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Anti-hyperlipidemic caffeoylquinic acids from the fruits of *Pandanus tectorius* Soland

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ABSTRACT

Ten caffeoylquinic acids (CQAs) were obtained from the anti-hyperlipidemic extract of fruits of *Pandanus tectorius*. All of the compounds were isolated and purified by various column chromatographies especially by preparative HPLC method. Their structures were determined under the aid of spectroscopic methods. All compounds were isolated from genus *pandanus* for the first time. The anti-hyperlipidemic activities of three compounds characterized with high content in the fruits were tested in HepG2 cells.The three CQAs all significantly reduced the intracellular content of TC and TG.

INTRODUCTION

Plants of genus *Pandanus* have been used as folk medicine as a treatment for leprosy, bronchitis, measles, dermatitis and urinary tract aliments (Vahirua-Lechat *et al*, 1996). In China, five species and one varied species belonging to this genus are spread in the subtropical and tropical regions including Hainan province, Guangdong province, and Taiwan province. The extract of fruits of *Pandanus tectorius* possessing many medicinal effects in treating influenza, hepatitis, and nephritis have been recorded in standardized and benchmarked Chinese Materia Medica of Guangdong province (Peng *et al*, 2010). Its fruits with the important anti-hyperlipidemic activites attracted our interest. Previously we confirmed the lipid lowering effects of the 70% ethanol extract of the fruits of *P. tectorius*. By a further step, we verified that the n-butanol fraction obtained from the 70% ethanol extract of the fruits was the active part. However, little information is available on the chemical constituents of this active fraction of the medicinal plants. Based on these, we carried out the studies on chemical principles as well as the lipid lowering effects of the isolates.

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During this course, ten caffeoylquinic acids were obtained and identified. Moreover, the anti-hyperlipidemic activities of three selected compounds of high content in the plant were tested.

MATERIALS AND METHODS

General Experimental Procedures

The NMR data were recorded on Bruker AV 600 (600MHz for ^{1}H and 150MHz for ^{13}C) in CD_3OD with TMS as internal standard. Chromatography was performed on silica gel column (200-300 mesh, Qingdao Haiyang) and Sephadex LH-20 column (GE Healthcare, Sweden). HPLC isolation was conducted on a lumtech K1001 analytic LC equipped with two pumps of K-501, a UV detector of K-2600, and a column of YMC-Pack ODS-A (250×10mm, 5μm). HepG2 cells originally from the American Type Culture Collection (ATCC) (Manassas, VA, USA), were obtained from the China Union Medical University.

Collection and preparation of plant material

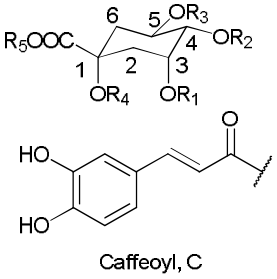
The medicinal material was collected at Hainan Province in July 2011 and identified by Prof. Weiyong Lai at the School of Pharmaceutical Science, Hainan Medical Unversity. A voucher specimen has been deposited there (Voucher specimen NO. PT20110714).

Extraction and Isolation

The dried fruits (6 Kg) of *Pandanus tectorius* were dried in shade and exhaustively extracted with 70% ethanol. The extract was filtered and concentrated on reduced pressure until only H_2O remained.

The remaining solution was sequentially partitioned with petroleum ether (34 g), $CHCl_3$ (42 g), EtOAc (53 g) and n-BuOH (100 g). The n-BuOH extract (42 g) was subjected to a column chromatography on silica gel using petroleum $CHCl_3$-MeOH as the mobile phase and six fractions (Fr.1-6) were obtained. Five fractions (Fr.2-6) were purified by a column chromatography of Sephadex LH-20 eluting with MeOH and then the eluent was concentrated *in vacuo*, respectively.

The obtained residues were further purified by semi-preparative HPLC using MeOH-H_2O as the eluent to yield the compounds **1**-**10** (Figure 1).



