



**HAL**  
open science

## **Antioxidant, haemolytic activities and HPLC-DAD-ESI-MSn characterization of phenolic compounds from root bark of *Juniperus oxycedrus* subsp *oxycedrus***

Tarik Mohammed Chaouche, Farah Haddouchi, Fouzia Atik-Bekara, Riadh Ksouri, Rachid Azzi, Zahia Boucherit, Choukri Tefiani, Romain Larbat

### ► To cite this version:

Tarik Mohammed Chaouche, Farah Haddouchi, Fouzia Atik-Bekara, Riadh Ksouri, Rachid Azzi, et al.. Antioxidant, haemolytic activities and HPLC-DAD-ESI-MSn characterization of phenolic compounds from root bark of *Juniperus oxycedrus* subsp *oxycedrus*. *Industrial Crops and Products*, 2015, 64, pp.182-187. 10.1016/j.indcrop.2014.10.051 . hal-01475928

**HAL Id: hal-01475928**

**<https://hal.univ-lorraine.fr/hal-01475928>**

Submitted on 23 Aug 2023

**HAL** is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

## **Antioxidant, haemolytic activities and HPLC-DAD-ESI-MS<sup>n</sup> characterization of phenolic compounds from root bark of *Juniperus oxycedrus* subsp. *oxycedrus***

Tarik Mohammed Chaouche <sup>a,\*</sup>, Farah Haddouchi <sup>a</sup>, Fouzia Atik-Bekara <sup>a</sup>, Riadh Ksouri <sup>b</sup>, Rachid Azzi <sup>c</sup>, Zahia Boucherit <sup>c</sup>, Chokri Tefiani <sup>d</sup>, Romain Larbat <sup>e,f</sup>.

<sup>a</sup> *Laboratoire des Produits Naturels, Département de Biologie, Université Aboubekr Belkaïd, B.P 119, Tlemcen, 13000, Algérie*

<sup>b</sup> *Laboratoire des Plantes Extrêmophiles, Centre de Biotechnologie de Borj-Cédria, B.P 901, Hammam-Lif, 2050, Tunisie*

<sup>c</sup> *Laboratoire Antibiotiques, Antifongiques: physico-chimie, synthèse et activité biologique, Département de Biologie, Université Aboubekr Belkaïd, B.P 119, Tlemcen, 13000, Algérie*

<sup>d</sup> *Département des sciences agronomique et des forets, Université Aboubekr Belkaïd, B.P 119, Tlemcen, 13000, Algérie*

<sup>e</sup> *INRA UMR 1121 "Agronomie & Environnement" Nancy-Colmar, TSA 40602, 54518 Vandoeuvre Cedex, France*

<sup>f</sup> *Université de Lorraine UMR 1121 "Agronomie & Environnement" Nancy-Colmar, TSA 40602, 54518 Vandoeuvre Cedex, France*

\*Corresponding author. Tel.: +213 772735216.  
E-mail address: kiratcqa@yahoo.fr (T. M. Chaouche).

### **ABSTRACT**

This study investigates the phenolic compound content and biological activities in four extracts (methanol, water, hexane and dichloromethane) of *Juniperus oxycedrus* subsp. *oxycedrus* root bark. The methanol extract was the most concentrated in total phenolics ( $76.1 \pm 2.8$  mg GAE/g DW), flavonoids ( $39.0 \pm 2.5$  mg CE/g DW) and tannins ( $31.3 \pm 2.1$  mg CE/g DW). HPLC-DAD-ESI-MS<sup>n</sup> analysis of the methanol extract led to the identification of proanthocyanidin oligomers, quercetin hexose, quercitrin, and isorhamnetin hexose. The highest antioxidant activities were found in the methanol extract which exhibited the lowest IC<sub>50</sub> in all the antioxidant assays *i.e.* DPPH, ABTS, oxygen singlet, hydroxyl radical and superoxide anion scavenging assays, inhibition of the  $\beta$ -carotene bleaching and iron reducing assays. Incubation of the four extracts (20 mg/ml) with human erythrocytes for one hour led

to haemolytic activities between 2.05% and 4.37%. The present findings suggest that the root bark could be used as food ingredient.

**Keywords:** *Juniperus oxycedrus*, root bark, antioxidant activity, haemolysis activity, HPLC-MS<sup>n</sup>.

### Highlights

- ▶ The methanol extract has the highest contents in phenolic, flavonoid and tannins
- ▶ The methanol extract contains proanthocyanidin oligomers and glycosylated flavonoids
- ▶ Antioxidant activities of extracts were tested by different methods
- ▶ The methanol extract presents high antioxidant activity
- ▶ *Juniperus oxycedrus* root bark extracts display a low haemolytic activity

## 1. Introduction

Antioxidants can be used in food industry to prevent oxidation or neutralize the free radicals and, consequently, to improve food quality by reducing nutritional losses and by extending the shelf life of food. The most used synthetic antioxidants are butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), butyl gallate (BG), dodecyl gallate (DG), octyl gallate (OG), propyl gallate (PG), tert-butylhydroquinone (TBHQ) and hydroxyl tetramethylchroman carboxylic acid (Trolox). Emerging toxicological data highlighted, however, potential health risks relative to the use of some synthetic antioxidants (Thompson & Moldeus, 1988, Gülçin, 2012). Hence, BHA and BHT have been shown to be carcinogenic, and BHT is associated with the internal and external haemorrhaging at high doses in rodents (Hocman, 1988). In this context, natural products appear healthier and safer than synthetic antioxidants. Nowadays, there is an emerging interest for identifying alternative natural and safe sources of food antioxidants, especially of plant origin, as phenolic compounds and flavonoids (Chaouche et al., 2013a; Gülçin., 2012; Lesjak et al., 2013)

*Juniperus oxycedrus* (Cupressaceae) is one of seventy species in the genus *Juniperus* throughout the world (Akkol, Guvenç, & Yesilada, 2009). *J. oxycedrus* is a shrub or small tree growing wild in stony places of the Mediterranean and Near East countries. Klimko et al., (2007) considered that *Juniperus oxycedrus* could be divided in 4 subspecies: subsp.

*oxycedrus*, subsp. *macrocarpa* Ball (Sibth. & Sm.), subsp. *badia* Debeaux (H.Gay), and subsp. *transtagana* Franco. These subspecies differ in habit, dimension of cones and width of needles. *Juniperus oxycedrus subsp. oxycedrus* is the most abundant in the Algeria.

