Flavonoids from *Psychotria serpens* L., a Herbal Medicine with Anti-Cancer Activity

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Abstract: In clinical, *Psychotria serpens* L. was often substitute for *Caulis trachelospermi* to treat cancer in China. Meanwhile, EtOAc and n-BuOH fractions of MeOH extract of *P. serpens* L. show power activity against H460, HepG2, Hela, and PC9/GR cell lines, and no toxic effects against normal 16HBE cell lines. In order to search significant anti-cancer active leading compounds, sevenetin (**5**), rutin (**6**), kaempferol-3- flavonoids, quercetin (**1**), tamarixetin-3-O-rutinoside (**2**), quercetin 3-*O*-(2^{G}-*β*-D- xylopyranosy- lrutinoside) (**3**), kaempferol (**4**), tamarixO- rutino- side (**7**) were isolated from *P. serpens* L., and their structures were identified through spectroscopic techniques including NMR (1D and 2D) and MS. **2-5** were the first isolated from genus *Psychotria*. All of compounds were the first isolated from *P. serpens*.

Keywords: *Psychotria serpens* L., anti-cancer, flavonoids.

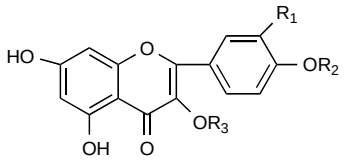
INTRODUCTION

In clinical, *Psychotria serpens* L. was often substitute for *Caulis trachelospermi* to treat cancer in China [1]. However, the chemical constituents of it were few reported. Meanwhile, *P. serpens* L. is one of four specimens which were as folk medicines to treat swelling and pain [2, 3], has ability to relax jinluo, strengthen bones, relieve rheumatic pains and colds, cool the blood, and disperse swelling. It was used to treat arthralgia due to wind-dampness, sciatica, swollen or pain of acne and throat. Moreover, *Psychotria* (Rubiaceae) is widespread in tropical and subtropical regions, and in China, 17 species and one variation have been found [2-4]. The most important is that EtOAc and n-BuOH fractions of MeOH ectract of *P. serpens* L. show powerful activity against H460, HepG2, Hela cell lines, reversal of tumor multidrug resistance activity to PC9/GR cell lines, and no toxic effects to normal 16HBE cell linesl in the preliminary pharmacological experiment (see Table **1**). As a continuation of our anticancer active chemical investigation on genus of *Psychotria* [5-9] from China, quercetin (**1**) [10], tamarixetin-3-O-rutinoside (**2**) [11], quercetin 3-*O*-(2^{G}-*β*- D-xylopyranosy- lrutinoside) (**3**) [12], kaempferol (**4**) [13], tamarixetin (**5**) [14], rutin (**6**) [15], kaempferol - 3- O -rutino- side (**7**) [15] were from n-BuOH fractions of MeOH ectract of it. Compounds **2-5** were the first isolated from genus *Psychotria*. All of compounds were the first isolated from *P. serpens*.

Table 1: Cytotoxic Activities of EtOAc and n–BuOH Fractions of MeOH ectract of *P. serpens* L. in Five Human Cancer Cell Lines (IC_{50}, μM)

Extracts	H460	HepG2	Hela	PC9/GR	16HBE
EA	45.00	45.00	45.00	45.00	150
BU	45.00	45.00	45.00	45.00	150

EA: Ethyl acetale of *P. serpens* L.; **BU**: n-butyl alcohol of *P. serpens* L. Cytotoxic activity was measured by MTT assay as shown in experimental section.



1 R_1=OH,R_2=H, R_3=H
2 R_1=OH,R_2=CH_3, R_3=rhamnosyl-(1➔6)-glucoside
3 R_1=OH,R_2=H, R_3=xylosyl-(1➔2)-rhamnosyl-(1➔6)-glucoside
4 R_1=H,R_2=OH, R_3=H
5 R_1=OH,R_2=CH_3,R_2=H
6 R_1=OH,R_2=H,R_2=rhamnosyl-(1➔6)-glucoside
7 R_1=H,R_2=H, R_3=rhamnosyl-(1➔6)-glucoside

Figure 1: Flavonoids from *P. serpens* L.

