PHYTOCONSTITUENTS FROM *DELONIX REGIA* RAF ROOTS AND THEIR BIOLOGICAL ACTIVITIES

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ABSTRACT

Phytochemical investigation of the roots of *Delonix regia* yielded six compounds. They were identified as, aliphatic alcohol; heptadecanol [1], two phenolic acids; vanillic acid [2] and *p*-hydroxy benzoic acid [3], a mixture of the ester isomers of the *trans*- and *cis*-ferulate glucopyranoside [4], one flavonol glycoside; Kampferol-3-*O*-*α*-L-arabinopyranoside [5] and one megastigmanes; (6*R*, 9*S*)-3-oxo-*α*-dihydro-ionyl-9-*β*-D-glucopyranoside (Blumenol C-*O*-*β*-D-glucopyranoside) [6]. Identification of these compounds was based on ESI-MS, 1D and 2D-NMR analysis. All six compounds are new to the constituents of genus *Delonix*. The isolated compounds (1-5) were evaluated for their cytotoxic activities against human promyelocytic leukemia HL-60 cells and human monocytic leukemia U937 cells. The antioxidant activities were also evaluated using 1, 1-diphenyl-2-picrylhydrazyl free radical (DPPH), assay method.

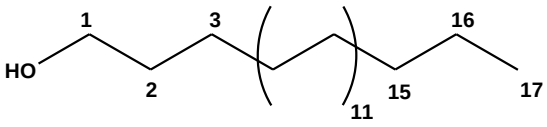
INTRODUCTION

Delonix regia (Boj.) Raf (Family Fabaceae) is an ornamental tree widely grown in tropical and subtropical regions. It is also known as the Royal Poinciana or Flamboyant. It produces clusters of orange-red flowers in April-May usually called "flame tree". Traditional healthy beverages were prepared from various parts of the tree (**Adje, *et al.*, 2008, Ali, *et al.*, 1999**). It is used in several countries to prepare extracts with antimicrobial, antifungal activities (**Ali, *et al.*, 1999, Sammour, El-Shanshoury, 1992**) and can be used as an antibiotic (**Aqil and Ahmad, 2003**). Anti-inflammatory and antioxidant activities of *Delonix regia* (Boj.) Raf flowers have been reported (**Su and Fan, 1997; Setharaman, *et al.*, 1984**). Concerning the chemical studies of this plant, several compounds such as flavonoid glycosides, anthocyanins, coumarins, fatty acids, sterols, triterpenes **(Adje, *et al.*, 2008**; **Abou Zeid, 2002; Hamdoon, 2009; Saleh and Ishak, 1976; Nabieh and Moheb, 1976**), condensed tannins (**Chai, *et al*, 2012; Farrukh, *et al*, 2006**), as well as protein, amino acids (**Evans and Bell, 1978; Sung and Fowden, 1968; Yeoh, *et al*, 1984**) and lectins (**Sammour and El-Shanshoury, 1992**) have been isolated from different parts of the plant. This report describes the isolation and identification of bioactive constituents from the roots of *Delonix regia*. The cytotoxic and the antioxidant activities of the isolated compounds (1-5) were also evaluated.

EXPERIMENTAL SECTION

General experimental procedures. Melting points were obtained on a Stuart SMP3 apparatus. UV spectra were determined with a Hitachi 340 spectrophotometer. IR spectra were carried out on a Nicolet 205 FT IR spectrometer connected to a Hewlett-Packard Color Pro. Plotter. ESIMS were measured on a TSQ Quantum (Thermo Electron Corporation) instrument. The ^{1}H- and ^{13}C NMR measurements were obtained with a Bruker DRX-500 NMR spectrometer operating at 500 MHz (for ^{1}H) and 125 MHz (for ^{13}C) in CD_3OD or $CDCl_3$ solution, and chemical

shifts were expressed in δ (ppm) with reference to TMS, and coupling constant (*J*) in Hertz. ^{13}C multiplicities were determined by the DEPT pulse sequence (135^{o}). COSY, HMBC, and HSQC NMR experiments were carried out using a Bruker DRX-500 high field NMR spectrometers. All 1D and 2D spectra were obtained using the standard Bruker software. Si gel (Si gel 60, Merck) and Sephadex LH-20 (Pharmacia) were used for open column chromatography. TLC was carried out on precoated silica gel 60 F_{254} (Merck) plates. Developed chromatograms were visualized by spraying with 1% vanillin-H_2SO_4, followed by heating at 100 ^{0}C for 5 min.



