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PHYTOCHEMICAL STUDY OF *JACARANDA OVALIFOLIA* R. BR. FAMILY BIGNONIACEAE CULTIVATED IN EGYPT

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تم فصل ثمانية مركبات من أوراق نبات الجكرندا اوفاليفوليا و قد تم التعرف عليها كالتالى: ٦'-إستر فينيل حامض الخليك للجلوكوز(١)، ميثيل بارا هيدروكسى خلات الفينيل (٢) ، زيزيبيوزيد I (٣) ، كحول فينيثيلى-٨-أ- بيتا-دى- جلوكوبيرانوزيل-(١←٢)-بيتا- دى-جلوكوبيرانوزيد (٤) ، اكتيوزيد (٥) ، أيزو اكتيوزيد (٦) ، أبيجينين-٧-أ- بيتا- دى-جلوكورونوبيرانوزيد (٧) ، جاكارانون (٨). و قد تم التعرف على هذه المركبات بإستخدام خواصها الطبيعية والوسائل الكيميائية و الطيفية المختلفة و أيضاً بمقارنتها بالنتائج المنشورة سابقا. المركب ١ تم فصله لأول مرة من مصدر طبيعى أما المركبات ٢،٣،٤،٦،٧ فقد تم فصلها لأول مرة من جنس الجكرندا ، والمركب ٨ تم فصله لأول مرة من هذا النبات ، أما المركب ٥ فقد تم فصله سابقاً من نفس النبات.

Eight compounds were isolated from the leaves of Jacaranda ovalifolia R. Br. and were identified as: 6`-phenyl acetic acid ester of glucose (1), methyl p-hydroxy phenyl acetate (2), zizybeoside I (3), phenethyl alcohol 8-O-β-D-glucopyranosyl-(1→2)-β-D-glucopyranoside (4), acteoside (5), isoacteoside (6), apigenin 7-O-β-D-glucuronopyranoside (7), Jacaranone (8). The structures of the isolated compounds were determined by physical, chemical and spectroscopic methods. Compound 1 is isolated here probably for the first time from a natural source, compounds 2,3,4,6 and 7 have been isolated for the first time from the genus Jacaranda, compound 8 was isolated for the first time from J. ovalifolia R. Br., and compound 5 was previously isolated from J. ovalifolia.

INTRODUCTION

The genus *Jacaranda* (Bignoniaceae) includes 50 species distributed from southern Mexico to Argentina. *Jacaranda ovalifolia* R. Br. (syn. *J. mimosaefolia* D. Don.), is a large deciduous tree, native to Brazil[1] and cultivated in Egypt as an ornamental plant. It has tiny but thick growing leaves and beautiful cluster of turquoise flowers, it is especially appreciated for its wood, which is used in making furniture.[2] Previous reports on the genus *Jacaranda* revealed the presence of flavonoids, hydroquinone, quinone, anthocyanins, mucilage, protein, triterpenes and acids.[3-15] It also revealed some biological activities as cytotoxic and antitumour,[10] cyclooxygenase and lipoxygenase inhibitory activities,[12,13] hypotensive, smooth muscle relaxant and antimicrobial activities.[5,6,16] Other activities include the effect of ursolic acid isolated from *J. decurrens* on the greenbug *Schizaphis graminum*,[11] the antiprotozoal activity and contact allergic dermatitis effect.[17,18] The present study deals with the isolation and identification of eight compounds from leaves of *Jacaranda ovalifolia*.

EXPERIMENTAL

Optical rotation was measured on Union PM-101 automatic digital polarimeter. Melting point was recorded on Yanaco PM-3 micro melting apparatus. Mass spectrum was recorded on JEOL JMS-SX 102 spectrometer. NMR spectra were recorded on JEOL JNM A400 spectrometer (400 MHz for ^{1}H-NMR and 100 MHz for ^{13}C-NMR) using TMS as internal standard. Preparative HPLC was carried out on

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column of ODS (150x20 mm i.d., YMC) with JASCO PU-1580 Pump, JASCO UV-975 UV/Visible detector and TOYO SODA RI-8000 refraction index detector. For Column chromatography, Kieselgel 60 (70-230 mesh, Merck), LiChroprep RP-18 (Merck) and Diaion HP-20 (Mitsubishi) were used. For TLC, Silica gel 60 precoated plates F-254 (Merck) were used. HPTLC was carried out using RP-18 precoated plates F-254s. Spots on TLC were visualized by spraying with 10% H_2SO_4 in 50% aqueous ethanol and followed by heating at 110°.

Plant material

Leaves of *Jacaranda ovalifolia* R. Br. were collected from Aswan Botanical Garden in October 1999; the plant was kindly identified by Prof. Dr. N. El-Keltawy, Prof. of Horticulture, Faculty of Agriculture, Assiut University. A voucher sample is kept in the herbarium of Dept. of Pharmacognosy, Faculty of Pharmacy, Assiut University, Assiut.