MATERIALS AND METHODS

General Experimental Procedures

Melting points were recorded on an X-6 micro-melting point apparatus, which was uncorrected.

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ISSN: 1929-2260 / E-ISSN: 1929-2279/15

Optical rotations were measured on a Schmidt + Haensch polaptronic hnqw5 polarimeter. NMR data were recorded on a Varian Unity INOVA spectrometer in Pyridine-d_5 at 400 MHz with TMS as internal standard. ESI-MS were obtained with MAT 95XP (Thermo) Mass Spectrometer,

Preparative TLC was performed with Si gel GF_{254}. Silica gel H (200-300 mesh, Qingdao Marine Chemical Plant) was used for flash chromatography. Sephadex™ LH-20 (25-100 mm) was supplied by GE Healthcare Bio-Sciences AB, Sweden. ODS (YMC GEL, RP-C_{18}, 50 mm) was from YMC. CO. LTO, Japan.

Cell Lines

Human lung cancer cell lines H460, human liver hepatocellular carcinoma cell lines HepG2, human cervical cancer cells Hela, human lung cancer resistance of strains induced by Gefitinib PC9/GR, human normal lung bronchial epithelial cell lines 16HBE were provided by Professor Zi Li of Guangzhou Medical University. The IC_{50} data were obtained by MTT.

Plant Material

Specimens of *Psychotria serpens* L. were collected in Luofu Mountain, Zhaoqing City, Guangdong province, Peoples′ Republic of China and identified by Dr. Guang-Tian Peng, Guangzhou University of Chinese Medicine. A voucher specimen (No. PSS-1) was deposited at the Lab of Natural Products Research, Guangzhou University of Chinese Medicine.

Extraction and Isolation

The whole *Psychotria serpens* L. (10.5 kg) was extracted with MeOH at room temperature. The combined methanol extracts were concentrated *in vacuum* to give a light brown residue (2.0kg). The residue was suspended in H_2O, and then partitioned with PE (200 g), EtOAc (900 g), and n-Buoy (350 g), successively. The n-BuOH fraction (350g) was