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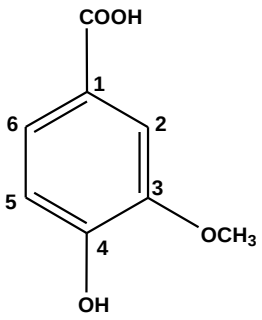

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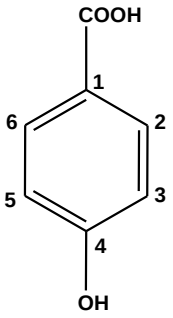

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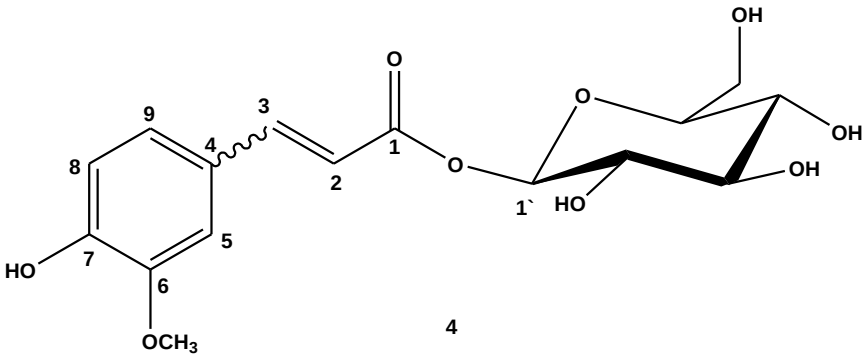

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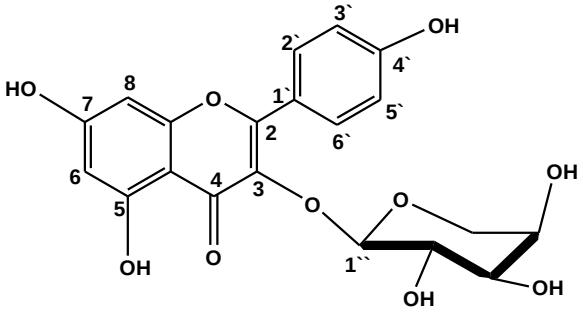

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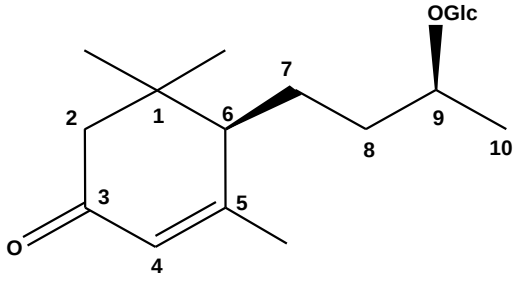

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Plant material

Delonix regia roots were collected from the garden of Al-Azhar University, Nasr city, Cairo, Egypt, in July 2007, and were kindly identified by Engineer Badeia Hassan Aly Dewan, Consultant of Egyptian Flora, Agricultural Museum, Dokki, Giza, Egypt, and by Mrs. Terase Labib, Taxonomist of Orman Garden, Giza, Egypt. A voucher specimen has been deposited in the Pharmacognosy Department, Faculty of Pharmacy, Al-Azhar University, Cairo, Egypt.

Extraction and isolation

The air dried powdered roots of *Delonix regia* (3 kg) were subjected to exhaustive extraction with 70% ethyl alcohol (7L x 3). The combined ethanolic extracts were concentrated under vacuum at 40^{o}C to dryness (370g). The concentrated ethanolic extract was suspended in distilled water (500 ml) and filtered. The water-soluble portion (255 g) was defatted with petroleum ether (166 g). The defatted crude extract was partitioned successively with ethyl acetate (12 g) and *n*-butanol (20.4 g). The EtOAc fraction (12 g) was applied to Si gel column and eluted with CH_2Cl_2-MeOH (100:0→70:30) to give five fractions of A (395 mg), B (60 mg), C (940 mg), D (98 mg) and E (560 mg). All fractions (A-E) were separately subjected to several runs of Si gel column chromatography using CH_2Cl_2-MeOH (100:0→85:15) to give A-1 (200 mg), B-1 (40 mg), C-1 (780 mg), D-1 (65 mg) and E-1 (340 mg). Fraction A-1 was rechromatographed over Si gel column eluted with petroleum ether-ethyl acetate (100:0→80:20) to give three sub-fractions of A-1a (35 mg), A-1b (50 mg) and A-1c (55 mg). Sub-fraction A-1a was repeatedly purified by Sephadex LH-20 column (MeOH) to give compound **1** (25 mg). Fraction C-1 was subjected to Si gel column eluted with CH_2Cl_2-MeOH (100:0→95:5) to give four sub-fractions of C-1a (65 mg), C-1b (70 mg), C-1c (150 mg) and C-1d (200 mg). Sub-fractions C-1a and C-1b were repeatedly purified by Sephadex LH-20 column (MeOH) to give compounds **2** (24 mg) and **3** (20 mg), respectively. Fraction D-1 was applied to Si gel column eluted with CH_2Cl_2-MeOH (100:0→80:20) followed by Sephadex LH-20 column (MeOH) to give compound **4** (45 mg). By the same method using Si gel column eluted with CH_2Cl_2-MeOH (100:0→75:25), fraction E-1 gives five sub-fractions of E-1a (95 mg), E-1b (60 mg), E-1c (40 mg), E-1d (30 mg) and E-1e (35). Sub fractions E-1b and E-1e were repeatedly purified by Sephadex LH-20 column (MeOH) to give compounds **5** (35 mg) and **6** (30 mg), respectively.