Extraction and Isolation

The air-dried leaves of *Jacaranda ovalifolia* R. Br. (2.5 kg) were extracted with methanol by maceration. The concentrated methanolic extract (700 g) was diluted with distilled water and fractionated successively with *n*-hexane, diethyl ether, ethyl acetate and *n*-butanol.

The dried *n*-butanol soluble fraction (109 g) was subjected to a column of Diaion-HP20 and eluted with water, 50% MeOH, 100% MeOH and acetone respectively. The 50% MeOH eluate (40 g) was chromatographed on a column of silica gel using CH_2Cl_2-MeOH-H_2O (85:15:1.5 to 60:40:10) gradient as solvent systems to give 3 fractions (F-1 to F-3).

F-1 (3.5 g) was chromatographed on LiChroprep RP-18 using 20% to 50% MeOH gradient as eluent to give two sub-fractions (F-1-1 and F-1-2). F-1-1 (250 mg) was subjected to HPLC on ODS column using 30% MeOH to afford compound **1** (40 mg). F-1-2 (100 mg) was subjected to HPLC on ODS column using 30% MeCN to give compound **2** (18 mg).

F-2 (8.0 g) was chromatographed on LiChroprep RP-18 using 20% to 50% MeOH gradient as eluent to give four sub-fractions (F-2-1, F-2-2, F-2-3 and F-2-4). F-2-2 was crystallized using methanol to afford compound **4** (27 mg). F-2-1 (100 mg) and F-2-3 (700 mg) were separately chromatographed on HPLC using ODS column and 30% MeOH and 40% MeOH as solvent systems respectively to afford compounds **3** (18 mg) and 5 (235 mg) respectively. F-2-4 (120 mg) was subjected to HPLC using ODS column and 45% MeOH to give compound **6** (20 mg).

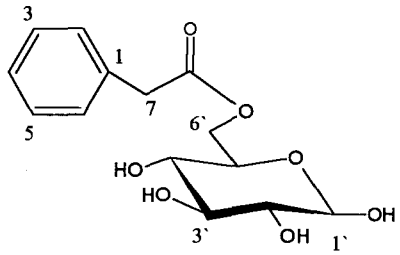
F-3 (2.0 g) was chromatographed on silica gel column using CH_2Cl_2-MeOH-H_2O (70:30:3) as eluent where compound **7** (193 mg) was isolated.

Five grams from the ethyl acetate soluble fraction were chromatographed on silica gel column using CH_2Cl_2-MeOH (30:1) then followed by reversed phase column chromatography on LiChroprep RP-18 using 50% MeOH to afford compound **8** (187 mg).

Compound (1): Obtained as brownish-yellow oil, (40 mg), molecular formula ($C_{14}H_{18}O_7$) from negative ion HR FAB-MS *m/z* 297 $[M-H]^-$, $[\alpha]_D^{22}$ +32.69° (c. 2.12 MeOH) with R_f= 0.34, from CH_2Cl_2-MeOH-H_2O (85:15:1.5). ^{1}H-NMR (CD_3OD, 400 MHz): aglycone: δ 7.12-7.22 (10 H, *m*, aromatic protons), 3.565, 3.562 (each 2H, *s*, H-7), sugar moiety: δ 4.37 (1H, *d*, *J*= 7.8 Hz, H-1` of β-D-glucose), 4.96 (1H, *d*, *J*= 3.7 Hz, H-1` of α-D-glucose), 4.10, 4.31 (each 2H, *m*, H-6` of α- and β-D-glucose) and 3.00-3.87 (*m*, other α- and β-D-glucose protons). ^{13}C-NMR (Tables 1 and 2).

Compound (2): Obtained as yellow oil, (18 mg), with R_f= 0.25, from (30% MeCN). ^{1}H-NMR (CD_3OD, 400 MHz): δ 7.05 (2H, *d*, *J*= 8.5 Hz, H-2, 6), 6.71 (2H, *d*, *J*= 8.5 Hz, H-3, 5), 3.51 (2H, *s*, H-7) and 3.64 (3H, *s*, H-9). ^{13}C-NMR (Table 1).

