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Flavonoids from two Cupressaceae Plants

Galal T. Maatooq, Saleh H. El-Sharkawy*, Mohamed S. Afifi, and Jack P. N. Rosazza[1]

University of Mansoura, Faculty of Pharmacy, Dept. of Pharmacognosy, Mansoura 35516 Egypt
[1]University of Iowa, College of Pharmacy, Center for Biocatalysis and Bioprocessing, Iowa City, IA 52242 USA

Abstract – Jaceidin, Jaceidin-7-O-methylether, and quercetin were isolated from *Juniperus phoenicea* L. alcoholic extract, however, Sequoiaflavone was isolated from *Cupressus semperiverns* L. In addition, the alcoholic extracts of both plants were found to contain also kaempferol-3-O-rhamnoside, quercetrin, myricitrin, cupressuflavone. The chemical identities of the isolated compounds were established using UV, IR, ^{1}H-and ^{13}C-NMR spectroscopy.

Key words – *Juniperus phoenicea* L. and *Cupressus semperiverns* L.

Introduction

Juniperus phoenicea L shrubs and trees occurs on the rocky areas of the Mediterranean region (Tackhom, 1974) indigenous to Egypt. *Cupressus semperiverns* L is an ornamental plant growing in Egypt. Juniperus was used for treatment of cough, hemorrhoids and antispasmodic (Issa-Bay, 1930, Al-Antaki, 1923). *J. verginiana* possess diuretic, antibacterial, abortificient and antihypertensive activity (Dallimore and Jackson, 1931), *J. communis* L. exerts diuretic and antiseptic properties and *C. sempervirens* L. was used for treatment of hemorrhoids, chronic cough and as a strong hair tonic (Hussein). Cupressuflavone and amentoflavone were isolated from the extracts of *C. torulosa* (Marti *et al.*, 1964). *C. macrocarpa* (Abul Qasim *et al.*, 1985).

J. macropoda (Ilyas. *et al.*, 1977, Fatma *et al.*, 1978), *J. phoenicea* (Roy *et al.*, 1984), and *J. drupaceae* (Sakar and Friedrich, 1984). Hinokiflavone was also isolated from different extracts of juniper species (Ilyas *et al.*, 1977, Fatma *et al.*, 1978, Roy *et al.*, 1984, Sakar and Friedrich, 1984, Pascual *et al.*, 1980). Sciadopitysin (7,4'4'''-trimethyl-amentoflavone) was isolated from *J. horizontalis* (Hameed *et al.*, 1973) and 7,7'''-di-O-methylcupressu-flavone from *J. recurva* (Hameed *et al.*, 1973). Amentoflavone, hinokiflavone, isocryptomerin, quercetagetin-3-O-rhamnoside, quercetin-3-O-rhamnoside and kaempferol-3-O-glucoside were also isolated from the leaves of *J. macropoda* (Ilyas *et al.*, 1977). Robustoflavone, and monomethylhinokiflavone were isolated from the leaves of *J. macropoda* (Fatma *et al.* 1978). It was also reported that the acetone extract of *C. jusitanica var. benthani* leaves contains podocarpusflavone, monomtheylhinokiflavone and 1-4'-O-methylcupresso-flavone (Taufeeq *et al.*, 1978). It was reported that the leaves of *J. phoenicea* contains in addition, robustoflavone, and monomethylhinokiflavone (Fatma *et al.*, 1978); While, *J. macropoda* contains carpusflavone-A and isocryptomerin. Monomethylamentoflavone, apigenin, robustoflavone and monomethylhinoki-

*Author for correspondence.

