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A NEW FLAVONOL GLYCOSIDE FROM *POLYGONUM BELLARDII* ALL. GROWING IN EGYPT

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تم فى هذا البحث فصل مركب فلافونول جليكوزيد جديد هو ميرستين ٣ ـ أ (٥" استيل الفا ـ اربينوفيورانوزيد) من الأجزاء الهوائية لنبات البوليجونم بيلاردى ألين النامى فى مصر مع مركبات أخرى هى كوراستين٣ ـ أ (٥" استيل الفا ـ اربينوفيورانوزيد) وكوارستين ٣ روتينوزيد (روتين) وألفا أميرين وبيتا سيتوستيرول وبيتا سيتوستيرول ٣ ـ أ بيتا جليكوزيد. وقد تم التعرف على المركبات المفصولة باستخدام الطرق الطيفية المختلفة.

A new flavonol glycoside named myricetin-3-O-(5''-acetyl α-*arabinofuranoside) has been isolated from Polygonum bellardii All. F. Polygonaceae growing in Egypt, together with quercetin-3-O-(5''*-acetyl α-*arabinofuranoside), quercetin-3-O-rutinoside* (rutin), α-amyrin, β-sitosterol and β-*sitosterol-3-O-*β-*glucoside. Their structures were elucidated using different spectral techniques.*

INTRODUCTION

The genus *Polygonum* (F. Polygonaceae) comprises about 200 species mostly distributed in the North temperate countries, a few are tropical or in the Southeren hemisphere.[1&2] Many species of the genus *Polygonum* had been reported to exhibit a variety of interesting biological activities such as: laxative, expectorant, diuretic, tonic, anti-inflammatory, anti-allergic, insect-icide, anthelmintic, astringent and haemostatic.[3-6] In addition, some species used in treatment of suppurative dermatitis, gonorrhea, chronic gastritis, dudinal ulcers, gout

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and haemorroids.[6-9] Nothing was mentioned about the folkloric uses of *Polygonum bellardii* All. in available literature. The plant grows in sandy places around Alexandria, Mandara, Abukir, Damietta, Tel-El-Kebir, Assiut, Ekhmim, Dendera, Farafra and Kharge. The plant is known locally as Maksus-El-Gariyla, Qardab or Qordob.[10] A variety of chemical constituents as flavonoids, anthraquinones, naphthoquinones, sesquiterpenoids, lignans, coumarins, and stilbenes have been reported in the genus *Polygonum*.[11-17] It was, this deemed of interest to investigate *Polygonum bellardii* All. growing in Egypt for its phytoconstituents.

EXPERIMENTAL

General procedures

Electrothermal 9100 Digital Melting Point Instrument (England Ltd., England) was used for determination of m.ps. Shimadzu Infrared-470 spectrophotometer (Japan) for recording infrared spectra. JEOL JMS 600 Hz for EI-MS (Japan). Varian mercury 300 MHz NMR Spectrometer (Oxford) for recording ^{1}H and ^{13}C-NMR using TMS as internal standard. Silica gel (70-230 mesh, E. Merck, Germany) for column chromatography. Moderate pressure liquid chromatography (MPLC); CIG column system (22 mm i.d. x 30 cm, Kusano Scientific Co., Tokyo, Japan) was used for final purification. Precoated silica gel $G_{60}F_{254}$ and RP-18 plates for TLC (E-Merck, Germany). Sheets of Whatman No. 1 filter paper (Whatman, Ltd., England) for paper chromatography. The following solvent systems were used:

I- n-Hexane-EtOAc (9:1 v/v)
II- EtOAc-MeOH (8.5:1.5 v/v)
III- $CHCl_3$-MeOH-H_2O (7.5:2.5:0.3 v/v)
IV- n-Butanol-AcOH-H_2O (4:1:2 v/v)
V- MeOH-H_2O (6:4 v/v).

Plates were visualized by spraying with 10% v/v H_2SO_4 in MeOH and/or 5% $AlCl_3$ in MeOH.

Plant material

The aerial parts of *Polygonum bellardii* All. was collected during flowring stage in April 2004 from the fields of Assiut governorate and kindly identified and authenticated by Prof. Dr. M. M. M. Zarea, Professor of Taxonomy, Faculty of Science, Assiut University.

