PHYTOCHEMICAL SCREENING AND ANTIOXIDANT ACTIVITY OF *Juniperus phoenicea* ssp. *phoenicea* L. EXTRACTS FROM TWO TUNISIAN LOCATIONS

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ABSTRACT

The antioxidant potency of *Juniperus phoenicea* ssp. *phoenicea* leaf extracts from two Tunisian localities was investigated. Total antioxidant activity was determined using an ABTS and DPPH assays. In addition, the content of phenols and total flavonoids were measured in the tested extracts. The methanol and ethyl acetate extracts exhibit potent scavenging activities towards ABTS and DPPH radical cations. Whereas, the petroleum ether extracts exhibit a low scavenging activity due to their poverty of total polyphenolic and flavonoid content. The antioxidant activities exhibited by *J. phoenicea* ssp. *phoenicea* depended on the chemical composition of the tested extracts.
1. Introduction

The use of natural products and/or compounds of natural origin, such as extracts from plant and fruit materials, in the health and food sectors, have been growing in the last years. Indeed, the extracts from these natural resources contain molecules with antioxidant and antibacterial properties, and therefore are beneficial for human health (Wiseman et al., 1999). Further to that, many drugs derived from plant secondary metabolites have been applied for the treatment and/or prevention of various diseases (Menichini et al., 2009). Polyphenols are a class of chemical compounds present in many plants and/or fruits. They are well known for their antioxidant activity (Shahidi and Wanasundara, 1992) and their potential against cardiovascular diseases (Ames et al., 1993). Their presence in natural plants is well known and widely reported in the literature.

The genus Juniperus is an important medicinal plant source, largely used in traditional medicine. Many Juniperus species were used traditionally to treat several diseases, especially diabetes, diarrhea, rheumatism, bronco-pulmonary diseases and as a diuretic (Bellakhder, 1997). J. phoenicea L. is a shrub or a small tree from the Mediterranean region (Bonnier and Douin, 1990). The species present a very large geographical distribution ranging from Portugal to Saudi Arabia (Meloni et al., 2006). Previous studies pointed out the chemical characterization of Juniperus phoenicea extracts and biological activities (Hayouni et al., 2007; Ennajar et al., 2009). However, very limited information’s are available about the antioxidant properties of J. phoenicea ssp. phoenicea from the south Mediterranean basin. Therefore, in present study chemical composition of J. phoenicea ssp. phoenicea leaves extract collected from two Tunisian localities and their antioxidant activities have been investigated.

2. Materials and Methods

2.1. Collection and preparation of plant extracts

Juniperus phoenicea ssp. phoenicea aerial parts (leaves) were collected in two Tunisian localities: Tabarka and J. Mansour, in January 2007. Botanical identification was performed by a taxonomic botanist from the High Institute of Biotechnology of Monastir, Tunisia, according to the flora of Tunisia (Cuédod, 1954). A voucher specimens (Jp-01-007) and (Jp-01-007) have been kept in the Laboratory of Pharmacognosy, Faculty of Pharmacy of Monastir-Tunisia, for future reference. The leaves were air-dried at room temperature under shade, and ground into powder using an electric grinder. The powder was packaged and stored in dry and well-ventilated room until use. Petroleum ether, ethyl acetate, and methanolic extracts with different polarities were obtained by Soxhlet apparatus (6 h). They were concentrated to dryness and kept at 4 °C in refrigerator and absence of light.

2.2. Preliminary phytochemical analysis

In order to test the presence of tannins, flavonoids and coumarins, we used the method described by Tona et al. (1998). Two milligrams of each extract were separately dissolved in 2ml of the adequate solvent. The identification of major chemical groups was carried by thin layer chromatography (TLC) on silica gel 60 F254 Merck (layer thickness0.25 mm) as follows; for flavonoids, TLC was developed in n-butanol/acetic acid/water 4:1:5 (top layer), spots were visualized with 1% aluminium chloride solution in methanol under UV (366 nm) (Harborne, 1974). Coumarins were detected under UV (366 nm) thanks to their blue fluorescence which becomes intense after spraying 10% potassium hydroxide solution in ethanol. The test for tannins was carried out with Fe Cl3. Each class of tannins gave a specific coloration.

