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Chemical composition and antioxidant activities of the essential oils from green and ripe berries of *Juniperus excelsa* growing in Lebanon

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Abstract

The essential oils of unripe and ripe berries have been obtained by hydrodistillation and identified using GC/MS revealing significant difference in compositions between the two essential oils. Thirty two compounds (86%) and thirty compounds (86.81%) have been identified in the unripe and ripe barriers essential oils respectively. While trans-nerolidol (23.76%), (Z,E)-farnesol (22.2%), and α-pinene (21.8%) have been the major compounds of the unripe berries' essential oil, α- pinene (44%) has been the major compound of the ripe berries' essential oil in addition to other compounds like β- myrcene (6.99%), (E,E)-farnesol (4.66%), and β- pinene (4.57 %). The antioxidant activities of the essential oils of green and ripe barriers, in addition to that of the positive control Butylated hydroxyltoulene (BHT) have been employed using 2, 2'-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging method. The results reveal limited antioxidant activities of the two essential oils compared to BHT.

Key-Words: *Juniperus excelsa*, essential oil, antioxidant activity

Introduction

Juniperus excelsa, or "Grecian Juniper", is a coniferous plant in the genus *Juniperus* of the cypress family Cupressaseae which is scattered widely through the northern hemisphere especially in Albania, Iran, Turkey, Greece, Lebanon, Syria, Serbia, and Caucus Mountains [1]. In Lebanon, This evergreen tree expands on all the northern part of the Western Mountain Chain and in some parts of the Eastern Mountain Chain [2] at an altitude varying between 800 m (Nahr Ibrahim/Qartaba) and 2800 m (Makmel Mountain Chain).

The medical use of *Juniperus excelsa* is well known in the Bosnian, Lebanese, and Turkish folk medicine. The berries are used to treat skin diseases like skin rash and eczema [3] in addition to a wide range of respiratory tract diseases like asthma, common cold, cough, bronchitis, throat inflammation, pneumonia and tuberculosis, [2 , 4, 5], urinary tract inflammations, renal and gall bladder stones, and rheumatism [6].

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Food quality is affected by several factors especially lipid oxidation which leads to flavor deterioration, biological damage, loss of nutritional and safety values. These effects are due to the formation of reactive oxygen species (ROS) that can cause damage to proteins, membranes and biological components, thus affecting vital cell functions [7]. In order to preserve food quality and prevent or delay lipid oxidation, synthetic antioxidants, like BHT, are commonly used. However, the safety of the use of these synthetic additives is of big concern especially that The International Agency for Research on Cancer, part of the World Health Organization, considers BHT to be possibly carcinogenic to humans [8].

In this study, the first report on the Lebanese *Juniperus excelsa*, the chemical composition of the essential oils of green and ripe berries of *J. Excelsa* has been determined and their antioxidant activities have been assessed. The results obtained justify the use of these berries in traditional medicine, however, the limited antioxidant activities observed do not encourage their use as food additives.

Material and Methods

Chemicals and Media

All chemical have been purchased from Sigma-Aldrich (Steinheim, Germany) and Fluka Chemie (Buchs, Switzerland).

Plant Material

1500 g of chosen unripe berries from *Juniperus excelsa* have been collected from Makmel Mountain, North Lebanon, in December, 2010.

Isolation of the Essential Oils of Green and Ripe Berries

The 1290 g of air dried green berries have been divided into two equal parts. The first part has been grounded and submitted to hydrodistillation for 3 hrs, using a Clevenger –type apparatus [9], to extract its essential oil. The second part has been left for one week to ripe and change to purple-brown. The ripe berries have been treated in the same way as the green berries for their essential oil's extraction and the obtained essential oils have been collected separately in dark glass vessels and stored at 4°C until analysis.

Analysis of the Essential Oils by GC/MS

A Schimadzu QP 2010 plus gas chromatography system interfaced to a 2010 mass spectrometer has been used for the analysis of the two essential oils. The separation has been performed on a 30 m x 0.25 mm i.d. (internal diameter) fused silica capillary column coated with 0.25 µm film Rtx-5MS. The injector and the detector temperatures have been respectively 250 and 280 °C. The oven temperature has been held at 40 °C for 5 min, and programmed from 40 to 100 °C at 4 °C min^{-1} then to 280 °C at 19 °C min^{-1} and finally maintained at 280 °C for 5 min. Split injection was conducted with a split ratio of 5:10. Helium has been used as carrier gas, and flow-rate has been 1.62 mL min^{-1}. The mass spectra have been recorded over a range of 30-1000 amu (atomic mass unit) at 0.5s $scan^{-1}$. Solvent cut time has been set to 3 min and the Ionization energy was 70 eV. The inlet and ionization source temperature have been 280 °C. Most constituents have been identified based on comparing their retention indices and mass spectra to the NIST library.

