

## Comparative chemical composition, antioxidant and hypoglycaemic activities of *Juniperus oxycedrus* ssp. *oxycedrus* L. berry and wood oils from Lebanon

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### Abstract

Juniper (*Juniperus oxycedrus*) is used in European cuisine for its distinguishing flavour. *J. oxycedrus* ssp. *oxycedrus* berry and wood essential oils were tentatively identified by GC and GC/MS. Fifty compounds were identified in the berry oil and 23 compounds were identified in the wood oil. The *J. oxycedrus* ssp. *oxycedrus* berry oil was characterised by high contents of  $\alpha$ -pinene (27.4%) and  $\beta$ -myrcene (18.9%). Other important compounds were  $\alpha$ -phellandrene (7.1%), limonene (6.7%), *epi*-bicyclosesquiphellandrene (2.3%) and  $\delta$ -cadinene (2.2%) while, in the wood oil,  $\delta$ -cadinene (14.5%) is a major main component, together with *cis*-thujopsene (9.2%) and  $\alpha$ -muurolene (4.9%). *In vitro* evaluation of antioxidant activity by the DPPH method showed a significant activity for both oils with IC<sub>50</sub> values of 1.45  $\mu$ l/ml for wood and 7.42  $\mu$ l/ml for berries. Hypoglycaemic activity was investigated through the inhibition of  $\alpha$ -amylase. The results revealed that oil obtained by hydrodistillation from *J. oxycedrus* ssp. *oxycedrus* wood exhibits an interesting activity with IC<sub>50</sub> of 3.49  $\mu$ l/ml.

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### 1. Introduction

*Juniperus oxycedrus* (Cupressaceae) is a shrub or small tree growing wild in stony places of the Mediterranean and Near East countries. Juniper berries are used as a spice, particularly in European cuisine, and also give gin its distinguishing flavour. According to one FAO document, juniper berries are the only spice derived from conifers. Juniper berries are used in northern European and particularly Scandinavian cuisine to “impart a sharp, clear flavour” to meat dishes, especially wild bird and game meats (Montagne, 1999). They also season pork, cabbage and sauerkraut dishes. Traditional recipes for choucroute

garnie, an Alsatian dish of sauerkraut and meats, universally include juniper berries.

Several studies have reported the chemical composition of solvent extracts and essential oil obtained by hydrodistillation of leaves and berries of *J. oxycedrus* (Adams, 1998; Guerra, Carmen, & Garcia, 1987; Milos & Radonic, 2002). *J. oxycedrus* wood oil has been studied less frequently (Boti, Bighelli, Cavaleiro, Salgueiro, & Casanova, 2006).

Another use of *J. oxycedrus* is to prepare the so-called oil of cade, known also as “juniper tar”. This oil was used in dermatology to treat chronic eczema and other skin diseases while the rectified oil was used as a fragrance component in detergents, soaps, creams and lotions (Leung & Foster, 1996). *J. oxycedrus* was used in folk medicine for the treatment of various diseases, such as hyperglycaemia,

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obesity, tuberculosis, bronchitis and pneumonia (Sanchez de Medina et al., 1994). Leaves and stems of *J. oxycedrus* ssp. *oxycedrus* have been found to reduce the blood pressure of normotensive rats, to inhibit the response to histamine, serotonin and acetylcholine, and to exhibit significant anti-inflammatory activity (Moreno, Bello, Beltrán, Calatayud, & Primo-Yu' fera, 1998). Several extracts of leaves, resins, barks and fruits of *J. oxycedrus* were found to inhibit the growth of several microorganisms (Karaman, Şahin, Güllüce, Oğütçü, & Şengül Adıgüzel, 2003). Other *Juniperus* members, e.g., *J. procera* and *J. chinensis*, were found to exhibit free-radical scavenging activity (Lim et al., 2002).