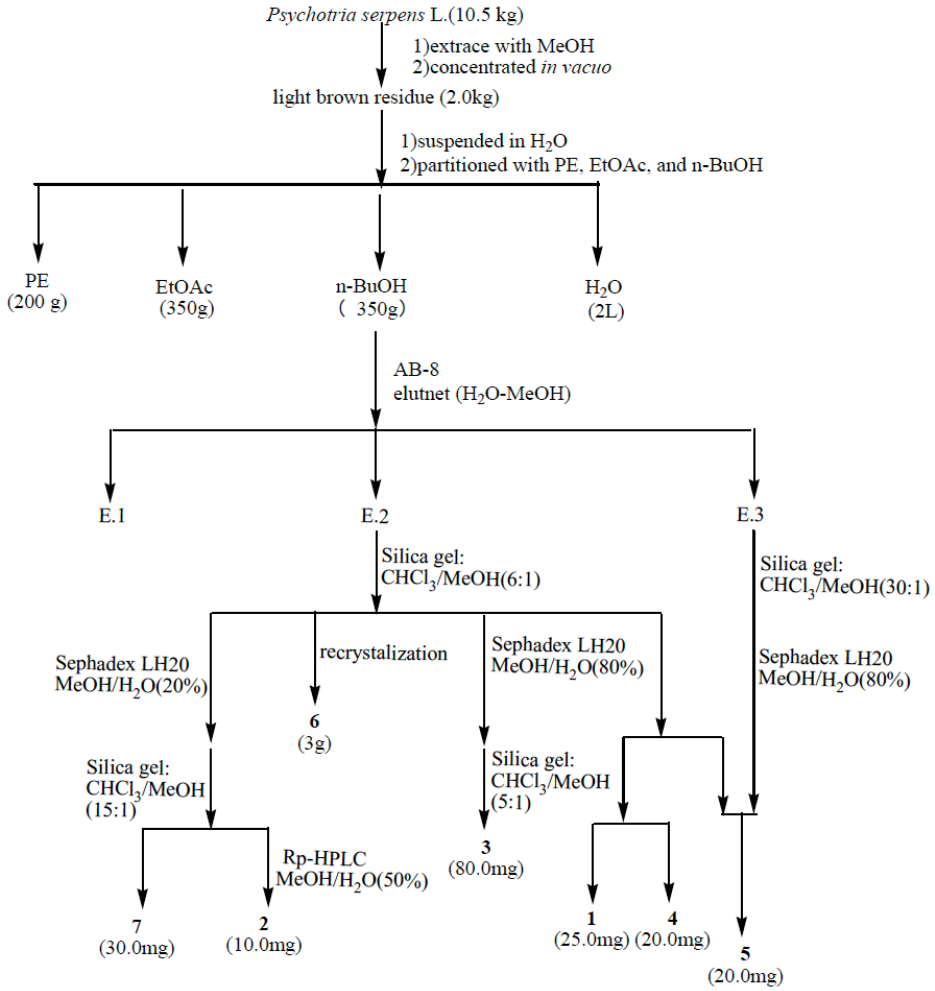

Chart 1 Extraction and isolation of *Psychotria serpens* L.

chromatographed by macroporous resin AB-8, silica gel, Sephadex LH-20, and ODS, 8 compounds were obtained (see Chart **1**).

RESULTS

Quercetin (1)

Yellow powder (MeOH), m.p. 307-310˚C, $C_{15}H_{10}O_7$, ESI-MS m/z 301 $[M-H]^+$. ^{1}H-NMR (CD_3OD, 400 MHz) *δ*: 6.16 (1H, d, *J*=2.0 Hz, H-6), 6.37 (1H, d, *J*=2.0 Hz, H-8), 7.72 (1H, d, *J*=2.0 Hz, H-2'), 7.62 (1H, d, *J*=8.4, 2.0 Hz, H-6'), 6.86 (1H, d, *J*=8.4 Hz, H-5'); ^{13}C-NMR data (CD_3OD, 100 MHz) see Table **2**.

5, 7, 3'-trihydroxy-4'-methoxyflavonol-3-O-rutinoside (2)

Yellow powder (MeOH), mp 168-169˚C, $C_{28}H_{32}O_{16}$, ESI-MS m/z 625 $[M+H]^+$. ^{1}H-NMR (CD_3OD, 400 MHz) *δ*: 6.20(1H, d, *J*=1.8 Hz, H-6), 6.38(1H, s, H-8), 8.00(1H, d, *J*=2.0 Hz, H-2'), 6.94(1H, d, *J*=8.4 Hz, H-5'), 7.67(1H, dd, *J*=8.4, 2.4 Hz, H-6'), 5.21(1H, d, *J*=6.8 Hz, H-1''), 4.56 (1H, s, H-1'''), 1.14 (3H, d, *J*=6.2 Hz, H-6'''), 4.00 (3H, s, -OCH_3), 3.20-3.85 (16H, m, sugar protons); ^{13}C-NMR data (CD_3OD, 100MHz) *see* Table **2**.

