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Antioxidant Capacity of Essential Oils of Two Juniperus Species from Northwest of Algeria

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Abstract: This study was performed to evaluate the *in vitro* antioxidant activities of *Juniperus phoenicea* and *Juniperus oxycedrus* essential oils by three different methods, revealing an important antioxidant potential for both species. The results of the total antioxidant capacity of the EO's were 212.42 mg AE / g DM for *J.phoenicea* and 88.14 mg AE / g DM for *J.oxycedrus*. The effective concentration that reduces 50% of the DPPH solution is 1.279 g / ml for the *J. phoenicea* EO's and 6.56 µg/ml for *J.oxycedrus* EO's. With the method of FRAP, the two essential oils tested showed moderate reducing activity and significantly lower than ascorbic acid. The results indicated that *Juniperus* species have shown antioxidant activity which could be potential candidates for preparation of natural antioxidant drug or additive preparation.

Keywords: Essential Oil, *J. oxycedrus* and *J. phoenicea*, Antioxidant Activities

1. Introduction

Reactive oxygen species (ROS) including superoxide radicals, hydroxyl radicals, singlet oxygen and hydrogen peroxide are often generated as byproducts of biological reactions, or from exogenous factors (Cerutti, 1991). They have the ability, either directly or indirectly, to damage all biomolecules, including proteins, lipids, DNA, and carbohydrates(Shacter, 2000), eventually leading to many chronic diseases such as atherosclerosis, cancer, diabetics, rheumatoid arthritis, post-ischemic perfusion injury, myocardial infarction, cardiovascular diseases, chronic inflammation, stroke and septic shock, aging and other degenerative diseases in humans (Fridovich, 1999; Fang et al., 2002). Currently, many kinds of synthetic antioxidants, such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), tertiary butyl hydroquinone (TBHQ), and propyl gallate (PG), which are commonly used in processed foods, are known to have toxic and carcinogenic effects on human health (Kutlu et al., 2014). Recently there has been a considerable interest in finding natural antioxidants from plant materials to replace synthetic ones (Çakmak et al., 2012). In searching for novel natural antioxidants, some plants have been investigated in the past few years for their antioxidant and radical scavenging components, but there is still a demand to find more information concerning the antioxidant potential of plant species (Chanda et al., 2010).

The genus *Juniperus* belongs to the family *Cupressaceae* consisting of 55 species, all of which occur throughout the northern hemisphere of the world (Farjon, 1998) except *Juniperus procera* which is the only species of the genus that grows naturally in the southern hemisphere (Adams et al., 1993). In traditional medicine, *Juniperus* species are used as remedies against the common cold, urinary infections, urticaria, dysentery, hemorrhage, and rheumatic arthritis and to relieve menstrual pain worldwide (Orhan et al., 2012). Aromatic oils from junipers have been used since antiquity for fragrance, flavouring, medicinal, Antimicrobial, insecticidal and cosmetic purposes (Derwich et al., 2010). Therefore, the objectives of present study was to evaluate the in vitro antioxidant activity of the essential oils of *Juniperus phoenicea* and *Juniperus oxycedrus* through different free radical scavenging assay.

2. Materials and Methods

2.1. Evaluation of Total Antioxidant Capacity

The total antioxidant capacity of essential oils of *Juniperus*

was evaluated by phosphomolybdenum method according to Prieto et al. (1999). Briefly, 0.3 ml of essential oils was added to 3 ml of reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The reaction mixture was incubated at 95ºC for 90 min. Then, the absorbance of the solution was measured at 695 nm against blank. In the case of the blank, 0.3 mL of the ethanol was used in place of sample. The antioxidant capacity of extracts was evaluated as equivalent of ascorbic acid (mg AAE g^{-1}). as mg ascorbic acid equivalent (AAE)/g dry weight of essential oil.

2.2. Determination of DPPH Radical - Scavenging Activity

The DPPH radical scavenging assay elucidated by Chan et al., (2007) with modifications was followed. Different dilutions of the essential oils (1,25; 2,5; 5; 10; 20 and 40 µg/ml) were prepared. DPPH solution was also prepared by dissolving 4 mg of DPPH in 100 ml ethanol. Then, 1 ml of essential oil from each dilution was added into the test tube containing 4 ml of DPPH solution. Control was prepared by adding 1 ml of ethanol to 4 ml of DPPH solution. Ascorbic acid was used as standard. The mixture was shaken vigorously and was left to stand in the dark for 30 min. The absorbance of the resulting solution was measured spectrophotometrically at max of 517 nm. The scavenging activity of each compound on DPPH radical was calculated using the following equation. Calculation: % scavenging activity = [absorbance of control absorbance of sample / absorbance of control] × 100

The IC_{50} value is defined as the amount of antioxidant necessary to inhibit DPPH radical formation by 50%.

