A NEW BENZOFURAN DERIVATIVE FROM THE LEAVES OF *FICUS PUMILA* L.

MỘT BENZOFURAN MỚI TỪ LÁ CÂY TRÂU CỔ (*FICUS PUMILA* L.)

Pham Thi Nhat Trinh[1], Nguyen Huu An[2], Pham Ngoc An[2], Mai Dinh Tri[3], Le Viet Tien[4], Phan Nhat Minh[3], and Le Tien Dung[2*]

[1] Faculty of Natural Sciences, Tiengiang University, Tiengiang, Vietnam
[2]Institute of Applied Materials Science, Vietnam Academy of Science and Technology, Ho Chi Minh City, Vietnam
[3]Department of Bioactive Compound, Institute of Chemical Technology, Academy of Science and Technology, Ho Chi Minh City, Vietnam
[4]Department of Organic Chemistry, Ho Chi Minh University of Science, Vietnam

Received 05/03/2017, Peer reviewed 03/5/2017, Accepted for publication 30/6//2017

ABSTRACT

*In Vietnam, people use Ficus pumila L. in cases of lumbago, rheumatism, anaemia, hematuria, and hypogalactia and there is a little study on its phytochemicals up to now. Using column chromatography with normal and reverse phase of silica gel as adsorbent, a new benzofuran derivative, pumiloside (**1**) together wiith seven known flavonoid glycosides, afzelin (**2**), astragalin (**3**), quercitrin (**4**), isoquercitrin (**5**), kaempferol 3-O-rutinoside (**6**), rutin (**7**) and kaempferol 3-O-sophoroside (**8**) were isolated from the leaves of Ficus pumila. The structures were established by spectroscopic data and comparison with the literature values.*

Keywords: *Pumiloside; Ficus pumila L.; benzofuran derivative; flavonoid glycoside.*

TÓM TẮT

*Cây trâu cổ được dùng để điều trị các bệnh đau thắt lưng, viêm khớp dạng thấp, thiếu máu, hạ huyết áp... cho đến nay có ít công trình nghiên cứu về thành phần hóa học trên loài này ở Việt Nam. Trong bài báo này, sử dụng các kỹ thuật sắc ký cột silica gel pha thường và pha đảo, một dẫn xuất benzofuran mới pumiloside (**1**), cùng 7 flavonoid glycoside, afzelin (**2**), astragalin (**3**), quercitrin (**4**), isoquercitrin (**5**), kaempferol 3-O-rutinoside (**6**), rutin (**7**) và kaempferol 3-O-sophoroside (**8**) đã được phân lập từ lá cây trâu cổ Ficus pimula. Cấu trúc các chất được xác định bằng phổ NMR, MS và kết hợp với tài liệu tham khảo*

Từ khóa: *Pumiloside; Ficus pumila L.; dẫn xuất benzofuran; flavonoid glycoside.*

1. INTRODUCTION

In folk medicine of Vietnam, *Ficus pumila* L. or creeping fig belonging in the Moraceae family is considered to be tonic and used in cases of lumbago, rheumatism, anaemia, hematuria, and hypogalactia. Various triterpenes, flavonoids, sesquiterpenoids, sterols and coumarins have been previously reported from *F. pumila* with antimicrobial and antioxidative activities [1,4-6,10-11]. In this paper, the isolation process and structure elucidation of one new benzofuran derivative (**1**), along with seven known flavonoid glycosides (**2-8**) from methanol extract of *F. pumila* leaves are reported.

2. METHODS

2.1. General experimental procedures

Specific rotation was measured on digital polarimeter (Kruss, Hamburg, German). NMR spectra were recorded on Bruker AM500 FTNMR spectrometer (Bruker, Karlsruhe, Germany) using TMS as an internal standard, Institute of Chemistry (Vietnam Academy of Science and Technology, Hanoi, Vietnam). HR-ESI-MS were performed on MicroOTOF-Q mass spectrometer (Bruker, Karlsruhe, Germany), University of Science (National University, HoChiMinh city, Vietnam). Fourier transform infrared (FT-IR) was performed on Nicolet-6700 spectrophotometer (Waltham, MA, USA). UV spectra were carried out on UV–vis spectrophotometer Jenway. 6315 (Bibby Scientific Limited, Staffordshire, UK). TLC was performed on silica gel 60 F254 (1.05554.0001, Merck, Darmstadt, Germany). The zones were detected using UV at 254 or 365 nm or a solution of $FeCl_3$/EtOH or H_2SO_4/EtOH. Column chromatography was performed on silica gel (240–430 mesh, Merck, Darmstadt, Germany) and Sephadex LH-20 (GE Healthcare Bio-Sciences AB, Uppsala, Sweden).