It is well known that diabetes mellitus is the commonest endocrine disorder that, according to the World Health Organization (WHO, 2004), affects more than 176 million people world wide. The term diabetes mellitus describes a metabolic disorder of multiple aetiologies and is characterised by chronic hyperglycaemia, with disturbances of carbohydrate, fat and protein metabolism, resulting from defects in insulin secretion, insulin action, or both. The causes of type 2 diabetes are either insulin resistance with relative insulin deficiency or predominantly an insulin secretory defect, with or without insulin resistance. Diabetes is a major risk factor for premature atherosclerosis, and oxidative stress plays an important role since diabetic monocytes produce increased superoxide anion ( $O_2^-$ ) (Venugopal, Devaraj, Yang, & Jialal, 2002). Therefore, antioxidants are very important for the defence of a living system against oxidative stress. The addition of antioxidants to food products earns increasing popularity as a powerful means for extending the shelf-life of products and for decreasing the nutritional losses by preventing or slowing the oxidation process (Tsuda, Ohshima, Kawakishi, & Osawa, 1994). The most commonly applied antioxidants in the food industry are synthetic phenols, such as butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA). Their safety, however, is doubtful, and there has been a general desire to replace synthetic food additives with natural alternatives (Howell, 1986). Therefore, intensive research for utilisation of natural antioxidants that may serve as potent candidates in combating carcinogenesis and aging processes, has been carried out.

As part of our continuing programme to investigate antioxidant and hypoglycaemic activities of spices (Conforti, Statti, Uznov, & Menichini, 2006; Statti, Loizzo, Nadjafi, & Menichini, 2006), in this work we report the antioxidant activity analyzed *in vitro* by DPPH test and hypoglycaemic activity by the inhibition of  $\alpha$ -amylase of *J. oxycedrus* ssp. *oxycedrus*, oils growing wild in Lebanon.

## 2. Materials and methods

### 2.1. Plant material

Hundred grams of casually chosen ripe berries and 100 g of wood from *J. oxycedrus* ssp. *oxycedrus* were collected on

Baskinta Mountain, Lebanon, in November, 2003 and authenticated by Prof. S. Safi, Biology Department, Faculty of Sciences II, Lebanese University. A voucher specimen was deposited in the Herbarium, Faculty of Sciences II, Lebanese University.

### 2.2. Isolation of essential oils

The plant material was submitted to hydrodistillation for 3 h, using a Clevenger-type apparatus (Clevenger, 1928). Each oil was dried over anhydrous sodium sulphate to remove traces of moisture to give yields of 0.68% (w/w) and 0.72% (w/w) for the wood and berries, respectively.

### 2.3. Analysis of the oils

The essential oils from seeds and wood of *J. oxycedrus* ssp. *oxycedrus* were analysed by gas chromatography (GC) and gas chromatography–mass spectrometry (GC–MS). GC analysis were performed on a Shimadzu GC17A gas chromatograph equipped with a flame ionisation detector (FID) and controlled by Borwin Software. The samples were analysed on a fused silica 30 m SE-30 capillary column with an internal diameter of 0.25 mm and a film thickness of 0.25  $\mu$ . Nitrogen was used as the gas vector at a constant flow of 1.0 ml/min; split ratio 1:30. Injector and detector were maintained at 250 and 280 °C, respectively. Column temperature was initially kept at 40 °C for 5 min, then gradually increased to 250 °C at 5 °C/min rate and finally held for 10 min at 250 °C. GC–MS analyses of the oils were carried out using a Hewlett–Packard 6890 gas chromatograph equipped with an SE-30 capillary column (100% dimethylpolysiloxane, 30 m length, 0.25 mm i.d., 0.25  $\mu$  film thickness) and interfaced with a Hewlett–Packard 5973 Mass Selective. Ionisation of the sample components was performed in electron impact mode (EI, 70 eV). The carrier gas was helium (1 ml/min) and the analytical conditions worked with the following programme: oven temperature was 5 min isothermal at 40 °C, then 40–250 °C at a rate of 5 °C/min; then held isothermal for 10 min. Injector and detector were maintained at 250 and 280 °C, respectively. The mass range from 50 to 550 amu was scanned at a rate of 2.9 scans/s. For analysis, oils were dissolved in dichloromethane (ca. 1 mg/ml) and aliquots (1  $\mu$ l) were directly injected. Analyses were also run by an HP-5 capillary column (5%-phenyl-methylpolysiloxane, 30 m length, 0.25 mm i.d., 0.25  $\mu$  film thickness). Gas chromatographic conditions were as given. Most constituents were tentatively identified by gas chromatography by comparison of their retention indices (*I*) with those of the literature (Adams, 1995) or with those of authentic compounds available in our laboratory. Retention indices for all compounds were determined by co-injection of the sample with a solution containing a homologous series of C9–C31 *n*-alkanes (Tranchant, 1995). Further tentative identification was achieved by comparison of their mass spectra on both columns with