Material and methods for determination of cytotoxic and antioxidant activities

Cell line culture assay

Human promyelocytic leukemia HL-60 cells and human monocytic leukemia U937 cells were used in this study as a model to investigate the cytotoxic activities of isolated compounds (1-5) in cell culture using MTT assay **(Mosmann, 1983; Green, 1984**).

Human promyelocytic leukemia HL-60 and human monocytic leukemia U937 cells were kindly provided by Prof. Dr**.** Alexandra K. Kiemer (Dept. of Pharmaceutical Biology, Saarland University, Germany**).** Cells were maintained in RPMI1640 medium supplemented with 10% heat-inactivated fetal calf serum (Grand Island Biological Co., Grand Island, NY, USA), 100 U/mL penicillin G, 100 μg/mL streptomycin, and 1 % l-glutamine (Sebac, Germany) in an atmosphere of 5 % CO_2 in humidified air at 37 °C. In all experiments, exponentially growing cells were used. In order to establish the activity of the test compounds, 2-day experiments were set up. On the first day, *one mL of cell suspension* was added in a concentration of 9×10^5 cell/mL to Eppendorfs (size 1.5 mL or 2 mL, Greiner Bio-One*).* To each cell suspension, *the compounds*

were added at different concentrations (with a DMSO concentration equal to 0.4%). To a 96 well plate (Greiner Bio-One) 100 µl of cell suspension with compounds were added. This micro plate was then incubated for 24 hours in an atmosphere of 5 % CO_2 in humidified air at 37 °C. On the second day, 10 μl of MTT stock solution was added to each well (final conc. 0.5 mg /mL), in the dark and under sterile conditions. The plate was then incubated at 37 °C for 2 hrs in the dark. 200 μl of DMSO was then added in each well to dissolve the formazane liberated. The plate was gently shaken and the color was immediately measured with a microplate reader at 550 nm (reference 690 nm).

Statistical analysis

Numerical results are presented as mean ± SE. Student's *t*-test was used to compare two groups while multiple groups were compared by one-way analysis of variance (ANOVA). $P < 0.05$ was taken as the level of significance.

DPPH radical-scavenging assay

The DPPH free-radical- scavenging assay is based on the abilities of compounds to quench stable DPPH free radicals. Reaction mixtures (200 µL) were prepared by combining multiple concentrations of test sample in methanol (10 µL) with DPPH (Aldrich, Milwaukee, WI) in MeOH (190 µL). The final DPPH concentration was 300 µM. The reaction mixtures were incubated in 96-well microtiter plates at room temperature for 30 min. After the reaction, reduction of the radical was then measured at 517 nm. Percent inhibition by sample treatment was determined by comparison with controls containing no test samples. Vitamin E and Tolox were used as positive controls. IC50 values denote the concentration of sample required to scavenge 50% DPPH free radicals (**Hosny, *et al.*, 2002; Lee, *et al.*, 1998**). All tests were performed in triplicate and the results averaged.

Percentage of DPPH color inhibition = 1-(Absorbance of sample / Absorbance of control) x 100.

Heptadecanol [1]: Colorless oil; IR (KBr) Cm^{-1}: 3350, 1055, 1060, 735, 720; ^{1}H NMR (*$CDCl_3$*, 500 MHz) δ 3.63 (2H, t, *J*=6.3 Hz, H-1), 1.52 (2H, m, H-2) 1.29 (28H, *brs*, H-3-H-16), 0.86 (3H, t, *J*=7.1 Hz, H-17); ^{13}C NMR (*CD_3OD*, 125 MHz) δ 63.11 (CH_2, C-1), 32.82 (CH_2, C-2), 31.92 (CH_2, C-15), 29.69-29.35 (CH_2, C4-C14), 25.73 (CH_2, C-3), 22.68 (CH_2, C-16), 14.10 (CH_3, C-17); ESIMS *m/z* 279 $[M + Na]^+$.

Vanillic acid [2]: An off-white powder [MeOH]; m.p. 208-210°C; UV λ_{max} (MeOH) nm: 242, 289, 320; IR υ_{max} (KBr) Cm^{-1}: 3550 (OH), 3200-2500 (OH), 1675 (CO), 1602, 1510 (aromatic C=C), 1285 (OMe); ^{1}H NMR (*CD_3OD*, 500 MHz) δ 7.55 (1H, d, *J*=8.1 Hz, H-6), 7.53 (1H, s, H-2), 6.80 (1H, d, *J*=8.1 Hz, H-5), 3.88 (3H, s, OCH_3); ^{13}C NMR (*CD_3OD*, 125 MHz) δ 168.50 (C, C=O), 151.00 (C, C-4), 147.20 (C-3), 123.90 (CH, C-6), 123.80 (C, C-1), 114.50 (CH, C-5), 112.50 (CH, C-2), 55.00 (OCH_3); ESIMS *m/z* 169 $[M + H]^+$, 153 $[M-15]^+$, 151 $[M -17]^+$.

