4.4 Hz, H-3), 2.38 (2H, ddd, *J* = 12.8, 4.4, 2.4 Hz, H-7), 1.95 (2H, dt, *J* = 12.8, 4.8 Hz, H-7), 1.74-1.48 (12H, m, H-1, 2, 6, 9, 11), 1.36 (2H, m, H-6), 1.26-1.11 (6H, m, H-1, 5, 11), 0.98 (6H, s, Me-14), 0.74 (6H, s, Me-15), 0.62 (6H, s, Me-11); ^{13}C-NMR (100 MHz, $CDCl_3$):δ 148.7 (C-8), 106.6 (C-13), 79.1 (C-3), 57.8 (C-5), 54.9 (C-9), 39.5 (C-4), 39.4 (C-10), 38.5 (C-1), 37.3 (C-7), 28.5 (C-2), 28.2 (C-14), 24.3 (C-6), 22.4 (C-12), 15.6 (C-15), 14.8 (C-11); Positive EIMS *m/z* (rel. int): 442 $[M]^+$ (25), 427 (70), 409 (37), 229 (71), 207 (100), 189 (56), 135 (62), 107 (42), calcd for $C_{30}H_{50}O_5$.

(Ⅷ) Pheophorbide A methyl ester (8)

Dark green amorphous powder from CH_2Cl_2/MeOH; UV-vis (EtOH) λ_{max} (log ε) 665 (4.21), 608 (3.75), 537 (3.81), 506 (3.78), 430-320 (flat top, 4.62) nm; ^{1}H-NMR (400 MHz, pyridine-d_5):δ 9.88 (1H, s, H-β), 9.70 (1H, s, H-α), 8.85 (1H, s, H-δ), 8.20 (1H, dd, *J* = 17.8, 11.6 Hz, H-2a), 6.82 (1H, s, H-10), 6.38 (1H, d, *J* = 17.8 Hz, H-2b), 6.19 (1H, d, *J* = 11.6 Hz, H-2b), 4.58 (1H, br d, *J* = 7.3 Hz, H-8), 4.47 (1H, br d, *J* = 9.0 Hz, H-7), 6.38 (1H, d, *J* = 17.8 Hz, H-2b), 3.93 (1H, s, H-10b), 3.60 (3H, s, H-1'), 3.68 (3H, s, H-5a), 3.69 (2H, q, *J* = 7.6 Hz, H-4a), 3.22 (3H, s, H-3a), 3.37 (3H, s, H-1a), 2.61 (1H, m, H-7b), 2.84 (1H, m, H-7b), 2.93 (1H, m, H-7a), 2.53 (1H, m, H-7a), 1.82 (3H, d, *J* = 7.3 Hz, H-8a), 1.68 (3H, t, *J* = 7.6 Hz, H-4b); ^{13}C-NMR (100 MHz, pyridine-d_5):δ 133.1 (C-1), 12.5 (C-1a), 136.9 (C-2), 129.8 (C-2a), 123.3 (C-2b), 137.4 (C-3), 11.6 (C-3a), 146.3 (C-4), 20.1 (C-4a), 18.1 (C-4b), 130.0 (C-5), 12.4 (C-5a), 135.4 (C-6), 52.3 (C-7), 30.8 (C-7a), 31.8

(C-7b), 174.0 (C-7c), 52.5 (C-7d), 52.0 (C-1'), 50.8 (C-8), 23.6 (C-8a), 190.3 (C-9), 65.9 (C-10), 170.8 (C-10a), 53.3 (C-10b), 137.4 (C-11), 156.5 (C-12), 151.0 (C-13), 138.9 (C-14), 149.5 (C-15), 162.7 (C-16), 173.6 (C-17), 142.9 (C-18), 98.4 (C-α), 105.6 (C-β), 106.8 (C-γ), 94.7 (C-δ); FABMS *m/z* 606 (38, $[M]^+$), 547 (8.5), 459 (4.5), calcd for $C_{36}H_{38}N_4O_5$.

(IX) Acid hydrolysis of flavonoids 2-6

Compounds **2-6** (1.0 mg) were each treated with 2 N methanolic HCl (2 mL) under conditions of reflux at 90°C for 1 h, respectively. The mixture obtained was extracted with CH_2Cl_2. The aqueous hydrolysate was neutralized with Na_2CO_3 and filtered. After cooling, the solution was evaporated with a stream of N_2. D-glucose and L-rhamnose were determined by TLC on cellulose in pyridine/EtOAc/HOAc/H_2O (5:5:1:3) its R_f values 0.4 and 0.6, respectively.