2.3. Determination of total phenolic compounds and flavonoid contents

The amount of phenolic compounds present in the J. phoenicea leaf extracts was determined by Folin Ciocalteu reagent (Duh et Yeng, 1997) using gallic acid as standard according to Yuan et al., 2005. Each extract (100 μl) were mixed with 2 ml of 2% Na2CO3 and incubated at room temperature for 2 min. of 100 μl 50% Folin-Ciocalteu’s phenol reagent were added, the reaction tubes were the allowed to stand for 30 min at room temperature, before absorbance at 720 nm was measured (Genesys 10UV scanning spectrophotometer). The total amount of phenolic compounds was determined from the calibration curve, and expressed as percent of gallic acid in the extract (Capeaka et al., 2005). The amount of flavonoids was determined by the method of (Zhishen et al., 1999) using the Quercetin as the standard. A known volume of the extracts was placed in a 10 ml volumetric flask. Distilled water was added to make the volume to 5 and then mixed with 0.3 ml NaNO2 (1.20 w/v). Three milliliters of 10% aluminium trichloride (1:10 w/v) was added 5 min later. 6 min later, we added 2 ml of 1 N NaOH. The total absorbance was measured at 510 nm. The flavonoid content was determined from the calibration curve, and expressed as percent of quercetin in the extract (Eberhardt et al., 2000; Luximon-Rammon et al., 2000).

2.4. Antioxidant activity

DPPH radical scavenging assay

The DPHH free radical-scavenging assay was carried out, as previously reported by (Cheel et al., 2007) with some modifications, the extracts of J. phoenicea leaves at various concentrations (1, 3, 10, 100 and 150 μg/ml) were added to a 0.06mM DPPH* solution in ethanol and the reaction mixture was shaken vigorously. After incubation for 30 min at room temperature, the absorbance at 517nm was recorded spectrophotometrically. A control solution without the tested compound was prepared in the same manner as the assay mixture. The degree of decolourisation indicates the free-
radical scavenging efficiency of the substances. The antioxidant activity of extracts *J. phoenicea* leaves was calculated as an inhibitory effect (Scavenging activity %) of the DPPH radical formation as follows:

Scavenging activity (%) = \((100 \times (A_{517\text{ sample}} - A_{517\text{ control}})/A_{517\text{ control}})\)

\((A_{517\text{ sample}})\) and \((A_{517\text{ control}})\) were the absorbance at 517nm of samples with and without extracts, respectively. The IC\(_{50}\) (%) value was defined as the concentration in (mg/ml) of the compound required to scavenge the DPPH radical by 50%. The data were analyzed using analysis of variance (ANOVA).

**Measurement of the ABTS scavenging activity**

We adopted the method of Re et al., 1999 to determine using ABTS** free radical scavenging assay with some modifications. A stock solution ABTS solution (7mM) and \(K_2S_2O_8\) (2.45mM) was prepared. The mixture was allowed to stand for 15 h in the dark at room temperature. The ABTS solution was then diluted with ethanol to obtain the absorbance of 0.7±0.2 units at 734 nm. In order to test the antioxidant activity of extracts, the following concentrations (0.5, 2.5, 4.5, 7.5 and 9.5 mg/ml) was obtained by dissolving the extract samples with methanol. 10 ml of each sample at various concentrations was added to 990 ml of diluted ABTS** . The absorbance was taken at 734 nm and stopped after 30 min using the spectrophotometer. all measurements were performed in triplicate. The percent scavenging of ABTS** was calculated by the following formula.

\[\text{Scavenging activity (%) = } (A_x - A_0)/(A_x)\]

\(A_x\) and \(A_0\) were the absorbance at 734nm of samples with and without essential oils, respectively. IC50 values, defined as the inhibiting concentrations of substrate that causes 50% loss of ABTS activity (colour), were calculated by regression analysis.
3. Results and discussion

3.1 Phytochemical study

The results of the Juniperus phoenicea ssp. phoenicea phytochemical screening are reported in table 1. The methanol and ethyl acetate extracts were rich in flavonoids and tannins. The petroleum ether extracts are devoid of tannins and coumarins. Whereas small quantities of coumarins were detected only in the methanol extracts.