those stored in Wiley 138 and Wiley 275 libraries. The percentage composition of *J. oxycedrus* ssp. *oxycedrus* oils was computed by the normalisation method from the GC peak areas, related to GC peak area of an external standard injected into the GC–MS equipment under conditions identical to the above. Percentage of total area was obtained by their addition. All determinations were performed in triplicate and averaged.

#### 2.4. Antioxidant activity (DPPH assay)

Free radical-scavenging activity was determined, using a rapid TLC screening method based on the reduction of a methanolic solution of the coloured free radical, 1,1-diphenyl-2-picrylhydrazyl (DPPH<sup>•</sup>). After developing and drying, TLC plates were sprayed with a 0.2% DPPH<sup>•</sup> solution in MeOH. The plates containing the essential oils were examined 30 min after spraying. The samples with antioxidant activity appeared as yellow spots against a purple background.

In order to determine the radical-scavenging potency, the samples which exhibited antioxidant activity were investigated with an experimental procedure that was adapted from Wang et al. (1998). To an ethanol solution of 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical (final concentration was  $1.0 \times 10^{-4}$  M), test samples were added at different concentrations. The reaction mixtures were shaken vigorously and then kept in the dark for 30 min. The absorbances of the resulting solutions were measured in 1 cm cuvettes, using a Perkin–Elmer Lambda 40 UV/Vis spectrophotometer at 517 nm against blank, which was without DPPH<sup>•</sup>. All tests were run in triplicate and averaged. Ascorbic acid was used as a positive control.

#### 2.5. Hypoglycaemic activity ( $\alpha$ -amylase inhibition)

The bio-assay for  $\alpha$ -amylase inhibition was adopted from Sigma–Aldrich and modified (Conforti et al., 2005). Both control oils and seed ethanol extract were mixed with starch solution and left to react with an  $\alpha$ -amylase solution under alkaline conditions at 25 °C. The reaction was measured over 3 min. The generation of maltose was quantified by the reduction of 3,5-dinitrosalicylic acid to 3-amino-5-nitrosalicylic acid. This reaction is detectable at 540 nm. In the presence of  $\alpha$ -amylase inhibitors, less maltose would be produced and the absorbance value would be decreased. Preliminary experiments were carried out to establish optimal conditions and these were found to be: starch 0.25% (w/v);  $\alpha$ -amylase 1 U/ml; inhibitor concentration 1 mg/ml. The most widely described drug, Acarbose (Bayer, Italy), was used as positive control.

#### 2.6. Statistical analysis

All the results were expressed as means  $\pm$  standard deviation (SD) of three independent measurements and the sta-

tistical significance was assessed by Student's *t* test. The confidence limits were set at  $P < 0.05$ .

