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Antioxidant flavonol rhamnosides from *Rhamnus disperma* roots

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Abstract

Four flavonol rhamnosides were isolated from *Rhamnus disperma* roots and identified on the basis of their spectroscopic data as rhamnocitrin 3-*O*-*α*-L-rhamnopyranoside [**1**], quercetin 7-*O*-*α*-L-rhamnopyranoside [**2**], Kampferol 3, 7-di-*O*-*α*-L-rhamnopyranoside [**3**] and quercetin 3, 4'-di-*O*-*α*-L-rhamnopyranoside [**4**]. Occurrence of compounds **1**-**3** in *Rhamnus disperma* was reported here for the first time. All compounds [**1**-**4**] showed good antioxidant activity against DPPH radical with IC_{50} values 12.85, 6.82, 12.01 and 11.89 μg/ ml,respectively.

1. INTRODUCTION

Flavonoids have gained recent interest because of their broad pharmacological activity and their benefit role among all body tissues including liver, brain and heart[1]. In addition, the putative therapeutic effects of many traditional medicines may be ascribed to the presence of flavonoids[2]. The biological and pharmacological effects of flavonoids may depend upon their behavior as antioxidant and or prooxidant effects[3]. Therefore, the antioxidant activity of flavonoid aglycones and its glycosides may be give information about sugar effect on the aglycone divers activities. Family Rhamnaceae consists of 59 genera distributed all over the world with about 900 species, the most famous genus is *Rhamnus* which comprises 110 species[4]. *Rhamnus* species are known to be rich with anthraquinones[5] and flavonoid glycosides[6]. *Rhamnus disperma* one of only two *Rhamnus* species present in Egypt[7] with only two phytochemical investigation studies on the fruits, arial parts and roots[6, 8], these two studies reveled presence of various types of flavonoid aglycons and glycosides. In the present study, the flavonoid glycosides isolated from *Rhamnus disperma* have been tested for the scavenging activity and they ability to quench the free radical in compared to the quercetin aglycone, vitamin C and butaylated hydroxyl anisol as standard antioxidant compounds.

2. MATERIALS AND METHODS

2.1 General experimental procedures

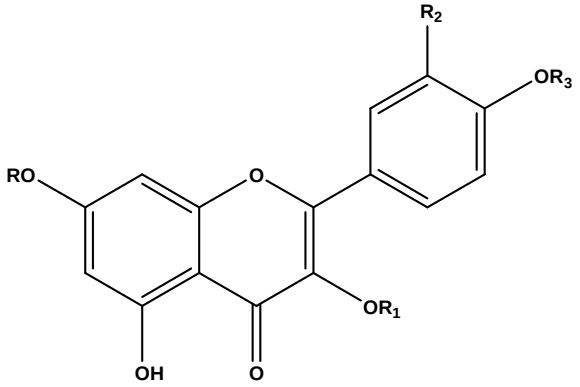
UV spectra were determined with a Hitachi 340 spectrophotometer. IR spectra were carried out on a Nicolet 205 FT IR spectrometer connected to a Hewlett-Packard Color Pro. Plotter. EIMS was carried on Scan EIMS-TIC, VG-ZAB-HF, X-mass (158.64, 800.00) mass spectrometer (VG Analytical, Inc.). The ^{1}H-, ^{13}C and ^{13}C-APT NMR measurements were obtained with a Bruker Avance III NMR spectrometer operating at 400 MHz (for ^{1}H) and 100 MHz (for ^{13}C-APT) in DMSO-d_6 solution, and chemical shifts were expressed in δ (ppm) with reference to TMS, and coupling constant (*J*) in Hertz. Sephadex LH-20 (Pharmacia) and Si gel (Si gel 60, Merck), were used for open column chromatography. Solid phase extraction was performed on SPE-C_{18} cartridges (Strata columns). 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.

2.2 Plant material

Rhamnus disperma Ehrenb. roots were collected from Saint Kathrin Protectorate, South Sinai, Egypt in April 2013. The plant was identified by Dr. Ibraheem Elgarf, Professor of Plant Taxonomy, Faculty of Science, Cairo University, Egypt. A voucher specimen has been deposited in the Pharmacognosy Department, Faculty of Pharmacy, Al-Azhar University, Cairo, Egypt.