Compound (3): Isolated as white amorphous powder, (18 mg), with R_f= 0.35, from $CHCl_3$-MeOH-H_2O (75:25:2.5). ^{1}H-NMR (C_5D_5N, 400 MHz): aglycone: δ 7.47 (2H, *br. d*, *J*= 7.6 Hz, H-2, 6), 7.18 (2H, *br. dd*, *J*= 7.6, 7.6 Hz, H-3, 5), 7.04 (1H, overlapped with solvent signal, H-4), 4.60 and 4.90 (each 1H, *d*, *J*= 12.0 Hz, H-7). β-D-glucose: δ 5.16 (1H, *d*, *J*= 7.8 Hz, H-1`), 3.70 (2H, *m*, H-5`, H-5``), 4.04 (1H, *m*, H-2`), 4.34 (1H, *dd*, *J*= 2.0, 12.0 Hz, $H_a$-6`), 4.81 (1H, *d*, *J*= 7.6 Hz, H-1``), 3.93 (1H, *t*, *J*= 8.4



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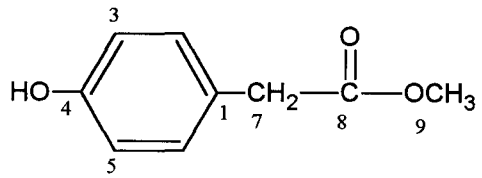

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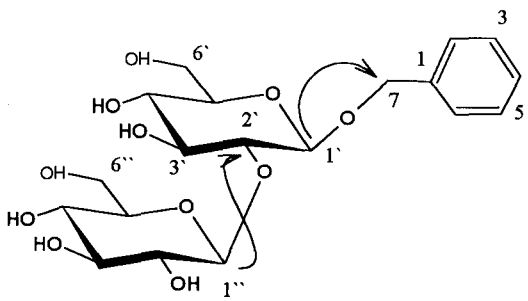

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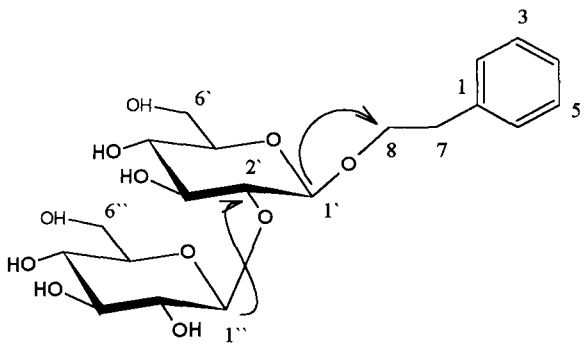

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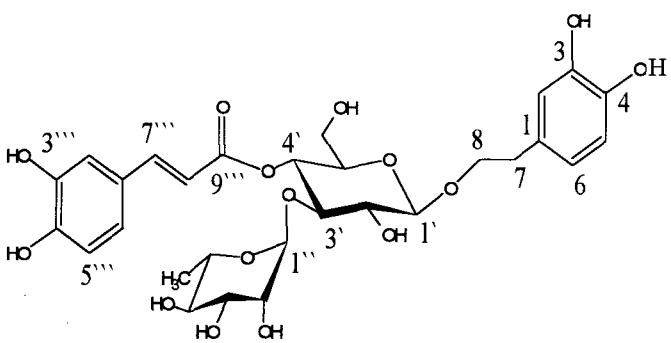

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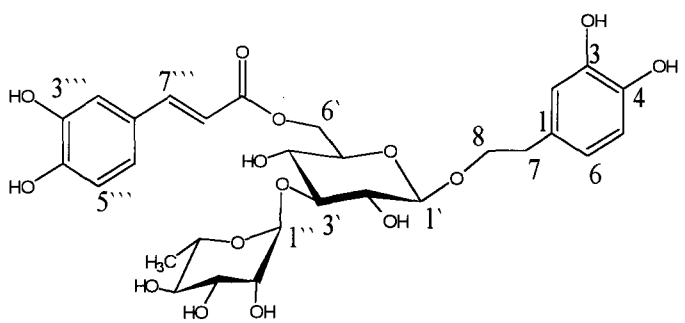

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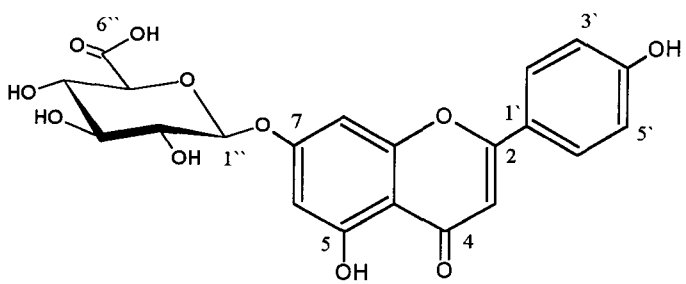

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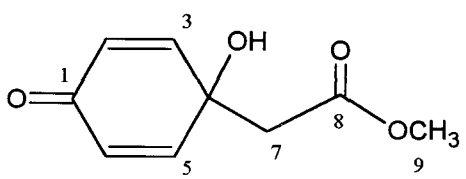

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Table 1: ^{13}C-NMR spectral data of compound **2** and **8** and the aglycone part of compounds **1, 3-7** (100 MHz).