(X) Cytotoxicity assay

Cytotoxicity against KB (human mouth epidermoid carcinoma) and Hela (human cervical epitheloid carcinoma) cells was measured using an MTT [3-(4,5-dimethylthiazole-2-yl)-2,5-diphenyltetrazolium bromide] assay, based on reported methods[22]. Briefly, the cells were cultured in RPMI-1640 medium supplemented with serum in an atmosphere of 5% CO_2 incubated at 37°C. Test samples and the control drug standard were prepared at concentrations of 1, 10, 20, and 40 μg/mL. After seeding 2880 cells/well in a 96-well microplate for four h, 20 μL of sample or standard agent was placed in each well and incubated at 37°C for 3 days. Twenty μL of MTT were added, and incubation continued for five hours. After removing the medium and adding DMSO (200 μL/well) into the microplate with shaking for 10 min, the formazan crystals (the product of MTT reacting with dehydrogenase existing in mitochondria) were re-dissolved, and their absorbance was measured on a model MR 7000 microtiter plate reader (Dynatech International Corporation, Edgewood, New York) at a wavelength of 550 nm. The ED_{50} was defined as the concentration of test sample resulting in 50% reduction of the absorbance found with the untreated cells. Mitomycin *c* was as a positive control.

(XI) Antioxidant assays

Measurement of the scavenging activity of DPPH radicals[23]. The test sample (150 μL) dissolved in methanol at various concentrations was mixed with 37.5 μL of methanolic solution containing 0.75 mM DPPH (Sigma) radicals. The mixture was shaken vigorously and left to stand for 30 min in the dark, following which the absorbance was measured at 517 nm against a blank (Shimada et al. 1992). The scavenging ability was calculated as follows: Scavenging activity (%) = [(ΔA_{517} of control-ΔA_{517} of sample)/ΔA_{517} of control] × 100. A curve of sample concentration against % DPPH was generated to estimate the concentration of sample needed to cause a 50% reduction of the initial DPPH concentration. This value is known as the ED_{50} (dose effective in 50% of test samples/subjects, also called oxidation index) and was expressed in terms of milligrams per milliliter. This assay was performed in triplicate for each sample, and the mean values were used to calculate the ED_{50}. α-tocopherol was used as the positive control.

Results and Discussion

The EtOAc and *n*-hexane layers of *S. nepalensis* were chromatographed successively on silica gel,

Sephadex LH-20, RP SPE column, and HPLC to afford seventeen known compounds, including six flavonoids, quercetin (**1**)[8], quercetin-3-*O*-α-L-rhamnoside (**2**, quercitrin)[12], quercetin-3-*O*-β-D-glucoside (**3**)[8], isorhamnetin-3-*O*-β-D-glucoside (**4**)[8], kaempferol-3-*O*-β-D-glucoside (**5**)[13], rutoside (**6**)[12], three pheophorbides, one triterpene, (+)-α-onocerin (**7**)[14], three pheophorbides, pheophorbide A methyl ester (**8**)[15], methyl (10*S*)-hydroxypheophorbide A (**9**)[15], (10*S*)-hydroxypheophorbide A (**10**)[15], two steroids, β-sitosterol (**11**)[16], stigmasterol (**12**)[13], two benzenes, *p*-coumaric acid (**13**)[17], caffeic acid (**14**)[18], together with three fatty acids, myristic acid (**15**)[19], palmitic acid (**16**)[20], and linolenic acid (**17**)[21]. Their structures of isolated compounds were established by interpretation and full assignments of 1D, 2D spectroscopic data, and comparison with literature.

Among the seventeen isolates, compounds **1**-**6** exhibited spectral feature closely resembling those of flavonoid glycosides[8,12,13]. The molecular formulas $C_{15}H_{10}O_7$ for **1**, $C_{21}H_{20}O_{11}$ for **2**, $C_{21}H_{20}O_{12}$ for **3**, $C_{22}H_{22}O_{12}$ for **4**, $C_{21}H_{20}O_{11}$ for **5**, and $C_{27}H_{30}O_{16}$ for **6** were determined by ^{1}H, ^{13}C NMR analysis and ESI-MS data. Their ^{1}H and ^{13}C NMR spectra data indicated that the aglycone of **2**-**4**, and **6** were quercetin (**1**) and the aglycone of **5** was kaempferol.