2.3 Extraction and isolation

The air dried powdered roots of *Rhamnus disperma* (1kg) were subjected to exhaustive extraction with 70% ethyl alcohol (3Lx3). The combined ethanolic extracts were concentrated under vacuum at 40^{o}C to dryness (160g). The concentrated ethanolic extract was suspended in distilled water (300ml) and filtered. The water-soluble portion was defatted with petroleum ether (46g). The defatted crude extract was partitioned successively with ethyl acetate (25g) and *n*-butanol (50g). The ethyl acetate fraction was applied to Si gel column and eluted with CH_2Cl_2-MeOH (100:0→70:30) to give three fractions of A (317mg), B (1.25g) and C (840mg). Fraction B was subjected to Si gel column chromatography using CH_2Cl_2-MeOH (100:0→80:20) to give B-1 (300mg), B-2 (91mg), B-3 (290mg) and B-4 (210mg). Fraction B-1 was rechromatographed over Si gel column eluted with CH_2Cl_2-MeOH (100:0→85:15) to give two sub-fractions of B-1a (110mg) and B-1b (70mg). Sub-fractions B-1a and B-1b were repeatedly purified by Sephadex LH-20 column (MeOH) followed by Si gel C_{18} column chromatography to give compound **1** (30mg) and compound **2** (32mg), respectively, from sub-fraction B-1a and compound **3** (12mg) and **4** (19mg), respectively, from sub-fraction B-1b.



1 R = CH_3, R_1 = Rha, R_2= H, R_3 =H
2 R = Rha, R_1 = H, R_2= OH, R_3 =H
3 R = Rha, R_1 = Rha, R_2= H, R_3 =H
4 R = H, R_1 = Rha, R_2= OH, R_3 =Rha

Fig. 1: Structures of compounds 1-4

Rhamnocitrin 3-*O*-*α*-L-rhamnopyranoside [**1**]: Yellow amorphous powder [MeOH]; UV λ_{max} (MeOH) nm: 244sh, 266, 315sh, 350, λ_{max} (MeONa) nm: 246, 269, 302sh, 351sh, 389, λ_{max} ($AlCl_3$) nm: 256sh, 275, 302, 354, 400, λ_{max} ($AlCl_3$/HCl) nm: 275, 298sh, 348, 399, λ_{max} (AcONa) nm: 264, 318sh, 358, 405sh, λ_{max} (AcONa/boric acid) nm: 264, 319sh, 352; IR u_{max} (KBr) cm^{-1}: 3430, 1650, 1599, 1512; ^{1}H NMR (DMSO-d_6, 400 MHz) aglycone δ 8.12 (2H, d, *J*=8.8 Hz, H-2`, H-6`), 6.95 (2H, d, *J*=8.8 Hz, H-3`, H-5`), 6.82 (1H, d, *J*=1.6 Hz, H-8), 6.42 (1H, d, *J*=1.6 Hz, H-6), 3.85 (3H, s, 7-OCH_3); sugar moiety δ 5.55 (1H, d, *J*=1.2 Hz, H-1``), 3.79 (1H, brs, H-2``), 3.65 (1H, dd, *J*=9.2, 3.2 Hz, H-3``), 3.44 (1H, t, *J*=9.5 Hz, H-4``), 3.31 (1H, dd, *J*=9.6, 6.1 Hz, H-5``), 1.13 (3H, d, *J*=6.0 Hz, H-6``); EIMS *m/z* 300 $[aglycone]^+$.