***p*-hydroxybenzoic acid [3]:** Pale yellow needles [EtOAc]; m.p. 211-214°C; UV λ_{max} (MeOH) nm: 270; IR υ_{max} (KBr) Cm^{-1}: 3500 (OH), 3200-2500 (OH), 1665 (CO), 1600, 1585, 1515; ^{1}H NMR (*CD_3OD*, 500 MHz) δ 7.85 (2H, *d*, *J*=8.5 Hz, H-2, H-6), 6.78 (2H, *d*, *J*=8.5 Hz, H-3, H-5); ^{13}C NMR (*CD_3OD*, 125 MHz) δ 170.0 (C, C=O), 162.5 (C, C-4), 132.96 (CH, C-2, C-6), 122.10 (C-1), 115.99 (C-3, C-5); ESIMS *m/z* 139 $[M + H]^+$.

Ferulate glucopyranside [4]: White amorphous powder [MeOH]; UV λ_{max} (MeOH) nm: 210, 226, 315. IR υ_{max} (KBr) Cm^{-1}: 3385, 1710, 1675, 1620, 1604, 1590, 1515. ^{1}H and ^{13}C NMR data

(500 MHz for ^{1}H and 125 for ^{13}C, *CD_3OD*), are shown in **Table 1**. ESIMS *m/z* 357 $[M+H]^+$, 713 $[2M+H]^+$, 194 [Ferulic acid]$^+$.

Kampferol-3-*O*-*α*-L-arabinopyranoside [5]: Yellow amorphous powder [MeOH]; m.p. 179-181°C; UV λ_{max} (MeOH) nm: 266, 356, λ_{max} (MeONa) nm: 270, 320, 410, λ_{max} ($AlCl_3$) nm: 275, 305, 400, λ_{max} ($AlCl_3$/HCl) nm: 300, 350, 395, λ_{max} (AcONa) nm: 276, 320, 385, λ_{max} (AcONa/boric acid) nm: 265, 355; IR υ_{max} (KBr) Cm^{-1}: 3450 (OH), 1645 (CO). ^{1}H NMR (*CD_3OD*, 500 MHz) aglycon δ 7.95 (2H, d, *J*=8.9 Hz, H-2`, H-6`), 6.79 (2H, d, *J*=8.6 Hz, H-3`, H-5`), 6.27 (1H, br s, H-8), 6.08 (1H, br s, H-6); sugar moiety δ 5.00 (1H, d, *J*=6.6 Hz, H-1``), 3.79 (1H, dd, *J*=7.8, 6.6 Hz, H-2``), 3.69 (1H, m, H-4``), 3.66 (1H, brd, *J*=10.0 Hz, H-5``b), 3.53 (1H, dd, *J*=8.3, 3.8 Hz, H-3``), 3.30 (1H, brd, *J*=10.0 Hz, H-5``a); ^{13}C NMR (*CD_3OD*, 125 MHz) aglycon δ 179.44 (C, C-4), 167.00 (C, C-7), 162.95 (C, C-5), 161.64 (C, C-4`), 158.72 (C, C-2), 158.55 (C, C-9), 135.54 (C, C-3), 132.24 (CH, C-2`, 6`), 122.65 (C, C-1`), 116.17 (CH, C-3`, 5`), 105.32 (C, C-10), 100.36 (CH, C-6), 95.08 (CH, C-8); sugar moiety δ 104.54 (CH, C-1``), 74.05 (CH, C-3``), 72.78 (CH, C-2``), 68.95 (CH, C-4``), 66.79 (CH_2, C-5``); ESIMS *m/z* 418 $[M]^+$.

3-oxo-*α*-dihydro-ionyl-9-*β*-D-glucopyranoside (Blumenol C-*O*-*β*-D-glucopyranoside) **[6]:** White amorphous powder [MeOH]; UV λ_{max} (MeOH) nm: 235; IR υ_{max} (KBr) Cm^{-1}: 1680 (C=O), 3450 (OH); ^{1}H NMR (500 MHz *CD_3OD*) ionyl moiety δ 5.67 (1H, s, H-4), 4.71 (1H, m, H-9), 2.40 (1H, d, *J*=17.5 Hz, H-2$_{\beta}$), 2.00 (1H, d, *J*=17.5 Hz, H-2$_{\alpha}$), 1.89 (1H, dd, *J*=9.7, 6.3 Hz, H-6), 1.58 (1H, m, H-8), 1.53 (1H, m, H-7), 1.95 (3H, s, H-13), 1.15 (3H, d, *J*=6.2 Hz, H-10), 0.98 (3H, s, H-11), 0.92 (3H, s, H-12)); sugar moiety δ 4.22 (1H, d, *J*=7.8 Hz, H-1`), 3.46-3.81 (4H, m, H-2`-H-5`), 3.75 (1H, dd, *J*=11.9, 2.0 Hz, H-6$_{\alpha}$), 3.56 (1H, dd, *J*=11.8, 5.2 Hz, H-6$_{\beta}$); ^{13}C NMR (125 MHz, *CD_3OD*) ionyl moiety δ 202.47 (C, C-3), 169.32 (C, C-5), 125.55 (CH, C-4), 77.64 (CH, C-9), 52.58 (CH, C-6), 48.16 (CH_2, C-2), 37.48 (CH_2, C-8), 37.40 (C, C-1), 29.06 (CH_3, C-12), 27.47 (CH_3, C-11), 26.69 (CH_2, C-7), 24.19 (CH_3, C-13), 21.93 (CH_3, C-10); sugar moiety δ 103.95 (CH, C-1`), 78.31 (CH, C-5`), 77.48 (CH, C-3`), 75.33 (CH, C-2`), 71.78 (CH, C-4`), 62.89 ($CH_2$, C-6`); ESIMS *m/z* 373 $[M+H]^+$, 210 $[M-162]^+$.