*Juniperus oxycedrus* is used to prepare empyreumatic oil, obtained from the destructive distillation of the branches and wood of the plant, which is widely employed in human and veterinary dermatology to treat chronic eczema and other skin diseases (Bouhlal et al., 1988). Furthermore, this plant is also used as a folk remedy to treat various illness, such as hyperglycaemia, obesity, tuberculosis, bronchitis, and pneumonia (WHO, 1980).

*Juniperus* extracts exhibit many biological activities *i.e.* antioxidant (Chaouche, Haddouchi, Ksouri, Medini, & Atik-Bekara, 2013b), antiseptic (Miceli et al., 2009, Taviano et al., 2013), antiviral (Sassi, Harzallah-Skhiri, Bourgougnon, & Aouni, 2008), anti-inflammatory (Akkol et al., 2009; Lesjak et al., 2013), analgesic (Akkol et al., 2009), anticancer (Kusari, Zuhlke, & Spiteller, 2010), antidiabetic (Orhan et al. 2012), neuroprotective (Tavares et al., 2012). *Juniperus* extracts are also useful in the treatment of psoriasis (Koerfgen, 1964) and neurasthenic neurosis (Jonkov & Naidenov, 1974). The chemical composition of the essential oil from diverse *Juniperus* sp. has attracted significant medical interest (Loizzo et al., 2007; Orav, Koel, Kailas, & Müürisepp, 2010), however, much less attention has been paid to other secondary compounds. Some reports have highlighted the presence of phenolic compounds including flavonoids and biflavonoids in berries of several *Juniperus* species (Innocenti et al., 2007, Miceli et al., 2009, Taviano et al., 2013), however the phenolic composition in other plant parts and especially roots is still largely unknown.

The objectives of this study were to characterize (i) the biological properties and (ii) the phenolic composition in root bark of *Juniperus oxycedrus subsp. oxycedrus*. *Juniperus* root bark was sequentially extracted with four solvents differing in their hydrophobicity. Each extract was then measured for total phenolic, flavonoid and tannin contents and characterized for their antioxidant capacity using several *in vitro* assays. The methanol extract, which exhibited the highest total phenolic content and the highest antioxidant capacity, was then submitted to HPLC-DAD-ESI-MS<sup>n</sup> analysis to determine its phenolic composition. In addition the haemolytic properties of the four extracts were determined.

## 2. Materials and methods

### 2.1. Plant material and extract preparation

The root bark of *Juniperus oxycedrus subsp. oxycedrus* was snatched in April 2012 from north-west of Algeria (Tlemcen). It was identified in the Laboratory of Natural Products, Department of Biology, University of Tlemcen (Algeria). Voucher specimen was deposited at the herbarium of the laboratory. Ten grams of root bark was ground to a fine powder and then successively extracted with hexane (100 ml), dichloromethane (DCM, 100 ml), methanol (MeOH, 100 ml), and water (100 ml), in a Soxhlet apparatus for 24 h. After filtration of each solvent, the organic phases were concentrated under a vacuum by evaporating to dryness. The percentage of yield for each extract was calculated from the following formula: (weight of extract/weight of dried plant material)  $\times$  100.

## 2.2. Quantification of phenolic classes

The total phenolic content (TPC) was determined using the Folin-Ciocalteu reagent as described by Dewanto, Wu, Adom, and Liu (2002). TPC was expressed as mg of gallic acid equivalents per gram of dry weight (mg GAE/g DW) through a calibration curve (0–400  $\mu$ g/ml range).

Total flavonoid content (TFC) was measured as described by Dewanto et al. (2002). TFC was expressed as mg of (+)-catechin equivalent per gram of dry weight (mg CE/g DW), through a calibration curve of (+)-catechin (0–400  $\mu$ g/ml range).

The total tannin content (TTC) was determined using the vanillin-methanol solution as described by Sun, Richardo-da-Silvia, and Spranger (1998). TTC was expressed as mg of (+)-catechin equivalents per gram of dry weight (mg CE/g DW) through a calibration curve (0 - 400  $\mu$ g/ml range).

## 2.3. HPLC-DAD-ESI-MS<sup>n</sup> analysis

The phenolic composition of the methanol extract (50 $\mu$ l) was analysed on a HPLC-DAD-ESI-MS<sup>n</sup> system consisting in a binary solvent delivery pump (Surveyor MS, Thermo-Finnigan, USA) connected to a diode array detector (Surveyor PDA plus, Thermo-Finnigan, USA) and a LTQ Orbitrap mass spectrometer (Thermo Scientific, Bremen, Germany), equipped with an atmospheric pressure ionisation interface and operated in ESI negative and positive ion modes. The LC separation was achieved on a C18 LichroCART (250 mm  $\times$  4.6 mm) column (Merck, Germany) using the following mobile phase: (A) water formic acid

0.1% and (B) MeOH, acetonitrile, formic acid (50/50/0.1). LC separation consisted in an isocratic step at 1% of B for 2 min followed by a linear gradient from 1% to 40% of B in 100 min, then to 70% of B in 25min and 90% of B in 55 min with a flow rate at 700  $\mu$ l/min. For the mass spectrometer, the conditions were the same as described in Chougui et al. (2013).

The data were processed using the XCALIBUR software program (version 2.1, <http://www.thermoscientific.com>). Experimental exact masses and MS<sup>2</sup> fragmentation data were compared to metabolomics data banks (Pubchem Compound: <http://pubchem.ncbi.nlm.nih.gov/>, ReSpect: <http://spectra.psc.riken.jp/>, Mass Bank: <http://www.massbank.jp>) and available literature in order to identify the nature of the phenolic compounds.

#### *2.4. Determination of antioxidant activities*

There are several methods for the determination of antioxidant activities. The chemical complexity of extracts, often a mixture of dozens of compounds with different functional groups, polarity and chemical behavior, could lead to scattered results. Therefore, an approach with multiple assays for evaluating the antioxidant potential of extracts would be more informative and even necessary. In all tests antioxidants, BHT is used as a positive control.

##### *2.4.1. DPPH assay*

The ability of the corresponding extracts to donate hydrogen atoms or electrons was measured from the bleaching of purple colored methanol solution of 1,1-diphenyl-2-picrylhydrazyl (DPPH), according to the method described by Masuda et al. (1999). Inhibition of DPPH radical was calculated from the following formula: Inhibition (%) =  $[A_0 - A_1/A_0] \times 100$  where  $A_0$  and  $A_1$  are the absorbance at 30 min of the control and the extract, respectively. The antiradical activity was expressed as IC<sub>50</sub> ( $\mu$ g/ml), the extract dose required to cause a 50% decrease of the absorbance at 517 nm. A lower IC<sub>50</sub> value corresponds to a higher antioxidant activity.