Quercetin 7-*O*-*α*-L-rhamnopyranoside [**2**]: Yellow amorphous powder [MeOH]; UV λ_{max} (MeOH) nm: 255, 269sh, 373, λ_{max} (MeONa) nm: 242sh, 290, 267, 456, λ_{max} ($AlCl_3$) nm: 259sh, 274, 340, 457, λ_{max} ($AlCl_3$/HCl) nm: 267, 302sh, 365, 325, λ_{max} (AcONa) nm: 285, 378, 329sh, λ_{max} (AcONa/boric acid) nm: 261, 288sh, 387; IR u_{max} (KBr) cm^{-1}: 3350, 1660, 1610, 1585; ^{1}H NMR (DMSO-d_6, 400 MHz) 7.73 (1H, d, *J*=2.1 Hz, H-2`), 7.60 (1H, dd, *J*= 8.3, 2.1 Hz, H-6`), 6.90 (1H, d, *J*=8.8 Hz, H-5`), 6.79 (1H, d, *J*=1.6 Hz, H-8), 6.41 (1H, d, *J*=2.0 Hz, H-6), sugar moiety δ 5.55 (1H, d, *J*=1.2 Hz, H-1``), 3.85 (1H, dd, *J*=3.0, 1.5 Hz, H-2``), 3.65 (1H, dd, *J*=9.4, 3.3 Hz, H-3``), 3.58 (1H, dd, *J*=9.6, 6.1 Hz, H-5``), 3.44 (1H, t, *J*=9.5 Hz, H-4``), 1.14 (3H, d, *J*=6.1 Hz, H-6``), EIMS *m/z* 302 $[aglycone]^+$.

Kampferol 3, 7-di-*O*-*α*-L-rhamnopyranoside [**3**]: Yellow amorphous powder [MeOH]; UV λ_{max} (MeOH) nm: 243sh, 265, 315sh, 349, λ_{max} (MeONa) nm: 245, 270, 300sh, 350sh, 388, λ_{max} ($AlCl_3$) nm: 256sh, 274, 300, 355, 400, λ_{max} ($AlCl_3$/HCl) nm: 274, 298sh, 349, 398, λ_{max} (AcONa) nm: 264, 319sh, 357, 405sh, λ_{max} (AcONa/boric acid) nm: 265, 350; IR u_{max} (KBr) cm^{-1}: 3445, 1655, 1600, 1510; ^{1}H NMR (DMSO-d_6, 400 MHz) aglycone δ 7.80 (2H, d, *J*=9.1 Hz, H-2`, H-6`), 6.94 (2H, d, *J*=8.8 Hz, H-3`, H-5`), 6.78 (1H, d, *J*=2.1 Hz, H-8), 6.45 (1H, d, *J*=2.1 Hz, H-6), sugar moieties δ 5.55 (1H, d, *J*=1.2 Hz, H-1``), 5.31 (1H, d, *J*=1.2 Hz, H-1```), 3.66 (2H, dd, *J*=3.0, 1.5 Hz, H-2``, H-2```), 3.51 (2H, dd, *J*=9.4, 3.3 Hz, H-3``, H-3```), 3.32 (2H, dd, *J*=9.6, 6.1 Hz, H-5``, H-5```), 3.15 (2H, t, *J*=9.5 Hz, H-4``, H-4```), 1.14 (3H, d, *J*=6.1 Hz, H-6``), 0.82

(3H, d, *J*=6.1 Hz, H-6```); ^{13}C-APT NMR (DMSO-d_6, 100 MHz) aglycone δ 178.40 (C, C-4), 162.14 (C, C-7), 161.41 (C, C-5), 160.63 (C, C-4`), 158.30 (C, C-2), 156.55 (C, C-9), 135.05 (C, C-3), 131.26 (CH, C-2`, 6`), 120.82 (C, C-1`), 115.87 (CH, C-3`, 5`), 106.25 (C, C-10), 99.94 (CH, C-6), 95.05 (CH, C-8), sugar moieties δ 102.82 (CH, C-1``), 98.93 (CH, C-1```), 72.07 (CH, C-4``), 71.57 (CH, C-4```), 71.16 (CH, C-3``), 70.79 (CH, C-3```), 70.71 (CH, C-5``), 70.54 (CH, C-2``, C-5```), 70.27 (CH, C-2```), 18.39 (CH_3, C-6```), 17.94 (CH_3, C-6```), EIMS *m/z* 578 [M]+.