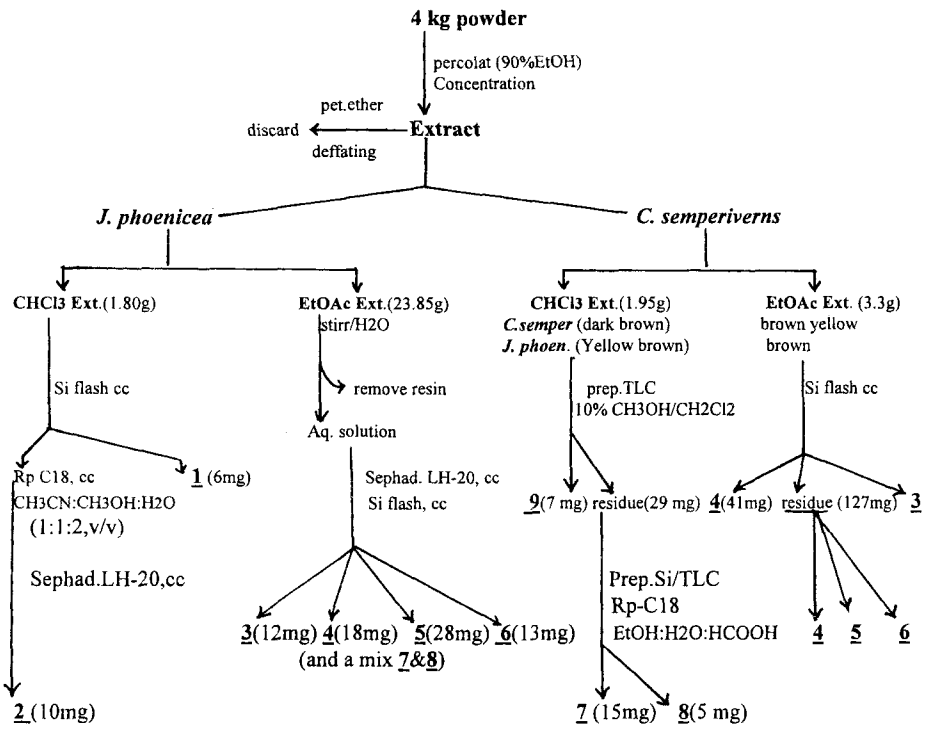
flavone were isolated from the leaf extract of *C. australis* (syn. *Callitris rhamboidea*) (Vidyapati *et al.*, 1979). 6-hydroxyapigenin (scuttellarin)-6-xyloside and 6-hydroxyluteolin-6-xyloside were isolated from *J. communis* fruits (Sethi *et al.*, 1981). Treatment of its ether extract with $NaHCO_3$ resulted in the isolation of hinokiflav one, and cupressuflavone (Pascual *et al.*, 1980). Five isoflavones and three glycosidal isoflavones were isolated from the alcohol extract of *J. macropoda*. Agathisflavone and isocryptomerin were isolated from *J. verginiana* leaves extracts (Roy *et al.*, 1984), while amentoflavone, hinokiflavone, cupressuflavone and quercetin were the major constituents of *J. drupaceae* (Sakar and Friedrich, 1984). Amentoflavone, cupressuflavone, and sequoiaflavone were isolated from *C. gracilis* and *C. macrocarpa* (Abul Qasim *et al.*, 1985). Amentoflavone was found universally distributed among all species and 4'''-monomethylamentoflavone, 7,4'-dimethylamentoflavone, and cupressuflavone were detected in most species (Gadek and Quin, 1985).

Juniperus and Cupressus are generally containing amentoflavone, cupressuflavone, and absence of any methylated biflavonyl (Gadek and Quin, 1985). Six biflavonoids were isolated from *J. indica* and five from *J. verginiana* (Balbaa *et al.*, 1976).

This study deals with a thorough investigation of *J. phoniceae* and *C. sempervirens* (Family Cupressaceae) extracts growing in Egypt. The chemical identity of the isolated flavonoids are reported herein using different spectroscopic techniques.

Experimental

Materials and Methods – Aerial parts of *J. phoniceae* and *C. sempervirens* were collected early September 1988 from localities near Mansoura, Egypt. The plants were identified and authenticated by Dr. Ali Hamza, Prof. of ornamental plants, Faculty of Agriculture, University of Mansoura, Egypt.



Scheme 1. Protocol of Isolation of *J. phoenicea* L. and *C. semperiverns* L. flavonoids.

Chemicals – All solvents used for extraction and chromatography were of analytical reagent grades. Anisidine phthalate (0.10 M of p-anisidine and phthalic acid in 95% EtOH) (Balbaa *et al.*, 1976). All authentic sugars were obtained from E. Merck, Germany). L-rhamnose (BDH Chemical Co., Pool, UK) α-Glucosidase and β-glucurinadase were purchased from Sigma Chemical Co., St. Louis, Mo, USA.

Instruments – Melting points were determined in open-ended capillary tubes using a Thomas-Hoover melting point apparatus and are uncorrected. IR spectra were determined on a Nicolet 5DXB FT-IR spectrometer (Shimadzu Corporation, Japan). Mass spectra were obtained on Kratos MS-50 triple analyzer, using xenon as a Carrier gas and 3-nitrobenzyl alcohol (3-NBA). ^{1}H- and ^{13}C-NMR, DEPT, HMBC and Selective INEPT experiments were obtained on Bruker WM-360 FT and AMX 600 FT spectrometer. Chemical shifts values are in ppm and J values in Hz and TMS as internal standard.