R_1=C, R_2=R_3=R_4=R_5=H (**1**)
R_2=C, R_1=R_3=R_4=R_5=H (**2**)
R_3=C, R_1=R_2=R_4=R_5=H (**3**)
R_1=R_2=C, R_3=R_4=R_5=H (**4**)
R_2=R_3=C, R_1=R_4=R_5=H (**5**)
R_1=R_3=C, R_2=R_4=R_5=H (**6**)
R_1=R_4=C, R_2=R_3=R_5=H (**7**)
R_1=R_2=C, R_5=CH_3, R_3=R_4=H (**8**)
R_1=R_3=C, R_5=CH_3, R_2=R_4=H (**9**)
R_1=R_2=R_3=C, R_4=R_5=H (**10**)

Fig. 1: Chemical structures of the obtained CQAs.

Determination of the Anti-hyperlipidemic Fraction

To identify the active fractions of *P. tectorius* fruit with blood lipid-lowering effects, the extracts of obtained in the extraction and isolation procedure was evaluated. The fractions of *P. tectorius* fruit extracted by petroleum ether, $CHCl_3$, EtOAc and the remaining residue showed no effect on serum TC and TG, while the *n*-butanol fraction was quite effective in decreasing these blood lipids. Accordingly, the n-butanol extract was assigned as the active extract.

Cell Culture

HepG2 cells were grown to 70%-80% confluence and then incubated in 0.02% BSA (Sigma-Aldrich) / DMEM (Gibco-BRL, Grand Island, NY, USA) for 12 h. Cells were then washed and incubated with 1 μM of various CQAs or 1 mM of the AMPK activator AICAR (Sigma-Aldrich, China) in 0.02% BSA + 100 μM oleic acid/DMEM or in 0.02% BSA + 100 μM oleic acid/DMEM alone for 6 h. Subsequently, the cells were subjected to oil-red O staining or TC and TG determination.

RESULTS AND DISCUSSIONS

Structure Identification of the Isolates

Compound 1

NMR data: ^{1}H NMR (600MHz, CD_3OD): δ: 7.56 (1H, d, *J*=15.6 Hz, H-7'), 7.04 (1H, brs, H-2'), 6.96 (1H, brd, *J*=7.8 Hz, H-6'), 6.78 (1H, d, *J*=7.8 Hz, H-5'), 6.28 (1H, d, *J*=15.6 Hz, H-8'), 5.35 (1H, m, H-3), 4.18 (1H, m, H-5), 3.72 (1H, m, H-4), 2.20~2.22 (4H, m, H-2, 6); the ^{13}C NMR (150 MHz, CD_3OD) spectral data were listed in Table. These spectral data showed basically agreement with the compound of chlorogenic acid (Ge, *et al*, 2007).

Compound 2

NMR data: ^{1}H NMR (600MHz, CD_3OD): δ: 7.63 (1H, d, *J*=15.6 Hz, H-7'), 7.06 (1H, s, H-2'), 6.98 (1H, d, *J*=7.8 Hz, H-6'), 6.76 (1H, d, *J*=7.8 Hz, H-5'), 6.37 (1H, d, *J*=15.6 Hz, H-8'), 4.84 (1H, dd, *J*=9.6, 2.4 Hz, H-4), 4.24 (2H, m, H-3, 5), 1.95~2.20 (4H, m, H-2, 6); ^{13}C NMR (150 MHz, CD_3OD) were listed in Table 1. These spectral data showed basically agreement with the reported literature of 4-O-caffeoylquinic acid (Ge, *et al*, 2007).

Compound 3

NMR data: ^{1}H NMR (600MHz, CD_3OD): δ: 7.56 (1H, d, *J*=15.6 Hz, H-7'), 7.02 (1H, s, H-2'), 6.94 (1H, d, *J*=7.8 Hz, H-6'), 6.74 (1H, d, *J*=7.8 Hz, H-5'), 6.20 (1H, d, *J*=15.6 Hz, H-8'), 5.14 (1H, m, H-3), 3.82 (1H, dd, *J*=10.8, 5.6 Hz, H-4) , 3.36 (1H, m, H-5), 1.82~2.20 (4H, m, H-2, 6); ^{13}C NMR (150 MHz, CD_3OD) spectral data were listed in Table 1. According to these spectral data, compound **3** was identified as 5-O-caffeoylquinic acid (Ge, *et al*, 2007).