C	1	2	3	4	5	6	7	8
1	135.6 *s* (135.5)	126.3 *s*	138.9 *s*	139.3 *s*	131.5 *s*	131.4 *s*	-	187.3 *s*
2	129.51 *d* (129.49)	131.3 *d*	127.9 *d*	129.5 *d*	117.1 *d*	117.1 *d*	164.3 *s*	128.1 *d*
3	130.4 *d*	116.3 *d*	128.6 *d*	128.6 *d*	144.7 *s*	144.6 *s*	102.8 *d*	152.5 *d*
4	128.0 *d*	157.5 *s*	127.6 *d*	126.4 *d*	146.1 *s*	146.1 *s*	181.9 *s*	67.9 *s*
5	130.4 *d*	116.3 *d*	128.6 *d*	128.6 *d*	116.5 *d*	116.5 *d*	161.9 *s*	152.5 *d*
6	129.51 *d* (129.49)	131.3 *d*	127.9 *d*	129.5 *d*	121.3 *d*	121.3 *d*	99.5 *d*	128.1 *d*
7	41.8 *t* (41.7)	40.9 *t*	70.8 *t*	36.6 *t*	36.6 *t*	36.6 *t*	163.0 *s*	45.5 *t*
8	173.51 *s* (173.46)	174.6 *s*		70.8 *t*	72.2 *t*	72.4 *t*	94.6 *d*	170.8 *s*
9		52.4 *q*					156.9 *s*	52.4 *q*
10							105.3 *s*	
1`							120.5 *s*	
2`							128.4 *d*	
3`							116.0 *d*	
4`							161.0 *s*	
5`							116.0 *d*	
6`							128.4 *d*	

Cpds 2, 5, 6, 8 were measured in CD_3OD.
Cpds 3, 4 were measured in C_5D_5N.
Cpd 7 was measured in DMSO-d_6
The data between parentheses for the isomer of **1**

Table 2: ^{13}C-NMR spectral data of the sugar part of compound **1** and compounds **3-7** (100 MHz).

C	1	3	4	5	6	7
β-D-glucose						
1`	98.2 *d* (94.0 *d*)	102.3 *d*	103.0 *d*	104.2 *d*	104.4 *d*	
2`	75.3 *d* (73.7 *d*)	84.3 *d*	84.0 *d*	76.2 *d*	75.4 *d*	
3`	77.9 *d* (74.7 *d*)	78.0[a] *d*	78.1[a] *d*	81.6 *d*	83.9 *d*	
4`	70.7 *d* (71.7 *d*)	71.4[b] *d*	71.5[b] *d*	70.6[a] *d*	70.0[a] *d*	
5`	76.2 *d* (71.8 *d*)	78.4[a] *d*	78.3[a] *d*	76.0 *d*	75.7 *d*	
6`	65.2 *t* (65.3 *t*)	62.5[c] *t*	62.4[c] *t*	62.4 *t*	64.6 *t*	
1``		106.6 *d*	106.4 *d*			
2``		76.9 *d*	76.7 *d*			
3``		78.0[a] *d*	77.9[a] *d*			
4``		71.2[b] *d*	71.2[b] *d*			
5``		78.6[a] *d*	78.7[a] *d*			
6``		62.5[c] *t*	62.7[c] *t*			
α-L-rhamnose						
1``				103.0 *d*	102.7 *d*	
2``				72.3[b] *d*	72.3[b] *d*	
3``				72.1[b] *d*	72.2[b] *d*	
4``				73.8 *d*	74.0 *d*	
5``				70.4[a] *d*	70.4[a] *d*	
6``				18.4 *q*	17.9 *q*	
Caffeoyl moiety						
1```				127.7 *s*	127.7 *s*	
2```				115.3 *d*	115.1 *d*	
3```				149.8 *s*	149.6 *s*	
4```				146.8 *s*	146.7 *s*	
5```				116.5 *d*	116.4 *d*	
6```				123.2 *d*	123.1 *d*	
7```				148.0 *d*	147.2 *d*	
8```				114.7 *d*	114.8 *d*	
9```				168.3 *s*	169.1 *s*	
β-D-glucuronic acid						
1``						99.5 *d*
2``						72.9 *d*
3``						76.4 *d*
4``						71.9 *d*
5``						73.9 *d*
6``						172.7 *s*

Cpds 1, 5, 6 were measured in CD_3OD.
Cpds 3, 4 were measured in C_5D_5N.
Cpd 7 was measured in DMSO-d_6
a, b, c Chemical shift values may be interchangeable.
Values between parentheses for α-D-glucose

Hz, H-2``), 4.28 (1H, *dd*, *J*= 2.5, 13.9 Hz, $H_a$-6``), 4.10-4.21 (2H, *m*, H_b-6` and H_b-6``), 4.01-4.21 (*m*, remaining glucose protons). ^{13}C-NMR (Tables 1 and 2).

