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New Flavone from the Aerial Parts of Bougainvillea Glabra

Dr. Amal Hussein Ahmed
Pharmacognosy Department, Faculty of Pharmacy, Al- Azhar University, Cairo, Egypt

ABSTRACT

New flavone was isolated for the first time from the methanolic extract of the aerial part of Bougainvillea glabra (Nyctaginaceae) & identified as luteolin-7-O-[2''-O-(5'''-O-feruloyl)- b-D-apiofuranosyl]- b-D-glucopyranoside in addition to five flavonoids isolated& identified after comparing their spectra with reported ones as vitexin, isovitexin, chrysoeriol, apigenin& luteolin .

Key Words: *Bougainvillea Glabra, Nyctaginaceae, flavones, luteolin-7-O-[2''-O-(5'''-O-feruloyl)- b-D-apiofuranosyl]- b-D-glucopyranoside& flavonoids.*

I. INTRODUCTION

Bougainvillea (family: *Nyctaginaceae*) is a very common ornamental plant grown almost all over the world in tropical and subtropical gardens [1]. It is grown as a shrub as well as a climber [2] . *Bougainvillea* was named after the world traveler, Louis de Bougainville, who discovered it in Brazil in 18th century and brought it to Europe where it became both widespread and popular [3] .Genus *Bougainvillea* (Four-o'clock) includes eighteen species native of tropical and subtropical regions of south America from Brazil west to Peru and south to southern Argentina [4] .The most horticulturally important three species of Bougainvillea are *B. spectabilis* Willdenow, *B. glabra* Choisy, and *B. peruviana* [5] . The leaves are alternate, simple ovate acuminate, 4-13 cm long [3] . The actual flower of the plant is small and generally white but each cluster of three flowers are surrounded of three or six bracts with the bright colors associated with plant, including pink, magenta, purple, red orange, white or yellow [4-6] . *Bougainvillea glabra* 'choicy' was first identified by Swiss botanist Jacques Denys Choisy in 1850. *B.glabra* which is also known as Lesser Bougainville, Snow White & Paper flower , has been used in a variety of disorders including diarrohea, acidity ,cough and sore throat. Decoction of dried flowers used for leucorrhoea and decoction of the stem used in hepatitis[7&8]. The main used part of the plant is leaves [8] . The reported constituents in leaf of *Bougainvillea glabra* 'Choicy' are alkaloids, flavonoids, tannins, saponins and proteins [7] . The leaves of *Bougainvillea glabra* are reported to have anti-inflammatory activities [8] , anti-hyperglycemic activity [9] , insecticidal activity , anti hyperglycemic activity ,anti ulcer , antimicrobial and antidiarrhoeal activity [10-14] . Hydroalcoholic extract of *Bougainvillea glabra* leaves showed inhibitory effect against all gram positive and gram negative bacteria except *Bacillus subtilis* and *Micrococcus leuteus* [15&16] .

Experimental Section

Plant Material

The plant materials (leaves) were collected during Feb.-March 2009 from Al- Orman garden, Giza, Egypt. The botanical identity of plant was kindly authenticated by Engineer Tereesa, Herbal specialist in Al-Orman garden, Egypt.

General experimental procedures

Ultraviolet spectrophotometric analysis

Chromatographically pure materials 1 mg each were dissolved in analytically pure methanol then subjected to UV spectroscopic investigation in 4 ml capacity quartz cells 1 cm thick using a Carl Zeiss spectrophotometer PMQ II. AlCl3, AlCl3/HCl, fused NaOAc / H3BO3 and NaOMe reagents were separately added to the methanolic solution of investigated material and UV measurements were then carried out.

Nuclear magnetic resonance spectroscopic analysis

The NMR spectra were recorded on a Varian Mercury VX-300 NMR spectrometer. 1H- spectra run at 300 MHz and 13C- spectra were run at 75.46 MHz in deutrated dimethylsulphoxide (DMSO-d6). Chemical shifts are quoted in γ and were related to that of the solvents.