Compound 4

NMR data: ^{1}H NMR (600MHz, CD_3OD): δ: 7.62 (1H, d, J=15.6 Hz, H-7''), 7.54 (1H, d, *J*=15.6 Hz, H-7'), 7.06 (1H, d, *J*=1.8 Hz, H-2''), 7.04 (1H, d, *J*=1.8 Hz, H-2'), 6.96 (1H, dd, *J*=7.8, 1.8 Hz, H-6'), 6.92 (1H, dd, *J*=7.8, 1.8 Hz, H-6''), 6.76 (1H, d, *J*=7.8 Hz, H- 5'), 6.78 (1H, d, *J*=7.8 Hz, H-5''), 6.32 (1H, d, *J*=15.6 Hz, H-8''), 6.26 (1H, d, *J*=15.6 Hz, H-8'), 5.64 (1H, dt, *J*=12.6, 7.2 Hz, H-3), 5.16 (1H, dd, *J*=10.8, 3.6 Hz, H-4), 4.34 (1H, dt, *J*=6.0, 3.6 Hz, H-5), 3.73 (3H, s, -OMe), 2.14~2.36 (4H, m, H-2, 6); ^{13}C NMR (150 MHz, CD_3OD) spectral data were listed in Table 1. According to these spectral data, compound **4** was identified as 3, 4-di-O-caffeoylquinic acid (Basnet, *et al*, 1996).

Compound 5

NMR data: ^{1}H NMR (600 MHz, CD_3OD) δ: 7.64 (1H, d, *J*=15.6 Hz, H-7″), 7.56 (1H, d, *J*=15.6 Hz, H-7'), 7.02 (1H, d, *J*=1.8 Hz, H-2″), 7.06 (1H, d, *J*=1.8 Hz, H-2'), 6.92 (1H, dd, *J*=7.8, 1.8 Hz, H-6'), 6.96 (1H, dd, *J*=7.8, 1.8 Hz, H-6″), 6.82 (1H, d, *J*=7.8 Hz, H- 5'), 6.76 (1H, d, *J*=7.8 Hz, H-5″), 6.30 (1H, d, *J*=15.6 Hz, H-8'), 6.26 (1H, d, *J*=15.6 Hz, H-8''), 5.12 (1H, dd, *J*=7.8, 3.2 Hz, H-4), 4.34 (1H, q, *J*=3.2 Hz, H-3), 2.10~2.42 (4H, m, H-2, 6); ^{13}C NMR (150 MHz, CD_3OD) spectral data were listed in Table 1. According to these spectral data, compound **5** was identified as 4, 5-di-O-caffeoylquinic acid (Chiang *et al*, 2004).

Compound 6

NMR data: ^{1}H NMR (600MHz, CD_3OD): δ: 7. 62 (1H, d, *J*=15.6 Hz, H-7''), 7.56 (1H, d, *J*=15.6Hz, H-7'), 6.94 (1H, dd, *J*=7.8, 1.8 Hz, H-6'), 6.96 (1H, dd, *J*=7.8, 1.8 Hz, H-6''), 6.80 (1H,

d, *J*=7.8 Hz, H-5'), 6.78 (1H, d, *J*=7.8 Hz, H-5''), 6.34 (1H, d, *J*=15.6 Hz, H-8''), 6.25 (1H, d, *J*=15.6 Hz, H-8'), 5.40 (2H, m, H-3, 5), 3.98 (1H, dd, *J*=7.2, 3.2 Hz, H-4), 2.12~2.48 (4H, m, H-2, 6); ^{13}C NMR (150 MHz, CD_3OD) spectral data were listed in Table 1. According to these spectral data, compound **6** was identified as 3, 5-di-O-caffeoylquinic acid (Chiang, *et al*, 2004).

