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Flavonoid Glycosides from Fruit Shells of *Phoenix dactylifera* L.

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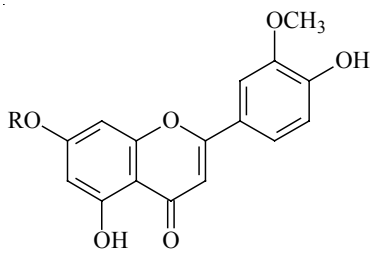
Two new natural flavonoids, chrysoeriol-7-O-β-L-arabinofuranosyl (1→2)-β-D-apiofuranoside and isorhamnetin 3-O-β-(4"-*p*-hydroxybenzoyl) galactopyranoside have been isolated from the acetone extract of the fruit shells of *Phoenix dactylifera* along with seven known flavonoids. The structure of the isolated compounds was elucidated on the basis of chemical analyses and spectral evidence. The antioxidant activity of the extract was also evaluated.

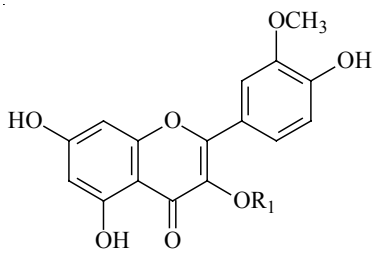
Key Words: *Phoenix dactylifera*, Palmae, Flavonoid glycosides, Spectroscopic analyses, Antioxidant activity.

INTRODUCTION

Dates are an important food in the Mediterranean region and are known to provide extracts which have curative properties of various illnesses and have been used in traditional medicine as tonic, while others consider it to be an aphrodisiac[1]. The flowers of the plant are used as a purgative and the pollen grains of the date palm have been used by Egyptians to improve fertility in women[2]. The extracts from dates were found to have protective effect on CCl_4 induced hepatotoxicity in rats[3]. Previous phytochemical investigations of the Phoenix species resulted in the isolation of sulphated flavonoids, glycosylated flavones and flavonols, procyanidin and taxifolin from different parts of the plant[4-6]. Despite the valuable researches made through studies of the constituents of several Palmae species, many of these plants remain virtually unexplored from a chemical point of view. In view of it, the present study was concerned by studying the chemical constituents which led to the identification of two new natural compounds *i.e.*, chrysoeriol-7-O-β-L-arabinofuranosyl (1→2)-β-D-apiofuranoside (**1**) and isorhamnetin 3-O-β-(4"-*p*-hydroxybenzoyl)galactopyranoside (**2**) with other seven known compounds elucidated *viz.*, chrysoeriol-7-glucoside, 7-rutinoside, 7-galactoside, luteolin-7-glucoside, 7-rutinoside, luteolin and chrysoeriol, using different chemical and spectroscopic methods. The antioxidant activity of the acetone extract of the fruit shells of *Phoenix dactylifera* has also been studied.



(**1**)
R = arabinofuranosyl
(1→2)-β-D-apiofuranoside



(**2**)
R_1 = 4"-*p*-Hydroxybenzoyl)
galactopyranoside

EXPERIMENTAL

The NMR were recorded on a Varian GEMINT-200 spectrometer, operating at 200 MHz for ^{1}H and 50 MHz for ^{13}C for using DMSO-d_6 as solvent. The UV data were recorded on Shimadzu spectrophotometer model UV-240; column chromatography was performed using polyamide 6S and Sephadex LH-20; paper chromatography was carried out on Whatmann No.1 or/and 3 MM using solvent systems (1) BAW (*n*-BuOH:HOAc:H_2O, 6:1:2); (2) H_2O; (3) 15 % AcOH (AcOH:H_2O, 15:85); (4) BAW (*n*-BuOH: HOAc:H_2O, 4:1:5) and visualized under UV light using $FeCl_3$, $AlCl_3$ and Naturstoff reagent as spraying reagents; Aniline hydrogen phthalate was used as specific reagent for sugar analysis.