Plant Extraction – A total of 4 kg of air-dried powdered plants were extracted according to the protocol (Scheme 1).

Results and Discussions

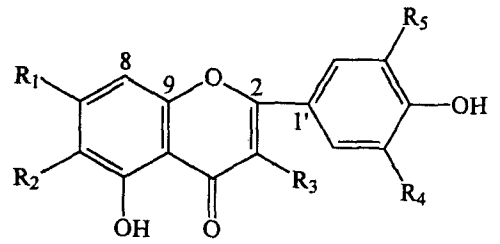
Jaceidin 1, Jaceidin-7-O-methylether 2, Quercetin 3, were isolated from *J. phoenicea* alcoholic extract, however, the biflavonoid; sequoiaflavone 9, was isolated from *C. semperiverns* L extracts. Both plants were found to contain also kaempferol-3-O-rhamnoside 4, quercitrin 5, myricitrin 6, cupressuflavone 7, and amentoflavone 8. The protocol of extraction and purification of these compounds are described in scheme 1. Compounds 3-6 and 9 were identified as previously reported (Al, 1923; Dallimore *et al.*; Abul *et al.*, 1985).

Compound 1 – Obtained as a yellow amorphous powder, mp 135-136°C. Its flavonoidal nonglycosidal nature was indicated by giving red color with Shinoda's and negative Molish's tests (Balbaa *et al.*, 1976). UV spectral data (CH_3OH) 274.5 nm (band II) and 353 nm (band I). $AlCl_3$ and $AlCl_3$/HCl spectra gave 36 and 19 nm batho-and hypsochromic shifts of band I, respectively. This reveals the absence of ortho-diOH (ring B) and presence of free 5 OH group and an oxygenation of position 6 (Mears and Mabry, 1972). NaOAc spectrum showed a 58 nm bathochromic shift of band I, no significant effect on band II and absence of any shoulder at 332-334 nm confirming the absence of free 7-OH (Mabry *et al.*, 1970). MS(CI/CH_4) m/z 403 (M^++29.12%), 375(M^++1,100%), 374 (M^++43%), 360(M^++1-CH_3, 4%), 359(M^++-CH_3, 10%), 345 (M^++1-2CH_3, 4%), 344(M^+-2CH_3, 2%), 330(M^+-CH_3, 1%), 275(30%), and 259(36%). ^{1}H-NMR spectra showed 6 singlets, two at δ_H 7.68 (H_2), 6.93(H_3 or H_8) and four at δ_H 3.93, 388, 3.82, and 3.74 integrated for 3 protons and assigned for OCH_3 at positions 3,6 and 3', respectively. These findings were confirmed using HMBC spectral data which showed that the signal at δH 6.93 crossed each of the signals at C_6, C_7, C_9, C_{10} and $C_{3'}$ (not C_4)-OCH_3 based on two criteria a) It showed a crossing interaction with δc 147.32($C_{3'}$) and 115.48($H_{5'}$). b) No crossing with $H_{6'}$ signal indicating its location at 3 bond away. ^{13}C-NMR displayed four signals at δc 55.65, 56.30, 59.57, and 59.89 for four OCH_3 groups. The signals δc 91.28(C_8 unsubstituted), 131.40(C_6-OH), 137.56(C_3-OH) confirms that the signal at δc 178 of C4 and flavonol ring. Nine oxygenated carbons were counted between 131-178 ppm i.e.six hydroxylated, two ether, and one carbonyl. These results confirmed 1 as Jaceidin-7-methylether (chrysoplenetin).

Compound 2 – Obtained as amorphous powder, mp 148-149°C, it is confirmed as a nonglycosidal flavonoid (Balbaa *et al.*, 1976). UV spectral data showed 269 nm (band II) and 351 nm (band I). $AlCl_3$ and $AlCl_3$/HCl spectra gave 36 and 17 nm bathochromic shifts of band I revealing the absence of ortho di-OH (ring B) and the presence of free 5-OH group and an oxygenated C_6 position (Mears