RESULTS AND DISCUSSION

Compound [1] was isolated as colorless oil. Its molecular formula was concluded to be $C_{17}H_{36}O$, from the molecular ion peak at m/z 279 $[M+Na]^+$ in the ESI-MS spectrum together with the data obtained from its ^{13}C NMR. The ^{1}H NMR spectral data of [1] exhibited signals for an aliphatic linear chain, which was characterized by an intense broad signal at δ 1.29 (H-3-H-16) and by a three proton triplet at δ 0.86 (*J*=7.1 Hz, H-17). A two proton resonance at δ 1.52 (m, H-2) correlated in the COSY spectrum with two geminal protons at δ 3.63 (t, *J*=6.3 Hz, H-1). ^{13}C-DEPT NMR showed one methylene bearing oxygen function at δ 63.11 (C-1) and one methyl carbon at δ 14.10 (C-17), which attached to their corresponding protons H-1 and H-17, respectively, in the HSQC spectrum. There was no resonance for a carbonyl in the ^{13}C NMR spectrum and, therefore, the chain was assumed to correspond to a saturated linear alcohol. Therefore 1 was elucidated as heptadecanol.

Compound [**2**] was obtained as an off-white powder. Its IR spectrum showed the presence of phenolic hydroxyl, hydroxyl of carboxylic acid, carbonyl and aromatic ring, whilst the UV spectrum showed absorption maxima at 242, 289, 320 nm. The compound showed molecular weight of 168, whilst its molecular formula was determined to be $C_8H_8O_4$ by ESI-MS and NMR

data. The ^{1}H NMR spectrum of [**2**] was characterized by the presence of signals of three aromatic protons as an ABX-type at δ 7.55 (1H, *d*, *J*= 8.1 Hz, H-6), 7.53 (1H, *s*, H-2) and 6.80 (1H, *d*, *J*= 8.1 Hz, H-5) and one methoxyl protons appeared as a singlet at δ 3.88. The ^{13}C NMR spectrum of [**2**] was in good agreement with values estimated for vanillic acid. It showed eight carbon signals; one carbonyl at δ 168.50 (COOH), two oxygenated aromatic carbons at δ 151.00 (C-4) and 147.20 (C-3), four non oxygenated aromatic carbons at δ 123.90 (C-6), 123.80 (C-1), 114.50 (C-5) and 112.50 (C-2) and one methoxyl at δ 55.00. Therefore, and by direct comparison with corresponding spectroscopic data published previously (**Kuo and Shue, 1991, Scott, 1972; Sakushima *et al.*, 1995**) compound [**2**] was identified as vanillic acid.

Compound [**3**] was obtained as pale yellow needles. Its UV spectrum showed an absorption band at 270 nm. Its molecular formula was concluded to be $C_7H_6O_3$ from the molecular ion peak at m/z 139 $[M+H]^+$ in the ESI-MS spectrum together with the data obtained from its ^{13}C NMR. In the ^{1}H NMR spectrum of [3], the presence of an AA`BB` spin system in the aromatic region at δ 7.85 (d, *J*=8.5) integrating for two protons (H-2 and H-6) and δ 6.78 (d, *J*=8.5) integrating for two protons (H-3 and H-5) indicating the presence of 1, 4-disubstituted benzene ring, together with signals for a carbonyl carbon at δ 170.00, two pairs of equivalent non-oxygenated carbon methins at δ 132.96 (C-2, C-6) and δ 115.99 (C-3, C-5), one oxygenated quaternary carbon at δ 162.50 (C-4) and one non-oxygenated quaternary carbon at δ 122.00 (C-1) in the ^{13}C and ^{13}C-DEPT NMR spectra, confirming [3] to be *p*-hydroxy benzoic acid and in good agreement with the reported literatures (**Kuo and Shue, 1991**). The assignment of all protons and carbons was confirmed by HSQC and HMBC experiments.