Authentic reference materials

β-sitosterol, β-sitosterol glucoside, α amyrin, quercetin, myricetin and rutin were obtained from the Pharmacognosy Department, Faculty of Pharmacy, Assiut University, Assiut, Egypt. Authentic sugars: D-glucose, L-rhamnose, L-arabinose and L-galactose were provided by El-Naser Pharmaceutical and Chemical Co., Egypt (ADWIC).

Extraction and isolation

The air-dried powdered aerial parts (500 g) of *Polygonum bellardii* All. was extracted by maceration and percolation with methanol till complete exhaustion. The combined

methanolic extract was concentrated under reduced pressure to a syrupy consistency. The solvent-free residue (45 g) was subjected to successive solvent fractionation with n-hexane, chloroform and ethyl acetate till complete exhaustion in each case and different fractions were dried over anhydrous sodium sulphate.

The n-hexane-soluble fraction (15 g) was chromatographed on silica gel column. Elution was started with n-hexane followed by n-hexane - EtOAc gradient (fractions 100 ml, each were collected). Fractions eluted with n-hexane - EtOAc 9.5:0.5 afforded compound **P1** (40 mg), while fractions eluted with n-hexane - EtOAc 9:1 afforded compound **P2** (80 mg).

The chloroform-soluble fraction (8 g) was chromatographed on silica gel column. Elution was started with $CHCl_3$ followed by $CHCl_3$-MeOH gradient (fractions 100 ml, each were collected). Fractions eluted with $CHCl_3$-MeOH (9:1) afforded compound **P3** (100 mg).

The ethyl acetate-soluble fraction (10 g) was chromatographed on silica gel column. Elution was started with $CHCl_3$ followed by $CHCl_3$-MeOH gradient (fractions 100 ml, each were collected). Fractions 12-20 eluted with $CHCl_3$-MeOH (8.5:1.5) were subjected to MPLC for final purification using RP-18 pre-packed column and MeOH - H_2O gradient. Fractions eluted with MeOH-H_2O (6.5:3.5) afforded compound **P4** (60 mg), fractions eluted with MeOH-H_2O (6:4) afforded compound **P5** (30 mg) and fractions eluted with MeOH-H_2O (4:6) afforded compound **P6** (25 mg). The fractions were checked for purity using system V as a developer.

Acid hydrolysis[18&19]

About 20 mg of the glycoside to be hydrolyzed was dissolved in 10 ml methanol to which an equal volume of 10% sulphuric acid was added. The mixture was refluxed on a boiling water bath for 3 hours during which samples were withdrawn and tested chromatographically to ensure complete hydrolysis. The hydrolyzate was shaken with ether (50 ml x 3). The ether was distilled off and the aglycone was crystallized from methanol and subjected to TLC and PC. The acidic mother liquor containing the sugar moiety(s) was neutralized with barium carbonate and filtered. The filtrate was concentrated under reduced pressure and separately spotted alongside with authentic sugars "D-glucose, L-rhamnose, L-arabinose and L-galactose" on Whatman No. 1 sheets developed in system IV.

Compound PI (α-amyrin): obtained as colourless needles from acetone, m.p 184-186°, R_f = 0.6 (system I). IR ν_{max} (KBr) cm^{-1} showed the following absorption bands: 3450 (OH), 2940 (C-H), 1385 (C-O) and 1051 (C-OH).

Compound P2 (β-sitosterol): obtained as colourless needles from MeOH, m.p 135-137°, R_f = 0.48 (system I). IR ν_{max} (KBr) cm^{-1} showed

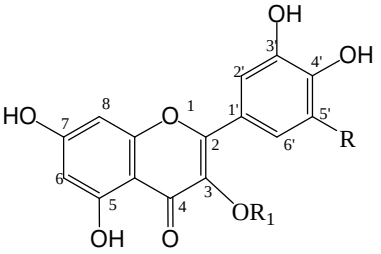
the following absorption bands: 3435 (OH) and 2930 (C-H).