and Mabry, 1972). NaAc spectrum showed a 43 and 5 nm bathochromic shift of band I and band II, respectively, indicating free C_4-OH and C_7-OH groups, respectively. The presence of a shoulder at 333 nm in the CH_3ONa spectrum confirmed the free C_7-OH. MS (CI/CH_4) m/z 389(M^++29, 7%), 361(M^++1.42%), 360(M^+, 23%), 345(M^++1-CH_3, 17%), 331(M^++1-2CH_3, 19%), 275(30%), 259(36%), and 85(100%). ^{1}H-NMR spectral data showed that it differs from 1 in the following 1) Presence of three OCH_3 signals at δc 3.73(C_6), 3.79(C_3), and 3.85 (C_3 2). The 6.47(H_8) singlet was shielded by 0.46 ppm consistent with the free C_7-OH assignment. ^{13}C-NMR spectral data is identical to 1 with few differences a) Absence of C_7-OCH_3 signal at dc 56.36 b) C_8 signal at δc 94.32 is shifted 3 ppm downfield from its corresponding of 1(ortho to C_7-OH). c) C_{10} signal at δc 104.20(meta to C_7-OH) is shifted upfield by 1.20 ppm. From this, it is concluded that compound 2 is 3,5,6,7,3',4'-hexahydroxyflavone-3,6,3'-trimethylether(7-demethyl derivatives of 1).

Compound 7– Amorphous powder, mp>360, its flavonoidal nonglycosidal nature was con-



Compound	R_1	R_2	R_3	R_4	R_5
1. Jaceidin-7-O	OCH3	OCH3	OCH3	OCH3	H
methylether	OH	OCH3	OCH3	OCH3	H
2. Jaceidin	OH	H	OH	OH	H
3. Quercetin	OH	H	O-Rhamn	H	H
4. Kaempferol-3-O-Rhamn.	OH	H	O-Rhamn	OH	H
5. Quercitrin		H	O-Rhamn	OH	H
6. Myricitrin					

Fig. 1. Structure of Isolated Flavonoids.

Table 1. ^{13}C-and ^{1}H-NMR Spectral Data of the Isolated Biflavonoids

C#	7		8		9		H#	7		8		9	
	I	II	I	II	I	II		I	II	I	II	I	II
2	163.1	163.10	165.20	165.20	165.20	165.20	3	6.81	6.81	6.90	6.85	6.90	6.79
								s	s	s	s	s	s
3	102.1	102.10	103.48	103.48	102.41	102.41							
4	181.7	181.80	180.50	181.75	181.75	181.82	6	6.49	6.49	6.34	6.47	6.38	6.37
								s	S	d,J=1	s	d,J=1	s
5	160.5	160.50	162.00	161.00	161.81	161.01	8			6.71	7.62,d	6.78	
6	99.9	100	100.4	100.1	97.98	100.70				d,J=1	J=8.7	d,J=1	
7	161.0	161.0	164.20	163.15	164.30	163.00	2'	7.52,d	7.52,d	8.10,d	7.62,d	8.06,d	7.58,d
								J=8.7	J=8.7	J=1.5	J=8.7	J=1	J=8.5
8	99.90	99.90	95.26	104.05	92.70	104.21							
9	154.50	154.50	157.22	156.16	157.16	154.70	3'	6.76,d	6.76,d		6.82,d		6.71,d
								J=8.7	J=8.7		J=8.7		J=8.5
10	102.90	102.90	105.40	105.42	106.20	105.31							
							5'	6.76,d	6.76,d	7.22,d	6.82,d	7.13,d	6.71,d
								J=8.7	J=8.7	J=8.5	J=8.7	J=8.2	J=8.5
1'	121.00	121.00	123.18	123.20	121.54	121.62	6'	7.52,d	7.52=d	8.02,dd	7.62,d	8.04,d	7.58,d
								J=8.7	J=8.7	J=1.5,8.5	J=8.7	J=1.8,2	J=8.5
2'	127.50	127.50	128.90	129.22	126.99	128.08							
3'	115.60	115.60	121.90	116.92	122.04	115.57	7-					3.83	
4'	161.00	161.00	159.10	161.78	160.44	161.62	OCH3						
5'	115.60	115.60	117.73	116.96	118.26	115.57							
6'	127.50	127.50	132.87	129.22	131.42	128.08							
7-OCH3					56.00								

^{13}C-and ^{1}H NMR at 90.56 and 360 MHz, respectively (DMSO-d_6).

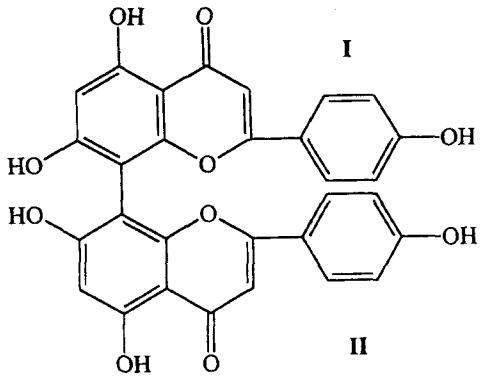
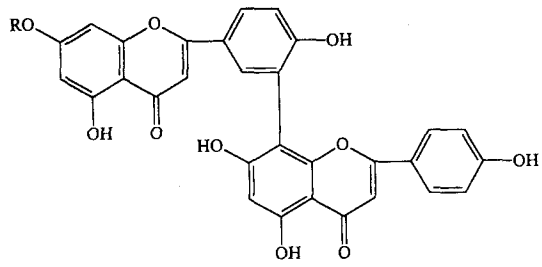


Fig. 2. Structure of Cupressuflavone 7 (C_8-C_8 linkage).



