Arabian Journal of Chemistry (2010) 3, 85–88

King Saud University

Arabian Journal of Chemistry

www.ksu.edu.sa
www.sciencedirect.com

ORIGINAL ARTICLE

First isolation of a flavonoid from *Juniperus procera* using ethyl acetate extract

Adil A. Mujwah [a,*], Mohammed A. Mohammed [b], Mohammed H. Ahmed [c]

[a] *Department of Chemistry, Teachers College, King Saud University, P.O. Box 4341, Riyadh 11491, Saudi Arabia*
[b] *Department of Chemistry, Faculty of Science, Sudan University of Science and Technology, Khartoum, Sudan*
[c] *Department of Chemistry, Faculty of Science and Technology, Ummdurman Islamic University, Sudan*

Received 4 April 2009; accepted 5 October 2009
Available online 10 February 2010

KEYWORDS

Juniperus procera;
Isolation;
Extraction;
Phenolic compound;
Flavonoids

Abstract Phytochemical investigation of the ethyl acetate extract of the leaves of *Juniperus procera* growing in south Saudi Arabia Enemas region led to the isolation of a new flavonoid using different chromatographic methods (i.e. paper, thin layer and column chromatography). The isolated flavonoid was identified and established by m.p., ^{1}H-NMR, ^{13}C-NMR, UV and MS spectral analysis. The isolated compound was identified as 3′,4′,3,7-tetrahydroxyflavone.

1. Introduction

Flavonoids are phenolic compounds widely spread in plants and foods of plant origin (Markham, 1982; Harborne, 1973; Nuutila et al., 2002; Argaez et al., 2007). They contain fifteen carbon atoms in their basic nucleus–flavan, arranged in a C_6–C_3–C_6 configuration consisting of two aromatic rings (A and B) linked by a three carbon unit which may or may not form a third ring (C) Markham, 1982. Rings (A and C) are chromane ring bearing a second aromatic ring B in position 2, 3 or 4. Flavonoids encompass a large group of polyphenolic substances that has antibacterial, anti-inflammatory, antiallergic, antimutagenic, antiviral, antineoplastic and vasodilator effects (Zheng et al., 2007; Mediavilla et al., 2007; Coelho et al., 2006).

Juniperus procera called Arar commonly known in English as African *Juniperus* is a coniferous tree native to the mountain of eastern Africa from east Sudan to Zimbabwe, and southwest of the Arabian Peninsula (widely distributed throughout the southern part of Saudi Arabia) (Gaber et al., 1992). The Arar tree has two kinds of leaves, spreading needle-like and imprecated scale-like (Migahid et al., 1978). It is medium-sized tree reaching 20–25 m (rarely 40 m) (Migahid et al., 1978). It is used locally for the traditional remedy of tuberculoses and Jaundice (Gaber et al., 1992).

It was found in the literature, that a lot of work dealing with *J. procera a*s general studies. While in this study we are focusing only on the isolation of flavonoids from leaves of *J. procera*. Only one flavonoid was isolated and could be identified as; 3′,4′,3,7-tetrahydroxyflavone **1**. This flavones has been isolated from this plant for the first time.

* Corresponding author.
E-mail address: amujwah@ksu.edu.sa (A.A. Mujwah).

1878-5352 © 2010 King Saud University. All rights reserved. Peer-review under responsibility of King Saud University.
doi:10.1016/j.arabjc.2010.02.003