Table 2, presents the yields (%), total polyphenol content and total flavonoid content of J. phoenicea ssp. phoenicea extracts. Values are expressed in (%) as gram per dry leaves. The yields of extracts varied widely in plant material and ranged from 4.28 to 6.27% in the petroleum ether extracts, 10.24 to 22.13% in ethyl acetate extracts and 28.77 to 39.23% in methanol extracts for J. phoenicea ssp. phoenicea leaves of Tabarka and J. Mansour, respectively. On the whole, chemical extracts of J. phoenicea ssp. phoenicea leaves of J. Mansour are richer in total polyphenolic and total flavonoid contents. Methanol, ethyl acetate and petroleum ether extracts had a significantly different total polyphenol contents. The petroleum ether extracts showed the lowest values (0.8-0.7%) and (7.9-11.02%) of the total polyphenolic content and total flavonoid content, respectively. The methanolic extract showed the highest polyphenolic content (32.23-48.2%) and total flavonoid content (41.18-65.23%).

3.2 Antioxidant activity

DPPH radical scavenging activity

The radical scavenging activity of J. phoenicea ssp. phoenicea extracts, against the DPPH radical, is shown in table 3. The extracts of J. phoenicea ssp. phoenicea leaves from J. Mansour have stronger antioxidant activity against DPPH radical than Tabarka’s extracts. The methanol extract is the most potent radical scavenger with a percentage decrease versus the absorbance of DPPH standard solution of 72.15% and 95.89% for J. phoenicea ssp. phoenicea extract of Tabarka and J. Mansour, respectively at a concentration of 100μg/ml and a respectively IC50 values of 20.38μg/ml and 8.77μg/ml. Ethyl acetate extracts showed significant scavenging activity with a percentage inhibition of the decrease of DPPH radical of respectively 56.45% and 76.28% at a concentration of 100μg/ml, and IC50 values of respectively 90.52μg/ml and 59.12μg/ml. it can be noticed that the methanol and ethyl acetate extracts showed significant antiradical activity, as measured by their capacity to scavenge the stable free radical DPPH, while petroleum ether extracts reduced dimly the absorbance at 517nm even at high concentration of the extracts.

The positive control vitamin C was a very active radical scavenger showing percentage decrease against DPPH radical of 100% at 100 μg/ml and an IC50 value of 3.1 μg/ml. These results indicate that Methanol tested extract (IC50 =8.77μg/ml) of J. Mansour showed comparable antioxidant activities than to that of the positive control (vitamin C).

The second evaluation of the antioxidant activity using the ABTS radical corresponds to the measure of the moles of the ABTS free radical scavenged by a test solution, independently of the antioxidant present in the mixture (Ghiselli et al., 2000). The ABTS values for the J. phoenicea ssp. phoenicea extracts are shown in table 4. In the whole, the results accord with those of the DPPH radical scavenging activity. Extracts from J. phoenicea ssp. phoenicea of J. Mansour which are richer in polyphenolic constituents are more potent than those of Tabarka. Both methanol and ethyl acetate extracts reduced the ABTS radical. Indeed methanol extracts was significantly more active than ethyl acetate. The IC50 values of methanol extracts were 2.8 and 0.6mg/ml versus 5.21 and 3.1mg/ml of ethyl acetate extracts, for Tabarka and J. Mansour, respectively. However, the petroleum ether extracts exhibit a low antioxidant potential with an IC50 higher than 10mg/ml.