Fruits of the *Phoenix dactylifera* were collected from south Sinai, Egypt. The fruits were then peeled to collect the fruit shells. Authentication was performed by Dr. M. El-Gebali, former researcher of botany at the National Research Center. A voucher specimen is deposited in the National Research Centre Herbarium.

Extraction and isolation: The cutted shells (1 kg) of fruits of the *Phoenix dactylifera* were exhaustively extracted with acetone several times (five extractions each with 3 L) at room temperature. The combined acetone extracts were concentrated under pressure to give a dry residue (100 g). The resulting extract was fractionated on a Sephadex LH-20 column using an eluting system of ethanol followed by different percentage of ethanol/water giving rise to six fractions. Compound **1** was separated from fractions 4 (335 mg, eluted by 40 % ethanol) by preparative PC, using BAW as a solvent. While fraction **5** (524 mg) eluted with 20 % ethanol gave rise to the pure compound **2** by further fractionation on Sephadex LH-20 using water for elution.

Antioxidant activity measurements: The recording of ESR spectra were performed with a Varian 109 ESR spectrometer, the ESR parameters were set at 100 KHz, X-band microwave frequency of 9.77 GHz, microwave power of 20 mW.

This technique is used to detect the free electrons in the samples either in organic compounds (free radical) or transition elements (paramagnetic centers) whereby a free electron (on its own) has a characteristic signal at g value of 2.0023 while the decrease of this signal characterize the antioxidant efficacy of the sample according to the percentage of the decrease. Also the resonance of the free electron at the commonly used frequency of 9.75 GHz (known as X-band microwave radiation and thus giving rise to X-band spectra) occurs at a magnetic field of *ca.* 3400 gauss.

Chrysoeriol-7-O-β-L-arabinofuranosyl(1→2)-β-D-apiofuranoside (1): R_f-values ×100: 30(1), 59(2), 32(3) 35(4); UV λ_{max} nm (MeOH): 251, 265, 342; +NaOMe: 263,400; + NaOAc: 260, 290sh, 396; +NaOAc/H_3BO_3: 265, 344; + AlCl3: 262, 296sh, 342sh, 378; +AlCl3 / HCl: 259, 296sh, 342sh, 378; ^{1}H NMR (DMSO-d_6): aglycone moiety: δ (ppm) 7.59 (d, *J* = 2 Hz, H-2'); 7.49 (dd, *J* = 2 & 8 Hz, H-6'); 7.04 (d, *J* = 8 Hz, H-5'); 6.88 (s, H-3); 6.77 (d, *J* = 2.1 Hz, H-8); 6.34 (d, *J* = 2.1 Hz, H-6); 3.87 (s, OCH_3). Sugar moieties: δ (ppm) 5.6 (d, *J* = 3.16 Hz, H-1"); 5.18 (d, *J* = 4 Hz, H-1'"); 4.205 (d, *J* = 3.4, H-2"); 4.06 (d, *J* = 9.48 Hz, H-4"a); 3.76 (d, *J* = 9.27 Hz, H-4"b); 3.07-3.8 (m, sugar protons); ^{13}C NMR (DMSO-d_6): aglycone moiety: δ (ppm) 164.2 (C-2); 103.5 (C-3); 181.9 (C-4); 161.1 (C-5); 99.2 (C-6); 162.9 (C-7); 95.6 (C-8); 156.9 (C-9); 105.5 (C-10); 127.6 (C-1'); 110.8 (C-2'); 150.9 (C-3'); 148.1 (C-4'); 116.0 (C-5'); 120.6 (C-6'); 56.2 (OCH_3). Sugar moieties: δ (ppm) 109.1 (C-1"); 78.1 (C-2"); 79.0 (C-3"); 74.5 (C-4"); 64.4 (C-5"); 108.4 (C-1'"); 82.1 (C-2'"); 77.3 (C-3'"); 86.2 (C-4'"); 61.4 (C-5'").