Production and hosting by Elsevier

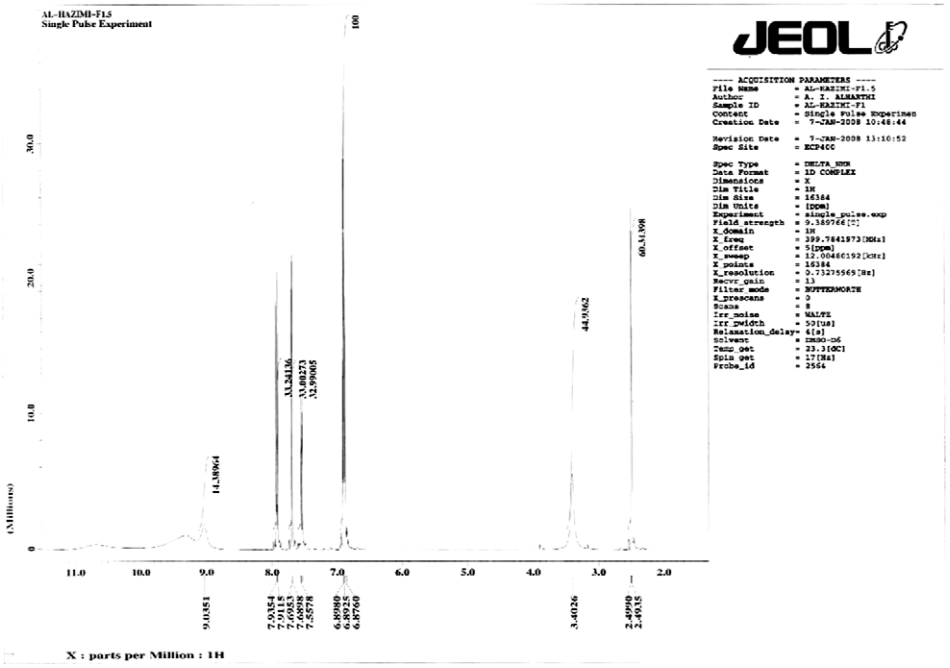

Figure 1 The ^{1}H-NMR chemical shift of flavonoid compound **1** in DMSO-d_6.

2. Materials and methods

The leaves part of this plant was collected from Enemas region south of Saudi Arabia during November 2006 and identified by Dr. Jacob Thomas, Department of Botany, King Saudi University, Riyadh. The plant sample was collected, air dried in shade, separated, grinded to fine powder.

3. Phytochemical study

3.1. Extraction

Dry leaves of *J. procera* (3.75 kg) were percolated with 85% methanol (15 L) at ambient temperature for 5 days. The extract was filtered and the solvent was removed under vacuum. Then, the residue was collected by filtration. The solvent was evaporated in the vacuum to yield 150 g of crude extract (CE) dissolved in 500 ml aqueous mehanol. The aqueous suspension of this extract was partitioned successively with petroleum ether (40–60), ethyl acetate and *n*-butanol. TLC and PC investigation showed that ethyl acetate fraction contained mainly many flavonoids but some of them were traces and others present in butanol fraction.

Table 1 ^{1}H and ^{13}C spectral values δ of compound **1**.

Carbon no.	^{1}H-NMR	^{13}C-NMR
2	–	145.56
3	–	116.11
4	–	172.49
5	7.92 d, J = 9.5 Hz	156.82
6	6.87 d, J = 8.8 Hz	114.75
7	–	162.78
8	6.89 d, J = 8.8 Hz	102.36
9	–	147.77
10	–	115.20
1′	–	127.00
2′	7.68 d, J = 2.20 Hz	145.56
3′	–	115.49
4′	–	123.04
5′	7.55 dd, 2.20, 8.8 Hz	137.71
6′	7.96 dd, 2.20, 8.8 Hz	120.18
OH	9.03	–

3.2. Isolation

A sample of 8 g of ethyl acetate extract was chromatographed on silica gel (400 g, 60 mesh 70–230, Astem) for column (100 × 5 cm) using MeOH/$CHCl_3$ in a ratio of 3:7 as eluent. The column fractions were combined on the bases of their PC and TLC patterns, fractions (F_4–F_{12}), 400 ml were collected and evaporated under reduced pressure to give 13 mg of solid compound **1**, m.p. 250 °C.

3.3. Experimental

Analytical grade solvents were used. The UV spectra were recorded on a Perkin Elmer – Lambda 2 spectrophotometer and UV lamp used for localization of fluorescent spots on TLC and PC. The IR spectra were measured on a Shimadzu IR-8400 spectrophotometer. Nuclear magnetic resonance spectra were recorded on a JEOL DELTA ESP400 MHz NMR spectrophotometer. Melting points (Mps) were determined on a Kofler hot-stage apparatus and uncorrected Mass spectra were recordeded on a SHIMADZU GC/MS-GP5050 spectrophotometer.