Compound (4): Obtained as colourless feathery crystals (27 mg), m. p. 189-191, with R_f= 0.38, from $CHCl_3$-MeOH-H_2O (75:25:2.5). ^{1}H-NMR (C_5D_5N, 400 MHz): aglycone: δ 7.09-7.32 (5H, *m*, aromatic protons), 3.03 (2H, *m*, H-7), 4.15 (1H, *m*, H-8), 3.75 (1H, *dd*, *J*= 8.8, 15.1 Hz, H-8). β-D-glucose: δ 4.85 (1H, *d*, *J*= 7.8 Hz, H-1`), 4.10 (1H, *m*, H-2`), 4.20 (2H, *m*, H-3`, H-4`), 3.83 (1H, *m*, H-5`), 4.25, 4.48 (each 2H, *m*, H-6`, 6``), 5.30 (1H, *d*, *J*= 7.6 Hz, H-1``), 4.08 (1H, *m*, H-2``), 4.30 (1H, *m*, H-3``), 4.13 (1H, *m*, H-4``), 3.91 (1H, *m*, H-5``). ^{13}C-NMR (Tables 1 and 2).

Compound (5): Isolated as yellowish-brown amorphous powder, (235 mg), with R_f= 0.40, from $CHCl_3$-MeOH-H_2O (75:25:2.5). ^{1}H-NMR (CD_3OD, 400 MHz): aglycone: δ 6.65 (1H, *d*, *J*= 2.0 Hz, H-2), 6.63 (1H, *d*, *J*= 8.1 Hz, H-5), 6.51 (1H, *dd*, *J*= 2.0, 8.1 Hz, H-6), 2.74 (2H, *t*, *J*= 7.1 Hz, H-7), 3.67 and 4.00 (each 1H, *dd*, *J*= 7.1, 17.1, H-8), caffeoyl moiety: δ 7.00 (1H, *d*, *J*= 2.0 Hz, H-2```), 6.73 (1H, *d*, *J*= 8.3 Hz, H-5```), 6.90 (1H, *dd*, *J*= 2.0, 8.3 Hz, H-6```), 7.54 and 6.22 (each 1H, *d*, *J*= 15.9 Hz, H-7```, H-8```) respectively. Sugar moiety: δ 4.33 (1H, *d*, *J*= 7.8 Hz, H-1`), 3.34 (1H, *dd*, *J*= 7.8, 9.2 Hz, H-2`), 3.78 (1H, *t*, *J*= 9.2 Hz H-3`), 4.87 (1H, *t*, *J*= 9.3 Hz, H-4`), 3.45 (1H, *m*, H-5`), 3.47–3.59 (2H, *m*, H-6`), 5.14 (1H, *d*, *J*= 1.7 Hz, H-1``), 3.87 (1H, *dd*, *J*= 1.7, 3.2 Hz, H-2``), 3.55 (1H, *m*, H-3``), 3.25 (1H, *m*, H-4``), 3.50 (1H, *m*, H-5``) and 1.04 (3H, *d*, *J*= 6.3 Hz, H-6``). ^{13}C-NMR (Tables 1 and 2).

Compound (6): Isolated as light brown amorphous powder, (20 mg), with R_f= 0.42, from $CHCl_3$-MeOH-H_2O (75:25:2.5). ^{1}H-NMR (CD_3OD, 400 MHz): aglycone: δ 6.62 (1H, *d*, *J*= 2.0 Hz, H-2), 6.59 (1H, *d*, *J*= 8.1 Hz, H-5), 6.48 (1H, *dd*, *J*= 2.0, 8.1 Hz, H-6), 2.73 (2H, *t*, *J*= 7.1 Hz, H-7), 3.62-3.72, 3.88-4.00 (each 1H, *m*, H-8), caffeoyl moiety: δ 6.98 (1H, *d*, *J*= 2.0 Hz, H-2```), 6.72 (1H, *d*, *J*= 8.3 Hz, H-5```), 6.84 (1H, *dd*, *J*= 2.0, 8.3 Hz, H-6```), 7.51 and 6.24 (each 1H, *d*, *J*= 15.9 Hz, H-7```, H-8```) respectively. Sugar moiety: δ 4.28 (1H, *d*, *J*= 7.8 Hz, H-1`), 3.46-3.54 (2H, *m*, H-2`, 3`), 3.92-4.00 (1H, *m*, H-4`), 3.30 (1H, *m*, H-5`), 4.30 (1H, *dd*, *J*= 6.1, 12.0 Hz, $H_b$-6`), 4.45 (1H, *dd*, *J*= 2.0, 12.0 Hz, H_a-6`), 5.13 (1H, *br. s*, H-1``), 3.62 - 3.72 (2H, *m*, H-2``, 3``), 3.32 - 3.40 (2H, *m*, H-4``, 5``) and 1.20 (3H, *d*, *J*= 6.4 Hz, H-6``). ^{13}C-NMR (Tables 1 and 2).