Compound	R
8. Amentoflavone	H
9. Sequoiaflavone	CH_3

Fig. 3. Structure of Isolated C_5-C_8 biflavonoods

firmed a s indicated under 1. UV spectral data showed 272 nm (band II), and 305 (sh) an 328 nm (band I) for flavone compounds. $AlCl_3$ and $AlCl_3$/HCl spectra were similar to 1 indicating the absence of ortho di-OH (ring B) and presence of free C_4-OH and C_7-OH and a 9 nm bathochromic shift confirming free C_7 (Mabry *et al.*, 1970). MS(CI/CH_4) m/z 567(M^++29.5%), 539(M^++1, 17%), 538(M^+, 8%), and 521(M^++1-H_2 O, 4%). ^{1}H-NMR spectral data (Table 1) showed 2 singlets integrated for two protons each at δ_H 6.8(H_3), and 6.49(H_6). The absence of the third singlet signal and the presence of two doublets at δ_H 7.52($H_{2,6}$-I&II) and 6.70($H_{3,5}$-I& II), J=8.70 Hz, suggesting a possible dimer. ^{13}C-NMR spectral data (Table 1) displayed six oxygenated carbons (154-182 ppm), two ethers at C_2 & C_9, a C_4-carbonyl and 3 C-OHs at C_5, C_7 and $C_{4'}$ positions. The presence of C_4 signal at δ_c 181.70 and presence of C_3 at δ_c 102.10 confirming the flavone skeleton. The signal at δ_c 99.90 was assigned for C_6-I&II and C_8I&II, while 127.50 and 115.60 were for $C_{2,6}$-I&II and $C_{3,5}$-I&II, respectively. The overall spectra of 7 (Fig. 2) are identical to the reported for cupressuflavone (Harborne and Mabry, 1982).

Compound 8 – Yellow amorphous powder, mp 258-259°C (lit. 260°C)(Dora and Edwards, 1991), its UV and MS spectra were similar to those of 7 indicating the presence of non-glycosidal flavonoid(M^+, m/z 538) with three free OH groups at C_5, C_7 and $C_{4'}$. ^{1}H-NMR spectral data (Table 1) showed two sets of AB and ABC systems and the overall integration of 12 protons suggesting possible c-c flavonoid dimer. Seven doublets were observed i.e. three-m-coupled doublets at δ_H 8.1(d, H_2, J=1.5 Hz), 6.71 (d, H_8-I, J=1.00 Hz) and 6.34(d, H_6-I, J=1.00 Hz) in addition to the doublets at 8.02(dd, H_6-I, J=8.50, 1.50 hZ), 7.22(d, H_5-I, J= 8.50 Hz), 7.62(d, $H_{2,6}$-II, J=8.7). The presence of a singlet at δ_H 6.47(H6-II) and absence of H_8-II and H_3-I signals suggested the possible engagement in the biflavonoidal link. ^{13}C-NMR spectral data (Table 1) showed two singlets at dc 129.22(C_2) and 116.96($C_{2,6}$-I&II and $C_{3,5}$-I&II) confirming the AB system in II. The carbonyl signal at 180.50 and 181.00 ppm confirm the biflavone skeleton. The presence of 12 oxygenated carbon signals between 155-181 ppm confirm the presence of six hydroxylated ones at C_5, C_7 and $C_{4'}$ of I and II, four ether linked carbons (C_2&C_9-I&II) and two C_4 carbonyls-I& II. The 104.05 ppm signal was assigned to C_9-II confirming its incoporation in the interflavonoidal linkage. The overall spectroscopic data are consistent with those of amentoflavone (Fig. 3) (Dora and Edwars, 1991).

Compound 9 – Yellow amorphous powder, mp 337-339°C (lit. 300°C) . it was identified as sequoiaflavone (Fig. 3) based on the spectroscopic analyses which are similar to reported data (Abul *et al.*, 1985).

This study revealed the c-c methylated biflavonoid, sequoiaflavone was isolated from

C. sempervirens. Cupressuflavone and amentoflavone are common flavonoid pre- sent in both *J. sphoenicea* L and *C. semperiverns* L.

Acknowledgments

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