##### *2.4.2. Iron reducing power*

The reductive potential of the extracts was determined according to the method of Oyaizu (1986). EC<sub>50</sub> value is the effective concentration at which the absorbance was 0.5 for reducing power.

#### 2.4.3. *ABTS* assay

The determination of ABTS<sup>•+</sup> radical scavenging was carried out as reported by Re et al. (1999). The values of IC<sub>50</sub> were determined as reported above.

#### 2.4.4. *β-carotene bleaching test*

This method is based on the de-coloration of β-carotene by the peroxides generated during the oxidation of linoleic acid (a free radical chain reaction) at elevated temperature. The protocol used is described in Koleva, Teris, Jozef, Linssen, & Lyuba (2002).

#### 2.4.5. *Hydroxyl radical scavenging activity*

The ability of the extracts to scavenge hydrogen peroxide was determined according to the method of Lopes, Schulman, and Hermes-Lima (1999). Hydrogen peroxide was generated with EDTA and 2-deoxyribose.

#### 2.4.6. *Singlet oxygen inhibition*

The capacity of extracts to scavenge singlet oxygen was measured as described by Chandrasekara and Shahidi (2011). Singlet oxygen was generated with DPN and Histidine.

#### 2.4.7. *Superoxide anion radical scavenging activity*

Measurement of superoxide anion scavenging activity was based on the method described by Ani, Varadaraj, and Akhilender Naidu (2006). Superoxide anions were generated in a non-enzymatic phenazine methosulfatenicotinamide adenine dinucleotide (PMS-NADH) system by oxidation of NADH and assayed by reduction of nitroblue tetrazolium (NBT).

#### 2.4. Haemolytic activity

Haemolytic activity of plant extracts was checked by the reported method of Lee et al. (2002). Freshly collected human red blood cells from healthy individuals were employed in this assay. Aliquots of 7 ml of blood were washed two times with sterile isotonic phosphate-buffered saline (PBS) solution (150 mM, pH 7.4) by centrifugation at 4000 rpm for 5 min. The cell suspension was prepared by finally diluting the pellet to 2% in saline solution. A volume of 0.5 ml of the cell suspension was mixed with 0.5 ml diluent containing 5, 10, 15 and 20 mg/ml concentrations of extracts in saline solution. The mixtures were incubated for 60 min at 37 °C and centrifuged at 4000 rpm for 5 min. The free haemoglobin in the supernatants was measured spectrophotometrically at 548 nm. Saline and distilled water were included as minimal and maximal haemolytic controls. Percent of haemolysis was calculated by the following formula:

$$\% \text{ haemolysis} = [\text{absorbance (extract - minimal control)} / \text{maximal control absorbance}] \times 100$$

#### 2.5. Statistical protocol

For all assessed tests, samples were analyzed in triplicates. Results were expressed as means  $\pm$  standard deviation. Statistical differences between treatments were determined through one-way ANOVA with a level of significance at  $P < 0.05$ .

### 3. Results and discussion

#### 3.1. Phytochemical Investigations

##### 3.1.1. Percent yield, total phenolic, flavonoid and tannin contents

The extraction yields vary from 12.1% to 1.4%, methanol (MeOH) giving the higher values before water, hexane and dichloromethane (DCM) (Table 1). It shows that root bark extract mainly consists of polar compounds with less than 3.5% of non polar constituents.

The MeOH extract exhibits the highest TPC ( $76.2 \pm 2.8$  mg GAE/g DW), TFC ( $39.0 \pm 2.5$  mg CE/g DW) and TTC ( $31.3 \pm 2.1$  mg CE/g DW) which are 3 to 4 times higher than in the



water extract (Table 1). On the other hand, the hexane and DCM extracts display very low values corresponding to less than 5% of those found in the MeOH extract. These results indicate that most of the phenolic compounds are extracted in polar solvents (water and MeOH) which is in agreement with previous studies on different plant species (Abdille, Singh, Jayaprakasha, & Jena, 2005; Norhaizan, Hern, Ismail, & Yee, 2010; Stankovic, Topuzovic, Solujic, & Mihailovic, 2010; Turkmen, Sari, & Velioglu, 2006).

Nevertheless, flavonoid were not the major phenolic compounds present in the root bark extracts as reflected by the TFC value (ranging from  $1.0 \pm 0.3$  to  $39.0 \pm 2.5$  mg CE/g DW) compared to TPC value (ranging from  $1.8 \pm 0.1$  to  $76.2 \pm 2.8$  mg GAE/g DW). Other compounds in the phenolic group such as phenolic acid and tannins might contribute to the high TPC value.

The TPC and TTC are higher than that of the same specie previously studied by Chaouche et al. (2013b) whereas TFC is similar.

### *3.1.2. Identification of phenolic compounds by HPLC-DAD-ESI-MS<sup>n</sup> analysis*

The identification of the phenolic compounds presented in the MeOH extract from root bark was done by HPLC-DAD-ESI-MS<sup>n</sup> analyses in negative and positive ion modes. The UV chromatogram recorded at 280 nm (Figure 1) revealed a high quantity of peak indicating a high diversity in the phenolic composition of the MeOH extract. Molecule identification was based on the UV spectrum, the exact mass and the MS/MS fragmentation pattern summarized in Table 2. Using this procedure, 17 phenolics compounds, were characterized.

Compounds **1** to **8** and **10** exhibited closely related  $\lambda_{\max}$  and MS/MS fragmentation patterns indicating that they belong to the same structural family. Compounds **2**, **3** and **7** displayed a calculated exact mass of 578.1408 Da corresponding to the formula C<sub>30</sub>H<sub>26</sub>O<sub>12</sub> and major fragments in negative mode ( $m/z$  425, 407 and 289) allowing their identification as proanthocyanidin dimers (Li & Deinzer, 2007; Touriño et al., 2008). Compounds **1**, **4** and **6** exhibited the same [M-H]<sup>-</sup> and [M+H]<sup>+</sup> with a  $m/z$  of 865.1936 and 867.2131, respectively. The major [M-H]<sup>-</sup> fragments at 577 and 287 revealed that compounds **1**, **4** and **6** are composed by proanthocyanidin dimer with the addition of one (epi)catechin. Thus, they were assigned as proanthocyanidin trimers.