Table 2: ^{13}C-NMR Spectral Data of 1-7

No.	1	2	3	4	5	6	7
2	148.2, s	148.8, s	157.0, s	146.7, s	148.8, s	158.6, s	157.2, s
3	138.1, s	138.2, s	133.6, s	135.9, s	138.3, s	135.8, s	134.1, s
4	177.5, s	178.0, s	178.1, s	176.1, s	177.7, s	179.5, s	178.0, s
5	162.6, s	161.6, s	161.6, s	161.1, s	157.8, s	163.0, s	161.6, s
6	99.4, d	98.7, d	98.4, d	97.9, d	99.6, d	100.1, d	98.7, d
7	165.7, s	164.8, s	164.3, s	164.2, s	166.0, s	166.1, s	164.8, s
8	94.6, d	93.6, d	93.4, d	93.0, d	94.8, d	95.0, d	93.6, d
9	158.4, s	157.4, s	157.3, s	156.9, s	162.8, s	159.4, s	158.0, s
10	104.7, s	104.3, s	104.4, s	103.2, s	104.8, s	105.7, s	104.2, s
1'	124.4, s	122.6, s	121.9, s	122.4, s	123.1, s	123.2, s	121.4, s
2'	116.2, d	117.9, d	116.2, d	129.3, d	117.0, d	117.9, d	131.0, d
3'	146.4, s	146.9, s	144.5, s	114.9, d	147.9, s	149.9, s	114.7, d
4'	148.9, s	149.5, s	148.3, s	159.1, s	149.7, s	145.9, s	160.1, s
5'	116.4, d	113.1, d	114.9, d	114.9, d	113.1, d	116.2, d	114.7, d
6'	121.8, d	121.6, d	122.3, d	129.3, d	122.9, d	123.7, d	131.0, d
OCH_3		55.5, q			56.4, q		
1''		103.1, d	99.5, d			104.9, d	103.3, d
2''		74.5, d	80.5, d			75.9, d	74.4, d
3''		76.8, d	76.5, d			78.3, d	76.8, d
4''		70.2, d	70.7, d			72.4, d	70.1, d
5''		76.0, d	75.7, d			77.3, d	75.8, d
6''		67.2, t	66.7, t			68.7, t	67.2, t
1'''		101.3, d	100.7, d			102.5, d	101.0, d
2'''		70.7, d	70.7, d			72.2, d	70.7, d
3'''		70.9, d	70.8, d			71.5, d	70.9, d
4'''		72.5, d	72.5, d			74.1, d	72.5, d
5'''		68.4, d	68.3, d			69.8, d	68.3, d
6'''		16.4, q	16.4, q			18.0, q	16.5, q
1''''			103.7, d				
2''''			73.3, d				
3''''			75.4, d				
4''''			69.7, d				
5''''			65.1, d				

Quercetin-3-*O*-(2^G-*β*-D-xylopyranosylrutinoside (3)

Yellow powder (MeOH), $C_{32}H_{38}O_{20}$, ESI-MS m/z 741 $[M-H]^+$. ^{1}H-NMR (CD_3OD, 400 MHz) *δ*: 6.21(1H, d, *J*=1.6 Hz, H-6), 6.40(1H, d, *J*=1.6 Hz, H-8), 7.65(1H, br. s, H-2'), 6.90(1H, d, *J*=7.2 Hz, H-5'), 7.63(1H, d, *J*=1.6 Hz, H-6'), 5.42(1H, d, *J*=6.0 Hz, H-1''), 4.50(1H, s, H-1'''), 1.11(3H, d, *J*=4.8 Hz, H-6'''), 4.80(1H, d, *J*=5.6 Hz, H-1''''), 3.20-4.15(27H, m, sugar protons); ^{13}C-NMR data (CD_3OD, 100MHz) *see* Table **2**.

Kaempferol (4)

Yellow powder (MeOH), mp 276-278˚C, $C_{15}H_{10}O_6$, ESI-MS m/z 286 $[M]^+$. ^{1}H-NMR (CD_3OD, 400 MHz) *δ*: 6.20(1H, d, *J*=1.6 Hz, H-6), 6.41(1H, d, *J*=2.0 Hz, H-8), 8.10(2H, d, *J*=7.2 Hz, H-2', 6'), 6.92(2H, d, *J*=7.2 Hz, H-3', 5'); ^{13}C-NMR data (CD_3OD, 100 MHz) see Table **2**.