Compound 7

NMR data: ^{1}HNMR (600MHz, CD_3OD): *δ*: 7.62 (1H, d, *J*=15.6 Hz, H-7''), 7.56 (1H, d, *J*=15.6 Hz, H-7'), 7.02 (1H, d, *J*= 1.8 Hz, H-2'), 7.05 (1H, d, *J*=1.8 Hz, H-2''), 6.97 (1H, dd, *J*= 7.8, 1.8 Hz, H-6''), 6.86 (1H, d, *J*=7.8 Hz, H-5''), 6.79 (1H, dd, *J*=7.8, 1.8 Hz, H-6'), 6.36 (1H, d, *J*=15.6 Hz, H-8''), 6.24 (1H, d, *J*=15.6 Hz, H-8'), 5.42 (1H, q, *J*=3.2 Hz, H-3), 4.34 (1H, ddd, *J*= 10.8, 9.6, 3.2 Hz, H-5), 3.94 (1H, dd, *J*=9.5, 3.0 Hz, H-4), 2.92 (1H, dt, *J*=15.6, 3.2 Hz, H-2b), 2.48 (1H, dt, *J*=13.2, 3.2 Hz, H-6b), 2.36 (1H, dd, *J*=15.6, 3.2 Hz, H-2a), 2.08 (1H, dd, *J*=13.2, 10.8 Hz, H-6a); ^{13}C NMR (150 MHz,CD_3OD) spectral data were listed in Table 1. According to these spectral data, compound **7** was identified as 1, 3-di-O-caffeoylquinic acid. (Dini, *et al*, 2006).

Compound 8

NMR data: ^{1}H NMR (600 MHz, CD_3OD): *δ*: 7.63 (1H, d, *J*=15.6 Hz, H-7''), 7.58 (1H, d, *J*=15.6 Hz, H-7'), 7.02 (1H, d, *J*=1.8 Hz, H-2''), 7.06 (1H, d, *J*=1.8 Hz, H-2'), 6.92 (1H, dd, *J*=7.8, 1.8 Hz, H-6'), 6.94 (1H, dd, *J*=7.8, 1.8 Hz, H-6''), 6.82 (1H, d, *J*=7.8 Hz, H-5'), 6.80 (1H, d, *J*=7.8 Hz, H-5''), 6.32 (1H, d, *J*=15.6 Hz, H-8''), 6.26 (1H, d, *J*=15.6 Hz, H-8'), 5.54 (1H, dt, *J*=12.0, 7.2 Hz, H-3), 5.12 (1H, dd, *J*=12.0, 3.2 Hz, H-4), 4.34 (1H, dt, *J*=6.4, 3.2 Hz, H-5), 3.74 (3H, s, -OMe), 2.10~2.36 (4H, m, H-2, 6); ^{13}C NMR (150 MHz, CD_3OD) spectral data were listed in Table 1. According to these spectral data, compound **8** was identified as 3, 4-di-O-caffeoylquinic acid methyl ester (Basnet P, *et al*, 1996).

Compound 9

NMR data: ^{1}H NMR (600M Hz, CD_3OD): *δ:* 7.64 (1H, d, *J*=15.6 Hz, H-7''), 7.56 (1H, d, *J*=15.6 Hz, H-7'), 7.02 (1H, d, *J*=1.8 Hz, H-2''), 7.05 (1H, d, *J*=1.8 Hz, H-2'), 6.98 (1H, dd, *J* =7.8, 1.8 Hz, H-6'), 6.96 (1H, dd, *J* =7.8, 1.8 Hz, H-6''), 6.80 (1H, d, *J* =7.8 Hz, H-5''), 6.78 (1H, d, *J*=7.8 Hz, H-5'), 6.32 (1H, d, *J*=15.6Hz, H-8''), 6.26 (1H, d, *J*=15.6 Hz, H-8'), 5.34 (1H, m, H-3), 5.42 (1H, dd, *J*=7.2, 3.2 Hz, H-5), 4.02 (1H, dt, *J*=6.0, 3.2 Hz, H-4), 3.73 (1H, s, -OMe), 2.15~2.36 (4H, m, H-2, 6); ^{13}C NMR (150 MHz,CD_3OD) spectral data were listed in Table 1. According to these spectral data, compound **9** was identified as 3, 5-di-O-caffeoylquinic acid methyl ester. (Chiang, *et al*, 2004).