Compounds **8** and **10** exhibited an expected exact mass at 1154.2657 which corresponds to the formula  $C_{60}H_{50}O_{24}$ . The major  $[M-H]^-$  fragments at 865, 577 and 289 are characteristic of proanthocyanidin tetramers (Li & Deinzer, 2007).

Compound **5** has an expected exact mass at 290.0779 and characteristic  $[M+H]^+$  fragments at  $m/z$  123, 139 and 165 allowing its identification as catechin (Li & Deinzer, 2007).

Compounds **9**, **11**, **13**, **14** and **16** exhibited the same  $[M-H]^-$  and  $[M+H]^+$  with  $m/z$  at 573.1007 and 575.1183, respectively. The expected exact mass at 574.1095 Da would correspond to the formula  $C_{30}H_{22}O_{12}$ , however none of these information was sufficient to identify exactly the nature of these molecules. The  $\lambda_{max}$  at 279nm may indicate that they could be close to the proanthocyanidins oligomers, however additional structural analyses in NMR would be necessary to correctly identify them.

Compound **12** displayed  $[M-H]^-$  parent ion at  $m/z$  463.0849 corresponding to the formula  $C_{21}H_{20}O_{12}$ . This, together with the major  $[M-H]^-$  fragment at  $m/z$  300 allowed to identify this compound as quercetin hexose (Ek, Kartimo, Mattila, & Tolonen, 2006).

Compounds **15** exhibited a  $[M+H]^+$  parent ion at  $m/z$  479.1116 corresponding to the formula  $C_{22}H_{22}O_{12}$ . Its fragmentation pattern matched that of isorhamnetin hexose. (Matsuda et al., 2009; Sawada et al., 2009).

Compound **17** exhibited a  $[M-H]^-$   $m/z$  at 447.2211. Its fragmentation pattern in negative mode was characteristic of quercetin-rhamnosyl also named quercitrin (Matsuda et al., 2009).

No previous work has been found on the identification of phenolic compounds in the root bark of *J. oxycedrus*. Root bark phenolic composition differs from that of Juniperis needles and berries. Indeed, root bark contains a high diversity of proanthocyanidin oligomers almost absent in berries and needles, whereas on the opposite, none of the biflavone found in the aerial parts (i.e. amentoflavone, cupressoflavone, biflavone...) were detected in the root bark (Innocenti et al, 2007. Miceli et al, 2011; Lesjak et al, 2013; Tavares et al ., 2012; Taviano et al ., 2013).

### 3.2. Determination of antioxidant activity

The DPPH test measures the capacity of the extracts to donate hydrogen to the radical. For all the extracts, the DPPH test displayed a good dose-dependent response, however, the low response of the hexane and DCM extracts did not allow to calculate an  $IC_{50}$ .  $IC_{50}$  of the water extract was similar to that of the BHT control. However, the  $IC_{50}$  of the MeOH extract was 10

times lower than the control, indicating a very high DPPH radical scavenging activity (Table 3).

The reducing power assay was realized by evaluating the ability of the extract to reduce  $\text{Fe}^{3+}$  to  $\text{Fe}^{2+}$ . Similar to the results obtained from the DPPH assay, the reducing power of all extracts was proportional to their concentrations. The MeOH extract showed relatively strong ferric ion-reducing activity as indicated by its low  $\text{EC}_{50}$  in comparison to the control BHT (Table 3). The water extract was however less efficient than the control.  $\text{EC}_{50}$  values of apolar extracts were not defined for the reducing power assay, because of the low reducing activity of these extracts. The reducing power of phenolic compounds was recently positively correlated with the presence of catechol nucleus in their structure (De Graft et al., 2007). The high reducing activity of the MeOH extract is thus probably linked to its phenolic composition. Indeed, among the phenolics characterized, the proanthocyanidin oligomers and quercetin isomers contain a catechol nucleus.

The potential of MeOH extract and water extract to scavenge free radicals was also assessed by other methods such as,  $\text{ABTS}^{\cdot+}$ ,  $\beta$ -carotene, oxygen singlet, hydroxyl radical, superoxide anion. Table 3 depicts the results, expressed as  $\text{IC}_{50}$  values and in comparison with BHT. As evidence by the  $\text{IC}_{50}$ , the MeOH extract showed higher activity than the BHT control for the ABTS radical-scavenging,  $\beta$ -carotene bleaching test and singlet oxygen scavenging (Table 3). The water extract was however less active than BHT.

Concerning the ABTS assay, the  $\text{IC}_{50}$  obtained with the MeOH extract ( $27.7\mu\text{g/ml}$ ) is in agreement with those reported by Al-Mustafa and Al-Thunibat (2008). Conversely,  $\text{IC}_{50}$  for the hydroxyl radical and superoxide anion are smaller than the ones measured with *J. sibirica* extracts and *J. foetidissima* (Lesjak et al., 2011, 2013).

All the antioxidant assays led to the same conclusion that the MeOH extract displays the highest antioxidant activity among the four Juniperus root bark extracts. The methanolic extract from Juniperus root bark exhibits also higher antioxidant properties than a hydro-methanolic extract obtained from Juniperus needles (Chouche et al., 2013b). This high antioxidant activity correlates with the high TPC, TFC and TTC, confirming the link already shown between phenolic contents and antiradical activity (Abdille et al., 2005; Oueslati et al., 2012). In addition, the composition of the MeOH extract may explain at least in part the antioxidant activity. Indeed, several reports have highlighted the antioxidant properties of proanthocyanidin oligomers, and quercetin and isorhamnetin isomers (Wolfe & Liu, 2008;

Musialik, Kuzmicz, Pawtowski, & Litwinienko, 2009). Moreover, with the exception of the superoxide anion and hydroxyl assays, the MeOH extract exhibited clearly higher antioxidant activity than the reference synthetic antioxidant BHT, making this extract and more generally Juniperus root bark a very interesting candidate for the development of natural antioxidant for the food industry.

### 3.3. haemolytic activity

Haemolytic activity was analysed against human red blood cells using distilled water as positive control. This study was evaluated because, even if the plant possesses potent antioxidant activity, its use in food and pharmaceutical preparations will be impossible in the presence of haemolytic effects which may confirm the presence of saponins (Sparg, Light, & van Staden, 2004).

Haemolytic activity after 1 h of treatment of blood with various concentrations (5, 10, 15 and 20 mg/ml) of various extracts by root bark of *J. oxycedrus*, was shown in Table 4. MeOH extract showed the highest hemolytic effect (2.5% to 4.4%), followed by water (2.3% to 3.5%), DCM extract (1.9% to 2.2%) and hexane extract (1.5% to 2.0%) at concentrations of 5 to 20 mg/ml.