Quercetin 3, 4'-di-*O*-α-L-rhamnopyranoside [**4**]: Yellow amorphous powder [MeOH]; UV λ_{max} (MeOH) nm: 254, 269, 349, λ_{max} (MeONa) nm: 272, 329, 376, λ_{max} ($AlCl_3$) nm: 275, 298sh, 355, 400, λ_{max} ($AlCl_3$/HCl) nm: 265sh, 279, 298sh, 348, 399, λ_{max} (AcONa) nm: 271, 350, λ_{max} (AcONa/boric acid) nm: 254, 267, 350; IR u_{max} (KBr) Cm^{-1}: 3340, 1640, 1605, 1565; ^{1}H NMR (DMSO-d_6, 400 MHz) aglycone δ 7.75 (1H, d, *J*=8.8 Hz, H-5`), 7.33 (1H, d, *J*=2.1 Hz, H-2`), 7.26 (1H, dd, *J*=8.3, 2.1 Hz, H-6`), 6.39 (1H, d, *J*=2.0 Hz, H-8), 6.19 (1H, d, *J*=2.0 Hz, H-6), sugar moieties δ 5.38 (1H, d, *J*=1.6 Hz, H-1``), 5.30 (1H, d, *J*=1.2 Hz, H-1```), 3.98 (2H, dd, *J*=3.0, 1.5 Hz, H-2``, H-2```), 3.78 (2H, dd, *J*=9.4, 3.3 Hz, H-3``, H-3```), 3.32 (2H, dd, *J*=9.6, 6.1 Hz, H-5``, H-5```), 3.15 (2H, t, *J*=9.5 Hz, H-4``, H-4```), 1.12 (3H, d, *J*=6.1 Hz, H-6``), 0.80 (3H, d, *J*=6.1 Hz, H-6```); EIMS *m/z* 302 [aglycone]$^+$.

2.4 Acid hydrolysis of compounds [1-4]

Five mg of each compound was separately refluxed with 2M HCl in MeOH (5 ml) at 80 ^{0}C for 5 h in a water bath. The reaction mixture was evaporated, and the hydrolysate after dilution with H_2O (10 ml) was extracted with $CHCl_3$ (3 x10 ml). The $CHCl_3$ extracts were evaporated to afford the aglycons, which were identified as 7-methoxy kampferol (rhamnocitrin) in **1**, quercetin in **2** and **4** and kampferol in **3** and by comparison with authentic samples, respectively. The aqueous layer was neutralized with sodium carbonate and concentrated to 1 ml under reduced pressure. The residue was compared with standard sugars by Si gel TLC [($CHCl_3$-MeOH-H_2O:30:12:4), 9 ml of lower layer and 1 ml of HOAc], which indicated the sugars of **1**-**4** to be rhamnose.

2.5 DPPH radical-scavenging assay

The free radical scavenging activities of compounds **1**-**4** were measured against 2,2-diphenyl-1-picryl-hydrazyl (DPPH) radical as described by Hosny *et al.*, 2002[9], with some modification. 1.9ml of DPPH-ethanol solution (300μM) was mixed with 0.1ml of different concentrations (6.5–500μg/ml) of isolated compounds. The reaction mixture was vortexes thoroughly and left in the dark at room temperature for 30min. The absorbance of the mixture was measured spectrophotometrically (Spectro UV-VIS double, 110V, 60Hz, Labomed, Inc. U.S.A.) at 517nm. Quercetin, vitamin C and butylated hydroxyl anisole (BHA) were used as standard drugs. The percentage of free radical scavenging was calculated according to the following equation: 1-(Absorbance of sample / Absorbance of blank) x 100.

2.6 Statistical analysis

The experimental results were expressed as mean ± standard deviation (SD) of three replicates.

3. RESULT AND DISCUSSION

From the ethyl acetate fraction of *Rhamnus disperma* roots, four simple flavonoid glycosides were isolated and have been identified by different spectroscopic methods (NMR, UV, IR and mass). Compounds 1-3 were reported here as a first time isolated from *Rhamnus disperma*, while compound 4 was isolated previously from *Rhamnus disperma* arial parts[6].