Compound 10

NMR data: ^{1}HNMR (600MHz, CD_3OD): *δ:* 7.48 (1H, d, *J*=15.6 Hz, H-7''), 7.46 (1H, d, *J*=15.6 Hz, H-7'), 7.45 (1H, d, *J*=15.6 Hz, H-7'''), 7.02 (1H, d, *J*=1.8 Hz, H-2''), 7.05 (1H, d, *J*=1.8 Hz, H-2'), 7.04 (1H, d, *J*=1.8 Hz, H-2'''), 6.98 (1H, dd, *J*=7.8, 1.8 Hz, H-6'), 6.96 (1H, dd, *J*=7.8, 1.8 Hz, H-6''), 6.96 (1H, dd, *J*=7.8, 1.8 Hz, H-6'''), 6.79 (1H, d, *J*=7.8 Hz, H-5''), 6.77 (1H, d, *J*=7.8 Hz, H-5'), 6.75 (1H, d, *J*=7.8 Hz, H-5'''), 6.32 (1H, d, *J*=15.6Hz, H-8''), 6.26 (1H, d, *J*=15.6 Hz, H-8'), 6.28 (1H, d, *J*=15.6 Hz, H-8'''), 5.45 (1H, m, H-3), 5.42 (1H, dd, *J*=7.2, 3.2 Hz, H-5), 5.24 (1H, dt, *J*=6.0, 3.2 Hz, H-4), 2.04~2.40 (4H, m, H-2, 6); ^{13}C NMR (150 MHz, CD_3OD) : *δ* 173.2 (C-7), 165.8 (C-9'), 165.6 (C-9''), 165.4 (C-9'''), 149.0 (C-4'), 148.6 (C-4''), 148.8 (C-4'''), 147.2 (C-7'), 147.0 (C-7''), 146.7 (C-7'''), 146.0 (C-3'), 146.3 (C-3''), 146.1 (C-3'''), 126.2 (C-1'), 126.0 (C-1''), 126.7 (C-1'''), 122.2 (C-6'), 121.8 (C-6''), 122.1 (C-6'''), 116.2 (C-5'), 116.0 (C-5''), 115.8 (C-5'''), 115.2 (C-2'), 115.0 (C-2''), 115.0 (C-2'''), 114.2 (C-8'), 113.8 (C-8''), 114.0 (C-8'''), 74.2 (C-1), 69.8 (C-4), 68.8 (C-3, 5), 35.8 (C-2, 6). According to these spectral data, compound **10** was identified as 3, 4, 5-tri-O-caffeoylquinic acid. (Kim *et al*, 2011).

Table. 1: ^{13}C NMR spectral data of compouds **1-9** (CD_3OD, *δ* in ppm).

No.		1	2	3	4	5	6	7	8	9
1		75.6	75.4	73.4	75.3	75.8	74.9	80.6	75.4	74.8
2		38.2	38.1	38.3	37.2	38.4	36.5	33.2	37.2	36.6
3		72.2	67.2	69.2	70.4	69.4	72.8	73.2	70.3	72.9
4		70.8	73.6	70.8	76.5	75.8	70.7	75.6	76.4	70.6
5		67.9	68.8	71.5	66.0	69.2	72.2	68.2	66.2	72.2
6		37.6	37.8	37.2	41.8	39.4	38.2	41.2	42.3	38.3
7		175.6	175.2	175.3	178.0	176.8	177.3	176.2	176.4	176.8
-OMe									52.6	52.4
1'	1''	127.4	127.6	127.6	127.6	127.6	127.8	127.6	127.6	127.5	127.4	127.5	127.5	127.5	127.7	127.6
2'	2''	115.3	115.2	115.3	115.3	115.2	115.2	115.3	115.2	115.2	115.0	115.6	115.1	115.0	115.1	115.0
3'	3''	146.6	146.7	146.6	146.5	147.2	146.8	146.5	147.2	146.7	146.6	146.8	146.6	147.4	147.3	146.5
4'	4''	149.7	149.8	149.7	149.8	149.6	149.5	149.8	149.6	149.7	149.5	149.8	149.5	149.7	149.7	149.6
5'	5''	116.6	116.6	116.6	116.5	116.6	116.5	116.5	116.6	116.2	116.0	116.5	116.3	116.4	116.5	116.1
6'	6''	122.8	123.0	123.1	123.0	123.1	123.0	123.0	123.1	123.1	122.8	123.0	123.1	123.0	123.0	123.2
7'	7''	147.0	147.2	147.3	147.4	147.4	147.2	147.4	147.4	147.3	147.5	147.8	147.6	147.5	147.5	147.2
8'	8''	115.0	115.0	115.0	115.1	115.2	115.0	115.1	115.2	115.4	115.5	115.6	115.0	115.1	115.3	115.0
9'	9''	168.2	168.3	166.2	168.4	168.5	168.4	168.4	168.5	168.4	167.6	168.6	168.6	168.4	168.6	168.5