GC-MS

The mass spectra were recorded on a Shimadzu GCMS-QP-1000 EX mass spectrometer at 70 eV. Identification and determination of the absolute configuration of monosaccharide units were performed on a Shimadzu 5050A quadrupol mass spectrometer .

Preparation of Plant Extract

The dried leaves of *Bougainvillea glabra* (1Kg) were powdered and extracted successively with Methanol. The methanolic extract (5.0 g) was subjected to column chromatography& eluted with gradient mixtures of methanol: water giving 3 fractions; fraction eluted with 7:3 methanol: water mixture (1.7 g), fr. 3:1 (0.81 g) and fr. Eluted with pure MeOH (0.5 g), respectively. The fr. Eluted with Methanol: H2O 3:1 ,was chromatographed on Sephadex LH-20 with H_2O:MeOH (1:1) as eluent. Fractions (each 5 mL) were collected and checked by TLC [Silica gel plates, $CHCl_3$:MeOH:PrOH:H_2O (5:6:1:4), organic phase]. Same fractions collected together, giving 413 fractions. Five flavonoids were isolated from fractions no. 52-59, 60-63, 156-160, 234-256 and 284-301& were identified as vitexin (15 mg), isovitexin (28 mg), chrysoeriol (8 mg), apigenin (8 mg) and luteolin (5 mg), respectively. Fraction 74-82 (60 mg) was further purified by semi-preparative HPLC leading to the isolation of a pure new compound named compound I.

RESULTS& DISCUSSION

Five flavonoids were isolated from the methanolic extract of the aerial part of Bougainvillea glabra& identified after comparing their spectra with reported ones as vitexin, isovitexin, chrysoeriol, apigenin& luteolin in addition to a pure compound (**I**).

Vitexin

Light yellow powder, melting point: 203-204 °C : UV : 335, 271 nm (MeOH).
1H-NMR : δ 3.53 (1H, m, H-5″), 3.54 (1H, m, H-3″), 3.57 (1H, m, H-4″), 3.74 (1H, dd, J = 12.3, 5.5 Hz, H-6a″), 3.77 (1H, dd, J = 12.3, 2.0 Hz, H-6b″), 4.11 (1H, t, J = 9.0 Hz, H-2″), 4.85 (1H, d, J = 9.9 Hz, H-1″) 6.44 (1H, s, H-8), 6.60 (1H, s, H-3), 6.95 (2H, d, J = 8.6 Hz, H-3′, 5′), 7.85 (2H, d, J = 8.6 Hz, H-2′, 6′).

13C-NMR (125 MHz, CD3COCD3 + D2O): δ 61.7 (C-6″), 70.6 (C-4″), 72.0 (C-2″), 74.5 (C-1″), 79.2 (C-3″), 81.6 (C-5″), 95.3 (C-8), 103.4 (C-3), 104.3 (C-10), 108.7 (C-6), 116.7 (C-3′, 5′), 122.4 (C-1′), 129.0 (C-2′, 6′), 157.7 (C-9), 161.0 (C-5), 162.0(C- 4′), 164.5 (C-7), 165.0 (C-2), 183.1 (C-4)

ESI-MS $[M+H]^+$ m/z 433.1

Comparing the above mentioned data with the published literature [17-21], it was concluded that this compound is vitexin.

Isovitexin

Yellow powder, mp 231-232. UV λ max (nm): 267, 332 (MeOH).

1H NMR (300MHz, DMSO-*d*6) *δ*: 6.78 (1H, s, 8-H), 6.51 (1H, s, 3-H), 6.93 (2H, d, *J*=8.8Hz, 3', 5'-H), 7.93 (2H, d, *J*=8.8Hz, 2', 6'-H), 4.82 (1H, m, glu 1-H), 3.15~4.84 (10H, m).

ESI MS (*m/z*): 431$[M-H]^-$. All data were identical to those of isovitexin [17, 22-25].