Isorhamnetin -3-O-β-(4''-*p*-hydroxybenzoyl)galactopyranoside (2): R_f-values ×100: 58(4), 60(2), 48(3); UV λ_{max} nm (MeOH): 255, 265sh, 300sh, 356; +NaOMe: 275,325, 410; +NaOAc: 270, 315sh, 380; +NaOAc/H_3BO_3: 255, 310sh, 357; + $AlCl_3$: 270, 308sh, 355sh, 400; +$AlCl_3$/HCl: 268, 308sh, 355sh, 398; ^{1}H NMR (DMSO-d_6): aglycone moiety: δ (ppm) 7.82 (d, *J* = 2 Hz, H-2');7.66 (dd, *J* = 2 & 8 Hz, H-6'); 6.96 (d, *J* = 8 Hz, H-5'); 6.45 (d, *J* = 1.8 Hz, H-8); 6.24 (d, *J* = 1.8Hz, H-6); 3.87 (s, OCH_3). Sugar moiety: δ (ppm) 5.8 (d, *J* = 7.5 Hz, H-1"); 3.2-3.8 (m, sugar protons); 7.61 (d, *J* = 8.4 Hz, H-2'"&6'"); 6.84 (d, *J* = 8.4 Hz, H-3'"&5'"). ^{13}C NMR (DMSO-d_6): aglycone moiety: δ (ppm) 157.1 (C-2); 133.3 (C-3); 177.3 (C-4); 161.2 (C-5); 98.7 (C-6); 164.0 (C-7); 94.4(C-8); 156.5 (C-9); 104.3 (C-10); 121.2 (C-1'); 113.7 (C-2'); 150.4 (C-3'); 147.3 (C-4'); 115.7 (C-5'); 122.4 (C-6'); 56.0 (OCH_3). Sugar moieties: δ (ppm) 101.9 (C-1"); 71.8 (C-2"); 72.4 (C-3"); 70.2 (C-4"); 75.0 (C-5"); 60.2 (C-6"); 124.2 (C-1'"); 136.4 (C-2'"); 116.8 (C-3'"); 164.5 (C-4'"); 116.5 (C-5'"); 136.3 (C-6'"); 169.0 (CO).

RESULTS AND DISCUSSION

The fruit shells of *Phoenix dactylifera* were extracted with absolute acetone several times. Fractionation of the concentrated acetone extract on a Sephadex LH-20 column afforded six fractions which were further subjected to chromatographic procedures to give the two new natural compounds **1** and **2** with the other known compounds which were identified by comparison of their spectral data with those published in the literature.

