

FLAVONOIDS FROM *Albizia chinensis* OF EGYPT

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ABSTRACT

The flavonoids kaempferol-3-O- α -L-rhamnopyranoside, quercetin-3-O- α -L-rhamnopyranoside together with luteolin, kaempferol and quercetin, were isolated from the methanolic extract of the leaves of *Albizia chinensis* collected from Egypt. Identification of the flavonoid constituents was carried by analysing their spectroscopic data and/or by comparing these data with those reported in the literature. The first three isolates were tested for their antimicrobial activity and the results revealed that the tested compounds exhibited moderate inhibiting activity against gram-positive and gram-negative bacteria while no antifungal activity was observed.

Key words: *Albizia chinensis*, Leguminosa, flavonoids, antimicrobial activity

RESUMEN

Se llevo a cabo el aislamiento de los flavonoides Kaempferol-3-O- α -L-rhamnopyranosida, quercetina-3-O- α -L-rhamnopyranosida, luteolina, kaempferol y quercetina, del extracto metanolico de las hojas de *Albizia chinensis* colectada de Egipto. La identificación de los compuestos se llevo a cabo mediante el análisis espectroscópico. Se valoro la actividad antimicrobiana de los tres primeros compuestos mostrando actividad moderada contra bacterias gram-positivas y gram-negativas. No se observo actividad antifungicida de estos compuestos.

Palabras clave: *Albizia chinensis*, Leguminosa, flavonoides, actividad antimicrobiana

INTRODUCTION

The genus *Albizia* (Leguminosa) 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 (Watt and Breyer-brandwijk, 1962). In traditional Chinese medicine, *Albizia* members are used for the treatment of insomnia, irritability, wounds and as antidiarrhetic, antiseptic and antitubercular (Chadha, 1985). Phytochemical studies carried out on plants of genus *Albizia*, have revealed

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them as sources of different groups of natural products (triterpenoid saponins, diterpenoids, lignans and pyridineglycoside). In particular, recently were reported eight compounds isolated from a 95% ethanolic extract from leaves of *A. chinensis* collected in China (Liu *et al.*, 2009). The compounds were identified as quercetin 3'-O- β -D-glucopyranosyl-3-O-rutinoside, kaempferol 3,7-di-O- β -D-glucopyranoside, rutin, D-pinitol, luteolin 7-O- β -D-glucopyranoside, (+)-lyoniresinol 3- α -O- β -D-glucopyranoside, (-)-lyoniresinol-3- α -O- β -D-glucopyranoside and syringin.

As a part of our continuous investigation on secondary metabolites from *Albizia* species (Melek *et al.*, 2007; Miyase *et al.*, 2010), we describe in this report the isolation of five flavonoids from the methanolic extract of *A. chinensis* leaves from Egypt.

MATERIALS AND METHODS

General experimental procedure

Column chromatography was run using silica gel 60 (Merck) and Sephadex LH-20 (Sigma). TLC and PTLC were carried out using silica gel (Merck) as stationary phase. Chromatograms were first visualized by observation under UV and then spraying with FeCl₃ spray reagent. UV spectra were obtained on a Shimadzu UV-240 spectrometer. NMR spectra were recorded on a JEOL EX 500 MHz and on a Varian GEMINT 200 MHz spectrometers. Chemical shifts were given on δ -scale with TMS as internal standard.

Plant material

The leaves of *A. chinensis* were collected from the zoological garden in Giza, Egypt in June 2009. Plant identification was confirmed by Mrs. T. Labib, head specialist for plant identification in El-Orman public garden, Giza, Egypt. The herbarium voucher specimen was deposited in the Herbarium of NRC (CAIRC).