Antioxidant Activity (DPPH Assay) of the Green and Ripe Essential Oils

The antioxidant activities of the essential oils have been assessed based on their radical scavenging effect on the stable DPPH radical. DPPH (0.0 2mM) has been mixed with a range of (40 µl – 500 µl) of the essential oil. The total volume has been adjusted to 4 ml by methanol so that the concentrations of the essential oil in the different test tubes ranged between 9.18 and 114.75 mg/ml. The reaction mixture has been shaken and then incubated at room temperature in dark for 60 minutes and the DPPH radical inhibition has been measured at 517 nm using a Shimadzu UV spectrophotometer. Using the same conditions, BHT has been used as a positive control to compare its results to those of the essential oil.

Results and Discussion

Chemical composition of the ripe and fresh essential oils

The results from GC/MS analyses of the essential oils of the green and ripe *Juniperus excelsa* berries are presented in Table 1. Thirty two compounds, representing 86 % of the total composition of the oil of the green berries, have been identified, whereas thirty compounds, representing 86.81% of the total composition of the oil of the ripe berries, have been identified.

The results show a remarkable difference in the composition of the two oils. Upon ripening, monoterpenes have exhibited a significant increase in percentage from 27.86 to 68.92%. Oxygenated monoterpenes and sesquiterpenes have increased but to a less extent. Conversely, oxygenated sesquiterpenes have decreased from 54.86 % to 8.48 %.

Since there is no indication in the literature regarding the conversion of sesquiterpenes into monoterpenes, it may be suggested that upon ripening, modifications in the activities of enzymes involved in secondary metabolism have lead to these variations.

The change in the composition upon ripening has been detected in *Juniperus oxycedrus* berries by Salido *et al.* [10]. The results obtained has shown that there has been an increment in the level of α- pinene,as in the case of our study. However, the variations of other compounds like α-terpineol, myrtenal, β -myrcene, D-limonene and terpinolene have been the opposite indicating a difference in mechanisms among different species of the same genus.

Based on composition, the obtained results are similar to the report of the chemical composition of the essential oil of the *Juniperus excelsa* grown in Turkey [11] where α-pinene has been the major component (55.5 %). Unlu *et al.* [11] have also reported the presence of α-cedrol (7.7 %) and verbenone (2.4%) which are not detected in our study. This difference in the oils' composition may result from geographical origin, edaphic factors, or harvesting time, in addition, Unlu *et al.* [11] have not specified whether the work has been carried out on ripe or green berries.

The elevated concentrations of the oxygenated sesquiterpenes, nerolidol (27.66%) and farnesol (22.43%), in the green berries are of great importance. Previous studies have shown that farnesol induced

effectively the apoptosis of carcinoma cells ([12] and was capable of suppressing tumorigenesis suggesting that it has a chemopreventative effect [13]. Similarly, nerolidol has been shown to induce cell death and arrest cell growth in human liver carcinoma cells [14].

On the other hand, the ripe essential oil contains molecules like β-Myrcene, D-Limonene, Farnesol, and Camphor along with the major constituent α –Pinene (Table 1) which are stated to have important health effects. Myrcene has been proved to have a dose dependent analgesic effect [15] and increase in the sleeping time [16, 17] have indicated that D-Limonene exhibit a chemopreventitive effect against hepatocarcinogenesis in mice. Limonene has shown the ability to attenuate the gastric carcinogenesis by increasing apoptosis [18] and to have a mild bronchoconstrictive effect. Similarly, camphor has been found to be effective against hepatic carcinogenesis [19] and stimulate error-free DNA repair processes [20]. Although α –Pinene is considered to be a primitive molecule with limited medicinal impact [21] Matsuo *et al.* [22] have given the first report that demonstrate the apoptotic effect of α –Pinene against Malignant melanoma (skin cancer).