Compound [**4**] was obtained as white amorphous powder. The molecular formula was determined as $C_{16}H_{20}O_9$ by mass spectrometry $[M+H]^+$ *m/z* 357 and $[2M+H]^+$ *m/z* 713. Furthermore, the presence of a peak at *m/z* 194, confirming the presence of a 4-hydroxy-3-methoxy cinnamic (ferulic) acid moiety. The UV spectrum of [4] showed absorption bands at 210, 226, 315 nm for a conjugated olefinic bond with aromatic system. The IR spectrum of the compound indicated the presence of alcoholic and ester groups at 3385 and 1710 cm^{-1}, respectively. The presence of an absorption band at 1675 cm^{-1} confirmed the presence of a conjugated ester group, which was supported by those at 1620 cm^{-1} (conjugated double bonds) and a phenyl ring at 1604, 1590 and 1515 cm^{-1}. ^{1}H- and ^{13}C-NMR data prove and fully elucidate the structure of [4] and suggests that [4] is the (*E*) and (*Z*)-ferulate glucopyranoside isomers. The same signals were observed in the ^{1}H- and ^{13}C-NMR spectra, but with slightly different chemical shifts. The signals of each isomer were selected and extracted from the ^{1}H- and ^{13}C-NMR spectra, based on their correlations observed in COSY, HSQC and HMBC spectra. The ^{1}H-NMR spectrum showed that, the aromatic signals was found with a pair of geometric isomers at δ 7.72 and δ 6.40 (each 1H, d, *J*=16.0 Hz, H-8 and H-7, respectively) for *trans* feruloyl moiety and δ 6.92 and δ 5.82 (each 1H, d, *J*=12.0 Hz, H-8 and H-7, respectively) for *cis* feruloyl moiety. The large vicinal coupling constant of 16.0 Hz between H-7 (*E*) and H-8 (*E*) confirm the *trans* geometry, where the coupling constant of 12.0 Hz between H-7 (*Z*) and H-8 (*Z*) confirm the *cis* geometry (**Bergman, *et al.*, 2001**). It can also be observed that two different ABX spin systems were observed for the feruloyl moiety, corresponding to the two different isomers as follow: δ 7.85 and δ 7.20 (each 1H, d, *J*=2.2 Hz) for H-2 (*Z*) and H-2 (*E*), respectively, δ 7.16 and δ 7.09 (each 1H, dd, *J*=8.4, 2.2 Hz) for H-6 (*Z*) and H-6 (*E*), respectively and δ 6.82 and δ 6.76 (each 1H, d, *J*=8.4 Hz) for H-5 (*E*) and H-5 (*Z*), respectively. Two sharp signals at δ 3.89 and δ 3.87 were also observed in the ^{1}H-NMR spectrum for the two methoxyl groups of the *E*- and *Z*-isomers, respectively. In the HSQC spectrum, a correlation was shown between the proton signal at *δ* 7.72 (H-8) and the

carbon signal at δ 148.26 (C-8) for the *E* isomer, while the next correlation can be observed for the proton signal at δ 6.92 (H-8) and the carbon signal at δ 147.47 for the *Z* isomer (C-8). A correlation for the two isomers in neighboring position of the double bond was also observed as follows: the correlation between the proton signals at δ 6.40 (H-7) and the carbon signal at δ 114.81 for the *E* isomer (C-7) and the correlation between the proton signals at δ 5.82 (H-7), and the carbon signal at δ 115.46 for the *Z* isomer (C-7). The coupling constants of the anomeric proton signals at δ 5.58 and 5.55 were 8.0 Hz (*E* isomer) and 7.8 (*Z* isomer), respectively, thus suggesting that one glucose unit was of the β-D-glucopyranoside type (**Baderschneider and Winterhalter, 2001**). COSY and HSQC experiments allowed the full identification of the spin system of each of β-D-glucose starting from the doublets at δ 5.58 and 5.55. In the HSQC spectra, correlations appeared between the anomeric proton signal at δ 5.58 (assigned to the 1`-position of glucose) and the carbon signal at δ 95.84 for the *E* isomer, and the anomeric proton signal at δ 5.55 (assigned to the 1`-position of glucose) also correlates to the carbon signal at δ 95.66 for the *Z* isomer. Furthermore, HMBC correlations were observed between the anomeric proton signal at δ 5.58 (H-1`) and the carbon signal at δ 167.75 (C-9) for the *E* isomer, and the anomeric proton signal at δ 5.55 (H-1`) and the carbon signal at δ 166.60 (C-9) for the *Z* isomer which confirmed the ester linkages. On the basis of signal intensities of ^{1}H NMR of both isomers the relative ratio of *E*- and Z- isomers was found to be 3:1. Based on the above evidences, compound [4] was found as a mixture of two isomers; *trans*- and *cis*-ferulate glucopyranoside (**Kim, *et al*., 2011**).

Table 1: NMR Data of compound [4] (500 MHz for ^{1}H and 125 MHz for ^{13}C-NMR, *CD_3OD*)

Position	^{1}H (*J* in Hz)		^{13}C		DEPT
	Trans (*E*)	*Cis* (*Z*)	*Trans* (*E*)	*Cis* (*Z*)	
1	-	-	127.62	127.88	C
2	7.20, d, 2.2	7.85, d, 2.2	111.91	115.23	CH
3	-	-	149.46	148.30	C
4	-	-	151.03	149.98	C
5	6.82, d, 8.4	6.76, d, 8.4	116.56	115.67	CH
6	7.09, dd, 8.4, 2.2	7.16, dd, 8.4, 2.2	124.35	127.41	CH
7	6.40, d, 16.0	5.82, d, 12.0	114.81	115.46	CH
8	7.72, d, 16.0	6.92, d, 12.0	148.26	147.47	CH
9	-	-	167.75	166.60	C
1`	5.58, d, 8.0	5.55, d, 7.8	95.84	95.66	CH
2`	3.40	3.40	74.07	74.01	CH
3`	3.45	3.45	78.05	78.31	CH
4`	3.38	3.38	71.15	71.15	CH
5`	3.42	3.42	78.86	78.49	CH
6`a 6`b	3.68 3.84	3.68 3.84	62.37	62.37	CH_2
OCH_3	3.89, s	3.87, s	56.48	56.48	CH_3