Extraction and isolation

Air-dried and powdered leaves (250g) of *A. chinensis* were extracted with MeOH (2L x 2) at room temperature. The total alcoholic extract was evaporated to dryness under reduced pressure. The residue (40 g) was suspended in distilled water (500 ml) and successively partitioned with CHCl₃ (500 ml x 2), EtOAc (700 ml x 3) and BuOH (700 ml x 3). Part of EtOAc fraction (3g) was subjected to silica gel column chromatography. The column was eluted first with CHCl₃ and then a CHCl₃-MeOH mixture with increasing the amount of MeOH up to 20%. A total of 30 fractions (100 ml each) were collected. The fractions were monitored by TLC using the solvent system CHCl₃-MeOH (5:1) and examined under UV followed by spraying with FeCl₃ reagent. The fractions eluted with 2-5% MeOH were found to be similar, so they were combined and found to contain a mixture of compounds **3-5** after TLC examination. The combined fraction was repeatedly chromatographed on preparative silica gel TLC plates eluted with CHCl₃-MeOH (5:1). Repeated purification of each component on Sephadex LH-20 column eluted with MeOH, afforded compounds **3** (37 mg), **4** (29 mg) and **5** (33 mg). The fractions eluted from the silica gel column with 6% MeOH, were combined after TLC analysis and contained compound **1** as a main component. The combined fraction was repeatedly chromatographed on Sephadex LH-20 column using methanol as eluent to yield compound **1** (42 mg). The fractions eluted with 8-20% MeOH were combined and found to contain a mixture of compounds **1** and **2** after TLC examination. The fraction was subjected to PTLC eluted with CHCl₃-MeOH (5:1) to afford **2**. Repeated purification of **2** on Sephadex LH-20 column eluted with MeOH yielded pure **2** (26 mg).

Kaempferol-3-O- α -L-rhamnopyranoside (**1**)

Amorphous yellow powder, UV λ_{\max} nm (MeOH): 265, 340, + NaOMe: 275, 388;

+AlCl₃: 275, 300 sh, 347, 398; + AlCl₃/HCl: 275, 300sh, 362; + NOAc: 274, 300sh, 362; + NaOAc/H₃BO₃: 262, 342. ¹H-NMR (500 MHz, acetone-d₆) δ: 12.67 (1H, s, OH-5), 7.82 (2H, d, *J*=8.4 Hz, H-2', H-6'), 6.99 (2H, d, *J*= 8.4 Hz, H-3', H-5'), 6.43 (1H, d, *J*=2.3Hz, H-8), 6.23 (1H, d, *J*=2.3 Hz, H-6), 5.49 (1H, d, *J*=1.5 Hz, H-1" Rha), 2.29-3.31 (4H, H₂"-H₅"), 0.86 (3H, d, *J*=5.6 Hz, Me-6"). ¹³CNMR (500 MHz, acetone-d₆) δ: 178.5 (C-4), 164.4 (C-7), 162.3 (C-5), 160.2 (C-4'), 157.7 (C-2), 157.2 (C-9), 134.9 (C-3), 130.8 (C-2'), 130.8 (C-6'), 121.5 (C-1'), 115.5 (C-3'), 115.5 (C-5'), 104.9 (C-10), 101.9 (C-1''), 98.8 (C-6), 93.7 (C-8), 71.3 (C-4''), 70.7 (C-3''), 70.6 (C-2''), 70.6 (C-5''), 16.9 (C-6'').

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

Amorphous yellow powder, UV λ_{max} nm (MeOH): 256, 265 sh, 350; +NaOMe: 270, 327, 394; + AlCl₃: 276, 304 sh, 334, 430; + AlCl₃/HCl 272, 301 sh, 353, 401; + NaOAc: 271, 322 sh, 372; + NaOAc/H₃BO₃: 261, 301 sh, 367. ¹HNMR (500 MHz, acetone-d₆) 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, H-1" Rha), 3.30-3.32 (4H, H₂" - H₅"), 0.88 (3H, d, *J*= 5.6 Hz, Me-6"). ¹³CNMR (500 MHz, acetone-d₆) 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'').