Luteolin

Yellow powder, mp 327-328. **IR (KBr) *ν*max**: 3402, 2629, 1653, 1618, 1510, 1356, 1301, 1259, 1165, 1031, 999, 947, 860, 829, 793, 739cm-1. **UV λ max** (nm): 261, 307 (MeOH); 265, 364 (NaOMe); 267, 412 (AlCl3); 265, 352 (AlCl3/HCl).
1H-NMR *δ*: 6.25 (1H, d, *J*=2.1Hz, 6-H), 6.54 (1H, d, *J*=2.1Hz, 8-H), 6.57 (1H, s, 3-H), 7.00 (1H, d, *J*=8.4Hz, 5'-H), 7.46 (1H, dd, *J*=2.1,8.4Hz, 6'-H), 7.52 (1H, d, *J*=2.1Hz, 2'-H), 13.01 (1H, s, 5-OH).
ESI-MS (*m/z*): 285 [M-H]-, 284,256.
All data were identical to those of luteolin [17, 20-22].

Chrysoeriol
Yellow powder, mp 327-328 . **IR (KBr) *ν*max**: 3352, 1651, 1624, 1564,1510, 1435, 1350, 1300, 1209, 1171, 1032, 993, 867, 835, 793, 764 cm-1. UV λ max (nm): 264, 341 (MeOH); 261, 402 (NaOMe); 274, 349, 387 (AlCl3); 272, 349, 385 (AlCl3/HCl).
1H-NMR : 6.25 (1H, d, *J*=2.1Hz, 6-H), 6.55 (1H, d, *J*=2.1Hz, 8-H), 6.69 (1H, s, 3-H), 7.00 (1H, d, *J*=8.3Hz, 5'-H), 7.60 (1H, dd, *J*=2.1,8.3Hz, 6'-H), 7.63 (1H, d, *J*=2.1Hz, 2'-H), 4.00 (1H, s, 4'-OCH3), 13.01 (1H, s, 5-OH).
ESI-MS (*m/z*): 299 [M-H]-, 284, 256. All data were identical to those of chrysoeriol [17, 24-25].

Apigenin
1H NMR : *γ*7.83 (2H, *d*, *J* = 8.8 Hz, H-2*0* and H-6*0*), 6.92 (2H,
d, *J* = 8.8 Hz, H-3*0* and H-5*0*), 6.83 (1H, *d*, *J* = 2.1 Hz, H-6), 6.71 (1H, *d*, *J* = 2.1 Hz, H-8), 6.58 (1H, *s*, H-3).
13C NMR: *γ* 180.45 (*s*, C-4), 164.94 (*s*, C-5), 164.41 (*s*, C-2), 162.60 (*s*, C-4*0*), 160.72 (*s*, C-9), 160.16 (*s*, C-7), 129.30 (*d*, C 2*0* and C-6*0*), 123.14 (*s*, C-1*0*), 117.06 (*d*, C-3*0* and C-5*0*), 109.39 (*s*, C-10), 106.57 (*d*, C-3), 104.83 (*d*, C-6), 99.34 (*d*, C-8). Comparing these data with the published ones [17-20] revealed that the product is Apigenin.

Compound (I) was isolated as a yellow amorphous solid (mp 252-254 ºC). The UV spectral data showed absorption bands at 248 nm and 334 nm. The IR spectrum presented bands at 3433 cm^{-1} (OH), at 1655 cm^{-1} (C=O) and 1605 cm^{-1} (C=C).

The molecular formula of compound (**I**) was calculated as $C_{36}H_{36}O_{18}$, showed a [M-H] at *m/z* 755 (calculated for $C_{36}H_{36}O_{18}$-H). Key fragmentation ions occurred at *m/z* 579 [M-*E*-feruloyl-H]$^-$, *m/z* 561 [M-*E*-feruloyl-H_2O-H]$^-$, *m/z* 447 [M-*E*-feruloyl-apiose-H]$^-$ and *m/z* 285 [M-*E*-feruloyl-apiose-glucose-H]$^-$.