Compound (7): Obtained as yellow amorphous powder, (193 mg), with R_f= 0.63 from (35% MeOH). ^{1}H-NMR (DMSO-d_6, 400 MHz): δ 6.74 (1H, *br. s*, H-3), 6.39 (1H, *br. s*, H-6), 6.74 (1H, *br. s*, H-8), 7.83 (2H, *d*, *J*= 8.3 Hz, H-2`, 6`), 6.87 (2H, *d*, *J*= 8.3 Hz, H-3`, 5`), 5.07 (1H, *d*, *J*= 6.6 Hz, H-1``) and 3.15-3.68 (*m*, other sugar protons). ^{13}C-NMR (Tables 1 and 2).

Compound (8): Obtained as yellowish-brown oil, (187 mg), with R_f= 0.70, from MeOH:H_2O (30:1). ^{1}H-NMR (CD_3OD, 400 MHz): δ 7.10 (2H, *dd*, *J*= 2.0, 8.3 Hz, H-3, 5), 6.17 (2H, *dd*, *J*= 2.0, 8.3 Hz, H-2, 6), 2.79 (2H, *s*, H-7) and 3.66 (3H, *s*, H-9). ^{13}C-NMR (Table 1).

RESULTS AND DISCUSSION

The leaves of *J. ovalifolia* were extracted with MeOH, and the extract was fractionated with *n*-hexane, diethyl ether, ethyl acetate and *n*-butanol. The *n*-butanol fraction was applied on a column of Diaion-HP 20 and eluted with water, 50% MeOH and 100% MeOH.

From 50% MeOH compounds **1-7** were isolated while from the ethyl acetate fraction compound **8** was isolated.

The ^{13}C-NMR including DEPT mode measurements (Tables 1 and 2) and ^{1}H-NMR spectral data of compound **1** (experimental section), showed signals at δ_C 98.2 with δ_H 4.37 (1H, *d*, *J*= 7.8 Hz) and δ_C 94.0 with δ_H 4.96 (1H, *d*, *J*= 3.7 Hz) corresponding to anomeric carbons and protons of β-glucopyranosyl and α-glucopyranosyl units respectively.[19]

The ^{1}H-NMR spectrum suggested the presence of two monosubstituted benzene rings from the signals at δ_H 7.12 to 7.22 (10 H, *m*). It also showed signals at δ_H 3.565 and 3.562 (each 2H, *s*) for two methylene protons.

The ^{13}C-NMR and DEPT ^{13}C spectra confirmed the presence of the two monosubstituted benzene rings from the signals

at δ_C 135.6 (1C, *s*), 135.5 (1C, *s*), 130.4 (4C, *d*), 129.51, 129.49 (each 2C, *d*) and 128.0 (2C, *d*). Other carbon signals at δ_C 173.51, 173.46 (each 1C, *s*), 41.8 (1C, *t*) and 41.7 (1C, *t*) were deduced for two carbonyl and two methylene carbons of the aglycone.

The attachment of the glucose to the aglycone was deduced to be at C-6` of glucose from the chemical shift values at $\delta_C$ 65.3 and 65.2 corresponding to C-6` of α- and β-glucopyranosyl units.[19-21]

The above mentioned data suggest that compound **1** is a mixture of diastereoisomers of phenyl acetic acid ester of glucose.

Previously, it was reported the presence of acylated flavonoid glycoside with an acyl group of phenyl acetic acid.[22]

To the best of our knowledge, compound **1** was isolated here for the first time from a natural source.

The ^{13}C-NMR spectral data including DEPT mode measurements of compound **2** (Table 1), showed signals at δ_C 157.5 (1C, *s*), 131.5 (2C, *d*), 126.3 (1C, *s*) and 116.3 (2C, *d*) corresponding to para disubstituted benzene ring which was confirmed through ^{1}H-NMR (experimental section), at δ_H 7.05 and 6.71 (each 2H, *J*= 8.5 Hz).[23] Other carbon signals at δ_C 174.6 (1C, *s*), 52.4 (1C, *q*) and 40.9 (1C, *t*) were deduced for carbonyl, methoxy and methylene groups respectively. In the ^{1}H-NMR spectrum, the methylene and methoxy groups were observed as singlet signals at δ_H 3.51 (2H, *s*), and at δ_H 3.64 (3H, *s*).