2.3. Ferric Reducing/Antioxidant Power (FRAP) Assay

The ferric reducing ability of different extracts was estimated by the method of Zhao et al. (2008). 0.5 ml of different concentrations of essential oils were mixed with 1.25 ml of 0.2 M phosphate buffer and potassium ferricyanide and 1.25 ml of 1% mixture was incubated at 50ºC for 20 min. After incubation, 2.5 ml of 10% trichloroacetic acid was added and the tubes were centrifuged foe 10 min at 3000rpm. The supernatant (1.25 ml) was mixed with 1.25 ml of distilled water and 250 µl of 0.1% ferric chloride solution. The solution absorbance was measured at 700 nm. Ascorbic acid was used as a positive control.

3. Result and Discussion

Several methods are commonly used to measure the antioxidant capacity of essential oils of *Juniperus phoenicea* and *Juniperus oxycedrus*.

As shown in Figure 1, the total antioxidant capacity was higher in essential oil of *Juniperus phoenicea* than *Juniperus oxycedrus* essential oil. It was determined to be 212.42 mg AE/g DM and 88.14 mg AE/g DM, respectively

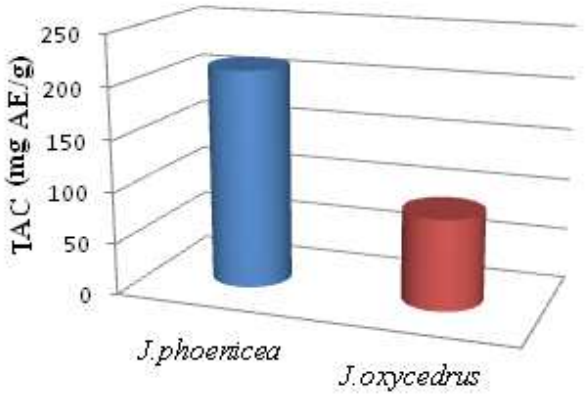

Figure 1. *The total antioxidant capacity of of Juniperus phoenicea and Juniperus oxycedrus essential oils.*

In DPPH method, the antioxidants react with the stable free radical. 1,1-diphenyl-2-picrylhydrazyl (deep violet color) and convert it to 1,1-diphenyl-2-picrylhydrazine with discoloration. The degree of discoloration indicates the free radical scavenging activities of the sample/antioxidant and it has been found that the known antioxidants such as cysteine, glutathione, ascorbic acid, tocopherol, and polyhydroxy aromatic compounds (hydroquinone, pyrogallol, etc.) reduce and decolorize 1,1-diphenyl-2-picrylhydrazyl by their hydrogen donating ability (Amiri, 2010). Regarding the IC_{50} values (Table 1 and fig 3), which are defined as the concentration of test material which is able to decrease the initial concentration of DPPH to half of its initial value (Erkan et al., 2008), it is possible to conclude that *J. phoenicea* oil has more pronounced antioxidant activity than *J. oxycedrus* oil, since its IC_{50} value is lower than the one of *J. oxycedrus*.

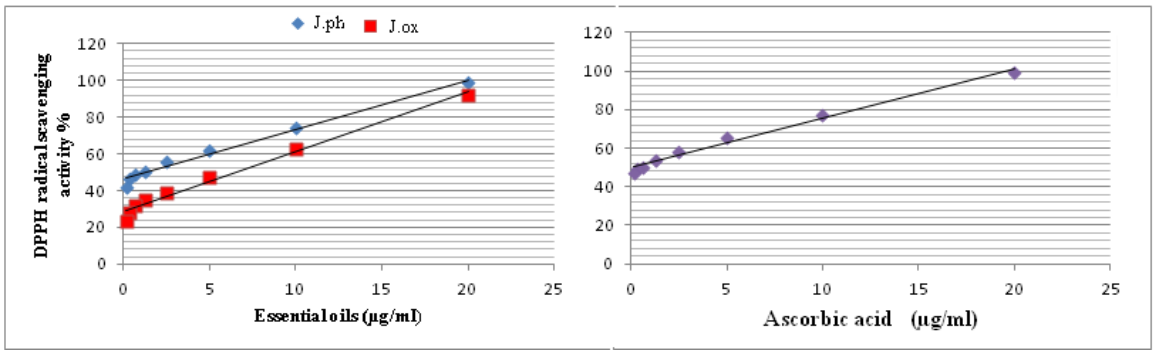

Figure 2. *The DPPH radical scavenging activity of Juniperus phoenicea and Juniperus oxycedrus essential oils at various concentrations.*

Table 1. *Antioxidant properties of the two Juniperus species essential oils measured by DPPH scavenging assay.*