The ^{1}H NMR spectrum (Table 1) showed signals at d 7.34 (1H, d, *J* 8.0 Hz), d 7.35 (1H, brs) and at d 6.88 (1H, d, *J* 8.0 Hz) assigned to H-6', H-2' and H-5' respectively, two doublets at d 6.68 (1H, d, *J* 2.0 Hz) and d 6.36 (1H, d, *J* 2.0 Hz), attributed to H-8 and H-6 of the A-ring, and one singlet at d 6.54 (1H, s) attributed to H-3 typical of a luteolin derivative. Signals at d 6.17 (1H, d, *J* 16 Hz, H-a), d 7.30 (1H, d, *J* 16 Hz, H-b), d 7.07 (1H, d, *J* 1.5 Hz, H-2""), d 6.69 (1H, d, *J* 8.0 Hz, H-5"") and d 6.87 (1H, d, *J* 8.0 and 1.5 Hz, H-6"") suggested the presence of an *E*-feruloyl unit.[26] A signal at d 3.74 (3H, s) indicates the presence of a methoxyl group.[27] NOESY experiment showed correlation between signal at d 3.74 (OMe) and at d 7.07, thus establishing the methoxyl group at position 3"" of the *E*-feruloyl unit.

A doublet at d 5.20 (1H, d, *J* 7.5 Hz, H-1") and a singlet at d 5.38 (1H, s) in the ^{1}H NMR spectrum revealed the presence of two anomeric hydrogen from two sugar units. The TOCSY experiment with irradiation at d 5.20 displayed the spin system of the b-D-glucopyranoside unit, whereas irradiation at d 5.38 resulted only in the singlet at d 3.75 (1H, s) suggesting an apiofuranosyl unit. The coupling constant of the anomeric proton at d 5.20 (1H, d, *J* 7.5 Hz) indicated that the present glucose unit has a b-configuration.[27]

The ^{13}C NMR experiment presented 35 signals, from which 15 were attributed to the aglycone, 9 to the *E*-feruloyl unit, 6 to the b-D-glucopyranosyl unit, and with 5 was possible determined a apiofuranosyl unit.[28]

The apiose unit was characterized through ^{1}H and ^{13}C NMR experiments compared to the literature data. In the ^{1}H NMR spectrum, apiose unit with OH linked to C-1''' and OH linked to C-2''' in *trans* configuration presents constant coupling $J_{1,2}$ 0-1 Hz, whereas *cis* configuration is characterized by $J_{1,2}$ 3-4 Hz.[16,17] The chemical shift of C-1 in ^{13}C NMR experiments in pyridine-d_5 to the a-D-apiofuranoside is d 105 and d 112 to b-D-apiofuranoside,[18] whereas in DMSO-d_6 these isomers produce signals at d 108 and d 109, respectively.[29-31] Thus, the apiose unit in (**1**) was identified as being a b-D-apiofuranoside.

The structure and bonds of these units on compound I was established from *g*HMQC and *g*HMBC experiments. *g*HMQC experiment showed direct correlations between carbons and the respective hydrogens (Table 1). *g*HMBC experiments showed long-range correlations between the hydrogen signal at d 5.20 (H-1" glucose) and the carbon signal at d 162.4 (C-7 aglycone), and between the hydrogen signal at d 3.52 (H-2" glucose) and the carbon signal at d 108.2 (C-1''' apiose). Besides, the chemical shift of the C-2" of the glucose (d 75.8) unit was clearly dishielded (+3) compared to the chemical shift of the analogous carbon resonance of a non-substituted glucose unit (d 72.9), supporting the glucose (1→2) apiose linkage.[28,29] The *g*HMBC experiment also showed correlation between the hydrogen signal at d 4.06 (H-5''' apiose) and the carbon signal at d 166.3 (*E*-feruloyl, C=O) thus evidencing the esterification at this position. The foregoing evidences in combination

with the downfield shift of the C-5''' apiofuranosyl (d 66.6) when compared to a non-acylated analogue (d 62.4) also supported this conclusion. Therefore, compound I was identified as luteolin-7-*O*-[2''-*O*-(5'''-*O*-feruloyl)- b-D-apiofuranosyl]- b-D-glucopyranoside (Figure 1).