Compound [1] was obtained as yellow amorphous powder. The UV spectrum of compound [1] showed two absorbance bands at 355 nm (band I) and 265 nm (band II), which are characteristic for flavones and flavonols[10]. The bathochromic shift of band **I** by about 50 nm after the addition of NaOMe indicates the presence of free 4`-hydroxyl group. The band II showed no bathochromic shift in the presence of AcONa suggested a flavonol with occupied 7-OH group. The bathochromic shift of band **I** by about 45 nm and band II by about 7 nm after the addition of $AlCl_3$ indicates the presence of free 5-hydroxyl group while 3-hydroxyl group is protected. The similarity between the spectra in the presence of $AlCl_3$ and $AlCl_3$/HCl, together with the absence of a significant shift after addition of AcONa/boric acid indicated the absence of an *O*-dihydroxy system on the B ring[10]. The EI-MS spectrum of compound [1] showed ion peak at *m/z* 300 [aglycone]$^+$. In the ^{1}H NMR spectrum of [1], two *meta*-coupled doublets at δ 6.42 and 6.82 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 8.12 ppm (2H, d, *J*=8.8 Hz, H-2`, H-6`) and δ 6.95 (2H, d, *J*=8.8 Hz, H-3`, H-5`) corresponding to four aromatic protons of B ring. In addition to one sharp singlet signal at δ 3.85 for one methoxyl group. These data indicated that the aglycon of [1] was rhamnocitrin[11]. The ^{1}H NMR spectrum of [1] exhibited one anomeric proton signal at δ 5.55 (brs, H-1``) of rhamnose. In addition the appearance of the strong sharp doublet signal at δ 1.13 ppm (3H, d, *J*= 6.0 Hz) for the secondary CH_3 of rhamnose confirmed the rhamnose unit. Acid hydrolysis of [1] afforded L-rhamnose as a sugar component and rhamnocitrin as the aglycone by comparison with authentic samples. Furthermore the

anomeric configuration for the sugar moiety was fully defined from its chemical shift and $^{3}J_{H1,H2}$ coupling constant. Accordingly the rhamnose was established to be in the *α*-configuration[12]. Therefore, compound [1] was identified as rhamnocitrin 3-*O*-*α*-L-rhamnopyranoside[13].

Compound [2] was obtained as yellow amorphous powder. The UV spectrum of [2] exhibited band I and band II absorbance maxima at 373 and 255 nm, respectively, which are characteristic for flavones and flavonols. The addition of NaOMe showed bathochromic shift by 56 nm of band **I** indicates the presence of free 4`-hydroxyl group. The addition of $AlCl_3$ showed bathochromic shift by 15 nm of band II and 74 nm of band I indicates the possibility of the presence of B ring *O*-dihydroxyl group at 3`and 4` and free 5-hydroxyl group. The bathochromic shift of band I caused by addition of $AlCl_3$ was decreased by 30 nm on the addition of HCl confirmed the presence of *O*-dihydroxyl group in B ring. The addition of NaOAc showed a bathochromic shift of band I by 29 nm indicative of the ionization of hydroxyl group located at position 4` while the absence of bathochromic shift of band **II** indicated the protection of hydroxyl group at position 7. Furthermore, the marked bathochromic shift of band **I** by 24 nm on the addition of a mixture of NaOAc/$H_3BO_3$ indicates the presence of *O*-dihydroxy group at position 3` and 4` in B ring[10]. The molecular formula of [2] was determined as $C_{21}H_{20}O_{11}$ from the ion peak in the EIMS *m/z* 302 [aglycone]$^+$. In the ^{1}H NMR spectrum of [2], two signals at δ 6.41(d, *J*=2.0 Hz, H-6) and 6.79 (d, *J*=1.6 Hz, H-8), were assigned to A ring protons. The ^{1}H NMR spectrum also exhibited a typical ABX system at δ 7.73 ppm (1H, d, *J*=2.1 Hz, H-2`), δ 7.60 (1H, dd, *J*= 8.3, 2.1 Hz, H-6`) and δ 6.90 (1H, d, *J*=8.8 Hz, H-5`) corresponding to three aromatic protons of B ring. These data indicated that the aglycone of [2] was in good agreement with quercetin[11]. The ^{1}H NMR spectrum of [2] exhibited one anomeric proton signal at δ 5.55 (1H, d, *J*=1.2 Hz, H-1``) and the strong sharp doublet signal at δ 1.14 (3H, d, *J*=6.1 Hz, H-6``-CH_3) confirmed the rhamnose unit. Acid hydrolysis of [2] afforded L-rhamnose as a sugar component and quercetin as the aglycone by comparison with authentic samples. Furthermore the anomeric configuration for the rhamnose moiety was established to be in the *α*-configuration from its chemical shift and $^{3}J_{H1,H2}$ coupling constant[12]. The downfield shift of H-6 and H-8 compared to those of quercetin indicates that, the rhamnose moiety was attached to C-7 position. Furthermore, the absence of bathochromic shift of band II with NaOAc confirmed the site of rhamnosylation. On the base of the above mentioned data compound [2] was distinguished as quercetin-7-*O*-*α*-L-rhamnopyranoside and in good agreement with the reported literatures[14]**.**