The results obtained were comparatively lower than many plant extracts previously screened and suggest its suitability for pharmacological purpose (Raja Kannan, Arumugam, Iyapparaj, Thangaradjou, & Anantharaman, 2013). It can be expected that the various extracts have a minor cytotoxicity (< 5.0 %) (Sharma & Sharma, 2001), which could explain the absence or low content of saponins in the extracts. Furthermore, it is important to note that the IC<sub>50</sub> and EC<sub>50</sub> values, found in the evaluation of the antioxidant activity of the methanolic extract (Table 3), do not exceed a concentration of 1mg/ml whereas at 5mg/ml the haemolytic activity is limited to 2.48%. It is therefore obvious that, within a concentration range sufficient to exhibit maximal antioxidant activity (0.5-3mg/ml), this root bark extract may be safe for human use.

## 4. Conclusion

The results of the present study revealed that root bark of *J. oxycedrus* is a rich source of phenolic compounds such as proanthocyanidin oligomers, quercetin hexose, quercitrin, and

isorhamnetin glucoside which are considered as valuable natural antioxidant. Overall, this paper established that the MeOH extract possesses high *in vitro* antioxidant potency. Haemolytic activity of four extracts against human erythrocytes was determined and it was found in safe range which indicated that root bark may be harmless for the use of pharmaceutical therapies and food industry.

## References

- Abdille, M. H., Singh, R. P., Jayaprakasha, G. K., & Jena, B. S. (2005). Antioxidant activity of the extracts from *Dillenia indica* fruits. *Food Chemistry*, *90*, 891–896.
- Akkol, E. K., Guvenc, A., & Yesilada, E. (2009). A comparative study on the antinociceptive and anti-inflammatory activities of five *Juniperus* taxa. *Journal of Ethnopharmacology*, *125*, 330–336.
- Al-Mustafa, A. H., & Al-Thunibat, O.Y. (2008). Antioxidant Activity of Some Jordanian Medicinal Plants Used Traditionally for Treatment of Diabetes. *Pakistan Journal of Biological Sciences*, *11*, 351–358.
- Ani, V., Varadaraj, M. C., & Akhilender Naidu, K. (2006). Antioxidant and antibacterial activities of polyphenolic compounds from bitter cumin (*Cuminum nigrum* L.). *European Food Research and Technology*, *224*, 109–115.
- Bouhlal, K., Meynadier, J. M., Peyron, J. L., Peyron, L., Marion, J. P., Bonetti, G., & Meynadier, J. (1988). Le cade en dermatology. *Parfums, Cosmetiques et Aromes*, *83*, 73–82.
- Chandrasekara, A., & Shahidi, F. (2011). Inhibitory activities of soluble and bound millet seed phenolics on free radicals and reactive oxygen species. *Journal of Agricultural and Food Chemistry*, *59*, 428–436.
- Chaouche, T. M., Haddouchi, F., Ksouri, R., Medini, F., El-Haci, I. A., Boucherit, Z., et al. (2013a). Antioxidant Potential of Hydro-methanolic Extract of *Prasium majus* L: An *in vitro* Study. *Pakistan Journal of Biological Sciences*, *16*, 1318-1323.
- Chaouche, T. M., Haddouchi, F., Ksouri, R., Medini, F., & Atik-Bekara, F. (2013b). *In vitro* evaluation of antioxidant activity of the hydro-methanolic extracts of *Juniperus oxycedrus* subsp. *oxycedrus*. *Phytothérapie*, *11*, 244–249.

- Chougui, N., Tamendjari, A., Hamidj, W., Hallal, S., Barras, A., Richard, T., et al. (2013). Oil composition and characterisation of phenolic compounds of *Opuntia ficus-indica* seeds. *Food Chemistry*, *139*, 796–803.
- De Graft-Johnson, J., Kolodziejczyk, K., Krol, M., Nowak, P., Krol, B., & Nowak, D. (2007). Ferric Reducing Ability Power of Selected Plant Polyphenols and their Metabolites: Implications for Clinical Studies on the Antioxidant Effects of Fruits and Vegetable Consumption. *Basic & Clinical pharmacology & Toxicology*, *100*, 345–352.
- Dewanto, V. X., Wu, K., Adom, K., & Liu, R. H. (2002). Thermal processing enhances the nutritional value of tomatoes by increasing total antioxidant activity. *Journal of Agricultural and Food Chemistry*, *50*, 3010–3014.
- Ek, S., Kartimo, H., Mattila, S., & Tolonen, A. (2006). Characterization of Phenolic Compounds from Lingonberry (*Vaccinium vitis-idaea*). *Journal of Agricultural and Food Chemistry*, *54*, 9834–9842.
- Gülçin, I. (2012). Antioxidant activity of food constituents: an overview. *Archives of Toxicology*, *86*, 345–391.
- Hocman, G. (1988). Chimio-prévention du cancer: phénoliques antioxydants (BHT, BHA). *International Journal of Biochemistry*, *20*, 639–651.
- Innocenti, M., Michelozzi, M., Giaccherini, C., Ieri, F., Vincieri, F. F., & Mulinacci, N. (2007). Flavonoids and Biflavonoids in Tuscan Berries of *Juniperus communis* L.: Detection and Quantitation by HPLC/DAD/ESI/MS. *Journal of Agricultural and Food Chemistry*, *55*, 6596–6602.
- Jonkov, S., & Naidenov, G. (1974). *Juniper* bath treatment of the neurasthenic neurosis. *Folia Medica (Plovdiv)*, *16*, 291–296.
- Klimko, M., Boratynska, K., Montserrat, J. M., Didukh, Y., Romo, A., Gómez, D., et al. (2007). Morphological variation of *Juniperus oxycedrus* subsp. *oxycedrus* (Cupressaceae) in the Mediterranean region. *Flora - Morphology, Distribution, Functional Ecology of Plants*, *202*, 133–147.
- Koerfgen, G. (1964). On the treatment of psoriasis. *Zeitschrift für Haut- und Geschlechtskrankheiten*, *36*, 254–255.
- Koleva, I. I., Teris, A. B., Jozef, P. H., Linssen, A. G., & Lyuba, N. E. (2002). Screening of plant extracts for antioxidant activity: a comparative study on three testing methods. *Phytochemistry Analysis*, *13*, 8–17.