### 3. Results and discussion

#### 3.1. Essential oils composition

By hydrodistillation of the berries and wood of *J. oxycedrus* ssp. *Oxycedrus*, a white–yellow oil, yields of 0.72% (w/w) and 0.68%, w/w (relative to dry weight material), respectively, were obtained. The results of their GC and GC–MS analyses are given in Table 1, where the compounds are listed according to their order of elution. Fifty constituents, representing 82.2% of the total components in the oil, were identified in the essential oil extracted from the berries of *J. oxycedrus* ssp. *oxycedrus*. The monoterpenes (68.4%) represent the main fraction of the oil and  $\alpha$ -pinene (27.4%) and  $\beta$ -myrcene (18.9%) were the main components of this fraction. Also,  $\alpha$ -phellandrene (7.1%) and limonene (6.7%) were present in considerable amounts. Epibicyclosesquiphellandrene (2.3%),  $\delta$ -cadinene (2.2%),  $\beta$ -caryophyllene (1.6%) and  $\alpha$ -muurolene (0.9%) were the main components of the sesquiterpenes fraction, which accounted for 13.1% of the oil. It is possible to find in the literature that the berry oil of *J. oxycedrus* is dominated by  $\alpha$ -pinene and  $\beta$ -myrcene, but the ratio of the two compounds may vary drastically. Moreover, oils of different origins could be differentiated by the occurrence of other components in appreciable contents. As can be seen, the oil of *J. oxycedrus* berry growing in Lebanon is also characterised by high contents of  $\alpha$ -pinene and  $\beta$ -myrcene, as also is the oil of *J. oxycedrus* berry growing in Europe (Milos & Radonic, 2002). The chemical composition of the oil isolated from *J. oxycedrus* ssp. *oxycedrus* wood is also shown in Table 1. Twenty-three compounds were identified, which accounted for 45.8% of the total oil composition. The sesquiterpenes represent the major fraction, in which  $\delta$ -cadinene (14.5%) and *cis*-thujopsene (9.2%) were the main components. Other important compounds were  $\alpha$ -muurolene (4.9%), cadalene (3.7%), eremophilene (2.5%), and  $\alpha$ -cedrol (2.2%). According to a previous work performed on *J. oxycedrus* wood oils from Spain, France and Italy,  $\delta$ -cadinene was the major component (Barrero et al., 1993). Marongiu et al. (2003) have analysed samples collected in Sardinia and they observed the presence, as the most abundant components, besides  $\delta$ -cadinene, of 1-epi-cubenol (12.5%), cubenol (10.5%),  $\alpha$ -muurolol (4.8%),  $\alpha$ -cadinol (3.7%) and  $\alpha$ -humulene (3.2%).

There is a large difference between *J. oxycedrus* ssp. *oxycedrus* berry oil and *J. oxycedrus* ssp. *oxycedrus* wood oil. It is of interest to note that a survey of the two types of oil revealed that they showed a wide variation in the number of identified compounds, their contents and chemical composition. This difference was reflected also in biological activities.

Table 1  
Composition (%) of berry and wood oils from *J. oxycedrus* ssp. *oxycedrus*