Compound [**5**] was obtained as yellow amorphous powder. The UV spectrum of [5] exhibited band I and band II absorbance maxima at 356 and 266 nm, respectively, which are

characteristic for flavones and flavonols (**Markham, 1978**). The UV-shift reagents indicate free 5-, 7- and 4`-hydroxyl groups and the absence of *O*-dihydroxy groups (**Markham, 1978**). The molecular formula of [5] was determined as $C_{20}H_{18}O_{10}$ from the molecular ion peak in the ESI-MS at *m/z* 418 $[M]^+$. In the ^{1}H NMR spectrum of [5], two broad singlets at δ 6.08 and 6.27, were assigned to H-6 and H-8 of the A ring respectively. The ^{1}H NMR spectrum also exhibited a typical AA`BB` system at δ 7.95 ppm (2H, d, *J*=8.9 Hz, H-2`, H-6`) and δ 6.79 (2H, d, *J*=8.6 Hz, H-3`, H-5`) corresponding to four aromatic protons of B ring. These data indicated that the aglycon of [3] was a 3, 5, 7,4` tetra-oxygenated flavonol derivative which is in good agreement with kampferol. The ^{1}H NMR spectrum of [5] exhibited one anomeric proton signal at δ 5.00 (d, *J*=6.6 Hz, H-1``) of arabinose. The ^{13}C-NMR spectrum of compound [5] supported these observations having the twenty carbon signals of which 5 were arising from sugar moiety. The remaining resonances were indicative for kampferol as aglycon (**Markham, *et al,* 1978**; **Wagner, *et al,* 1976**). The resonances for C-2`/C-6` and C-3`/C-5` were observed by the same chemical shifts at δ 132.24 and 116.17, respectively, confirming the *p*-substitution of ring B. It also revealed that sugar moiety consisted of one molecule of arabinose, based on the existence of one anomeric carbon signal at δ 104.54 ppm (CH, C-1``) and the signal at δ 66.79 ppm ($CH_2$, C-6``). The arabinose was determined to be in the pyranose form from its ^{13}C NMR data. Furthermore the anomeric configuration for the sugar moiety was fully defined from its chemical shift and $^3J_{H1,H2}$ coupling constant. Accordingly the arabinose was established to be in the *α*-configuration (**Gorin and Mazurek, 1975**). The glycosylation at C-3 of the aglycon was revealed from the upfeild shift of C-3 and the downfield shift of C-2 compared to those of C-3 and C-2 of kampferol (**Markham, *et al.,* 1978**; **Wagner, *et al,* 1976**). On the base of the above mentioned data compound [5] was distinguished as kampferol-3-*O*-*α*-L-arabinopyranoside and in good agreement with the reported literatures (**Salatino, 1999**).

Compound [**6**] was isolated as a white amorphous powder, and the molecular formula was determined as $C_{19}H_{32}O_7$ by ESI-MS and ^{13}C NMR. The ^{13}C NMR (Table 2) showed 19 carbon signals, among them 6 carbon signals were assigned to one *β*-D-glucose and the remaining 13 to the *α*-ionyl moiety. The ^{13}C-DEPT NMR indicate the presence of 3 quaternary, 8 methin, 4 methylene and 4 methyl carbons. The signal at δ 202.47 and the two signals at δ 169.32 and 125.55 suggesting the presence of one carbonyl (C-3) and one double bond, respectively. The ^{1}H NMR spectrum of 6 showed signals for three singlet tertiary methyl groups at δ 1.95 (C-13), 0.98 (C-11) and 0.92 (C-12), and one signal for a secondary methyl at δ 1.15 (d, *J*=6.2, C-10). The sugar was identified as a *β*-D-glucopyranose on the basis of ^{13}C NMR chemical shifts (δ 103.95, 78.31, 77.48, 75.33, 71.78 and 62.89), ^{1}H NMR chemical shift of the anomeric proton (δ 4.22) and of the $J_{1`,2`}$ coupling constant of 7.8 Hz. This information suggested that, compound 6 was the glucoside of C-13 megastigmane (*α*-dihydro-ionyl type) derivative (**Cui, *et al.,* 1993**). The absolute configuration at C-6 and C-9 was assigned to be *R* and *S*, respectively, based on their chemical shifts (**Pabst, *et al.,* 1992**). Consequently, compound 6 was elucidated as (6*R*, 9*S*)-3-oxo-*α*-dihydro-ionyl-9-*β*-D- glucopyranoside (Blumenol C-*O*-*β*-D-glucopyranoside) and in good agreement with the reported literatures (**Cui, *et al.,* 1993**).