Compound [**3**] was obtained as yellow amorphous powder. The UV spectrum of compound [**3**] exhibited band I and band II absorbance maxima at 349 and 265 nm, respectively, which are characteristic for flavones and flavonols. The results of UV shift reagents of compound [3] were similar to that of compound [1], indicating that, compound [3] has free 4`-hydroxyl group, free 5-hydroxyl group, occupied 3- and 7-OH groups and the absence of an *O*-dihydroxy system on the B ring[10]. The molecular formula of compound [**3**] was determined as $C_{27}H_{30}O_{14}$ from the molecular ion peak in the EI-MS at *m/z* 578 [M]$^+$. The ^{1}H NMR spectrum of compound [**3**], showed two signals at δ 6.45 ppm (1H, d, *J*=2.1 Hz) and δ 6.78 ppm (1H, d, *J*=2.1 Hz) corresponding 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 7.80 ppm (2H, d, *J*=9.1 Hz, H-2`, H-6`) and δ 6.94 ppm (2H, d, *J*=8.8 Hz, H-3`, H-5`), corresponding to four aromatic protons of B ring. These data indicated that the aglycon of [3] was in good agreement with kampferol[11]. Two anomeric proton signals at δ 5.55 ppm (1H, d, *J*=1.2 Hz, H-1``) and δ 5.31 ppm (1H, d, *J*=1.2 Hz, H-1```) of the two rhamnose units, in addition to the two doublets at δ 1.14 ppm (3H, d, *J*=6.1 Hz, H-6``), 0.82 ppm (3H, d, *J*=6.1 Hz, H-6```) of rhamnoses were observed in the ^{1}H NMR spectrum of compound [**3**]. The ^{13}C-APT NMR spectrum of compound [**3**] supported these observations having the twenty seven carbon signals of which 12 were arising from sugar moieties. The remaining resonances were indicative for kampferol as aglycon[15, 16]. It also revealed that the sugar moieties consisted of two molecules of rhamnose, based on the existence of two anomeric carbon signals at δ 102.82 ppm (CH, C-1``) and δ 98.93 ppm (CH, C-1```), respectively, and the signals at δ 18.39 ppm (CH_3, C-6```) and δ 17.94 ppm (CH_3, C-6```) of rhamnoses. The two sugars were determined to be in the pyranose form from their ^{13}C NMR data. Furthermore the anomeric configuration for the sugar units was fully defined from their chemical shift and $^{3}J_{H1,H2}$ coupling constants. Accordingly the two rhamnoses were established to be in the *α*-configuration[12]. The upfeild shifts of C-3 and C-7 of the aglycone and the downfield shift of C-2 compared to those of C-3, C-7 and C-2 of kampferol indicates that, one of the rhamnose moiety was attached to C-7 position and the other rhamnose moiety was attached to C-3 position[15, 16]. Furthermore, the results of UV shift reagents confirmed the sites of glycosylation to be C-3 and C-7 positions. Therefore, compound [**3**] was identified as Kampferol 3, 7-di-*O*-*α*-L-rhamnopyranoside and in good agreement with the reported literatures[11, 17]**.**