The ^{1}H NMR spectrum of **1** showed the signals at δ 7.79 (H-2', d, *J* =2.0 Hz), 7.61 (H-6', dd, *J* =8.8, 2.0 Hz), 6.87 (H-5', d, *J* = 8.8 Hz), 6.38 (H-8, d, *J* =1.2 Hz), and 6.18 (H-6, d, *J*=1.2 Hz), which was determined as quercetin, compared with reported data and the authentic sample. The ^{1}H NMR spectrum of **2** showed the similar signals as compound **1**, except for a sugar moiety signals and one anomeric proton at δ 5.34 (Rha-1, s) in **2**. This was further confirmed by the ^{13}C NMR spectrum, which exhibited 22 carbon signals, with 15 carbons representing the aglycone (quercetin), six carbon peaks for the rhamnopyranoside unit [δ 103.5 (Rha-1), 71.9 (Rha-2), 72.1 (Rha-3), 73.2 (Rha-4), 72.0 (Rha-5), 17.7 (Rha-6)]. The α-anomeric configuration for the rhamnopyranoside was judged by the coupling constants[21]. Accordingly, the NMR data of **2** were identical with those of reported quercetin 3-*O*-α-rhamnopyranoside.

The spectral data of **3** showed that it possessed the same aglycone (quercetin) as that of **2**, except for the sugar moiety. Comparison of the NMR data of compound **3** with the literature[8], the sugar moiety of **3** is glucopyranose instead of the rhamnopyranoside in **2**. Further checking the coupling constant of Glc-H-1 (*J* = 7.2), the β-linkage configuration of glucose in **3** was undoubtedly determined.

The spectral data of **4** was similar to that of **3**. The molecular weight of **4** was 14 mass units greater than that of **3** indicating that **4** had more CH_3 unit than **3**. The NMR data revealed that the methoxy group was attached to the C-3' of B-ring. Therefore, compound **4** was identified as isorhamnetin-3-*O*-β-D-glucoside by comparison with literature[8]. The ^{1}H NMR data of compound **5** exhibited the very close signals as **3** except for the A_2B_2 system signal of B ring instead of the ABX system signal in **3**. Thus, the structure of compound **5** was deduced as kaempferol-3-*O*-β-D-glucoside, which also was confirmed by comparison of spectral data with those in the literature[13].

The molecular weight of compound **6** was 146 mass units greater than that of **3**, indicating that **6** had one more rhamnopyranosyl unit. This was confirmed by its 1D [δ_H 5.09 (Glc-1, d, *J* =7.6 Hz)/δ_C 104.5, 4.54 (Rha-1, s)/δ_C 102.5, 1.10 (Rha-6, d, *J* = 6.4 Hz)/δ_C 17.6] NMR data, respectively. The rhamnose was attached to the hydroxyl group at the C-6 position of glucose

as judged from the downfield shift (ca. 6.0 ppm) of the C-6 signal. The ^{13}C NMR data also supported the attachment of rutinosyl moiety to the C-3 of quercetin (**1**). Furthermore, the NMR data of **6** were identified as rutoside by comparison of those data in the literature[12].

Acid hydrolysis of flavonoid glycosides **2-6**, respectively, obtained aglycones and D-glucoses from **3**, **4**, **5**, and **6**, L-rhamnoses from **2** and **6**, which of sugar moieties were determined by the ^1H NMR spectral data and TLC analyses.

Compound **7**, $[\alpha]^{25}_D$: +8.3° (*c* 0.1, $CHCl_3$), had a molecular formula of $C_{30}H_{50}O_2$ deduced from EIMS *m/z* 442 $[M]^+$. The IR spectrum revealed the presence of hydroxyl (3472 cm^{-1}) and exocyclic double bond (1644 and 1384 cm^{-1}) functionality. The ^{13}C NMR spectrum showed 15 carbon signals, indicating that compound **7** is a dimer of chemical structure of 8(12)-drimene with hydroxyl group at C-3, linked at C-12[17]. Based on the spectral data above and compared with the reported data[14], compound **7** was identified as (+)-α-onocerin.