Compound **1** was obtained as a yellow amorphous powder, it appeared on paper chromatograms as a dark brown spot changing by ammonia to fluorescent yellow and gave positive reaction with Naturstoff reagent. Its UV spectral data in MeOH and by using diagnostic reagents suggested that it's a 7,3'-di-O-substituted flavone[7], whereby the addition of NaOAc produced no shift in band (II) indicating the substitution of the 7-position. A bathochromic shift in band (I) without a decrease in intensity on the addition of NaOMe as well as the absence of any shift in band (I) on the addition of $NaOAc/H_3BO_3$ indicated the absence of an *ortho*-dihydroxyl group in ring B together with the substitution of 3'-position and not the 4'. On complete acid hydrolysis with 2 N HCl (1.5 h 100 °C) yielded the aglycone chrysoeriol identified by Co-PC, UV spectral data and ^{1}H NMR[8], the sugars arabinose and apiose were also obtained (Co-PC). Consequently, from acid hydrolysis we can state that the occupation of a methoxyl group at the 3'-position and the sugar moieties at 7-position. ^{1}H NMR spectrum showed two doublet signals at δ 6.77 and δ 6.34 ppm (each 1H, J = 2.1 Hz) assignable of H-8 and H-6, respectively, which were more downfield than that of chrysoeriol due to the glycosylation at the 7-position. The singlet signal at δ 6.88 (1H, s, H-3) confirmed its flavone type. The presence of three aromatic protons was confirmed through an ABX-type coupling at δ 7.59 (1H, d, J = 2 Hz, H-2'); 7.49 (1H, dd, J = 2 & 8 Hz, H-6'); 7.04 (d, J = 8 Hz, H-5'). The glycosidic nature of **1** was confirmed from the appearance of two anomeric protons; the first anomeric doublet signal at δ 5.6 with J = 3.16 Hz identical of an apiosyl moiety where the $J_{1,2}$ *ca.* 3-4 Hz confirmed the more stable β-D-erythrofuranoside form of the apiose[9,10] and the second upfield anomeric proton doublet signal at δ_H 5.18 (J = 4 Hz) typical for a O-β-L-terminal arabinofuranosyl unit. The attachment of the apoisyl moiety directly to C-7 with the linkage of the arabinosyl moiety at H-2" of it was suggested by the appearance of its H-2" signal at δ 4.20 (d, J = 3.4 Hz) more downfield than the unsubstituted one at (δ 4.18 ppm) together with the great downfield of H-8. ^{13}C NMR spectrum, were in good agreement with the reported values for 7-substituted chrysoeriol with the known substitution effects[7,11]. The two anomeric carbon signals appeared at δ 109.1 (C-1") and 108.4 (C-1'") constituent with the corresponding data

for the apiofuranoside and arabinofuranoside, respectively. The ^{13}C NMR shift of the apiosyl carbon C-2" which appeared at δ 78.1 more downfield than the unsubstituted one at (δ 76.5 ppm)[11], confirmed that the interglycosidic linkage to be apiofuranose (1→2) arabinofuranose. Thus, the structure of compound **1** was established as chrysoeriol-7-O-β-L-arabinofuranosyl (1→2)-β-D-apiofuranoside.

Compound **2** which was isolated and purified from fraction (III) showed a dark purple spot on paper chromatogram under UV light changing to dark yellow when fumed with ammonia vapours and has moderate migration in aqueous and organic solvents. The UV spectrum with absorption maxima at 255 nm (band II), 300sh and 356 nm (band I) indicated a flavonol skeleton[12], besides the addition of shift reagents (see experimental) confirmed a 3,3'-disubstituted flavonol with free hydroxyl groups at 5,7 and 4'-positions[13]. An acid hydrolysis of **2** with 2N HCl (1.5 h 100 °C) yielded the sugar galactose identified by comparative paper chromatography together with the aglycone isorhamnetin and *p*-hydroxybenzoic acid (Co-PC, UV spectral data and ^{1}H NMR). The ^{1}H NMR spectral data revealed one distinct anomeric hexose proton resonance at δ ppm 5.8 (d, *J* = 7.5 Hz) attributed to the anomeric proton of a galactosyl moiety attached at 3-position. The presence of a *p*-hydroxybenzoyl moiety was ensured by the characteristic proton signals which resonated at δ 7.61 (d, *J* = 8.4 Hz, H-2''' and 6'''); 6.84 (d, *J* = 8.4 Hz, H-3''' and 5'''). The spectra also showed the presence of an isorhamnetin moiety by the appearance of proton signals at δ 6.24 (d, *J* = 1.8 Hz, H-6) and 6.45 (d, *J* = 1.8 Hz, H-8) with the known chemical shifts of the B-ring (see experimental) and the singlet signal at δ 3.87 of the methoxyl group. Final confirmation of the structure of **2** was achieved by ^{13}C NMR whereby, the spectrum exhibited a β-anomeric carbon signal at δ 101.9 similar to that of galactopyranoside. Assignments of the aglycone carbons were aided by comparison with the reported values for 3-glycoside isorhamnetin[14]. The aromatic carbon signals at δ 136.4 (C-2''' and 6'''); 116.8 (C-3''' and 5''') and the carbonyl group at δ 169.0 were indicative of a *p*-hydroxybenzoyl moiety[11], which was assigned to be at the galactosyl C-4 on the basis of the galactosyl C-4 downfield shift as it appeared at δ 70.2 ppm with a consequent upfield shifts of both galactosyl C-3 and C-5 at δ 72.4 and 75 ppm, respectively than the unsubstituted carbons. Consequently, compound **2** was elucidated as isorhamnetin 3-O-β-(4"-*p*-hydroxybenzoyl)galactopyranoside.