Ascorbic Acid	*J. phoenicea* oils	*J. oxycedrus* oils
0.06	1.279	6.56

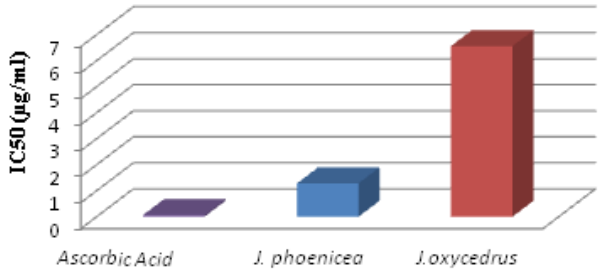

Figure 3. *IC_{50} Values.*

The FRAP assay, which provides fast reproducible results, measures the ability of an antioxidant to reduce the ferric tripyridyltriazine (Fe^{+3}-TPTZ) complex and produce the ferrous tripyridyltriazine (Fe^{+2}-TPTZ) complex, which is blue in color and can be detected at 700 nm (Benzie and Strain, 1996). Our results show that the FRAP activity of *Juniperus* essential oils was increased in a dose-dependent manner. Importantly, the activity of the *Juniperus phoenicea* essential oils was significantly higher than the activities of *Juniperus oxycedrus* essential oils (Figure 4).

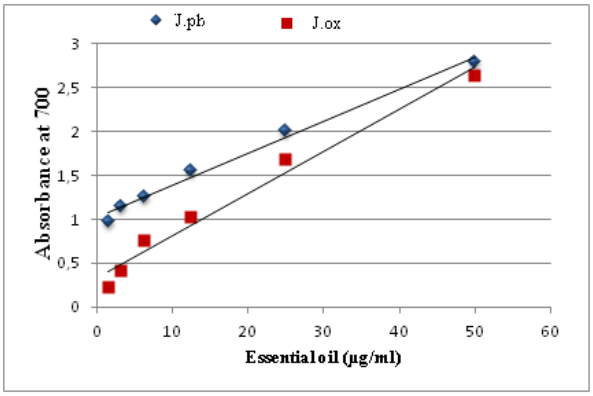

Figure 4. *FRAP activity of Juniperus phoenicea and Juniperus oxycedrus essential oils at various concentrations.*

Our results corroborate those of Menaceur et al. (2013) and who reported that *J. phoenicea* and *Juniperus oxycedrus* extract was found to be remarkably effective toward DPPH inhibition. In other study, higher antioxidant activities were recorded for Essential oils from *Juniperus thurifera*, *Juniperus oxycedrus* and *Juniperus phoenicea* collected in various areas in Morocco (Satrani et al., 2015). Loizzo et al. (2007) found a value of IC_{50} of 7.42 µg/ml in his study to the antioxidant capacity of the essential oils from the fruits of the Lebanon *J. oxycedrus*. Emami et al. (2007) reported an important antioxidant effect in several species of the *Juniperus* genus originating from Iran. Bouzouita et al. (2008) showed a good antioxidant potential of the essential oils of Tunisian *J. phoenicea*.

Recently, Elmhdwi et al. (2015), found that vegetal extracts is strongly among the three tested extracts from the Leaves of *Juniperus Phoenicea,* 70% acetone is the best solvent for scavenging activity and have exhibited the higher reducing power. El Jemli et al. (2016) found in his investigation of the antioxidant properties and phenolic contents of aqueous leaf extracts of *Juniperus thurifera*, *Juniperus oxycedrus*, *Juniperus Phoenicea,* and *Tetraclinis articulata* from Morocco that the aqueous extract of *Juniperus oxycedrus* showed the highest antioxidant activity as measured by DPPH, TEAC, and FRAP assays with IC_{50} values of 17.91 ± 0.37 μg/mL, 19.80 ± 0.55 μg/mL, and 24.23 ± 0.07 μg/mL, respectively. The antioxidant potential of *J. phoenicea* and *J. oxycedrus* essential oils was investigated using three different methods; the most frequent DPPH and FRAP assays and phosphomolybdenum method. As one can notice the antioxidant activity of related with their chemical composition. The most important antioxidant compounds are phenolics specially flavonoids which are presented in different kinds of plants (Moein and Moein, 2010). As a peculiarity, these essential oils contain important amounts of flavonoids. High concentrations of flavonoids are reflected in the significant scavenging properties (Yang et al., 2008; Bischin et al., 2011; Moussa-Ayoub et al., 2011; Medini et al., 2013; El Jemli et al., 2016).

4. Conclusion

In this study, the antioxidant activities of methanol extracts of *Juniperus phoenicea* and *Juniperus oxycedrus* essential oils cultivated in northwest of Algeria were investigated. We used various *in vitro* antioxidant activity measurement methods to determine that these *Juniperus* essential oils have significant antioxidant activity. Significantly, *Juniperus phoenicea* essential oils showed greater antioxidant activity compared with *Juniperus oxycedrus* essential oils. Based on the results described above, we conclude that two *Juniperus* oils might be a good candidate for further investigation in developing new antioxidant agents and can be used as a natural additive in food, cosmetic and pharmaceutical industries. However, the safety and toxicity of these compounds will need to be addressed.

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