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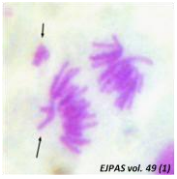

Flavonoids from *Albizia procera*

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The flavonoids luteolin, quercetin-3-O-α-L-rhamnopyranoside, quercetin-3-O-β-D-galactopyranoside, quercetin-3-O-β-D-glucopyranoside and quercetin-3-O-(2''-O-β-D-xylopyranosyl)-β-D-galactopyranoside, were isolated from the methanolic extract of the leaves of *Albizia procera* using chromatographic methods. Identification of the flavonoid constituents was carried by analyzing their spectroscopic data and/or by comparing these data with the reported ones in the literature.

Introduction

The genus *Albizia* (Leguminosae) comprises about 150 species widely distributed in Africa and Central and South America. In Africa, several *Albizia* species are used in folk medicine for the treatment of rheumatism, stomach trouble, cough, diarrhea, wounds and as anthelmintic[1].

In traditional Chinese medicine, *Albizia* members are used for the treatment of insominia, irritability, wounds and as antidysentric, antiseptic and antitubercular[2]. Phytochemical studies carried out on plants of genus *Albizia*, have revealed them as sources of different groups of natural products, triterpenoid saponins[3-11], diterpenoids[12], flavonoids[13-22], lignans[21,23], phenolic glycosides[24,25] and pyridineglycoside[26].

As a part of our continuous investigation on secondary metabolites from *Albizia* species[9,11], we describe in this report the isolation of five flavonoids from the methanolic extract of *A. procera*.

Materials and Methods

General experimental procedure

Column chromatography was run using silica gel 60 (Merck) and Sephadex LH-20 (Sigma). TLC was carried out using silica gel (Merck) as stationary phase. Chromatograms were first visualized by observation under UV and then spraying with $FeCl_3$ spray reagent. NMR spectra were recorded on a JEOL EX 500 MHz and a Varian GEMINT 200 MHz spectrometers. Chemical shifts were given on δ-scale with TMS as internal standard.

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Plant material

The leaves of *A. procera* were collected from the zoological garden in Giza, Egypt. Plant identification was confirmed by Mrs. T. Labib, head specialist for plant identification in El-Orman public garden, Giza, Egypt.

Extraction and Isolation

Air-dried and powdered leaves (750 g) of *A. procera* were extracted with chloroform (2L x 2) and then with MeOH (2L x 2) at room temperature. The total alcoholic extract was evaporated to dryness under reduced pressure. The residue (30 g) was suspended in distilled water (500 ml) and successively partitioned with CH_2Cl_2 (500 ml x 2), EtOAc (700 ml x 3) and BuOH (700 ml x 3). The BuOH fraction (10 g) was subjected to silica gel column chromatography. The column was eluted first with CH_2Cl_2 and then with CH_2Cl_2-MeOH mixture with increasing amount of MeOH to 20%. A total 40 fractions 100 ml each were collected. The fractions were monitored by silica gel TLC plates using solvent system $CHCl_3$-MeOH-H_2O (60: 30: 5) and examined under UV light followed by spraying with $FeCl_3$ reagent. The fractions which eluted with 10% MeOH were combined after TLC analysis contained compound **1** as a major component. The combined fraction was repeatedly chromatographed on Sephadex LH-20 column using methanol as eluent to yield pure **1** (40 mg). The fractions which eluted from silica gel column with 12-14% MeOH were combined contained compound **2** as a major component. The combined fraction was repeatedly chromatographed on Sephadex LH-20 column using methanol as eluent to yield pure **2**

(50 mg). The fractions which eluted with 15% MeOH were combined. The combined fraction was subjected to preparative paper chromatography eluted with solvent system n-Butanol - Acetic acid – Water (4: 1: 5, upper layer) followed by repeated purification on Sephadex LH-20 column eluted with MeOH to yield inseparable mixture of **3** and **4** (30 mg). The fractions which eluted from the silica gel column with 17% methanol were combined after TLC analysis and found to contain compound **5** as a major constituent. The combined fraction was chromatographed on Sephadex LH-20 column chromatography using methanol as eluent to yield pure **5** (25 mg).

Luteolin (1)

Amorphous yellow powder. ^{1}HNMR (500 MHz, acetone-d_6) δ: 12.96 (1H, s, OH-5), 7.46 (1H, d, *J*=2.3 Hz, H-2'), 7.44 (1H, dd, *J*=8.4, 2.3 Hz, H-6'), 6.96 (1H, d, *J*= 8.4 Hz, H-5'), 6.55 (1H, s, H-3), 6.49 (1H, d, *J*= 2.3Hz, H-8), 6.21 (1H, d, *J*= 2.3 Hz, H-6), (OH-7 & OH-4' were not detected).