Compound P3 (β-sitosterol-3-O-β-glucoside): obtained as a white amorphous powder from MeOH, m.p 288-289°, R_f = 0.53 (system II). IR ν_{max} (KBr) cm^{-1} showed the following absorption bands: 3455 (OH) and 2935 (C-H).

Compound P4 [Quercetin-3-O-(5''-acetyl α-arabinofuranoside)]: obtained as a yellow powder from MeOH, R_f = 0.50 (system, II). The UV spectral data in MeOH and with different ionizing and complexing reagents are listed in Table 1. The ^{1}H-NMR spectral data (DMSO-d_6, 300 MHz) are listed in Table 2, while the ^{13}C-NMR spectral data (DMSO-d_6, 75 MHz) are compiled in Table 3. EI-MS showed peak at m/z 302 corresponding to quercetin aglycone.

Compound P5 [Myricetin-3-O-(5''-acetyl α-arabinofuranoside)]: obtained as a yellow powder from MeOH, R_f = 0.45 (system, II). The UV spectral data in MeOH and with different ionizing and complexing reagents are listed in Table 1. The ^{1}H-NMR spectral data (DMSO-d_6, 300 MHz) are listed in Table 2, while the ^{13}C-NMR spectral data (DMSO-d_6, 75 MHz) are listed in Table 3. EI-MS of the compound showed a peak at m/z= 318 corresponding to myricetin aglycone.

Compound P6 [Quercetin-3-O-rutinoside (Rutin)]: obtained as a yellow powder from MeOH, R_f = 0.38 (system, III). The UV spectral data in MeOH and with different ionizing and complexing reagents are listed in Table 1. The ^{1}H-NMR spectral data (DMSO-d_6, 300 MHz) are compiled in Table 2. EI-MS of the compound showed a peak at m/z 302 = $[M - (glucose + rhamnose)]^+$ ascribable to quercetin aglycone and a peak at m/z 274 assigned to $[M-(glucose+rhamnose+CO)]^+$.



Compound P4	Compound P5	Compound P6
R = H	R = OH	R = H
R_1 = acetyl arabinose	R_1 = acetyl arabinose	R_1 = rutinose

Table 1: UV Spectral Data of Compound **P4 - P6** in methanol and with Different Ionizing and Complexing Reagents.

Comp.	Band	λ_{max} and $\Delta\lambda_{max}$ nm										
		MeOH	NaOMe		$AlCl_3$		$AlCl_3$/HCl		NaOAc		NaOAc/H_3BO_3	
		λ_{max}	λ_{max}	$\Delta\lambda$	λ_{max}	$\Delta\lambda$	λ_{max}	$\Delta\lambda$	λ_{max}	$\Delta\lambda$	λ_{max}	$\Delta\lambda$
P4	II	257	291	+34	264	+7	265	+8	275	+18	262	+5
	I	351	400	+49	422	+71	392	+41	374	+23	371	+20
P5	II	251	281	+30	263	+12	262	+11	270	+19	255	+4
	I	352	405	+53	415	+63	395	+43	380	+28	272	+20
P6	II	258	288	+30	275	+17	272	+14	273	+15	266	+8
	I	358	419	+61	425	+67	400	+42	381	+23	374	+16

Table 2: ^{1}H-NMR Spectral Data of Compounds **P4 - P6** (DMSO-d_6, 300 MHz).