The above mentioned data indicated that compound **2** is methyl *p*-hydroxy phenyl acetate. This compound was previously isolated from *Lactuca perennis* (Asteraceae) with molecular formula $C_9H_{10}O_3$.[24]

The ^{13}C, DEPT ^{13}C (Tables 1 and 2) and ^{1}H-NMR spectral data (experimental section), of compound **3** including ^{1}H-^{1}H COSY and HSQC showed signals at δ_C 102.3 with δ_H 5.16 (1H, *d*, *J*= 7.8 Hz), and δ_C 106.6 with δ_H 4.81 (1H, *d*, *J*= 7.6 Hz), corresponding to anomeric carbons and protons of two β-glucopyranosyl units. The ^{1}H-NMR spectrum and comparing with related compound[19] suggested the presence of mono-substituted benzene ring from the signals at δ_H 7.47 (2H, *br. d*, *J*= 7.6 Hz, H-2, 6), 7.18 (2H, *dd*, *J*= 7.6, 7.6 Hz, H-3,5) and 7.04 (1H, overlapped with the solvent signal, H-4). Also it showed signals at δ_H 4.60 and 4.90 (each 1H, *d*, *J*= 12.0 Hz) for methylene protons.

The ^{13}C and DEPT ^{13}C-NMR spectra confirmed the presence of the methylene carbon at δ_C 70.8 (2H, *t*), and the monosubstituted benzene rings from the signals at δ_C 138.9 (1C, *s*), 128.6 (2C, *d*), 127.9 (2C, *d*), and 127.6 (1C, *d*). The attachment of the glucose to the aglycone and between the two glucose units were deduced from the chemical shift of the methylene group (C-7) at δ_C 70.8 and from the downfield shift of C-2` of glucose to $\delta_C$ 84.3.[25-27] It was also confirmed from HMBC experiment, where correlations were observed between methylene carbon of the aglycone and the anomeric proton of the glucose and between C-2` of glucose with H-1`` of the terminal glucose.

The above mentioned data indicated that compound **3** is benzyl alcohol 7-O-β-D-glucopyranosyl-(1→2)-β-D-glucopyranoside (zizybeoside I), which has molecular formula $C_{19}H_{28}O_{11}$ which was previously isolated from *Zizyphus jujuba* Mill (var. *inemis* Bunge) (Rhamnaceae)[25] and from *Foeniculum vulgare* Miller (Umbelliferae).[26]

The ^{13}C-NMR spectrum including DEPT mode measurement of compound **4** (Tables 1 and 2), showed eighteen signals equivalent to twenty carbon atoms including aromatic and two sugar moieties. The data showed similarity to that of compound **3** (zizybeoside I),[26] except an additional methylene group at δ_C 36.6 was observed suggesting the presence of phenethyl alcohol moiety instead of benzyl alcohol moiety of zizybeoside I. This suggestion was confirmed through ^{1}H-^{1}H COSY and HSQC where the two methylene groups at δ_C 36.6 and 70.8 were attached to each other. The attachment between the two glucose units and between the glucose and the aglycone were deduced from the chemical shift of C-8 at δ_C 70.8, downfield shift of C-2` to $\delta_C$ 84.0[26] and confirmed through HMBC correlations between C-8 and H-1` and between C-2` and H-1``. So, compound **4** was assigned as phenethyl alcohol 8-O-β-D-glucopyranosyl-(1→2)-β-D-glucopyranoside, which was previously isolated from *Bupleurum falcatum* L. (Umbelliferae), with molecular formula $C_{20}H_{30}O_{11}$.[27]

The NMR data of compound **5** (Tables 1 and 2), suggested the presence of a bioside of an aromatic compound. The ^{13}C-NMR including

DEPT mode measurements and ^{1}H-NMR spectral data of **5** (experimental section), showed signals at δ_C 104.2 with δ_H 4.33 (1H, *d*, *J*= 7.8 Hz) corresponding to β-D-glucopyranosyl anomeric carbon and proton, and at δ_C 103.0, 18.4 with δ_H 5.14 (1H, *d*, *J*= 1.7 Hz) and δ_H 1.04 (3H, *d*, *J*= 6.3 Hz) respectively corresponding to C-1`` and C-6`` of α-L-rhamnopyranosyl unit.[28]

The ^{1}H-NMR spectrum suggested the presence of two trisubstituted benzene rings with ABX system from the signals at δ_H 6.65 (1H, *d*, *J*= 2.0 Hz), 6.63 (1H, *d*, *J*= 8.1 Hz), 6.51 (1H, *dd*, *J*= 2.0, 8.1 Hz) for the first trisubstituted ring and at δ_H 7.00 (1H, *d*, *J*= 2.0 Hz), 6.73 (1H, *d*, *J*= 8.3 Hz), 6.90 (1H, *dd*, *J*= 2.0, 8.3 Hz) for the second trisubstituted ring. It also showed signals at δ_H 2.74 (2H, *t*, *J*= 6.2 Hz) and at δ_H 3.67, 4.00 (each 1H, *m*) for two methylene protons and at δ_H 7.54, 6.22 (each 1H, *d*, *J*= 15.9 Hz) for two *trans* olefinic protons. The first trisubstituted ring with the two methylene groups suggested the presence of 3,4-dihydroxy phenethyl alcohol moiety, while the second trisubstituted ring with the two *trans* olefinic protons suggested the presence of *trans* caffeoyl moiety.