2.2 Plant material

The leaves of *F. pumila* were collected in Lam Dong province, Vietnam in August 2015 and authenticated by Luu Hong Truong (Southern Institute of Ecology, Vietnam Academy of Science and Technology). A voucher specimen (No 101) has been preserved in the Institute of Applied Materials Science.

2.3 Extraction and isolation

Dried leaves of *F. pumila* (3.5 kg) were extracted with ethanol 96% (4 × 10 L). The extract was evaporated to dryness (750 g) under reduced pressure. The ethanol extract was partitioned by column chromatography with solvents of increasing polarity starting with *n*-hexane, followed by chloroform, ethyl acetate and methanol, respectively. The *n*-hexane (210 g), chloroform (88 g), ethyl acetate (128 g) and methanol (212 g) extracts were obtained after rotary evaporation process.

The methanol fraction was fractionated by column chromatography on silica gel using mixture of EtOAc:MeOH (1:0 to 0:1, v/v) to yield five fractions (M1–5). Fraction M2 (32.8 g) was subjected to silica gel column chromatography eluted with $CHCl_3$:MeOH (50:1 to 0:1, v/v) to obtained five subfractions (M2.1-M2.5). Subfraction M2.3 was purified by a series of Sephadex LH-20 column in methanol and followed by column chromatography with elution of $CHCl_3$:MeOH (20:1, v/v) repeatedly to afford **2** (3.0 mg), **3** (3.0 mg), **4** (5.0 mg) and **5** (10.0 mg). Fraction M3 (47.3 g) was repeatedly chromatographed over silica gel column and eluted with $CHCl_3$:MeOH (25:1 to 0:1, v/v) to give four subfractions (M3.1-M3.4). Subfraction M3.2 was purified by a series of of silica gel column chromatography eluted with $CHCl_3$:MeOH (15:1, v/v) to give **6** (7.2 mg). Subfraction M3.3 (9.6 g) was rechromatographed on a silica gel column and eluted by $CHCl_3$:MeOH (10:1, 7:1, v/v) to give **1** (5.8 mg), **7** (15.0 mg) and **8** (3.7 mg).

2.4. Pumiloside (1)

Amorphous powder; $[\alpha]^{25}_D$ - 22,0 (*c* 0,01, methanol); ^1H and ^{13}C NMR spectroscopic data, see Table 1; HR-ESI-MS *m*/*z*: 419.0924 $[M+Na]^+$ (calcd. for $C_{18}H_{20}O_{10}Na$, 419.0951).

3. RESULTS AND DISCUSSION

The ethanol extract was fractionated by flash columns chromatography eluting with *n*-hexane, chloroform, ethyl acetate and

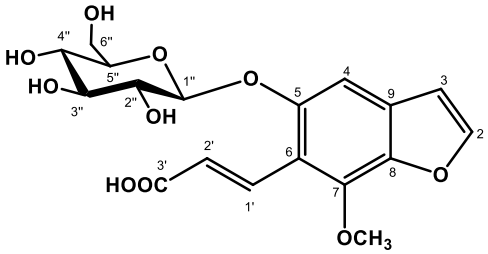
methanol to give four fractions. The methanol fraction was subjected to repeated silica gel column chromatography to yield a new compound named pumiloside (**1**), along with afzelin (**2**) [3], astragalin (**3**) [9], quercitrin (**4**) [9], isoquercitrin (**5**) [12], kaempferol 3-*O*-rutinoside (**6**) [9], rutin (**7**) [5] and kaempferol 3-*O*-sophoroside (**8**) [2] (Figure 1).