Assignment	**P4**		**P5**		**P6**	
	(δ) ppm (No. of proton, multiplicity)	J (Hz)	(δ) ppm (No. of proton, multiplicit)	J (Hz)	(δ) ppm (No. of proton, multiplicity)	J (Hz)
5-OH	12.61, (1H, s)	-	12.59, (1H, s)	-	12.57, (1H, s)	-
Phenolic OH groups	10.84, 9.68, and 9.24, (1H, s, each)	-	-	-	-	-
H-6	6.20, (1H, d)	2.1	6.20, (1H, d)	2.1	6.18, (1H, d)	1.8
H-8	6.40, (1H, d)	2.1	6.40, (1H, d)	2.1	6.37, (1H, d)	1.8
H-2'	7.48, (1H, d)	2.4	7.04, (1H, s)	-	7.54, (1H, d)	2.1
H-5'	6.86, (1H, d)	8.1	-	-	6.84, (1H, d)	9.3
H-6'	7.45, (1H, dd)	8.1, 2.4	7.04, (1H, s)	-	7.51, (1H, dd)	2.1, 9.3
H-1"(arab.)	5.47, (1 H, d)	1.5	5.42, (1H, br.s)	-	-	-
H-1" (glc.)	-	-	-	-	5.33, (1H, d)	-
H-1'"(rham.)	-	-	-	-	4.37, (1H, br. s)	7.0
Other sugar protons	3.15-3.80 (m)	-	3.16-3.94 (m)	-	3.2-3.75 (m)	-
CH_3-CO	1.91 (3H, s)	-	1.89, (3H, s)	-	-	-
CH_3 (rham.)	-	-	-	-	0.98, (3H, d)	6.0

Table 3: ^{13}C-NMR Spectral Data of Compound P4 and P5 (DMSO-d_6, 75 MHz).

Carbon No.	Chemical shift (δ ppm)		Carbon No	Chemical shift (δ ppm)	
	P4	**P5**		**P4**	**P5**
2	157.51	157.81	4'	148.46	136.54
3	133.36	133.26	5'	115.67	145.45
4	177.61	177.61	6'	121.65	108.17
5	161.31	161.24	arab.		
6	98.77	98.79	1''	108.40	107.80
7	164.25	164.23	2''	82.05	81.72
8	93.68	93.65	3''	77.73	77.58
9	156.46	156.45	4''	82.05	81.95
10	104.10	104.20	5''	63.94	63.30
1'	121.05	119.94	$\underline{C}H_3$-CO	20.48	20.40
2'	115.53	108.17	CH_3-$\underline{C}O$	170.03	170.21
3'	145.77	145.45			

RESULTS AND DISCUSSION

The methanolic extract of *Polygonum bellardii* All. aerial parts was subjected to solvent fractionation followed by different chromatographic techniques to yield six compounds labelled **P1-P6**.

Compound P1: was identified as α-amyrin from its physical, chemical and chromatographic characters as well as IR spectral data.[20&21] This was confirmed by co-chromatography with authentic sample of α-amyrin.

Compound P2: was identified as β-sitosterol from its physical, chemical and chromatographic characters. This was confirmed by co-chromatography with authentic sample of β-sitosterol.

Compound P3: was identified as β-sitosterol-3-O-β-glucoside from its physical, chemical and chromatographic characters as well as IR spectral data.[20&21] This was confirmed by co-chromatography with authentic sample of β-sitosterol-3-O-β-glucoside. Acid hydrolysis of compound **P3** yielded aglycone and sugar moities. The aglycone was identified as β-sitosterol (m.p, m.m.p and co-chromatography with authentic sample of β-sitosterol using system I as a developer) and the sugar was identified as glucose (co-chromatography with authentic sample of sugars using PC and system IV as a developer).

Compound P4: The UV spectral data of this compound revealed the presence of free OH group at C-4' from the bathochromic shift with NaOMe in band I ($\Delta\lambda_{max}^{NaOMe}$ = +49 nm) with increase in absorbance. A

free hydroxyl group at C-7 was revealed by the bathochromic shift with NaOAc in band II (λ_{max}^{NaOAc} = +18 nm). Presence of orthodihydroxy groups in ring B and free hydroxyl group at C-5 was revealed by the bathochromic shift with $AlCl_3$ in band I ($\Delta\lambda_{max}^{AlCl_3}$ = +71 nm), which decreased upon addition of hydrochloric acid ($\Delta\lambda_{max}^{AlCl_3+HCl}$ = +41 nm). The bathochromic shift with NaOAc-H_3BO_3 mixture in band I ($\Delta\lambda_{max}^{NaOAc+H_3BO_3}$ = +20 nm) confirmed the orthodihydroxy groups in ring B.