- Kusari, S., Zuhlke, S., & Spiteller, M. (2010). Chemometric evaluation of the anticancer pro-drug podophyllotoxin and potential therapeutic analogues in *Juniperus* and *Podophyllum* species. *Phytochemical Analysis*, 22, 128–143.
- Lee, J. Y., Cho, P. Y., Kim, T. Y., Kang, S. Y., Song, K. Y., & Hong, S. J. (2002). Hemolytic activity and developmental expression of pore-forming peptide, clonorin. *Biochemical and Biophysical Research Communications*, 296, 1238–1244.
- Lesjak, M. M., Beara, I. N., Orcic, D. Z., Anackov, G. T., Balog, K. J, Franciškovic, M. M., et al. (2011). *Juniperus sibirica* Burgsdorf as a novel source of antioxidant and anti-inflammatory agents. *Food Chemistry*, 124, 850–856.
- Lesjak, M. M., Beara, I. N., Orcic, D. Z., Risti, J. D., Anackov, G. T., Bozin, B. N., et al. (2013). Chemical characterisation and biological effects of *Juniperus foetidissima* Willd. 1806. *LWT - Food Science and Technology*, 53, 530–539.
- Li, H. J., & Deinzer, M. L. (2007). Tandem mass spectrometry for sequencing proanthocyanidins. *Analytical Chemistry*, 79, 1739–1748.
- Loizzo, M. R., Tundis, R., Conforti, F., Saab, A. M., Statti, G. A., & Menichini, F. (2007). Comparative chemical composition, antioxidant and hypoglycaemic activities of *Juniperus oxycedrus* ssp. *oxycedrus* L. berry and wood oils from Lebanon. *Food chemistry*, 105, 572–578.
- Lopes, G. K., Schulman, H. M., & Hermes-Lima, M. (1999). Polyphenol tannic acid inhibits hydroxyl radical formation from Fenton reaction by complexing ferrous ions. *Biochimica et Biophysica Acta*, 1472, 142–152.
- Masuda, T., Yonemori, S., Oyama, Y., Takeda, Y., Tanaka, T., Andoh, T., et al. (1999). Evaluation of the antioxidant activity of environmental plants: Activity of the leaf extracts from seashore plants. *Journal of Agricultural and Food Chemistry*, 47, 1749–1754.
- Matsuda, F., Yonekura-Sakakibara, K., Niida, R., Kuromori, T., Shinozaki, K., & Saito, K. (2009). MS/MS spectral tag-based annotation of non-targeted profile of plant secondary metabolites. *Plant Journal*, 57, 555–577.
- Miceli, N., Trovato, A., Dugo, P., Cacciola, F., Donato, P., Marino, A., et al. (2009). Comparative Analysis of Flavonoid Profile, Antioxidant and Antimicrobial Activity of the Berries of *Juniperus communis* L. var. *communis* and *Juniperus communis* L. var. *saxatilis* Pall. from Turkey. *Journal of Agricultural and Food Chemistry*, 57, 6570–6577.

- Miceli, N., Trovato, A., Marino, A., Bellinghieri, V., Melchini, A., Dugo, P., et al. (2011). Phenolic composition and biological activities of *Juniperus drupacea* Labill. berries from Turkey. *Food and Chemical Toxicology*, *49*, 2600–2608.
- Musialik, M., Kuzmicz, R., Pawtowski, T. S., & Litwinienko, G. (2009). Acidity of hydroxyl groups: An overlooked influence on antiradical properties of flavonoids. *Journal of Organic Chemistry*, *74*, 2699–2709.
- Norhaizan, M. E., Hern, S. E., Ismail, A., & Yee, C.L. (2010). Antioxidant activity in different parts of roselle (*Hibiscus sabdariffa* L.) extracts and potential exploitation of the seeds. *Food Chemistry*, *122*, 1055–1060.
- Orav, A., Koel, M., Kailas, T., & Müürisepp, M. (2010). Comparative analysis of the composition of essential oils and supercritical carbon dioxide extracts from the berries and needles of Estonian juniper (*Juniperus communis* L.). *Procedia Chemistry*, *2*, 161–167.
- Orhan, N., Aslan, M., Demirci, B., & Ergun, F. (2012). A bioactivity guided study on the antidiabetic activity of *Juniperus oxycedrus* subsp. *oxycedrus* L. leaves. *Journal of Ethnopharmacology*, *140*, 409–415.
- Oueslati, S., Ksouri, R., Falleh, H., Pichette, A., Abdelly, C., & Legault, J. (2012). Phenolic content, antioxidant, anti-inflammatory and anticancer activities of the edible halophyte *Suaeda fruticosa* Forssk. *Food Chemistry*, *132*, 943–947.
- Oyaizu, M. (1986). Studies on products of the browning reaction prepared from glucose amine. *Japanese Journal of Nutrition*, *44*, 307–315.
- Raja Kannan, R. R., Arumugam, R., Iyapparaj, P., Thangaradjou, T., & Anantharaman, P. (2013). *In vitro* antibacterial, cytotoxicity and haemolytic activities and phytochemical analysis of seagrasses from the Gulf of Mannar, South India. *Food Chemistry*, *57*, 1017–1022.
- Re, R., Pellegrini, N., Proteggente, A., Pannala, A., Yang, M., & Rice-evans, C. (1999). Antioxidant activity applying an improved ABTS radical decolorization assay. *Free Radical Biology and Medicine*, *26*, 1231–1237.
- Sassi, A. B., Harzallah-Skhiri, F., Bourgougnon, N., & Aouni, M. (2008). Antiviral activity of some Tunisian medicinal plants against herpes simplex virus type 1. *Natural Product Research*, *22*, 53–65.
- Sawada, Y., Akiyama, K., Sakata, A., Kuwahara, A., Otsuki, H., Sakurai, T., et al. (2009). Widely targeted metabolomics based on large-scale MS/MS data for elucidating metabolite accumulation patterns in plants. *Plant and Cell Physiology*, *50*, 37–47.