3.3.1. 3′,4′,3,7-Tetrahydroxyflavone

This was obtained as yellow crystals, 13 mg, R_f = 0.76, mp. 250 °C. UV–Vis λ_{max} in MeOH: (nm) 248, 281 sh, 307 sh 318 sh 362; ($AlCl_3$) 269 sh, 299, 319 sh, 423; ($AlCl_3$/HCl) 264,

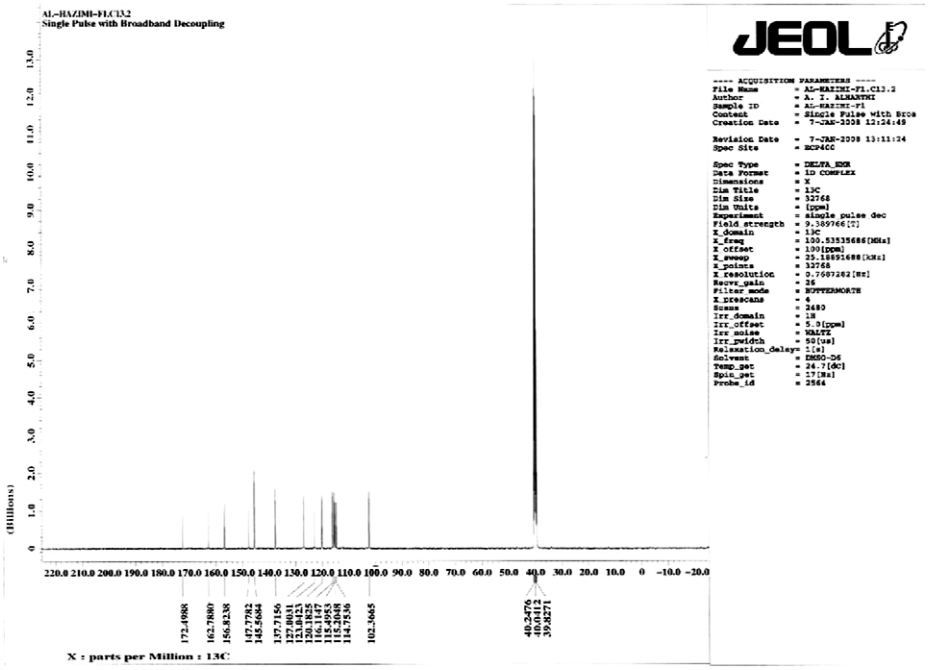

Figure 2 The ^{13}C-NMR chemical shift of flavonoid compound **1** in DMSO-d_6.

296 sh, 320, 458; (NaOAc) 252 sh, 292, 331, 379; (NaOAc/H_3BO_3) 263 sh, 315, 381; (NaOMe) 252, 299, 331, 361, 410. IR (KBr), v = 605, 700 (C–H, Ar), 1097 (C–O, ethe), 1512, 1570 (C═C, Ar), 1604 (C═O) and 3354 cm^{-1} (OH). ^{1}H-NMR (DMSO-d_6), Fig. 1, δ = 6.89 (d, J = 8.8 Hz, 2H, H-6, H-8), 7.54 (dd, J = 2.2 Hz, J = 8.8 Hz, H5′, H-6′), 7.69 (d, J = 2.20 Hz, H-2′), 7.92 (d, J = 9.5 Hz, 1H, H-5), 10–14 ppm (broad singlet, 4-OH), Table 1.

The ^{1}H–1H Cosy correlation between δ_H 6.89 and 7.69 and δ_H 7.19 and 7.52. ^{13}C-NMR (DMSO-d_6), Fig. 2: δ 172.49 (C-4); 162.78 (C-7); 156 (C-5); 147.77 (C-9); 145.56 (C-2 and C-2′); 137.71 (C-5′); 127.00 (C-1′); 123.04 (C-4′); 120.00 (C-6′); 116.11 (C-3); 115.49 (C-3′); 115.20 (C-10); 114.75 (C-6); 102.36 (C-8), Table 1.