Compound **8** was obtained as a dark green amorphous powder, and its molecular formula $C_{36}H_{38}N_4O_5$ was deduced from its FABMS (*m/z* 606, $[M]^+$) and ^{13}C NMR data. The UV spectrum of **8** showed absorptions at λ_{max} 665 (sh), 608, 537, 506 and 430-320 (br) nm, suggesting that **8** could be a pheopheorbide-type compounds[24]. The ^1H and ^{13}C NMR spectra exhibited the characteristics of the signals for three singlet olefinic protons (δ_H 9.88, 9.70, 8.85), monosubstituted double bond (δ_H 8.20, 6.38, 6.19; δ_C 129.8, 123.3), four singlet methyls (δ_H 3.68, 3.37, 3.22, 1.82; δ_C 12.4, 11.6, 23.6, 12.5), one triplet methyl (δ_H 1.68; δ_C 18.1), two oxymethyls (δ_H 3.60, 3.93; δ_C 52.5, 53.3), and two NH protons (δ_H 0.89, -1.35), indicating that compound **8** was a pheopheorbide. Furthermore, literatures survey revealed that the ^1H- and ^{13}C-NMR spectroscopic data of **8** showed close similarity with those reported for pheophorbide A methyl ester[15]. Also, compounds **9** and **10** were identified as (10*S*)-hydroxypheophorbide A (**9**), and (10*S*)-hydroxypheophorbide A (**10**), respectively, by MS analyses and comparison of their ^1H and ^{13}C NMR spectroscopic data with those in the literature[15].

In addition, seven known common compounds, β-sitosterol (**11**)[16], stigmasterol (**12**)[16], *p*-coumaric acid (**13**)[17], caffeic acid (**14**)[18], myristic acid (**15**)[19], palmitatic acid (**16**)[20], linolenic acid (**17**)[21] were also confirmed by the spectroscopic data and comparison with those in the literature.

Moreover, the isolated compounds (**1-17**) were evaluated for cytotoxic actvivity. Bioassay results revealed that the isolated pheophorbide derivatives (**8-10**) possessed significant cytotoxicity against KB (ED_{50} = 4.5, 5.2, 3.8 μg/mL for **8**, **9**, and **10**, respectively) and Hela (ED_{50} = 4.2, 3.7, 3.4 μg/mL for **8**, **9** and **10**, respectively) human tumor cells. In addition, the radical-scavenging activities of flavonoids **1-6** and benzenes **13-14** were measured by DPPH scavenging test (Table 3). Of these isolates,

Table 3. DPPH radical-scavenging activity of compounds 1-6 and 13-14.

Compound	ED_{50} (μg/mL)[a]
1	5.92 ± 0.13
2	7.10 ± 0.26
3	6.01 ± 0.01
4	> 100
5	> 100
6	> 100
13	> 100
14	10.49 ± 0.15
α-tocopherol	12.49 ± 0.19

[a]ED_{50} value (μg/mL) were calculated from the isolated compounds which has DPPH radical-scavenging activity greater than 50% when test concentration in 100 μg/mL.

compounds **1**, **2**, **3**, and **14** showed significant anti-oxidative activity with EC_{50} at 5.92, 7.10, 6.01, and 10.49 μM, respectivly. α-Tocopherol (vitamin E) was used as a standard, with EC_{50} value at 12.49 μM.

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台灣產東方肉穗野牡丹（*Sarcopyramis nepalensis*）之化學成分研究

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東方肉穗野牡丹（*Sarcopyramis nepalensis*）為野牡丹科肉穗野牡丹屬植物，其屬全草具清熱解毒、清肝瀉火、肺熱咳嗽與腫毒等之效。從本植物之酒精萃取物經一系列色層分離純化得到十七個化合物，包括六個黃酮類化合物 quercetin (**1**)、quercetin-3-*O*-α-L-rhamnoside (**2**)、quercetin-3-*O*-β-D-glucoside (**3**)、isorhamnetin-3-*O*-β-D-glucoside (**4**), kaempferol-3-*O*-β-D-glucoside (**5**)、rutoside (**6**)，一個三萜類化合物 triterpene, (+)-α-onocerin (**7**)，三個 pheophorbides 類化合物 pheophorbide a methyl ester (**8**)、methyl (10*S*)-hydroxypheophorbide a (**9**)、and (10*S*)-hydroxypheophorbide a (**10**)，兩個固醇類化合物 β-sitosterol (**11**)、stigmasterol (**12**)，以及 *p*-coumaric acid (**13**)、caffeic acid (**14**)、myristic acid (**15**)、palmitic acid (**16**)、linolenic acid (**17**) 等五個化合物。所有化學結構均藉由各種光譜資料（核磁共振光譜、質譜、紅外線光譜）分析確立。細胞毒殺活性測試結果顯示，化合物 **8** 和 **9** 針對 KB and Hela 癌細胞株具有毒殺效果，其 ED_{50} 值約分別為 3.8-5.2 μg/mL 與 3.4-4.2 μg/mL。除此之外，化合物 **1**、**2**、**4** 與 **14** 於清除自由基活性測試中顯示具有良好的抗氧化活性。

關鍵字：東方肉穗野牡丹、黃酮類、脫植醇脫鎂葉綠素、細胞毒殺活性、抗氧化活性

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