- Sharma, P., & Sharma, J.D. (2001). *In vitro* hemolysis of human erythrocytes by plant extracts with antiplasmodial activity. *Journal of Ethnopharmacology*, 74, 239–243.
- Sparg, S. G., Light, M. E., & Van Staden, J. (2004). Biological activities and distribution of plant saponins. *Journal of Ethnopharmacology*, 94, 219–243.
- Stankovic, S. M., Topuzovic, M., Solujic, S., & Mihailovic V. (2010). Antioxidant activity and concentration of phenols and flavanoids in the whole plant and plant parts of *Teucrium chamaerdys* L. var *glanduliferum* Haussk. *Journal of Medicinal Plant Research*, 4, 2092–2098.
- Sun, B., Richardo-da-Silvia, J. M., & Spranger, I. (1998). Critical factors of vanillin assay for catechins and proanthocyanidins. *Journal of Agricultural and Food Chemistry*, 46, 4267–4274.
- Tavares, L., Gordon, J., Fortalezasa, S., Stewart, D., Ricardo, B. F., & Cláudia, N. (2012). The neuroprotective potential of phenolic-enriched fractions from four *Juniperus* species found in Portugal. *Food Chemistry*, 135, 562–570.
- Taviano, M. F., Marino, A., Trovato, A., Bellinghieri, V., Melchini, A., Dugo, P., et al. (2013). *Juniperus oxycedrus* L. subsp. *oxycedrus* and *Juniperus oxycedrus* L. subsp. *macrocarpa* (Sibth. & Sm.) Ball. “berries” from Turkey: Comparative evaluation of phenolic profile, antioxidant, cytotoxic and antimicrobial activities. *Food and Chemical Toxicology*, 58, 22–29.
- Thompson, D., & Moldéus, P. (1988). Cytotoxicity of butylated hydroxyanisole and butylated hydroxytoluene in isolated rat hepatocytes. *Biochemical Pharmacology*, 37, 2201–2207.
- Touriño, S., Fuguet, E., Jáuregui, O., Saura-Calixto, F., Cascante, M., & Torres, J. L. (2008). High-resolution liquid chromatography/electrospray ionization time-of-flight mass spectrometry combined with liquid chromatography/electrospray ionization tandem mass spectrometry to identify polyphenols from grape antioxidant dietary fiber. *Rapid Communications in Mass Spectrometry*, 22, 3489–3500.
- Turkmen, N., Sari, F., & Velioglu, Y. S. (2006). Effects of extraction solvents on concentration and antioxidant activity of black and black mate tea polyphenols determined by ferrous tartrate and Folin-Ciocalteu methods. *Food Chemistry*, 99, 835–841.
- WHO. (1980). Expert Committee on Diabetes Mellitus Second Report. Technical Report Series 646. World Health Organization, Geneva, p. 66.
- Wolfe, K. L., & Liu, R. H. (2008). Structure-activity relationships of flavonoids in the cellular antioxidant activity assay. *Journal of Agriculture and Food Chemistry*, 56, 8404–8411.

Yao, H. W., Li, J., Chen, J. Q., & Xu, S. Y. (2004). Inhibitory effect of leflunomide on hepatic fibrosis induced by CCl<sub>4</sub> in rats. *Acta Pharmacologica Sinica*, 25, 915–920.

**Table 1. Yield, TPC, TFC and TCC of root bark extracts obtained from the extraction solvents with different polarity.**

| Extract | Yield (%) | TPC (mg GAE/g DW) <sup>A</sup> | TFC (mg CE/g DW) <sup>B</sup> | TTC (mg CE/g DW) <sup>C</sup> |
|---------|-----------|--------------------------------|-------------------------------|-------------------------------|
| Hexane  | 3.5       | 3.2 ± 0.1 <sup>b</sup>         | 2.0 ± 0.1 <sup>b</sup>        | 1.0 ± 0.5 <sup>b</sup>        |
| DCM     | 1.4       | 1.8 ± 0.1 <sup>a</sup>         | 1.0 ± 0.3 <sup>a</sup>        | 0.5 ± 0.1 <sup>a</sup>        |
| MeOH    | 12.1      | 76.2 ± 2.8 <sup>d</sup>        | 39.0 ± 2.5 <sup>d</sup>       | 31.3 ± 2.1 <sup>d</sup>       |
| Water   | 11.4      | 25.4 ± 2.0 <sup>c</sup>        | 11.0 ± 1.0 <sup>c</sup>       | 8.3 ± 0.9 <sup>c</sup>        |

Data are expressed as mean±SD of three replicates. Data with different superscripts (a, b, c, d) in the same column are considered significant.

<sup>A</sup>Total phenolic content (TPC) is expressed as milligram of gallic acid equivalent per gram of extract (mg GAE/g extract).

<sup>B</sup>Total flavonoid content (TFC) is expressed as milligram of catechin equivalent per gram of extract (mg CE/g extract).

<sup>C</sup>Total tannin content (TTC) is expressed as milligram of catechin equivalent per gram of extract (mg CE/g extract).

**Table 2. Phenolic compounds identified by HPLC-MS<sup>n</sup> Orbitrap analysis for the methanolic extract of the root bark of *J. oxycedrus*. Peak number as in Fig. 1.  $\lambda_{max}$  is the absorbance maxima in the UV/visible range. The reported fragments were observed in source and verified through MS/MS analysis of the parental ion in both negative and positive mode. The reference column relates previous reports on metabolites in different plants.**