4. Results and discussion

4.1. Phytochemical study

The flavonoid compound **1** shown in Fig. 3, which isolated from ethyl acetate extracts of *J. procera*, was identified by comparing its ^{1}H-NMR, ^{13}C-NMR, ^{1}H–^{1}H Cosy NMR and UV spectrum in methanol with different diagnostic shift reagents (NaOMe, $AlCl_3$, $AlCl_3$/HCl, NaOAc, NaOAc/H_3BO_3) with

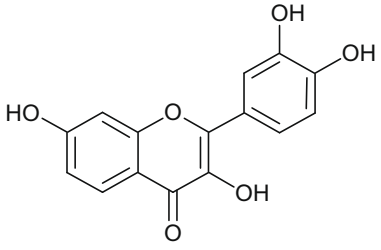

Figure 3 Isolated flavonoid compound **1**.

the published data in literature (Markham, 1982; Agrwal et al., 1989; Markham et al., 1989).

The isolated compound **1** was identified as a flavonoid this was clear from its UV–Vis absorption spectra in methanol with shift reagents. It was a flavonol possess hydroxyl group at position 3; this was clear from the absorption pattern of bands **I** and **II** in the UV–Vis spectra. The absence of free carbon at passion 3 was confirmed by the absence of one singlet at δ 6.79 ppm in ^{1}H-NMR spectrum.

The presence of 3′-OH, 4′-OH was observed from bathochromic shift (+48 nm) as appeared in band **I** with the decrease of intensity upon addition of sodium methoxide (NaOMe). From the ^{1}H-NMR spectrum the presence of the hydroxyl substitution at 3′,4′ was provided by the presence of one signal doublet–doublet at δ 7.54 ppm J = 2.2 Hz, J = 8.8 Hz representing the proton at carbon 5′,6′, respectively and the presence of the other signal as doublet at δ 7.69 ppm J = 2.20 Hz representing carbon at 2′.

The presence of the hydroxyl substitute at 7-position this was provided by the presence of signal doublet at δ 6.89 ppm J = 8.8 Hz representing the proton at carbon 6 and 8, respectively. The presence of signal doublet at δ 7.92 ppm J = 9.5 Hz representing the proton at C-5, which appear at low field due to the deshielding effect of the 4-keto function.

The bathochrom shift (58 +) nm at band **I** upon addition of aluminium chloride($AlCl_3$) which was indicating the presence (i) a catechol system, and when we added hydrochloric acid (HCl) we observed that no increasing of intensity of band **I** and **II**, this confirming the catechol system in B-ring.

Compound **1** substituted in position −7 as indicated by its UV–Vis spectra upon addition of diagnostic shift reagents (NaOAc). When we added boric acid (H_3BO_3) to methanolic sodium acetate bands **1** shifted (+19) nm indicating B-ring catechol system. However, flavonoids with 3′,4′ – hydroxylation pattern give two peaks in the UV–Vis spectrum band **II** has two peaks, the B-ring catechol moiety is probably