Component	$I^a/I^b$	Berry oil <sup>c</sup>	Wood oil <sup>c</sup>	ID method <sup>d</sup>
$\alpha$ -Pinene	936/933	27.4 ± 0.05		I, MS
Camphene	953/951	0.1 ± 0.002		I, MS
Sabinene	973/970	4.5 ± 0.001		I, MS
$\beta$ -Pinene	978/972	0.4 ± 0.005		I, MS
$\beta$ -Myrcene	986/981	18.9 ± 0.07		I, MS
$\alpha$ -Phellandrene	1005/1003	7.1 ± 0.002		I, MS
$\Delta^3$ -Carene	1012/1010	tr		I, MS
$\alpha$ -Terpinene	1016/1012	tr		I, MS
<i>p</i> -Cymene	1025/1023	0.5 ± 0.008	0.2 ± 0.004	I, MS
Limonene	1032/1028	6.7 ± 0.005	tr	I, MS, Co-GC
1,8-Cineole	1035/1033		tr	I, MS, Co-GC
$\gamma$ -Terpinene	1059/1057	0.1 ± 0.001		I, MS
Terpinolene	1089/1086	0.2 ± 0.004		I, MS, Co-GC
Linalool	1098/1092	0.4 ± 0.002	tr	I, MS, Co-GC
$\alpha$ -Campholene aldehyde	1128/1125	0.1 ± 0.006		I, MS
<i>trans</i> -Pinocarveol	1152/1139	0.1 ± 0.003		I, MS
Terpinen-4-ol	1178/1175	0.1 ± 0.005		I, MS
$\alpha$ -Terpineol	1189/1183	0.3 ± 0.008		I, MS
Verbenone	1216/1204	0.1 ± 0.001		I, MS
<i>trans</i> -Carveol	1217/1212	0.2 ± 0.003		I, MS
Citronellol	1228/1214	0.3 ± 0.005		I, MS
Carvone	1242/1241	0.1 ± 0.009		I, MS
Geraniol	1255/1235	0.1 ± 0.002		I, MS
Bornyl acetate	1291/1285	0.6 ± 0.004		I, MS
$\alpha$ -Terpinyl acetate	1358/1350	0.1 ± 0.003	tr	I, MS
$\alpha$ -Cubebene	1351/1340	0.5 ± 0.007	tr	I, MS
$\alpha$ -Ylangene	1373/1370	0.4 ± 0.005		I, MS
$\alpha$ -Copaene	1377/1375	0.3 ± 0.004	0.2 ± 0.007	I, MS
$\beta$ -Elemene	1379/1387		0.6 ± 0.002	I, MS
Longifolene	1415/1402	0.2 ± 0.002		I, MS
$\beta$ -Caryophyllene	1418/1415	1.6 ± 0.009	2.0 ± 0.005	I, MS, Co-GC
<i>cis</i> -Thujopsene	1429/1426	0.3 ± 0.002	9.2 ± 0.04	I, MS
$\beta$ -Gurjunene	1442/1432	0.4 ± 0.001		I, MS
<i>trans</i> - $\beta$ -Farnesene	1441/1444	0.3 ± 0.001		I, MS
$\alpha$ -Humulene	1454/1447	1.0 ± 0.005	1.3 ± 0.009	I, MS
<i>allo</i> -Aromadendrene	1461/1459		0.7 ± 0.001	I, MS
$\beta$ -Selinene	1484/1482	0.8 ± 0.008	0.2 ± 0.006	
Eremophilene	1486/1489	0.2 ± 0.003	2.5 ± 0.004	I, MS
Epi-bicyclosquiphellandrene	1495/1491	2.3 ± 0.002	1.9 ± 0.001	I, MS
$\alpha$ -Muurolene	1499/1496	0.9 ± 0.002	4.9 ± 0.003	I, MS
$\gamma$ -Cadinene	1515/1513	0.6 ± 0.007		I, MS
$\delta$ -Cadinene	1524/1522	2.2 ± 0.008	14.5 ± 0.08	I, MS
Cadina-1,4-diene	1529/1527	0.1 ± 0.004	0.5 ± 0.001	I, MS
$\alpha$ -Cedrol	1598/1596	0.3 ± 0.005	2.2 ± 0.006	I, MS
Cadalene	1673/1677	0.1 ± 0.002	3.7 ± 0.002	I, MS
( <i>E</i> , <i>E</i> )-Farnesol	1722/1720	0.6 ± 0.006		I, MS
Methyl palmitate	1934/1929	0.1 ± 0.004	0.8 ± 0.003	I, MS
Palmitic acid	1967/1963	0.2 ± 0.008		I, MS, Co-GC
Manoyl oxide	1989/1993	0.2 ± 0.003		I, MS
Ethyl palmitate	2005/1996	0.1 ± 0.007	tr	I, MS
Methyl stearate	2141/2133	0.1 ± 0.006	0.4 ± 0.009	I, MS
Ethyl linoleate	2159/2152	tr		I, MS
Ethyl stearate	2197/2190	tr		I, MS
Identified compounds		82.2	45.8	
Monoterpenes		68.4	0.2	
Sesquiterpenes		13.1	44.2	
Diterpene		0.2		
Fatty acids		0.5	1.4	

<sup>a</sup> SE-30 MS non-polar column.

<sup>b</sup> HP-5 MS non-polar column; compositional values less than 0.1% are denoted as traces (tr).

<sup>c</sup> Mean value ± standard error, *n* = three independent determinations.

<sup>d</sup> I, Retention index; MS, mass spectrum; Co-GC: co injection with authentic compound.



### 3.2. Determination of antioxidant activity

The antioxidant activity of berries and wood oils of *J. oxycedrus* ssp. *oxycedrus* was carried out using the DPPH<sup>•</sup> test. The model of scavenging stable DPPH free radicals can be used to evaluate the antioxidant activities in a relatively short time. The absorbance decreases as a result of a colour change from purple to yellow as the radical is scavenged by antioxidants. The effect of antioxidants on 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical-scavenging is thought to be due to their hydrogen-donating ability. This activity is given as % DPPH radical-scavenging that is calculated by the equation