Table 4. Antioxidant activity of extracts of J. oxycedrus ssp. macrocarpa leaves observed with ABTS** radical (%) measured at different concentrations.

<table>
<thead>
<tr>
<th>Concentration [µg/ml]</th>
<th>Tabarka Petroleum ether extract</th>
<th>Tabarka Ethyl acetate extract</th>
<th>Tabarka Methanol extract</th>
<th>Ibal Mansour Petroleum ether extract</th>
<th>Ibal Mansour Ethyl acetate extract</th>
<th>Ibal Mansour Methanol extract</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5</td>
<td>1.14</td>
<td>18.45</td>
<td>32.45</td>
<td>2.02</td>
<td>22.78</td>
<td>41.78</td>
</tr>
<tr>
<td>2.5</td>
<td>1.96</td>
<td>32.78</td>
<td>48.23</td>
<td>2.56</td>
<td>45.2</td>
<td>53.95</td>
</tr>
<tr>
<td>4.5</td>
<td>2.65</td>
<td>45.15</td>
<td>60.19</td>
<td>3.21</td>
<td>56.12</td>
<td>67.01</td>
</tr>
<tr>
<td>7.5</td>
<td>3.10</td>
<td>59.78</td>
<td>79.85</td>
<td>3.99</td>
<td>62.35</td>
<td>80.14</td>
</tr>
<tr>
<td>9.5</td>
<td>3.57</td>
<td>75.23</td>
<td>82.47</td>
<td>4.13</td>
<td>79.85</td>
<td>95.98</td>
</tr>
<tr>
<td>IC50</td>
<td>&gt;&gt;10</td>
<td>5.21</td>
<td>2.8</td>
<td>&gt;&gt;10</td>
<td>3.1</td>
<td>0.6</td>
</tr>
<tr>
<td>TEAC [mM]</td>
<td>-</td>
<td>0.21</td>
<td>0.73</td>
<td>-</td>
<td>0.56</td>
<td>1.26</td>
</tr>
</tbody>
</table>

http://www.jebas.org
There is an increasing interest in the potential health benefits of natural antioxidants contained in plants but little is known about their mechanism of action at the molecular level. Indeed, it is well known that the polyphenols and flavonoids have an important role in the antioxidant activity. Thus the methanol and ethyl acetate extracts exhibit stronger antioxidant potential when compared with the petroleum ether extracts which are poor from these compounds. These results support our hypothesis according to which scavenging potential for DPPH and ABTS radicals of methanol and ethyl acetate extracts would be ascribed to the presence of its polyphenolic compounds. Whereas the scavenging potential for DPPH and ABTS radicals of petroleum ether extract would be ascribed to the low content of their polyphenolic content (Ben Mansour et al., 2007).

In fact, polyphenols, particularly flavonoids, which are widely distributed in the plant kingdom and are present in considerable amounts in fruits, vegetables, spices, medicinal herbs, and beverages, have been used to prevent many human diseases, such as diabetes, cancers, and coronary heart diseases (Broadhurst et al., 2000). Moreover, flavonoids have been shown to exhibit antioxidative, antiviral, antibacterial, and antitoxic activities (Middleton and Kandaswami, 1993). The biological activities of these polyphenols in different systems are believed to be due to their redox properties, which can play an important role in absorbing and neutralizing free radicals, quenching singlet and triplet oxygens, or decomposing peroxides (Oswa, 1994).

4. Conclusion

The present study has demonstrated that J. phoenicea ssp. phoenicea extracts possess potent antioxidant activities, of which could be derived from compounds such as flavonoids, phenols and sterols. These antioxidants activities could have contributed, at least partly, to the therapeutic benefits of the certain traditional claims. Thus, J. phoenicea ssp. phoenicea may serve as an ideal candidate for a cost-effective, readily exploitable natural phytochemical preparation.

References