Quercetin-3-O-α-L-rhamnopyranoside (2)

Amorphous yellow powder. ^{1}HNMR (500 MHz, acetone-d_6) δ: 12.68 (1H, s, OH-5), 7.46 (1H, d, *J*=2.3 Hz, H-2'), 7.34 (1H, dd, *J*=8.4, 2.3Hz, H-6'), 6.95 (1H, d, *J*=8.4 Hz, H-5'), 6.43 (1H, d, *J*=2.3 Hz, H-8), 6.22 (1H,d, *J*= 2.3 Hz, H-6), 5.45 (1H, d, *J*= 1.5 Hz, Rha H-1"), 3.30-3.32 (Rha H-2" – H-5"), 0.88 (3H, d, *J*= 5.6 Hz, Rha Me-6"), (OH-7 & OH-4' were not detected). ^{13}CNMR (500 MHz, acetone-d_6) δ: 178.5 (C-4), 164.5 (C-7), 162.3 (C-5), 157.6 (C-2), 157.2 (C-9), 148.5 (C-4'), 145.1 (C-3'), 135.0 (C-3), 121.9 (C-1'), 121.6 (C-6'), 115.9 (C-2'), 115.3 (C-5') 105.0 (C-10), 102.0 (C-1"), 98.7 (C-6), 93.7 (C-8), 71.3 (C-4"), 70.6 (C-2"), 70.5 (C-3"), 70.5 (C-5"), 16.9 (C-6").

Quercetin-3-O-β-D-galactopyranoside (3)

Amorphous yellow powder. ^{1}HNMR (500 MHz, acetone-d_6+D_2O) δ: 7.93 (1H, d, J=2.3 Hz, H-2'), 7.52 (1H, dd, *J*=8.6, 2.3Hz, H-6'), 6.88 (1H, d, *J*=8.6 Hz, H-5'), 6.45 (1H, d, *J*=2.5 Hz, H-8), 6.20 (1H, d, *J*= 2.3 Hz, H-6), 5.12 (1H, d, *J*=7.7 Hz, Gal H-1"), 3.89 (1H, d, *J*=3.1 Hz, Gal H-5"), 3.82 (1H, t, *J*=7.7 Hz, Gal H-6"a), (OH-7, OH-4' & OH-5 were not detected). ^{13}CNMR (500 MHz, acetone-d_6+D_2O) δ: 178.1 (C-4), 164.8 (C-7), 161.4 (C-5), 157.3 (C-2), 157.0 (C-9), 148.8 (C-4'), 144.6 (C-3'), 134.5 (C-3), 121.6 (C-1'), 121.4 (C-6'), 116.9 (C-5'), 115.2 (C-2') 104.2 (C-10), 104.0 (C-1"), 99.0 (C-6), 94.0 (C-8), 75.6 (C-5"), 73.6 (C-3"), 71.8 (C-2"), 68.1 (C-4"), 60.2 (C-6").

Quercetin-3-O-β-D-glucopyranoside (4)

Amorphous yellow powder. ^{1}HNMR (500 MHz, acetone-d_6+D_2O) δ: 7.79 (1H, d, J=2.3 Hz, H-2'), 7.52 (1H, dd, *J*=8.6, 2.3 Hz, H-6'), 6.89 (1H, d, *J*=8.6 Hz, H-5'), 6.44 (1H, d, *J*=2.5 Hz, H-8), 6.20 (1H, d, *J*= 2.5 Hz, H-6), 5.21 (1H, d, *J*=7.6 Hz, Glc H-1"), 3.23 (1H, m, Glc H-5"), (OH-7, OH-4' & OH-5 were not detected). ^{13}CNMR (500 MHz, acetone-d_6+D_2O) δ: 178.1 (C-4), 164.9 (C-7), 161.4 (C-5), 157.3 (C-2), 157.0 (C-9), 148.8 (C-4'), 144.6 (C-3'), 134.5 (C-3), 121.6 (C-1'), 121.4 (C-6'), 116.9 (C-5'), 115.2 (C-2') 104.2 (C-10), 103.0 (C-1"), 99.0 (C-6), 94.0 (C-8), 76.8 (C-5"), 76.6 (C-3"), 74.3 (C-2"), 69.5 (C-4"), 61.0 (C-6").