The antioxidant activity of flavonoids *i.e.* their ability to scavenge reactive oxygen species (ROS) may be the most important function of flavonoids in the body because oxidative stress in biological systems have been proven to be responsible for several damages and diseases. Thus, the potential antioxidant activity of the acetone extract of the fruit shells of

Phoenix dactylifera was investigated by means of ESR[15] using 2,2-diphenyl-1-picrylhydrazyl) as stable radical, whereby the antioxidant efficacy of the extract were followed by their ability in scavenging these radicals which appear by decreasing the intensity of the characteristic absorbance signal of DPPH [its ESR signal with the g-value at the maximum absorption at 2.0023][16] and the more the percentage of the signal intensity decrease the stronger the antioxidant activity. The results presented here indicated a high radical scavenging activity as the inhabitation percentage of the acetone extract was 69 % and consequently we can say that it might be a valuable natural antioxidant source.

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REFERENCES

1. S.A. El-Mougy, S.A. Abdel-Aziz, M. Al-Shanawany and A. Omar, *J. Pharm. Sci.*, **5**, 156 (1991).
2. M. Zohget and A. Elsheikh, Wild Plants in the Region of Riyadh, Riyadh, Saudi Arabia; King Saud University Press, pp. 185-186 (2000).
3. E.S. Amin, O. Awad, M.A. Samad and M.N. Iskander, *Phytochemistry*, **9**, 295 (1969).
4. F.T. Lorente and F. Ferreres, *Revista de Agroqimica y Tecnologia de Alimentos*, **28**, 581 (1988).
5. S. Ouafi, R. Gaceb-Terrak, N.R. Bounaga and P. Lebreton, *Compt. Returns. Acad. Sci. Paris, Sér.III*, **306**, 399 (1987).
6. J. Kesselmeier and M. Staudt, *J. Atmospheric Chem.*, **33**, 23 (1999).
7. K.R. Markham, Techniques of Flavonoid Identification, Academic Press, London, pp. 36-51 (1982).
8. Y.Z. Chen, H.D. Zhang and S.M. Zhang, *Maxim. Chem. J. Chin. Univ.*, **10**, 260 (1989).
9. R. Ranganathan, S. Nagarajan, T. J. Mabry, L. Yong-Long and P. Neuman, *Phytochemistry*, **19**, 2505 (1980).
10. B.P. Silva, L.S.M. Velozos and J.P. Parente, *Fitoterapia*, **71**, 663 (2000).
11. P.K. Agrawal, Carbon 13-NMR of Flavonoids, Elsevier Science, Amsterdam, Oxford, Tokyo, New York (1989).
12. T.J. Mabry, K.R. Markham and M.B. Thomas, The Systematic Identification of Flavonoid, Springer Verlag, Berlin (1970).
13. J.B. Harborne and C.A. Williams, in eds.: J.B. Harborne, T.J. Mabry and H. Mabry, The Flavonoids, Chapman and Hall, London (1975).
14. K.R. Markham, B. Ternai, R. Stanley and H. Geiger and T.J. Mabry, *Tetrahedron*, **34**, 1389 (1978).
15. E. Niki, A. Kawakami, M. Saito, Y. Yamamoto, J. Tsuchiya and Y. Kamiya, *J. Biol. Chem.*, **260**, 2191 (1985).
16. C.B. Ahn, Y.J. Jeon, D.S. Kang, T.S. Shin and B.M. Jung, *Food Res. Int.*, **37**, 253 (2004).

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