Lipid Lowering Effects of Selected CQAs

Three CQAs of compounds **1**, **4**, and **5**, particularly **4** and **5**, significantly reduced the oil-red O staining and TC (Figure 2A) and TG (Figure 2B) accumulation in HepG2 cells, suggesting that CQAs play an important role in the anti-hyperlipidemic activity of the fruits of *P. tectorius*.

Considering their potent lipid-modulating activity and high concentration in the fruits of *P. tectorius*, caffeoylquinic acids are assigned as the principle active components accounting for its anti-hyperlipidemic effect.

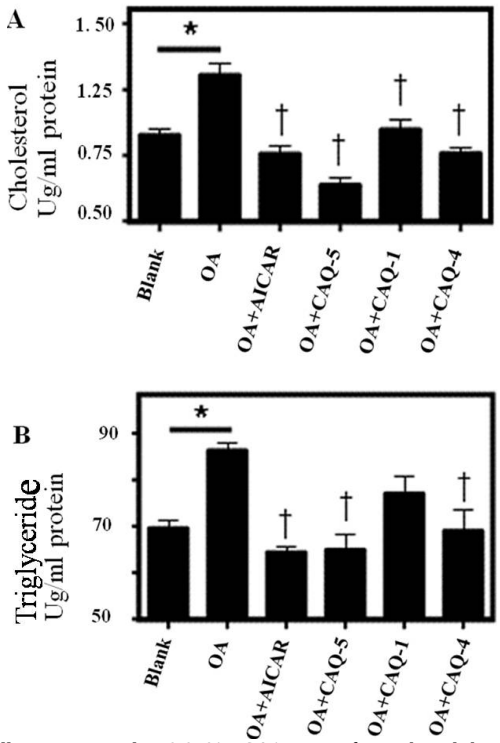


Fig. 2: Cells were starved in 0.02% BSA/DMEM for 12 h and then incubated with 1 μM of the individual CQAs or 1 mM AICAR in 0.02% BSA + 100 μM oleic acid (OA)/DMEM. Then the cells were subjected to TC (**A**) and TG (**B**) determination. **p*<0.05 OA group vs. normal group. †*p*<0.05 test group vs. OA group.

CONCLUSIONS

In present study, we systematically investigated the chemical constituents of the anti-hyperlipidemic fraction of the *n*-butanol obtained from the fruits of *P. tectorius*. During this course, ten CQAs were firstly isolated and throughly characterized and all of them were isolated from genus *Pandanus* for the first time. Furthermore, three selected compounds (**1**, **4**, and **5**) were evaluated for their lipid lowering effects, and they all displayed significant anti-hyperlipedmic activities. Based on these results, caffeoylquinic acids are firstly assigned as the principle active components accounting for the anti-hyperlipidemic effect of fruits of *P. tectorius*.

Therefore, our study will provide solid foundation for further investigation on the hypolipidemic effects of both the fruits of *P. tectorius* and the natural products of the CQAs.

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