Quercetin-3-O-(2"-O-β-D-xylopyranoxyl)-β-D-galactopyranoside (5)

Amorphous yellow powder. ^{1}HNMR (500 MHz, DMSO-d_6) δ: 12.67 (1H, s, OH-5), 7.68 (1H, d, *J*=8.4 Hz, H-6'), 7.47 (1H, bs, H-2'), 6.78 (1H, d, *J*=8.4 Hz, H-5'), 6.35 (1H, bs, H-8), 6.14 (1H, bs, H-6), 5.66 (1H, d, *J*=7.7 Hz, Gal H-1"), 4.53 (1H, d, *J*=6.9 Hz, Xyl H-1'"), (OH-7 & OH-4' were not detected). ^{13}CNMR (500 MHz, acetone-d_6+D_2O) δ: 178.3 (C-4), 164.4 (C-7), 161.5 (C-5), 156.9 (C-2, 9), 148.6 (C-4'), 144.7 (C-3'), 133.8 (C-3), 122.0 (C-1'), 122.6 (C-6'), 116.4 (C-5'), 115.4 (C-2') 104.4 (C-10), 104.2 (C-1'"), 100.0 (C-1"), 99.6 (C-6), 93.9 (C-8), 79.4 (C-2"), 75.4 (C-2"', 3"'), 73.7 (C-5"), 73.6 (C-3"), 69.5 (C-4"), 68.2 (C-4"'), 65.1 (C-5"'), 60.0 (C-6").

General method for acid hydrolysis:

Each glycoside (2 mg) in 3 ml 2N HCl and 3 ml methanol was heated at 100°C for 2h. The mixture was left to cool, diluted with H_2O and extracted twice with ethyl acetate. From the ethyl acetate layer, the aglycone was detected against reference sample by TLC (chloroform-methanol, 5: 1). The aqueous layer was repeatedly diluted with methanol and evaporated to dryness. The residue was investigated to detect the sugar by paper chromatography (PC) using solvent system n-BuOH-HOAc-H_2O (4: 1: 5, upper layer).

Results and Discussion

The methanolic leaf extract of *A. procera* was dissolved in water and the aqueous solution was successively extracted with chloroform, ethyl acetate then n-butanol. The material from the butanol extract was chromatographed on silica gel column chromatography. The collected fractions were examined by TLC and similar fractions were combined. The semi-purified compounds from the combined fractions were then purified using Sephadex-LH20 column chromatography and preparative paper chromatography to afford the flavonoids luteolin (**1**), quercetin 3-O-α-L-rhamnopyranoside (**2**) quercetin 3-O-β-D-galactopyranoside (**3**) quercetin 3-O-β-D-glucopyranoside (**4**) and quercetin 3-O-(2"-O-β-D-xyloxyl)-β-D-galactopyranoside **(5)**. The structures of the isolates were determined by acid hydrolysis, ^{1}H and ^{13}CNMR spectroscopic analysis and/or comparison with published data.

The flavonoid luteolin **1** was characterized by comparison of their spectral data with literature values[27].

In the ^{1}HNMR spectrum of Quercetin 3-O-α-L-rhamnopyranoside **2**, the presence of signals due to three *meta* – coupled protons at δ 6.22 (1H, d, *J*=2.3 Hz, H-6), δ 6.43

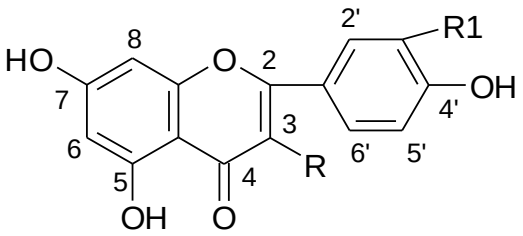
(1H, d, *J*= 2.3 Hz, H-8) and δ 7.46 (1H, d, *J*= 2.3 Hz, H-2') as well as two *ortho*-coupled protons at δ 6.95 (1H, d, *J*= 8.4 Hz, H-5') and δ 7.34 (1H, dd, *J*= 8.4, 2.3 Hz, H-6') along with carbon signal at δ 178.5 (C-4) in the ^{13}CNMR spectrum, indicated a typical 3', 4', 5, 7-tetrahydroxyflavonol skeleton. The two doublets in the ^{1}HNMR spectrum of **2** at δ 5.45 (1H, *J*= 1.5 Hz) and δ 0.88 (3H, *J*= 5.6 Hz) were assigned to H-1'' and H-6'' of a rhamnose unit[27]. The ^{13}CNMR data of **2** and the result of acid hydrolysis indicated α-L-rhamnopyranoside. The attachment of the moiety at C-3 position of the aglycone was revealed from the δ value of C-3 (135.0 ppm) and that of C-2 (157.6 ppm).