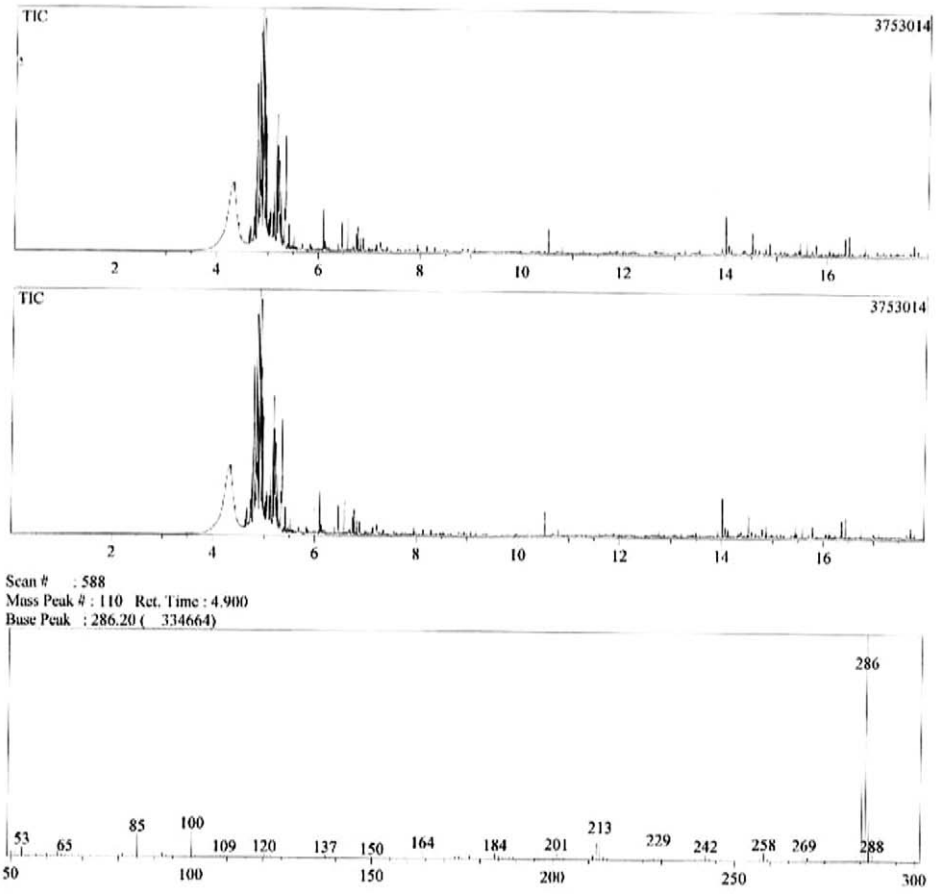

Figure 4 Mass spectrum of flavonoid compound **1**.

located at C-3′ and C-4′. Further evidence in favour of the above structure accumulated from the mass spectrum (Fig. 4) where the molecular ion M^+ 286.

Compound **1** was identified as 3′,4′,3,7 tetrahydroxy-flavone.

References

Agrwal, P.K. (Ed.), 1989. Carbon-13 NMR of Flavonoids, Elsevier Sci. Publishing Co., p. 581.

Argaez, B., Flowers, B.A., Gimenez-Turba, A., Ruiz, G., Waterman, P.G., Pena-Rodriguez, L.M., 2007. J. Phytmed. 11.

Coelho, R.G., Batista, L.M., Santos, L.C., Monteiro de Brito, A.R., Brasileria de Ciencias, W.V., 2006. J. Brazil. Farmaceut. Sci. 42 (3).

Gaber, S.M., Ilias, M., Farouk, S.E., Charles, D.H., 1992. J. Phytochem. 31, 2789.

Harborne, J.B. (Ed.), Phytochemical Methods: A guide to Modern Techniques of Plant Analysis. Chapman and Hall, London, 1973, pp. 1–74.

Markham, K.R. (Ed.), Techniques of Flavonoid Identification. Academic Press, London, 1982, pp. 1–60.

Markham, K.R., 1989. Methods in Plant Biochemistry, vol. 216, Acad. Press Ltd.

Mediavilla, V.G., Pilar, I.C., Collado, S., Esteller, A., Campos, S.S., Tunon, J., Gallego, J.G., 2007. Eur. J. Pharm. 557, 221.

Migahid, A.M., 1978. Flora of Saudi Arabia, vol. 1, Riyadh University Publisher.

Nuutila, A.M., Kammiovirta, K.M., Oksman-Caldentey, K.M., 2002. Comparison of methods for the hydrolysis of flavonoids and phenolic acids from onion and spinach for HPLC analysis. J. Food Chem. 76, 519.

Zheng, W., Gao, X., Chen, C., Tan, R., 2007. Int. Immunopharm. 7, 117.