Compound **1** was isolated as an amorphous powder. Its molecular formula, $C_{18}H_{20}O_{10}$, was obtained from analysis of the HR-ESI-MS ($[M+Na]^+$, *m/z* 419.0924). The ^{1}H NMR spectrum of **1** exhibited the resonance signals of two olefinic protons at δ_H [8.15 (1H, *d*, *J* = 16.0 Hz, H-1') and 6.91 (1H, *d*, *J* = 16.0 Hz, H-2') ppm], two olefinic protons of the furan ring at δ_H [7.65 (1H, *d*, *J* = 1.5 Hz, H-2) and 7.08 (1H, *br s*, H-3) ppm], one proton of benzene ring at δ_H [7.14 (1H, *s*, H-4) ppm], one methoxy group at δ_H [4.16 (3H, *s*, 7-OCH_3) ppm] and protons of *β*-glucose unit at δ_H [5.04 (1H, *d*, *J* = 8.5 Hz, H-1''); 3.92 (1H, *br d*, *J* = 12.0 Hz, H-6''a); 3.71 (1H, *dd*, *J* = 12.0 and 5.5 Hz, H-6''b); 3.60 (1H, *t*, *J* = 8.5 Hz, H-2''); 3.51 (1H, *m*, H-5''); 3.50 (1H, *m*, H-3''); 3.43 (1H, *t*, *J* = 8.5 Hz, H-4'') ppm]. The ^{13}C NMR together with DEPT spectra revealed 18 carbon signals including one carbonyl carbon at δ_C [173.4 (C-3') ppm], five methine carbons at δ_C [145.3 (C-2); 137.9 (C-1'); 121.2 (C-2'); 106.2 (C-3) and 94.6 (C-4) ppm], three oxygenated quaternary carbons at δ_C [159.2 (C-8); 156.9 (C-5) and 155.1 (C-7) ppm], two quaternary carbons at δ_C [113.9 (C-9) and 111.9 (C-6) ppm], one methoxy group [δ_C 60.6 (7-OCH_3) ppm] and six carbons of *β*-glucose unit at δ_C [102.7 (C-1''); 78.3 (C-5''); 78.2 (C-3''); 74.7 (C-2''); 71.2 (C-4'') and 62.5 (C-6'') ppm]. The ^{1}H NMR and ^{13}C NMR spectroscopic data confirmed that compound **1** has benzofuran skeleton.

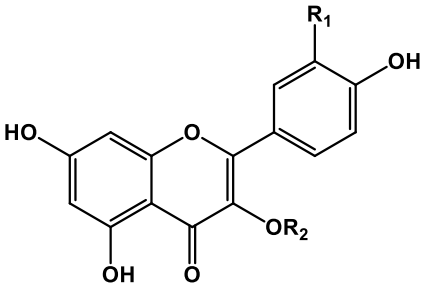
The NOESY spectrum analysis showed that the correlation between proton [δ_H 7.14 (*s*) ppm] and proton of furan ring [δ_H 7.08 (*br s*) ppm] identified those protons as H-4 and H-3. Hence, proton of furan ring [δ_H 7.65 (*d*, *J* = 1.5 Hz) ppm] was indicated as H-2. Moreover, olefinic proton [δ_H 8.15 (*d*, *J* = 16.0 Hz] shared HMBC correlation with carbonyl carbon (δ_C 173.4 ppm) and two oxygenated quaternary carbons (δ_C 156.9 and 155.1 ppm). Olefinic proton [δ_H 6.91 (*d*, *J* = 16.0 Hz)] shared HMBC correlation with carbonyl carbon (δ_C 173.4 ppm) and quaternary carbon (δ_C 111.9 ppm). Furthermore, proton H-4 shared a correlation with two oxygenated quaternary carbons (δ_C 159.2 and 156.9 ppm) and two quaternary carbons (δ_C 113.9 and 111.9 ppm) indicated that acid acrylic group attaching to C-6 (δ_C 111.9 ppm) of benzofuran skeleton. Carbon signals at (δ_C 159.2, 156.9, 155.1, 113.9 and 111.9 ppm) were indicated as C-8, C-5, C-7 and C-9, respectively. Additionally, HMBC data showed that protons of methoxy group [δ_H 4.16 (*s*) ppm] shared HMBC correlation with carbon C-7 and anomeric anomeric proton of *β*-glucose [δ_H 5.04 (*d*, *J* = 8.5 Hz)] shared correlation with carbon C-5. This confirmed that methoxy group attached to C-7 and *β*-glucose unit attached to C-5 of benzofuran skeleton. According to spectroscopic data enlist above, compound **1** was indicated as (*E*)6-(2-carboxyvinyl)-7-methoxy-5-hydroxy-benzofuran-5-*O*-*β* -D-glucopyranoside.

4. CONCLUSSION

From the methanol extract of *F. pumila* leaves in Vietnam, a new benzofuran glucoside, pumiloside (**1**) and seven known flavonoid glycosides, afzelin (**2**), astragalin (**3**), quercitrin (**4**), isoquercitrin (**5**), kaempferol 3-*O*-rutinoside (**6**), rutin (**7**) and kaempferol 3-*O*-sophoroside (**8**) were isolated and identified.