Compound [**4**] was obtained as yellow amorphous powder. Its UV spectrum showed two absorbance maxima at 349 and 254 nm corresponding to band I and band II, respectively, of and flavonols. The addition of NaOAc showed bathochromic shift by 16 nm of band **II** indicates the presence of free 7-hydroxyl group, while NaOMe shift reagent indicates occupied 4`-hydroxyl group. $AlCl_3$ shift reagent indicates free 7-hydroxyl group and occupied 3-hydroxyl group. Furthermore, $AlCl_3$\HCl and NaOAc/$H_3BO_3$ shift reagents indicate the absence of free *O*-dihydroxy groups at position 3` and 4` in B ring[10]. Its molecular formula was determined as $C_{27}H_{30}O_{15}$ after addition of two rhamnose moieties to the ion peak in the EIMS *m/z* 302 [aglycone]$^+$. The ^{1}H NMR spectrum of [4], showed signals for A ring and B ring typical for quercetin aglycone as in compound [2]. Furthermore, it showed two signals at δ 5.38 ppm (1H, d, *J*=1.6 Hz, H-1``), 5.30 ppm (1H, d, *J*=1.2 Hz, H-1```) corresponding to

the two anomeric proton signals of the two rhamnose units. In addition to two doublets at δ 1.12 (3H, d, *J*=6.1 Hz, H-6``), 0.80 (3H, d, *J*=6.1 Hz, H-6```) for the secondary CH_3 of the two rhamnose units. Acid hydrolysis of [4] afforded L-rhamnose as a sugar component and quercetin as the aglycone by comparison with authentic samples. Furthermore the anomeric configurations for the two rhamnose units were fully defined from their chemical shifts and $^3J_{H1,H2}$ coupling constants and were established to be in the *α*-configurations[12]. The sites of rhamnosylation were established to be C-3 and C-4` positions as indicated from the UV shift reagents. Therefore, compound [4] was identified as quercetin 3, 4'-di-*O*-*α*-L-rhamnopyranoside[6, 18].

The antioxidant activity of flavonoids and its glycosides has been considered as a play maker for its biological effects and its benefits in the preventing and/or treatment of a wide range of diseases. Presence of sugar in the flavonoids might be enhancing its solubility and bioavailability and consequently its pharmacological action. The present experimental work leading to isolation of four flavonoid glycosides with one (compounds **1** and **2**) or two sugar moieties (compounds **3** and **4**). Antioxidant comparison between isolated glycosidal compounds and flavonoid quercetin aglycon using DPPH stable free radical revealed the effect of sugar moiety on the scavenging activity of flavonoids as antioxidants. The result obtained in table 1, showed that, all isolated compounds (Glycoside form) coming late in the effect after quercetin (Aglycone form) and give a special concern for the importance of 3-OH free group in case of quercetin and compound **2** compared to other isolated compounds. On the other hand, replacing of hydrogen by methyl in position 7 and with rhamnose in position 3 in case of compound **1**, reduce its free radical scavenging effect compared to other isolated compounds and quercetin. Furthermore, compounds **3** and **4** (two sugar moieties) showing somewhat similar effect with a special concern to quercetin derivative (compound **4**) with IC_{50} value 11.89 *μ*g/ ml, over Kampferol derivative (compound 3) with IC_{50} value 12.01 *μ*g/ ml. The result in the table 1, give a clear sign for the powerful of all isolated compounds and queretin, if its compared to vitamin c or butaylated hydroxyl anisol.

Table 1: Antioxidant activity of compounds 1-4 against DPPH radical

Compounds	DPPH scavenging activity (IC_{50} in *μ*g/ ml)
1	12.85±3.16
2	6.82±2.77
3	12.01±2.91
4	11.89±0.66
Queretin	5.11±1.25
Vitamin C	13.49±3.31
BHA	16.92±2.56

4. CONCLUSION

The present study confirm the past research studies, that concerned with the structure activity relationship of antioxidant activity of flavonoids. Free OH group at 3-postion in flavonol is a point of decisive in the flavonoid structure, derivatization of this position with sugar reduce its activity. The second important decisive point is summarized in the number of free hydroxyl group that are distributed on the flavonoid compound. Further practical and comparison investigation for the isolated compounds to measure its abilities to chelate transition metals and antioxidant power in the biological systems might be the focus of the next research.

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