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Antioxidant Activity of the Green Essential Oil

The degree of inhibition has been calculated as a percentage using Equation 1.

$$\% \text{ DPPH inhibition} = (A_{\text{Blank}} - A_{\text{Sample}}) / A_{\text{Blank}} \times 100 \quad \text{(Equation 1)}$$

The essential oil of the green berries has exhibited weak antioxidant activity (Figure 1) having IC_{50} 36.15mg/ml compared to BHT with IC_{50} 4.09µg/ml (Figure 2). This is due to its major constituents, i.e. nerolidol, farnesol, and *α*-pinene, which are at best, weak antioxidants [23]. The limited antioxidant activity exhibited by the oil may be attributed to other constituents, which have been detected in low quantities, like terpinene (0.56%) [24] and terpinolene (0.67%) [25]. Similarly, the ripe essential oil has exhibited a weak antioxidant activity with an IC_{50} 29.76mg/ml (Figure 3) due to the dominance of *α*-pinene and β-pinene. However, the slightly enhanced activity of the ripe oil over the green oil may be related to the increment in the levels of D-limonene (3.49%) and β-myrcene (6.99%) that have been categorized by Santiago *et al.*[26], Ciftci *et al.* [27] and Bakkali *et al.* [28] as significant antioxidants.

Conclusion

This study gives a scientific explanation to justify the usage of the berries of *Juniperus excelsa* in folk medicine especially when used for healing skin, gastrointestinal, respiratory and cardiovascular diseases. The difference in the composition of green and ripe essential oils suggests that the berries can be used as ripe or green depending on the target disease.

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Table 1: Chemical composition of the E.O. of the green and ripe berries

Name	Rt	RI	Ripe Area %	Green Area %
Tricyclene	8.908	930	0.98	0.24
α -Pinene	9.317	933	44.4	21.8
Camphene	9.75	953	1.64	0.97
β -Pinene	10.592	978	4.57	0.99
β -Myrcene	11.092	991	6.99	1.73
α -Phellandrene	11.492	1007	-	-
3-Carene	11.683	1009	0.13	-
α -Terpinene	11.917	1018	0.21	-
p-Cymene	12.217	1025	1.06	0.14
D-Limonene	12.367	1030	3.49	0.76
Ocimene (*E*)	13.175	1046	-	-
γ -Terpinene	13.558	1052	2.33	0.56
Terpinolene	14.625	1086	3.12	0.67
α -Campholenal	15.908		1.26	-
trans-Pinocarveol	16.308	1141	1.22	-
Camphor	16.5	1149	2.66	0.41
cis-Pinocamphone	17	1176	0.33	-
Terpinen-4-ol	17.5	1180	0.24	-
α -Terpineol	17.9	1198	0.12	1.05
Myrtenal	18.075	1197	0.39	0.16
Verbenone	18.458	1208	0.31	-
trans-Carveol	18.7	1223	0.16	-
Bornyl acetate	20.492	1285	0.28	1.04
Myrtenyl acetate	20.842	1326	0.38	0.15
β -Cedrene	23.567	1423	1.58	0.38
cis-Thujopsene	23.975	1433	0.25	0.09
γ -Cadinene	24.958	1512	0.23	-
cis-Nerolidol	26.015	1562	-	3.9
trans- Nerolidol	26.346	1562	-	23.76
Nerolidylacetate	27.063		-	1.11
α -Cedrol	27.933	1610	3.61	0.87
Farnesol (*E,E*)	28.35	1716	4.66	0.23
Farnesol (*Z,E*)	29.467		-	22.2
Farnesal	29.708	1737	0.21	2.79
Monoterpenes (%)			68.92	27.86
Oxygenated monoterpenes (%)			7.35	2.81
Sesquiterpenes (%)			2.06	0.47
Oxygenated sesquiterpenes (%)			8.48	54.86
Total (%)			86.81	86