Compound **2** was then assigned the structure of quercetin 3-O-α-L-rhamnopyranoside based on the above spectral evidences and comparison with literature values[28,29].

Quercetin-3-O-β-D-galactopyranoside **3** and Quercetin-3-O-β-D-glucopyranoside **4** were obtained as inseparable mixture with compound **3** as the major component. The ^{1}HNMR spectrum of **3** and **4** showed two sets of signals due to quercetin moiety. One set of signals was at δ 7.93 (1H, d, J=2.3 Hz, H-2'), 7.52 (1H, dd, *J*=8.6, 2.3Hz, H-6'), 6.88 (1H, d, *J*=8.6 Hz, H-5'), 6.45 (1H, d, *J*=2.5 Hz, H-8), 6.20 (1H, d, *J*= 2.3 Hz, H-6). The other set was at δ 7.79 (1H, d, J=2.3 Hz, H-2'), 7.52 (1H, dd, *J*=8.6, 2.3 Hz, H-6'), 6.89 (1H, d, *J*=8.6 Hz, H-5'), 6.44 (1H, d, *J*=2.5 Hz, H-8), 6.20 (1H, d, *J*= 2.5 Hz, H-6). The spectrum also exhibited two proton signals at δ 5.12 (1H, d, *J*=7.7 Hz) and δ 5.21 (1H, d, *J*=7.6 Hz) assigned to anomeric proton of β-galactose and that of β-glucose, respectively. The ^{13}CNMR spectum of **3** and **4** showed two sets of signals due to the querctin moiety and two sets of signals attributable to a galactose and glucose moieties, respectively (experimental section). The signals of galactose moiety were at δ 104.0 (C-1''), 75.6 (C-5''), 73.6 (C-3''), 71.8 (C-2''), 68.1 (C-4'') and 60.2 (C-6'') while the signals due to the glucose moiety were at δ103.0 (C-1''), 76.8 (C-5''), 76.6 (C-3''), 74.3 (C-2''), 69.5 (C-4''), 61.0 (C-6''). Acid hydrolysis of **3** and **4** afforded quercetin identified by TLC comparison against authentic sample as well as the sugar component D-galactose and D- glucose recognized by paper chromatography against standard samples. The β-configuration of the anomeric centers was determined as β from the $J_{1,2}$ values and the ^{13}CNMR data indicated pyranose form for the two sugar units. The site attachment of the sugar moiety to C-3 position of quercetin, was established from the δ value of C-3 resonances at δ 134.5 and C-2 resonances at δ 157.3 for **3** and **4**. These values agreed well with the literature values[28,30] and the corresponding ones in the ^{13}CNMR data of compound **2** as well as other quercetin-3-O-glycosides[28,29].

Quercetin-3-O-(2''-O-β-D-xylopyranosyl)-β-D-galacto-pyranoside **5** is another quercetin 3-O-glycoside from the characteristic ^{1}H and ^{13}CNMR resonances as well as acid hydrolysis which afforded quercetin moiety and the sugar components D-galactose and D-xylose. The ^{13}CNMR spectrum of **5** showed in addition to the resonances of the quercetin moiety, eleven carbon resonances assigned to a 2-O-substituted β-galactopyranose (C-2", 79.2 ppm; C-1", 100.0 ppm) and a terminal β-D-xylopyranose units. The β-configurations of the anomeric protons of D-galactose and D-xylose were deduced from the $J_{1,2}$ values for both sugar units. The pyranose form for the sugar units was determined from their ^{13}CNMR data. The attachment of the terminal β-D-xylopyranosyl unit to the inner β-D-xylopyranosyl unit was established from the observed downfield position of C-2" and upfield position of C-1" of the inner sugar with reference to the corresponding values for compound **3** (δ C-2", 71.8; δ C-1", 104.0). The full structure of **5** was confirmed by comparing its spectral data with those reported in the literature[31].

Luteolin[20], quercetin 3-O-α-L-rhamnopyranoside[17,22] and quercetin-3-O-β-D-galactopyranoside[22] were previously reported from *A. julibrissin*. This is the first reported occurrence of quercetin-3-O-β-D-glucopyranoside and quercetin-3-O-(2''-O-β-D-xylopyranosyl)-β-D-galactopyranoside in genus *albizia*.



	R	R1
1	H	OH
2	O-α-L-Rhamnopyranosyl	OH
3	O-β-D-Galactopyranosyl	OH
4	O-β-D-Glucopyranosyl	OH
5	O-β-D-xylopyranosyl-(1→2)-β-D-galactopyranosyl	OH

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