| Peak | Rt   | $\lambda_{max}$ (nm) | [M-H] <sup>-</sup> | MS/MS [M-H] <sup>-</sup> | [M+H] <sup>+</sup> | MS/MS [M+H] <sup>+</sup> | Calculated mass | Formula   | Proposed molecule         | References                                     |
|------|------|----------------------|--------------------|--------------------------|--------------------|--------------------------|-----------------|---|---------------------------|--|
| 1    | 20.5 | 279                  | 865.1935           | 713<br>695<br>577<br>287 | 867.2129           | 715<br>577<br>579<br>289 | 866.2032        | C <sub>45</sub> H <sub>38</sub> O <sub>18</sub> | Proanthocyanidin trimer   | Li & Deinzer, 2007 ;<br>Tourinho et al., 2008  |
| 2    | 28.2 | 279                  | 577.1326           | 451<br>425<br>407<br>289 | 579.149            | 453<br>427<br>409<br>289 | 578.1408        | C <sub>30</sub> H <sub>26</sub> O <sub>12</sub> | Proanthocyanidin dimer    | Li & Deinzer, 2007<br>Tourinho et al., 2008    |
| 3    | 29.1 | 279                  | 577.1326           | 451<br>425<br>407<br>289 | 579.149            | 453<br>427<br>409        | 578.1408        | C <sub>30</sub> H <sub>26</sub> O <sub>12</sub> | Proanthocyanidin dimer    | Li & Deinzer, 2007 ;<br>Tourinho et al., 2008; |
| 4    | 32.3 | 279                  | 865.1935           | 713<br>695<br>577<br>287 | 867.2129           | 715<br>577<br>579<br>289 | 866.2032        | C <sub>45</sub> H <sub>38</sub> O <sub>18</sub> | Proanthocyanidin trimer   | Li & Deinzer, 2007 ;<br>Tourinho et al., 2008  |
| 5    | 32.7 | 279                  | 289.0703           | 289                      | 291.0857           | 123<br>139<br>165        | 290.0779        | C <sub>15</sub> H <sub>14</sub> O <sub>6</sub>  | Catechin                  | Li & Deinzer, 2007                             |
| 6    | 36.1 | 279                  | 865.1935           | 713<br>695<br>577<br>287 | 867.2129           | 715<br>577<br>579<br>289 | 866.2032        | C <sub>45</sub> H <sub>38</sub> O <sub>18</sub> | Proanthocyanidin trimer   | Li & Deinzer, 2007 ;<br>Tourinho et al., 2008  |
| 7    | 37.9 | 279                  | 577.1322           | 425<br>287<br>407        | 579.1484           | 427<br>409<br>301        | 578.1403        | C <sub>30</sub> H <sub>26</sub> O <sub>12</sub> | Proanthocyanidin dimer    | Li & Deinzer, 2007 ;<br>Tourinho et al., 2008  |
| 8    | 43.3 | 279                  | 1153.2557          | 865<br>577<br>289        | 1155.2765          | 867<br>579<br>291        | 1154.2671       | C <sub>60</sub> H <sub>50</sub> O <sub>24</sub> | Proanthocyanidin tetramer | Li & Deinzer, 2007                             |
| 9    | 47.6 | 279                  | 573.1009           | 447<br>285               | 575.1179           | 423<br>395               | 574.1094        | C <sub>30</sub> H <sub>22</sub> O <sub>12</sub> | Unknown compound          |  |
| 10   | 56.5 | 279                  | 1153.2545          | 865<br>577<br>289        | 1155.2765          | 867<br>579<br>291        | 1154.2672       | C <sub>60</sub> H <sub>50</sub> O <sub>24</sub> | Proanthocyanidin tetramer | Li & Deinzer, 2007                             |
| 11   | 57.1 | 280                  | 573.1007           | 447<br>283               | 575.1183           | 547<br>423<br>395        | 574.1095        | C <sub>30</sub> H <sub>22</sub> O <sub>12</sub> | Unknown compound          |  |
| 12   | 68.5 | 264, 365             | 463.0849           | 301                      | 465.1017           | 303<br>229               | 464.0933        | C <sub>21</sub> H <sub>20</sub> O <sub>12</sub> | Quercetin hexose          | Ek et al., 2006 ;<br>Sawada et al., 2009       |
| 13   | 70.3 | 279                  | 573.1011           | 447<br>283               | 575.1184           | 547<br>423<br>395        | 574.1095        | C <sub>30</sub> H <sub>22</sub> O <sub>12</sub> | Unknown compound          |  |
| 14   | 71.2 | 279                  | 573.1007           | 447<br>283               | 575.1183           | 547<br>423<br>395        | 574.1095        | C <sub>30</sub> H <sub>22</sub> O <sub>12</sub> | Unknown compound          |  |
| 15   | 76.1 | 255, 355             | 477.101            | 314.0415                 | 479.1116           | 317                      | 478.1062        | C <sub>22</sub> H <sub>22</sub> O <sub>12</sub> | Isorhamnetin-glucoside    | Sawada et al. 2009 ;<br>Matsuda et al., 2009   |
| 16   | 79.5 | 279                  | 573.1007           | 447<br>283               | 575.1183           | 547<br>423<br>395        | 574.1095        | C <sub>30</sub> H <sub>22</sub> O <sub>12</sub> | Unknown compound          |  |
| 17   | 91.2 | 255, 305, 372        | 447.2211           | 301<br>151               |                    |                          |                 | C <sub>21</sub> H <sub>20</sub> O <sub>11</sub> | Quercetin-rhamnosyl       | Matsuda et al., 2009                           |

**Table 3. IC<sub>50</sub> values for evaluated antioxidant assays and EC<sub>50</sub> values reducing power of water, MeOH extracts and BHT.**

|       | IC <sub>50</sub> /DPPH<br>µg/ml | EC <sub>50</sub> / Iron reducing<br>µg/ml | IC <sub>50</sub> /ABTS<br>µg/ml | IC <sub>50</sub> /β-carotene<br>µg/ml | IC <sub>50</sub> /Oxygen singulet<br>mg/ml | IC <sub>50</sub> /Hydroxyl<br>mg/ml | IC <sub>50</sub> /Superoxide anion<br>mg/ml |
|-------|---------------------------------|---|---------------------------------|---------------------------------------|--|-------------------------------------|---|
| MeOH  | 1.1                             | 72.0                                      | 27.7                            | 15.9                                  | 0.37                                       | 1.1                                 | 1.04  |
| Water | 10.3                            | 130.0                                     | 113.6                           | 125.9                                 | 1.0  | 3.2                                 | 3.21  |
| BHT   | 11.6                            | 392.3                                     | 73.1                            | 85                                    | 0.45                                       | 0.95                                | 0.56  |

IC<sub>50</sub>: Inhibition Concentration 50%, EC<sub>50</sub>: Effective Concentration at which the absorbance was 0.5, DPPH: 2,2-diphényl picrylhydrazyl, ABTS : 2,2-azino-bis-3-ethylbenzothiazoline-6-sulfonique acid, BHT: Butylated Hydroxytoluene,

**Table 4. Haemolytic activity, as a percentage of haemolysis caused by root bark of *J. oxycedrus*.**

| Extract         | 5 mg/ml | 10 mg/ml | 15 mg/ml | 20 mg/ml |
|-----------------|---------|----------|----------|----------|
| Hexane          | 1.52    | 1.62     | 1.84     | 2.05     |
| DCM             | 1.94    | 1.98     | 2.12     | 2.23     |
| MeOH            | 2.48    | 2.94     | 4.09     | 4.37     |
| Water           | 2.35    | 2.65     | 2.75     | 3.46     |
| Saline          | 0       | 0        | 0        | 0        |
| Distilled water | 100     | 100      | 100      | 100      |

Haemolytic percents of saline and distilled water were included as minimal and maximal haemolytic controls. All values represent the mean±standard of three separate experiments (p<0.05).

Figure caption:

**Figure 1. Chromatographic profiles at 280 nm of the methanol extract of the root bark. The numeration is that of Table 2.**