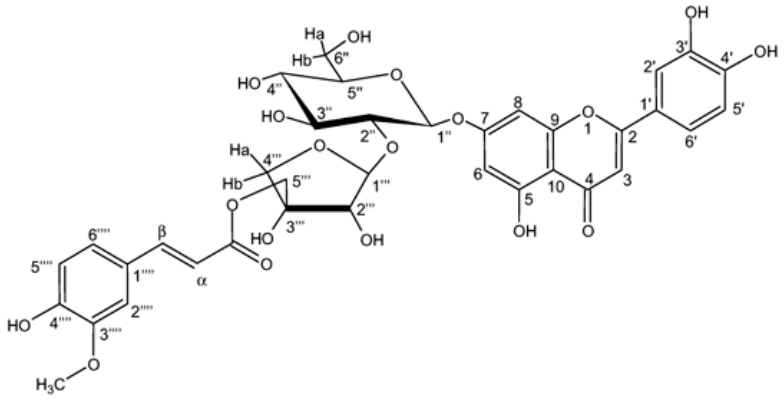

Figure 1. Structure of compound (**1**).

REFERENCES

[1]. N.A.SAHU& D. J. SAXENA, "*Int J Pharm Bio Sci. July; 3(3): (P) 247 – 250,* (2012) ".
[2]. A.Elumalai; M.C. Eswaraiah; K.M.Lahai& H.A.Shaik A, " *Asian J. Res. Pharm. Sci.; vol. 2; Issue 3. Page 85-87* (2012)*"*.
[3]. INTERNATIONAL JOURNAL OF DRUG DISCOVERY AND HERBAL RESEARCH (IJDDHR), " 2(1): January –March; 272 -274), (2012)**".**
[4]. J.M. JANCY RANI ;D.G.CHANDRAMOHAN& R.R. RENGANATHAN ,*" Int. J. Pharm Pharm Sci, Vol 4, Suppl 2, 12-16,* (2000) " .
[5]. V. I. Gupta; M.L.Georg;, L.M. Joseph; M.L. Singhal& H. P. Singh ,*" Journal of Chemical and Pharmaceutical Research,1(1): 233-237(*2009).
[6]. Research Journal of Pharmaceutical, Biological and Chemical Sciences, July – September, RJPBCS Volume 3 Issue 3 Page No. 605 (2012).
[7]. E.E. Sheeja ; E S. Edwin; A. R. Amal; V.B. Gupta& A.C. Rana *"Planta Indica. 1; 33-36*(2005) *"*.
[8]. S.N. Giri; A.K. Biswas; B.P. Saha& S.P. Pal.(1998): "*d. J. Pharm Sci. 50, 42-49, 1998*" .
[9]. Bolognesi A, Polito L, Olivieri F, Valbonesi P, Barbieri L, Battelli MG , "*Planta ,203: 422-429,1997* ".
[10]. Y. Schlein, R.L. Jacobson and G.C. Muller "*Am J Trop Med Hyg. ;65:300-303*"(2001).
[11]. E.S. Edwin, E.E. Sheeja, E. Toppo, V. Tiwari and K.R. Dutt. *"Ars pharm.;48(2):135-144*"(2007).
[12]. E.S. Edwin , E.E. Sheeja, A.Amalraj , R. Soni, G .Smita and V.B. Gupta, "*Planta Indica;2(3):25-26*, (2006) ".
[13]. Adebayo, OT Alabi, BV Owoyele, AO Soladoye. *"Nat. Prod.;3(4): 187-192*, (2009)*"* .
[14]. GI 14. H. Saikia & A. Lama: "*International Journal of Pharmaceutical Sciences and Drug Research; 3(2): 141-145,* (2011) *"* .
[15]. Mosmann, T: *"Journal of Immunological Methods, 65, 55-63,* (1983) *"* .
[16]. N. Anwer, S. Salik& D.Ahmed : "*Int. J. Agric. Biol.;11(5): 647-650* , (2009) *"* .
[17]. Agrawal PK,: *"Elsevier Science Publishing Co. Inc.: New York, pp. 283-364*, (1989) *"*.
[18]. Agrawal PK,: "*Phytochemistry 31(10): 3307- 3330,* (1992) *"* .
[19]. Francis A; Chul S; Masami T; Masahiro I& Michio H . *"Biochem. 64(2): 443-446*, (2000) *"* .
[20]. Fanshawe DB: " *East Afr. Agric.J. 32 :108*, (1966) *"* .
[21]. SU Ya-lun, Wang Yu-lan, YANG Jun-shan"*Chin Tradit Herb Drugs, 24(7): 343-344,* (1993) *"* .
[22]. Singab A. "*Natural medicines, 49(1): 96*, (1995) *"* .
[23]. LING Yun; ZHANG Yong-lin; CAI Shen. "*China J Chin Mater Med, 23 (4): 233-256,* (1998) *"* .
[24]. Lin, L.; Xie, N.; Chen, Z.H.; Wei, M.; Liu, X.F.; Liu, Y.P. *"J. Guangzhou Univ. Tradit. Chin. Med*. *16*, 46–51, (1999) *"*.
[25]. Horowitz, R.M " *B. Chem. Ind. (UK), 21, 498–498,* (1964) *"* .
[26]. Tronchet, J. M. J.; Tronchet, J. *"Carbohydr. Res. 34*, 263, (1974) *"* .
[27]. Kitagawa, I.; Sakagami, M.; Hashiuchi, F.; Zhou, J. L.; Yoshikawa, M.; Ren, J. *"Chem. Pharm. Bull., 37, 551,* **(**1989) *"*.
[28]. Jung, M. J.; Kang, S. S.; Jung, Y. J.; Choi, J. S. *"Chem. Pharm. Bull., 52, 1501, (2004) "*.
[29]. Mathias, L.; Vieira, I. J. C.; Braz-Filho, R.; Rodrigues-Filho, E. A. " *J. Nat. Prod., 61,* 1158, (1998) *"* .
[30]. Bashir, A.; Hamburger, M.; Gupta, M. P.; Solis, P. N.; Hostettmann, K. "*Phytochemistry, 30, 3781,* (1991) *"* .
[31]. Daniel Rinaldo; Clenilson M. Rodrigues; Juliana Rodrigues; Miriam Sannomiya; Lourdes C. dos Santos; Wagner Vilegas *J. Braz. Chem. Soc. vol.18 no.6*, (2007)" .