Luteolin (3)

Amorphous yellow powder, UV λ_{max} nm (MeOH): 254, 268, 349; +NaOMe: 230 sh, 266, 328, 403; +AlCl₃: 273, 300 sh, 424; +AlCl₃/HCl: 266 sh, 276, 294 sh, 355, 385; +NaOAc: 269, 384; +NaOAc/H₃BO₃: 260, 371. ¹HNMR (500 MHz, acetone-d₆) 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).

Kaempferol (4)

Amorphous yellow powder, UV λ_{max} nm (MeOH): 254 sh, 268, 322 sh, 365; +NaOMe: 275, 320, 412; +AlCl₃: 262 sh, 270, 352, 426; +AlCl₃/HCl 260, 271, 350, 426; +NaOAc: 275, 300, 385; +NaOAc/H₃BO₃: 269, 295 sh, 320 sh, 370. ¹HNMR (200 MHz, DMSO-d₆) δ: 8.00 (2H, d, *J*= 8.1 Hz, H-2', H-6'), 6.90 (2H, d, *J*=8.1 Hz, H-3', H-5'), 6.40 (1H, d, *J*= 2.5 Hz, H-8), 6.21 (1H, d, *J*= 2.5 Hz, H-6).

Quercetin (5)

Amorphous yellow powder, UV λ_{max} nm (MeOH): 258, 266 sh, 299 sh, 360; +NaOMe: 272, 327, 416, +AlCl₃: 275, 303 sh, 430; +AlCl₃/HCl: 271, 300, 364 sh, 402; +NaOAc: 270, 325, 393; +NaOAc/H₃BO₃: 262, 298, 387. ¹HNMR (200 MHz, DMSO-d₆) δ: 7.28 (m, H-6', H-2'), 6.88 (1H, d, *J*= 8.0 Hz, H-5'), 6.40 (1H, d, *J*= 2.5 Hz, H-8), 6.20 (1H, d, *J*= 2.5 Hz, H-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 H₂O and extracted twice with ethyl acetate. From the ethyl acetate layer, the aglycone was detected by TLC. The aqueous layer was repeatedly diluted with CH₃OH and evaporated to dryness. The residue was investigated to detect the sugar L-rhamnose by paper chromatography (PC) using solvent system BAW (n-BuOH-HOAc-H₂O, 4: 1: 5, top layer).

Antimicrobial activity

The antibacterial activity of the tested compounds were carried out against *Bacillus subtilis* NRRL B-543 and *Staphylococcus aureus* NRRL B-313 (Gram+ve bacteria) as well as *Escherichia coli* NRRL B-210 (Gram -ve bacteria) using nutrient agar medium.

The antifungal activity of these compounds was also tested against *Candida albicans* NRRL Y-477 using Sabouraud dextrose agar medium.

RESULTS AND DISCUSSION

As described in the experimental section, from the methanolic extract of *A. chinensis* leaves, the flavonoids kaempferol 3-O- α -L-rhamnopyranoside (**1**), quercetin 3-O- α -L-rhamnopyranoside (**2**) luteolin (**3**) kaempferol (**4**) and quercetin (**5**), were isolated. The structural identification of the isolates was elucidated by acid hydrolysis, UV, ^1H and ^{13}C NMR spectroscopic analysis and/or comparison with published data.

Kaempferol 3-O- α -L-rhamnopyranoside (**1**) was obtained as a yellow powder. The ^1H NMR spectrum of **1** showed one set of *meta*-coupled aromatic protons at δ 6.23 (1H, d, $J=2.3$ Hz) and δ 6.43 (1H, d, $J=2.3$ Hz) and two sets of *ortho*-coupled aromatic protons at δ 7.82 (2H, d, $J=8.4$ Hz) and δ 6.99 (2H, d, $J=8.4$ Hz). These signals were assigned to H-6, H-8, H-2' and H-6' and H-3' and H-5', respectively, of 4', 5, 7- trihydroxy flavonol skeleton. The two doublets at δ 5.49 (1H, $J=1.5$ Hz, H-1'') and δ 0.86 (3H, $J=5.6$ Hz, H-6''), suggested the presence of a rhamnose moiety in the structure of **1** (Markham, 1978). The nature of the sugar was confirmed by acid hydrolysis of the glycoside and paper chromatography of the obtained rhamnose against authentic sample. The attachment of the rhamnose moiety to C-3 position of the aglycone was deduced from the two carbon signals at δ 134.9 assigned to C-3 and δ 157.7 due to C-2, in the ^{13}C NMR spectrum of **1**. The anomeric center of the rhamnose unit was determined to have α -configuration based on $^3J_{\text{H-1}, \text{H-2}}$ value (1.5 Hz) and the pyranose form of the sugar was revealed from its ^{13}C NMR chemical shift values. These values were consistent with those of other flavonol 3-O-rhamnoside. Based on the