The ^{13}C-NMR and DEPT ^{13}C spectra confirmed the presence of the 3,4-dihydroxy phenethyl alcohol moiety from the signals at δ_C 131.5 (1C, *s*), 116.3 (1C, *d*), 144.7 (1C, *s*), 146.1 (1C, *s*), 117.1 (1C, *d*) and 121.3 (1C, *d*) with the methylene group at δ_C 36.6 and 72.2 (each 1C, *t*), and the caffeoyl moiety from the signals at δ_C 127.7 (1C, *s*), 115.3 (1C, *d*), 149.8 (1C, *s*), 146.8 (1C, *s*), 116.5 (1C, *d*), 123.2 (1C, *d*), 148.0 (1C, *d*) and 114.7 (1C, *d*) with the carbonyl carbon at δ_C 168.3 (1C, *s*).

Comparison of the above mentioned data with those reported, indicated that compound **5** is 3,4-dihydroxy phenylethyl alcohol 8-O-[(4`-O-caffeoyl)-α-rhamnopyranosyl-(1→3)]-β-glucopyranoside (acteoside) with molecular formula $C_{29}H_{36}O_{15}$.[29] It was previously isolated from *Jacaranda ovalifolia*.[30]

The NMR spectral data of compound **6** (Tables 1 and 2), is closely related to that of compound **5** (acteoside) except for the location of the *trans* caffeoyl moiety at C-6` of glucose instead C-4` of acteoside. This is based on the downfield shift of C-6` of glucose at the $^{13}$C-NMR spectrum ($\delta_C$ 64.6) comparing with C-6` of acteoside (δ_C 62.4). So that compound **6** was assigned as 3,4-dihydroxy phenylethyl alcohol 8-O-[(6`-O-caffeoyl)-α- rhamnopyranosyl-(1→3)]-β-glucopyranoside (isoacteoside),[28] which was previously isolated from *Leucosesptrum japonicum* (Miq.) with molecular formula $C_{29}H_{36}O_{15}$.[28]

The ^{13}C-NMR spectrum of compound **7** (Tables 1 and 2), exhibited nineteen signals equivalent to twenty one carbon atoms, six were assigned for one sugar moiety and the remaining fifteen for a flavonoid moiety. The ^{13}C-NMR including DEPT mode measurements and ^{1}H-NMR spectral data of **7** showed signals at δ_C 99.5 with δ_H 5.07 (1H, *d*, *J*= 6.6 Hz), four signals between δ_C 71.9-76.4, and one signal at δ_C 172.7 for carboxylic acid carbon indicateded the presence of β-glucuronic acid unit.

The ^{1}H-NMR spectral data (experimental section), suggested the presence of para disubstiuted benzene ring from the signals at δ_H 7.83 and 6.87 (each 2H, *d*, *J*= 8.3 Hz).[23] This suggestion was confirmed through carbon signals at δ_C 120.5 (1C, *s*), 128.4 (2C, *d*), 116.0 (2C, *d*) and 161.0 (1C, *s*). It also revealed the presence of three aromatic protons at δ_H 6.74, (2H, *br. s*) and 6.39, (1H, *br. s*).

The above mentioned data revealed that the aglycone part of compound **7** is apigenin.

From the above data and from the literatures,[31,32] compound **7** was elucidated as apigenin 7-O-β-D-glucuronopyranoside with molecular formula $C_{21}H_{18}O_{11}$. This compound was previously isolated from many plants as *Acanthus ebracteatus* (Acanthaceae).[32]

The ^{13}C-NMR and DEPT ^{13}C spectral data of compound **8** (Table 2), exhibited seven signals equivalent to nine carbon atoms including three quaternary at δ_C 67.9, 187.3 and 170.8, one methoxy at δ_C 52.4, one methylene δ_C 45.5 and four methine carbon atoms at δ_C 152.5, 128.1 (each 2C). The ^{1}H-NMR spectral data (experimental section), showed signals at δ 7.10 and 6.17 (each 2H, *dd*, *J*= 2.0, 8.3 Hz). Another two signals were observed at δ 2.79 (2H, *s*) and 3.66 (3H, *s*) for methylene and methoxy protons respectively. The above mentioned data indicated the presence of 1,4-benzoquinonoid derivatives where the signal at δ_C 187.3 is characteristic for the carbonyl

carbon C-1, while at δ_C 170.8 corresponding to ester carbonyl carbon, in addition, a signal at δ_C 52.4 with singlet signal in the ^{1}H-NMR for the methyl ester group.

From the above data and from the literatures,[14,33, 34] the compound was elucidated as 4-hydroxy-1-oxo-2,5-cyclohexadien-1-acetic acid methyl ester (Jacaranon). This compound was previously isolated from *Jacaranda caucana* Pittier and has molecular formula $C_9H_{10}O_4$.[34]

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