^{1}H-NMR spectrum showed two doublet signals at δ 6.40 and δ 6.20 (each 1H, d, J= 2.1 Hz), assigned to H-8 and H-6 in ring A, respectively. It also showed signals at δ 7.48 (1H, d, J= 2.4 Hz), δ 7.45 (1H, dd, J= 8.1, 2.4 Hz) and 6.86 (1H, d, J= 8.1 Hz), assigned to H-2', H-6' and H-5' in ring B, respectively. In addition, ^{1}H-NMR showed a signal at δ 5.47 (1H, d, J= 1.5 Hz) assigned to the anomeric proton of arabinose. The coupling constant (J= 1.5 Hz) of anomeric proton of arabinose indicated its α-configuration. The signals at δ 10.84, 9.68 and 9.24 were assigned to the hydroxyl groups at C-7, C-3' and C-4'.

The singlet at δ 1.91 (3H, s) was assigned to the protons of acetyl group attached to 5-OH group of the sugar moiety.[22]

The ^{13}C-NMR spectrum of compound **P4** showed twenty-one signals corresponding to twenty-two carbon atoms, the signals at δ 108.40 (C-1´´), 82.05 (C-2´´, C-4´´), 77.73 (C-3´´) and 63.94 (C-5´´) together with the signals at δ 20.48 and 170.03 were assigned for acetylated pentose sugar (acetylated arabinose). The other carbon signals were assigned for the aglycone part as cited in Table 3. The downfield shifts of C-5" at δ 63.95 and upfeild shift of C-4" at 82.05 of the sugar moiety compared with the published data[23-25] indicated that, the acetyl group was attached to the 5-OH group of the sugar.

Acid hydrolysis of compound **P4** yielded aglycone and sugar moities. The aglycone was identified as quercetin (m.p, m.m.p and co-chromatography with authentic sample of quercetin using system II as a developer) and the sugar was identified as arabinose (co-chromatography with an authentic sample using PC and system IV as a developer).

EI-MS showed a peak at m/z= 302 corresponding to the aglycone part (quercetin).[22] The EI-MS data together with the ^{13}C-NMR indicated that, compound **P4** is a flavoniod glycoside with the molecular formula $C_{22}H_{20}O_{12}$. From the above mentioned physical, chemical, chromatographic, spectral data (UV, ^{1}H-NMR, ^{13}C-NMR and EI-MS) and acid hydrolysis, compound **P4** was identified as quercetin-3-O-(5"-acetyl α-arabino-furanoside).[18,19,22&26] This compound was previously isolated from *Rodgersia podophylla* aerial parts as a hepatoprotective flavonol[27] and this is the first report of its

isolation from the family Polygonaceae.

Compound P5: The UV spectral data revealed the presence of free hydroxyl group at C-4', as indicated by the bathochromic shift with NaOMe in band I ($\Delta\lambda_{max}^{NaOMe}$ = +53 nm) with increase in absorbance. Presence of a free hydroxyl group at C-7 was revealed by the bathochromic shift with NaOAc in band II (λ_{max}^{NaOAc} = +19 nm). Presence of orthodihydroxy groups in ring B and free hydroxyl group at C-5 was revealed by the bathochromic shift with $AlCl_3$ in band I ($\Delta\lambda_{max}^{AlCl_3}$ = +63 nm), which decreased upon addition of hydrochloric acid ($\Delta\lambda_{max}^{AlCl_3+HCl}$ = +43 nm). The bathochromic shift with NaOAc-H_3BO_3 mixture in band I ($\Delta\lambda_{max}^{NaOAc+H_3BO_3}$ = +20 nm) confirmed the orthodihydroxy groups in ring B.

^{1}H-NMR spectrum of this compound indicated a penta-substituted flavonol. It exhibited two doublet signals at δ 6.40 and 6.20 (each 1H, d, J= 2.1 Hz), assigned to H-8 and H-6 in ring A, respectively and showed signal at δ 7.04 (2H, s), assigned to H-2' and H-6' in ring B. It also showed an anomeric sugar proton at δ 5.42 (1H, br.s) and a singlet signal at δ 1.89 (3H, s), ascribable to the protons of acetyl group attached to 5-OH group of the sugar moiety.[22]

The ^{13}C-NMR spectrum of compound **P5** showed twenty signals corresponding to twenty-two carbon atoms. The signals at δ 107.80 (C-1´´), 81.72 (C-2´´), 81.95 (C-4´´), 77.58 (C-3´´) and 63.30 (C-5´´) together with the signals at δ 20.40 and 170.21 were assigned to acetylated pentose sugar (acetylated arabinose). The other carbon signals were assigned to the aglycone part as cited in Table 3. The downfield shifts of C-5" at δ 63.30 and upfeild shift of C-4" at δ 81.95 of the sugar moiety compared with the published data[23-25] indicate that, the acetate group attached to the 5-OH group of the sugar.