Cytotoxic activity

Results displayed in Table 2 summarize the cytotoxic activities of the isolated compounds (1-5) using cultured human promyelocytic leukemia HL-60 cells and human monocytic leukemia U937 cells. Compounds were tested at six different concentrations ranging from 10 μM to 200 μM. A concentration of 200 μM was found to be most suitable to differentiate between activities

of compounds. Viability of the cells was measured in the MTT assay. The cytotoxicity of compounds was calculated as the percentage of cell viability readjusted according to the negative control (viability of cells in the negative control was set as 100 %). Among the compounds used, compounds 1 (long chain fatty alcohol), 2 and 3 (benzoic acid derivatives), exhibited the strongest cytotoxic activities against the HL-60 cells with a survival of 22, 18 and 13 %, respectively. The same compounds showed weak cytotoxic activities against the U937 cells with a survival of 51.07, 36.03 and 46.99 % respectively. In contrast, compounds 4 and 5 showed weak cytotoxic activities (survival 27 and 35 % respectively) against the HL-60 cells. Compound 4 was the most effective among the compounds as a cytotoxic agent against the U937 cells with a survival of 19.15 %, whilst compound [5] was the weakest.

Table 2: Results of cytotoxic and antioxidant activities of compounds (1-5) from *Delonix regia*.

Compound	Viability[1] of U937 cells after treatment with 400 μg/ ml of compounds	Viability[1] of HL-60 cells after treatment with 400 μg/ ml of compounds	DPPH scavenging activity IC_{50} in μg/ ml
1	22.15 ± 6.31	51.07 ± 3.29	*m*[2]
2	18.53 ± 1.11	36.03 ± 2.86	37.62± 7.14
3	13.01± 2.44	46.99 ± 6.07	76.01± 8.42
4	27.85 ± 4.93	19.15 ± 4.98	44.07 ± 3.00
5	35.12 ± 8.14	53.06 ± 2.56	37.17 ± 7.64
α-tocopherol	----	-----	72.66 ± 2.18
Trolox®	-----	-----	92.99 ± 3. 26

[1] Viability of untreated cells (Cell control) is set as 100 %.
[2] *m* = IC_{50} larger than 200 μg/ ml.

The data is presented as a mean of at least two independent measurements ± standard deviation.

Antioxidant activity

Table 2 shows the antioxidant activities of the isolated compounds (1-5) determined by DPPH radical-scavenging activity method, compared with those of the well-known antioxidants trolox® (6-hydroxy-2, 5, 7, 8-tetramethylchroman-2-carboxylic acid) and α-tocopherol at the same concentration and under the same condition. Among the compounds tested, the most active ones were [5], with an IC_{50} value of 37.17 ± 7.64 μg/ml, and [2] with an IC_{50} value of 37.62 ± 7.14 μg/ml, followed by [4] (IC_{50} = 44.07 ± 3.00 μg/ml). These compounds showed a higher DPPH scavenging activity superior to that of trolox® and α-tocopherol. The weakest antioxidant activity was exhibited by compound [3] (IC_{50} = 76.01± 8.42 μg/ml).

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مركبات فيتوكيميائيه من جذور نبات ديلونكس ريجيا و تأثيرها البيولوجى

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نتج عن الفحص الفيتوكيميائى لجذور نبات ديلونكس ريجيا ستة مركبات وتم تعريف هذه المركبات كالأتى: مركب كحول دهنى، هبتاديكانول (١) و مركبين من الأحماض الفينولية، حمض الفانيلليك (2) و حمض البارا هيدروكسى بنزويك (3) وخليط من استرات سيس و ترانس فريولات الجلوكوزايد (4) و مركب جلوكوزايد الفلافونولات، كامفيرول-٣-ارابينوزايد (5) ومركب ميجاستجمان، ٣-أكسو-داى هيدرو-الفا ايونول- ٩-جلوكوزايد (٦). وقد تم التعرف على هذه المركبات باستخدام طيف الكتلة والرنين النووي المغناطيسي لذرات الهيدروجين والكربون-١٣ أحادى وثنائي البعد. ومن الجدير بالذكر ان هذه المركبات يتم فصلها لاول مره من جنس نبات ديلونكس. كما تم دراسة التأثير المثبط لنمو الخلايا السرطانية الخاصة بسرطان الدم للمركبات المفصوله (١-٥) وقد وجد أن المركبات (١-٤) لهم تأثير قوى كمثبط لنمو الخلايا السرطانية الخاصة بسرطان الدم. كما تم ايضا دراسة الفاعلية المضاد للأكسدة لهذه المركبات (١-٥) باستخدام الراديكل الحر (دى بى اتش) ووجد أن للمركبات (٢، ٤، ٥) فاعلية مضادة للأكسدة. وهذه الدراسة تؤكد أن نبات ديلونكس ريجيا له أهمية كبيرة لاحتوائه على مواد طبيعية لها تأثير مثبط لنمو الخلايا السرطانية وتأثير مضاد للأكسدة.