spectral data and comparison with literature values, **1** was identified as kaempferol 3-O- α -L-rhamnopyranoside.

Quercetin 3-O- α -L-rhamnopyranoside (**2**) (quercitrin) was obtained as a yellow powder. In the ^1H NMR spectrum of **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}C NMR spectrum, indicated a typical 3', 4', 5, 7- tetrahydroxyflavonol skeleton. The two doublets in the ^1H NMR 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. The ^{13}C NMR data of **2** and the result of acid hydrolysis indicated α -L-rhamnoside. The attachment of the moiety at C-3 position of the aglycone was revealed from the δ value of C-3 (δ 135.0) and that of C-2 (δ 157.6) which are similar to the corresponding values of **1**. Compound **2** was then assigned the structure of quercetin 3-O- α -L-rhamnoside based on the above spectral evidences and comparison with literature values. The flavonoids luteolin (**3**), kaempferol (**4**) and quercetin (**5**) were characterized by comparison of their spectral data with the literature values (Harborne, 1993). Kaempferol was previously isolated from *Albizia versicolor* (Rukunga & Watermann, 1996) while luteolin was obtained from *A. julibrissin* (Jung *et al.*, 2004). Quercetin 3-O- α -L-rhamnoside was reported also from *A. julibrissin* (Lau *et al.*, 2007). In the case of quercetin and kaempferol 3-O- α -L-rhamnoside, to our knowledge this is the first reported occurrence of these compounds in the genus *Albizia*.

The activities assigned to the flavonoids identified in this work could explain some of the medicinal properties of *Albizia chinensis*.

The antibacterial activity for compounds

1-3 against the micro-organisms *Bacillus subtilis* NRRL B-543 and *Staphylococcus aureus* NRRL B-313 (Gram +ve bacteria) and *Escherichia coli* NRRL B-210 (Gram -ve bacteria) together with the antifungal activity against *Candida albicans* NRRL Y-477, were studied using the agar diffusion method (Cruickshank *et al.*, 1975). The results (Table 1) revealed that, in our experimental conditions, the tested compounds exhibited moderate inhibiting activity against gram-positive and gram-negative bacteria while no antifungal activity was observed.

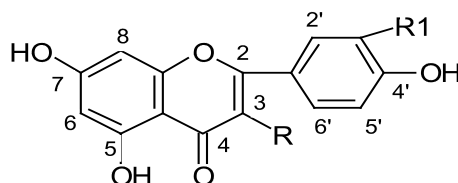
Table (1): Antimicrobial activity of compounds **1-3**

	1	2	3
Bacteria			
Gram-positive			
<i>Bacillus subtilis</i> NRRL B-543	17.5	16	16
<i>Staphylococcus aureus</i> NRRL B-313	17.5	16	16
Gram-negative			
<i>Escherichia coli</i> NRRL-210	17.5	16	16
Fungi			
<i>Candida albicans</i> NRRLY-477	-	-	-

- = No inhibition

Concentration 100 µg/ml

Experiments were performed by triplicated, the results represent the mean values of the data obtained. Inhibition diameter in mm



	R	R1
1	O- α -L-Rhamnopyranosyl	H
2	O- α -L-Rhamnopyranosyl	OH
3	H	OH
4	OH	H
5	OH	OH

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