5, 7, 3'-trihydroxy-4'-meth- oxyflavonol (5)

Yellow powder (MeOH), mp 260- 262℃, $C_{16}H_{12}O_7$, ESI-MS m/z 315 $[M-H]^+$. ^{1}H-NMR (pyr-d_5, 400 MHz) *δ*: 6.87(1H, d, *J*=2.0 Hz, H-8), 6.77(1H, d, *J*=2.0 Hz, H-6), 8.29(2H, d, *J*=2.4 Hz, H-2'), 8.18(2H, dd, *J*=8.4, 2.0 Hz, H-6'), 7.37 (1H, d, *J*=2.4 Hz, H-5'), 3.88(3H, s, -OCH_3); ^{13}C-NMR data (CD_3OD, 100 MHz) see Table **2**.

Rutin (6)

Yellow powder (MeOH), mp 185-186˚C, $C_{27}H_{30}O_{15}$, ESI-MS m/z 609 $[M-H]^+$. ^{1}H-NMR (CD_3OD, 400 MHz) *δ*: 6.14(1H, d, *J*=2.0 Hz, H-6), 6.32(1H, d, *J*=2.0 Hz, H-8), 7.62(1H, d, *J*=2.0 Hz, H-2'), 6.82(1H, d, *J*=8.4 Hz, H-5'), 7.57(1H, dd, *J*=8.4, 2.0 Hz, H-6'), 5.05(1H, d, *J*=7.6 Hz, H-1''), 4.48(1H, d, *J*=1.6 Hz, H-1'''), 1.07(3H, d, *J*=6.4 Hz, H-6'''), 3.20-3.85 (16H, m, sugar protons); ^{13}C-NMR data (CD_3OD, 100 MHz) see Table **2**.

Kaempferol-3-O-rutinoside (7)

Light yellow powder (MeOH), m.p. 223- 224˚C, $C_{27}H_{30}O_{15}$, ESI-MS m/z 595 $[M+H]^+$. ^{1}H-NMR (CD_3OD, 400 MHz) *δ*: 6.22(1H, d, *J*=1.6 Hz, H-6), 6.41(1H, br. s, H-8), 8.08(2H, d, *J*=8.8 Hz, H-2', 6'), 6.91(2H, d, *J*=8.8 Hz, H-3', 5'), 5.14(1H, d, *J*=7.2 Hz, H-1''), 4.54(1H, d, *J*=1.2 Hz, H-1'''), 3.01- 4.12 (16H, m, sugar protons), 1.14 (3H, d, *J*=6.0 Hz, H-6''') ; ^{13}C-NMR data (CD_3OD, 100 MHz) see Table **2**.

DISCUSSION

According to data of NMR and MS, Their structures of flavonoids were determined as quercetin (**1**), tamarixetin-3-O-rutinoside (**2**), quercetin 3-*O*-(2^G-*β*-D-xylopyranosy- lrutinoside) (**3**), kaempferol (**4**), tamarixetin (**5**), rutin (**6**), and kaempferol-3-O- rutinoside (**7**). All compounds were firstly obtained from *P. serpens* L. The aglyconeses of **3**, **6**, and **7**, those are quercetin (**1**) and kaempferol (**4**), were wildly anti-cancer activity [24-27]. According to literatures, flavonoids were also related to treat swelling and painrelieve rheumatic pains and colds [28-30].

CONFLICTS OF INTEREST STATEMENT

There are no conflicts of interest.

ACKNOWLEDGEMENT

This work was supported by the grant from the Key Program of Science and Technology Administration of Guangdong (No.2010A030100013); Open-end Fund in Public Lab of China Analytical Center, Guangzhou (001), and Fund of Startup pf Construction Troops of Teacher of Guangdong Province (A1033025). We would like to gratefully acknowledge Dr. Guang-Tian Peng, Guangzhou University of Chinese Medicine for identification of the plant.

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Received on 15-03-2015 Accepted on 02-04-2015 Published on 07-05-2015

DOI: http://dx.doi.org/10.6000/1929-2279.2015.04.02.3