DPPH radical-scavenging

$$= \text{sample absorbance/control absorbance} \times 100.$$

The DPPH<sup>•</sup> solution without sample was used as control. Acid ascorbic was used as positive control. All experiments were carried out in triplicate. Data were expressed as means  $\pm$  SD. The inhibitory concentration 50% (IC<sub>50</sub>) was calculated by a nonlinear regression curve with the use of Prism Graphpad Prism version 4.0 for Windows, GraphPad Software, San Diego, CA, USA ([www.graphpad.com](http://www.graphpad.com)). The dose–response curve was obtained by plotting the percentage of inhibition versus the concentrations. As shown in Table 2, the oil obtained from hydrodistillation of wood showed an interesting activity with an IC<sub>50</sub> value of 1.45  $\mu$ l/ml, while the oil obtained from hydrodistillation of berries exhibited an IC<sub>50</sub> value of 7.42  $\mu$ l/ml. A dose–response relationship was observed for *J. oxycedrus* ssp. *oxycedrus* oils (Fig. 1).

The antioxidant activity of different spices has been the subject of recently published research (Lee & Shibamoto, 2001; Mau, Ko, & Chyau, 2003; Zin, Abdul-Hamid, & Osman, 2002). In particular, some terpenes, such as carnosol (*Salvia officinalis*), eugenol (*Piper nigrum*), rosmanol (*Rosemarinus officinalis*), gingerol and zingerone (*Zingiber officinale*), thymol (*Thymus vulgaris*) and carvacrol (*Carum carvi*, *Origanum vulgare*), were found possess antioxidant activity through different mechanisms of action (Suhaj, 2006).

Table 2

IC<sub>50</sub> values ( $\mu$ l/ml) of antioxidant and hypoglycaemic activities of oils from *J. oxycedrus* ssp. *oxycedrus*

Plant material	Wood	Berry
Antioxidant activity (DPPH assay)	1.45 $\pm$ 0.05	7.42 $\pm$ 0.1
Hypoglycaemic activity ( $\alpha$ -amylase inhibition)	3.49 $\pm$ 0.09	>25

Data are given as means  $\pm$  SD ( $n = 3$ ). Ascorbic acid (IC<sub>50</sub> 0.002 mg/ml) and acarbose (IC<sub>50</sub> 50  $\mu$ g/ml) were used as positive controls for antioxidant and hypoglycaemic assay, respectively.

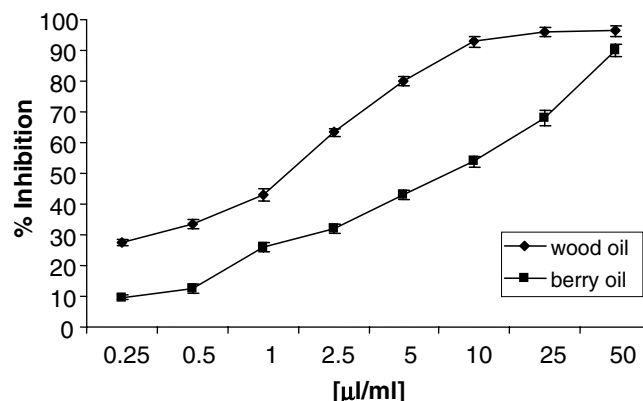


Fig. 1. Radical-scavenging activity of *J. oxycedrus* wood and berry oil using DPPH<sup>•</sup> assay. Each data point represents the mean  $\pm$  SD ( $n = 3$ ).

### 3.3. Determination of hypoglycaemic activity

The hypoglycaemic activity of *Juniperus oxycedrus* ssp. *oxycedrus* berry and wood essential oils were investigated by the inhibition of  $\alpha$ -amylase.

The absorbance ( $A$ ) due to maltose generated was calculated as

$$A_{540 \text{ nm}} \text{ control or Juniper oil} = A_{540 \text{ nm}} \text{ Test}$$

$$- A_{540 \text{ nm}} \text{ Blank.}$$

From the net absorbance obtained, the % (w/v) of maltose generated was calculated from the equation obtained from the maltose standard calibration curve (0–0.1%, w/v, maltose). Percent of inhibition was calculated as 100 – % reaction at  $t = 3$  min, whereby the % reaction = (mean maltose in sample/mean maltose in control)  $\times$  100.