Acid hydrolysis of compound **P5** yielded aglycone and sugar moities. The aglycone was identified as myricetin (m.p, m.m.p, co-chromatography with authentic sample of myricetin using system II as a developer) and the sugar was identified as arabinose (co-chromatography with authentic sample of arabinose using PC and system IV as a developer). EI-MS showed a peak at m/z= 318 corresponding to the myricetin.[22] The EI-MS data together with the ^{13}C-NMR indicated that, compound **P5** is flavoniod glycoside with molecular formula $C_{22}H_{20}O_{13}$. From the above mentioned physical, chemical, chromatographic, spectral data (UV, ^{1}H-NMR, ^{13}C-NMR and EI-MS) and acid hydrolysis, compound **P5** was identified as myricetin-3-O-(5"-acetyl α-arabinofuranoside) which is

reported for the first time in this work.

Compound P6: The UV spectral data of compound **P6** in MeOH and with different ionizing and complexing reagents revealed the presence of free hydroxyl group at C-4' as indicated by the bathochromic shift with NaOMe in band I ($\Delta\lambda_{max}^{NaOMe}$ = +61 nm) with increase in absorbance. Presence of free hydroxyl group at C-7 was revealed by the bathochromic shift with NaOAc in band II (λ_{max}^{NaOAc} = +15 nm). Presence of orthodihydroxy groups in ring B and free hydroxyl group at C-5 was revealed by the bathochromic shift with $AlCl_3$ in band I ($\Delta\lambda_{max}^{AlCl_3}$ = +68 nm), which decreased upon addition of hydrochloric acid ($\Delta\lambda_{max}^{AlCl_3+HCl}$ = +42 nm). Bathochromic shift with NaOAc-H_3BO_3 mixture in band I ($\Delta\lambda_{max}^{NaOAc+H_3BO_3}$ = +16 nm) confirmed the orthodihydroxy groups in ring B.

The ^{1}H-NMR spectrum showed a typical flavonol structure with trisubstituted benzene ring B and tetrasubstituted benzene ring A. It showed two doublet signals at δ 6.37 and 6.18 (each 1H, d, J= 1.8 Hz), assigned to H-8 and H-6 in ring A, respectively as well as signals at δ 7.54 (1H, d, J= 2.1 Hz), δ 7.51 (1H, dd, J= 2.1, 9.3 Hz) and 6.84 (1H, d, J= 9.3 Hz), ascribable to H-2', H-6' and H-5' in ring B, respectively. In addition, ^{1}H-NMR showed two anomeric sugar protons, one for glucose at δ 5.33 (1H, d, J= 7.0 Hz) and the other for rhamnose at δ 4.37 (1H, br.s) indicating its bioside nature. The appearance of a characteristic doublet at δ 0.98 (3H, d, J= 6.0 Hz) confirmed the presence of rhamnose moiety. The high coupling constant (J= 7.0 Hz) of anomeric proton of glucose indicated its β-configuration, while rhamnose bieng in α-configuration.

Acid hydrolysis of compound **P6** gave aglycone moiety and two sugar units. The aglycone was identified as quercetin (m.p, m.m.p and co-chromatography with authentic sample of quercetin using system II as a developer). The sugars were identified as glucose and rhamnose (co-chromatography with authentic samples using system IV as a developer). The EI-MS showed peak at m/z 302 corresponding to the aglycone part. From the above mentioned physical, chemical, chromatographic, spectral data (UV, ^{1}H-NMR, EI-MS) and acid hydrolysis, compound **P6** was identified as quercetin-3-O-rutinoside (Rutin).[18, 19, 22&26]

REFERENCES

1- L. Boulos and M. N. El-Hadidi, "The Weed Flora of Egypt", The American University, Cairo Press, 1984, pp. 21-34.