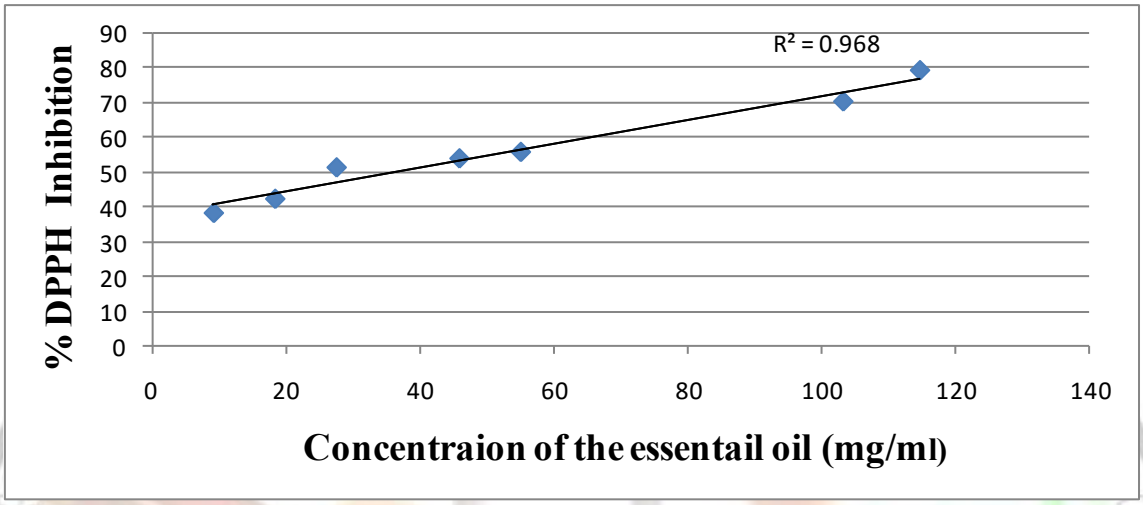

Fig. 1: DPPH scavenging capacity of green essential oil

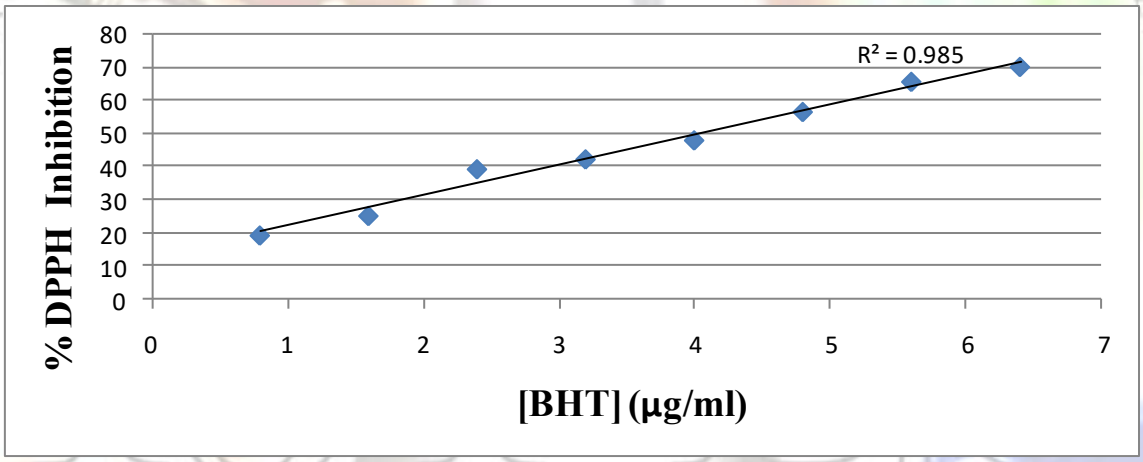

Fig. 2: DPPH Scavenging Capacity of BHT

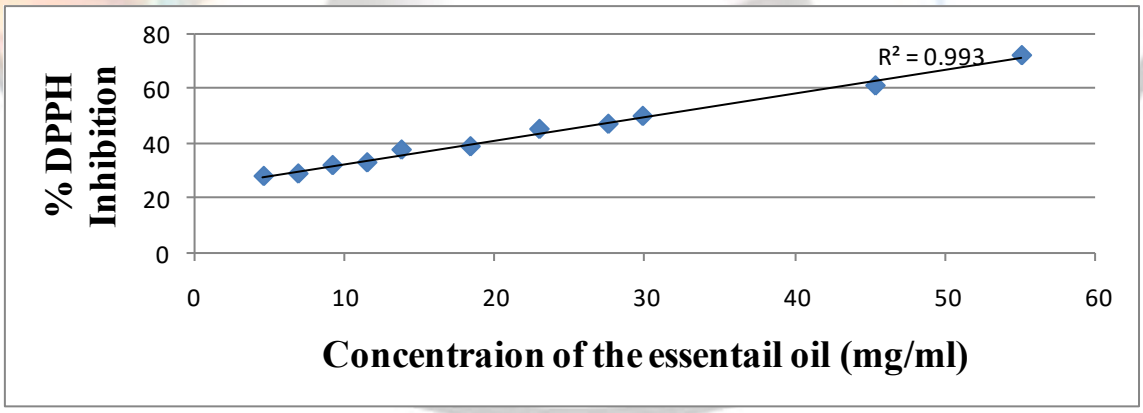

Fig. 3: DPPH Scavenging Capacity of Ripe Essential Oil