1



	R_1	R_2
2	H	Rha
3	H	Glc
4	OH	Rha
5	OH	Glc
6	H	Glc^{6}-^{1}Rha
7	OH	Glc^{6}-^{1}Rha
8	H	Glc^{2}-^{1}Rha

Figure 1. Chemical structure of compounds **1-8**

Table 1. ^{1}H and ^{13}C NMR spectral data of **1** (CD_3OD, 500 MHz)

Position	**δ_H, ppm (*J*, Hz)**	**δ_C, ppm**
Aglycone		
2	7.65 (*d*, 1.5 Hz)	145.3
3	7.08 (*br s*)	106.2
4	7.14 (*s*)	94.6
5		156.9
6		111.9
7		155.1
8		159.2
9		113.9
1’	8.15 (*d*, 16.0 Hz)	137.9
2’	6.91 (*d*, 16.0 Hz)	121.2
3’		173.4
7-OCH_3	4.16 (*s*)	60.6
Glc		
1’’	5.04 (*d*, 8.5 Hz)	102.7
2’’	3.60 (*t*, 8.5 Hz)	74.7
3’’	3.50 (*m*)	78.2
4’’	3.43 (*t*, 8.5 Hz)	71.2
5’’	3.51 (*m*)	78.3
6’’	3.92 (*br d*, 12.0 Hz); 3.71 (*dd*, 12.0 và 5.5 Hz)	62.5

REFERENCES

[1] L. C. N. Abraham, T. Masakuni, H. Isao, T. Hajime, Antioxidant flavonoid glycosides from the leaves of *Ficus pumila* L., *Food Chem,* 109, pp. 415–420, 2008.

[2] J. M. Hur, S. J. Park, J. G. Park, Y. H. Hwang, J. C. Park, T. Yokozawa, M. S. Kim, Flavonoids from the leaves of *Glycine max* showing anti-lipid peroxidative effect, *Natural Product Sciences*, 7, pp. 49-52, 2001.

[3] D.S. Jang, J. M. Kim, Y. M. Lee, J. L. Yoo, Y. S. Kim, J. H. Kim, J. S. Kim, Flavonols from *Houttuynia cordata* with protein glycation and aldose reductase inhibitory activity, *Nat. Prod. Sci,* 12, pp. 210-213, 2006.

[4] Juan E.A., Rideout J.A., Ragasa C.Y, Bioactive furanocoumarin derivatives from *Ficus pumila* (Moraceae), *Philipp. J. Sci*, 126, pp. 143-154, 1997.

[5] S. Y. Kim, J. J. Gao, W. C. Lee, K. S. Ryu, K. R. Lee, Y. C. Kim, Antioxidative flavonoids from the leaves of *Morus alba*, *Arch. Pharm. Res*, 22, pp. 81-85, 1999.

[6] J. Kitajima, K. Kimizuka, M. Arai, Y. Tanaka, Constituents of *Ficus pumila* leaves, *Chem. Pharm. Bull*, 46, pp. 1647-1649, 1998.

[7] J. Kitajima, K. Kimizuka, Y. Tanaka, New dammarane-type acetylated triterpenoids and their related compounds of *Ficus pumila* fruit, *Chem. Pharm. Bull*, 47, pp. 1138-1140, 1999.

[8] J. Kitajima, K. Kimizuka, Y. Tanaka, Three new sesquiterpenoid glucosides of *Ficus pumila* fruit, *Chem. Pharm. Bull*, 48, pp. 77-80, 2000.

[9] J. H. Lee, C. H. Ku, N. L. Baek, S. H. Kim, H. W. Park, D. K. Kim, Phytochemical constituents from *Diodia teres*, *Arch. Pharm. Res*, 27, pp. 40-43, 2004.

[10] L. Pistelli, E. E. Chiellini, I. Morelli, Flavonoids from *Ficus pumila*, *Bio. Sys. Ecol*, 28, pp. 287-289, 2000.

[11] C. Y. Ragasa, E. A. Juan, J. A. Rideout, A Triterpene from *Ficus pumila*, *J. Asian Nat. Prod. Res*, 1, pp. 269-275, 1999.

[12] L. O. Regasini, A. A. Lopes, D. H. S. Silva, M. Furlan, M. C. M. Young, D. A. Maria, E. J. Barreiro, V. S. Bolzani, Antiproliferative effect of *Pterogyne nitens* on melanoma cells, *Rev. Ciênc. Farm. Básica Apl*, 28, 335-340, 2007.

Corresponding author:
PhD. Le Tien Dung
Department of Pharmaceutical Biochemistry, Institute of Applied Materials Science, Vietnam Academy of Science and Technology, Ho Chi Minh City, Vietnam
Email: inpcdung@yahoo.com or ltdung@ict.vast.vn