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## ORIGINAL ARTICLE

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

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## KEYWORDS

*Juniperus procera*;  
Isolation;  
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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., <sup>1</sup>H-NMR, <sup>13</sup>C-NMR, UV and MS spectral analysis. The isolated compound was identified as 3',4',3,7-tetrahydroxyflavone.

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## 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<sub>6</sub>–C<sub>3</sub>–C<sub>6</sub> 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 tubercloses and Jaundice (Gaber et al., 1992).

It was found in the literature, that a lot of work dealing with *J. procera* as 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.

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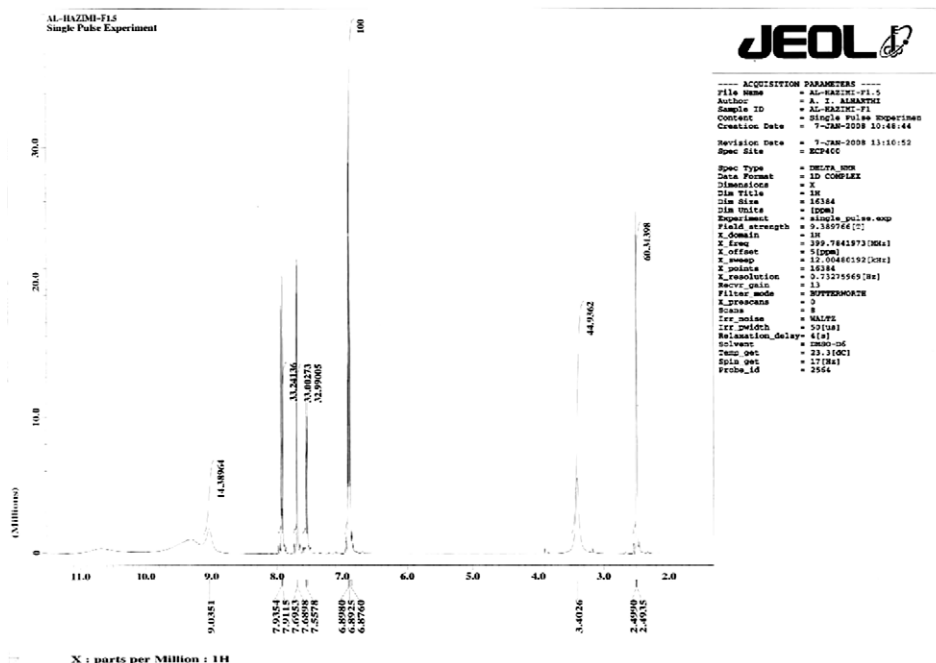


Figure 1 The  $^1\text{H}$ -NMR chemical shift of flavonoid compound **1** in  $\text{DMSO-d}_6$ .

## 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 methanol. 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.

### 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  $\text{MeOH/CHCl}_3$  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 recorded on a SHIMADZU GC/MS-GP5050 spectrophotometer.

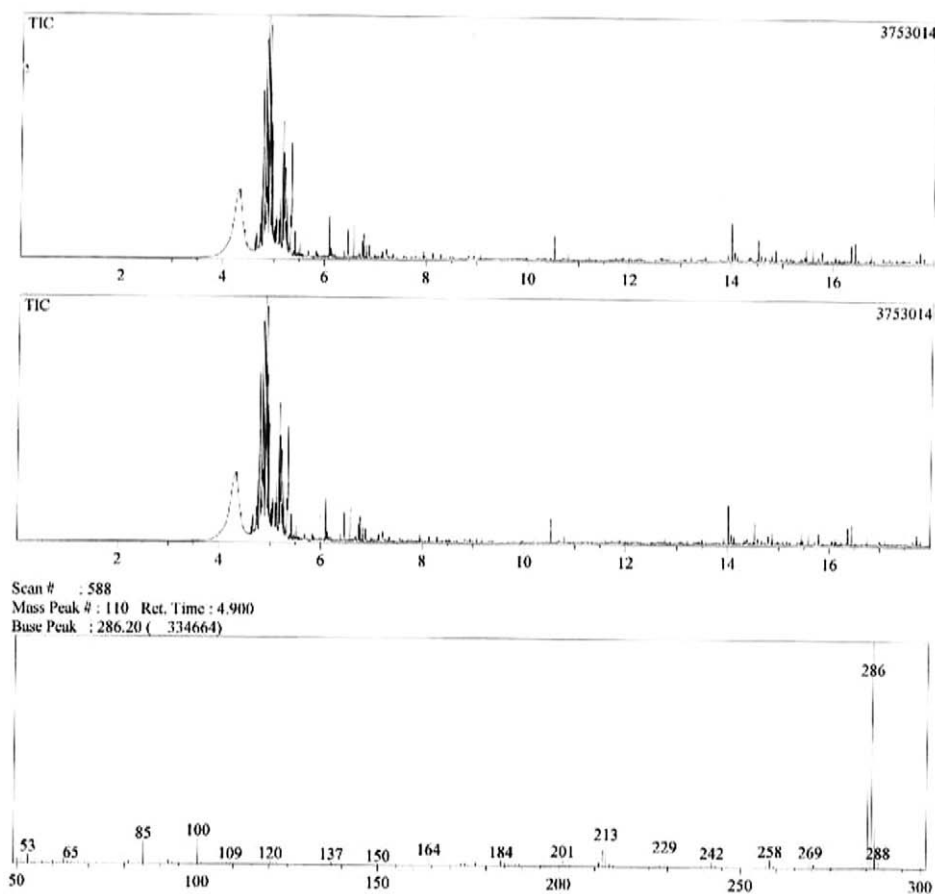
#### 3.3.1. 3',4',3,7-Tetrahydroxyflavone

This was obtained as yellow crystals, 13 mg,  $R_f = 0.76$ , mp. 250 °C. UV–Vis  $\lambda_{\text{max}}$  in MeOH: (nm) 248, 281 sh, 307 sh 318 sh 362; ( $\text{AlCl}_3$ ) 269 sh, 299, 319 sh, 423; ( $\text{AlCl}_3/\text{HCl}$ ) 264,

Table 1  $^1\text{H}$  and  $^{13}\text{C}$  spectral values  $\delta$  of compound **1**.

Carbon no.	$^1\text{H}$ -NMR	$^{13}\text{C}$ -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	–





**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.

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