The inhibitory concentration 50% (IC<sub>50</sub>) was calculated by nonlinear regression curve with the use of Prism Graphpad Prism version 4.0 for Windows, GraphPad Software, San Diego, CA, USA ([www.graphpad.com](http://www.graphpad.com)). The dose–response curve was obtained by plotting the percentage of inhibition versus the concentrations.

Results are summarised in Table 2, where data are given as means  $\pm$  SD. The enzyme inhibition exerted by *J. oxycedrus* ssp. *oxycedrus* wood oil followed a dose-dependent relationship (Fig. 2).

$\alpha$ -Amylase is the main enzyme in humans responsible for the breakdown of starch into simple sugars (dextrin, maltotriose, maltose and glucose). Although the activity of enzyme has not been directly involved in the aetiology of diabetes,  $\alpha$ -amylase inhibitors have been thought to improve glucose tolerance in diabetic patients (Lebovit, 1998). Extensive efforts have been made, over recent decades, to find a clinically effective  $\alpha$ -amylase inhibitor, with the aim of obtaining better control of diabetes (Jung, Matzke, & Stoltefuss, 1996). The results revealed that *J. oxycedrus* ssp. *oxycedrus* wood oil exhibited an interesting activity, with IC<sub>50</sub> of 3.49  $\mu$ l/ml, while oil obtained from berries exhibited a moderate activity, with IC<sub>50</sub> value > 25  $\mu$ l/ml. Recently, many studies have reported health

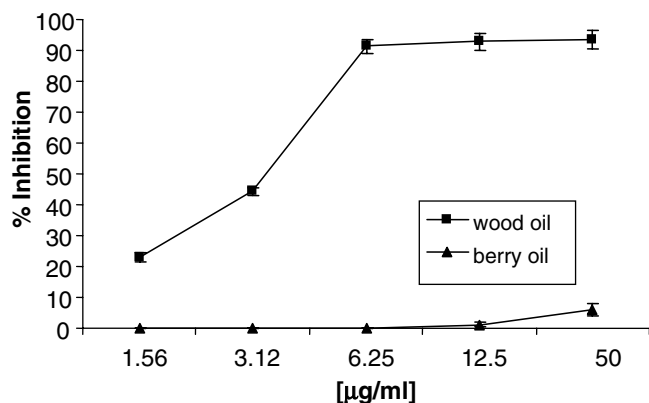


Fig. 2. Dose-dependent inhibition of  $\alpha$ -amylase by *J. oxycedrus* wood and berry oil. Each data represents the mean  $\pm$  SD ( $n = 3$ ).

benefits (against hyperglycaemia and diabetes) of some spices, such as *Salvia officinalis* through a metformin-like effect (Lima, Azevedo, Araujo, Fernandes-Ferreira, & Pereira-Wilson, 2006), *Rhus coriaria* and *Bunium persicum* (Statti et al., 2006), and grain, such as amaranth (*Amaranthus caudatus*) (Conforti et al., 2005) via the inhibition of  $\alpha$ -amylase. Another spice that could provide health benefits is *Origanum onites*, which might be effective in preventing, or at least in retarding, the development of some complications of diabetes mellitus (Lermioglu, Bagci, Onderoglu, Ortac, & Tugrul, 1997).

#### 4. Conclusion

Population-wide average dietary intake of common spices has been estimated at 0.5 g/person per day in Europe and 1.0 g/person per day in New Zealand. According to the American Spice Trade Association, per capita spice consumption in the United States was  $\sim 4$  g/person per day. In contrast, on the Indian subcontinent, turmeric consumption alone has been estimated at 1.5 g/person per day (Lampe, 2003).

Therefore, the capacity of spices to influence disease risk, within the context of culinary use, has not been completely evaluated. There is a strong need to understand the preventive effect of spices within the context of the total diet, where synergistic effects may be important.

Both wood and berry oils obtained from *J. oxycedrus* ssp. *oxycedrus* exerted antioxidant radical-scavenging effects when tested by DPPH $\cdot$  assay. However, hypoglycaemic activity, via the inhibition of  $\alpha$ -amylase, must be attributable to wood oil. Our work suggests *J. oxycedrus* ssp. *oxycedrus* as a promising future candidate supplement in food.

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