Table 1: NMR Spectral Data of Compound (I)

Position	1HNMR	13CNMR	gHMBC
2		164.5	H-3
3	6.52s	103	
4		181.5	
5	12.93	161.2	H-6
6	6.36 d	99.1	H-8
7		162.3	H-1"; H-6; H-8
8	6.69 d	94.5	H-6
9		156.9	H-8
10		105.4	H-6; H-3; H-8
1'		122.1	
2'	7.35 brs	113.6	H-6'
3'		149.4	H-5'
4'		145.1	H-2' ; H-6'
5'			
6'	7.34 d	119.2	
Glucose			
1"	5.2 d	97.8	H-2"
2"	3.52 t	75.8	
3"	3.43 t	76.7	H-2"
4"	3.21 t	69.8	H-2"
5"	3.48 m	77.1	H-4" ; H-3"
6"	a)3.5 m b)3.71 d	60.6	
Apiose			
1"'	5.38 s	108.3	H-2", H-2"'
2"'	3.77 s	76.5	
3"'		77.5	H-2"'; H-4"'
4"'	a)3.78 b)4.4	73.9	H-5"' ; H-2"
5"'	4.07 brs	66.7	H-2"'; H-4"'
E-Feruloyl			
α	6.16 d	113.7	H- β
β	7.30 d	144.9	H-6"" ; H-2""
1""		125.3	H-α ; H-5""
2""	7.06 d	110.7	H-6""; H- β
3""		147.7	-OCH3
4""		149.3	H-6""; H-2""
5""	6.68 d	115.4	H-6""
6""	6.87 dd	122.8	H-2"" ; H- β
-OCH3	3.74 s	55.5	
C=O		166.3	H-5""; H- β

Chemical shifts (γ) are in ppm