2- E. L. Core, "Plant Taxonomy", Prentice-Hall, INC., 1962, pp. 302-303.

3- J. M. Watt and M. G. Breyer-Brandwijk, "The Medicinal and Poisonous Plants of Southern and Eastern Africa", E. and S. Livingstone LTD, Edinburgh and London, 2nd. Ed., 1962, pp. 857-868.
4- K. R. Kirtikar, B. D. Basu and I. C. S. An, "Indian Medicinal Plants", Jayyed Press Delhi Reprint, 2nd. Ed., 1975,Vol. III, pp. 2094-2096.
5- H. Jayasuriya, N. M. Koonchanok, R. L. Geahlen, J. L. Mclaughlin and C.J. Chang, J. Nat. Prod., 55, 696 (1992).
6- M. A. El-Bashier, and A. F. S Ahmed, Egypt. J. Pharm. Sci., 34, 593 (1993).
7- M. L. Zimmermann and A. T. Snedens, J. Nat. Prod., 57, 236 (1994).
8- P. Tsai, J. Wang, C. Chang, S. Kuo and P. L. Chao, Phytochemistry, 49, 1663 (1998).
9- K. Xiao, L. Xuan, Y. Xu and D. Bai, J. Nat. Prod., 63, 1373 (2000).
10- R. Muschler, "A Manual Flora of Egypt", S.H Service Agency, INC., New York, 1970, Vol. I, pp. 255-268.
11- R. H. Thomson, "Naturally Occurring Quinones", Academic Press, London and New York, 2nd. Ed. (1971).
12- A. M. Rizk, "The Phytochemistry of the Flora of Qatar", Scientific and Applied Research Centre, University of Qatar, 1986, pp. 317-322.
13- L. L. Brown, S. R. Larson and A. T. Sneden, J. Nat. Prod., 61, 762 (1998).
14- I. Calis, A. Kuruüzüm, L. Ö. Demirezer, O. Sticher, W. Ganci, and P. Rüedi, ibid., 62, 1101 (1999).
15- X. Sun, M. L. Zimmermann, J. Camagne and A. T. Sneden, ibid., 63, 1094 (2000).
16- M. Takasaki, S. Kuroki, M. kuzuka and T. Konoshima, ibid., 64, 1305 (2001).
17- K. Wang, Y. Zhang and C. Yang, J. Ethnopharmacol., 96, 483 (2005).
18- T. J. Mabry, K. R. Markham and M. B. Thomas, "The Systematic Identification of Flavonoids": Springer Verlag, New York, Heidelberg and Berlin (1970).
19- J. Harborne, and T. Mabry, "The Flavonoids: Advances in Research", 1st Ed., University Press, Cambridge, 1982, pp. 98-99.
20- K. Yamaguchi, "Spectral Data of Natural Products", Elsevier Publishing Co., Amsterdam, London, New York (1970).
21- S. Budavari, The Merck Index: "An Encyclopedia of Chemicals, Drugs and Biologicals", Merck and co. INC, White House, N.J., U.S.A., 12th Ed. (1996).
22- V. Ossipov, K. Numri, J. Leponin, N. Prokopiev, E. Haukioja and K. Pihlaja, Biochemical Systematics and Ecology, 23, 213 (1995).

23- M. Aritomi, T. Komori and T. Kawasaki, Phytochemistry, 25, 231 (1986).
24- E. Breitmaier and. W. Voelter "Carbon-13 NMR Spectroscopy; High Resolution Methods and Application in Organic Chemistry and Biochemistry, New York, NY: VCH (1987).
25- K. R. Markham, B. Ternia, R. Stanly, H. Geiger, and T. J. Mabry, Tetrahedron, 34, 1389 (1978).
26- A. Hasan, I. Ahmed, M. Jay and B. Voirin, Phytochemistry, 39, 1211 (1995).
27- Y. Chin, S. Lim, Y. Kim, S. Choi, K. Lee and J. Kim, Planta Med., 70, 576 (2004).