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Comparison of Chemical Composition and Antioxidant Activity of Four Essential Oils Extracted from Different Parts of *Juniperus excelsa*

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ABSTRACT: The chemical contents and compositions of four essential oils extracted from leaves, branches, fruits and gum-resin of *Juniperus excelsa* from Jabal Al-Akhdhar in the Sultanate of Oman were studied using infra-red (IR) spectroscopy and gas chromatography-mass spectrometry (GC-MS). IR analysis revealed the presence of C-H, O-H, C=C and C=O functionality bonds with varied intensities. The GC-MS study showed the occurrence of 35 different monoterpenes of which 11 (31.43%) monoterpenes were shared by the four oils. α-Pinene was detected in branches and gum-resin oils as a major compound with different proportions (80.50% in gum oil and 79.03% in branches oil). The major component in leaf and fruit oils was the monoterpene limonene with proportions of 50.46% and 42.51%, respectively. Sesquiterpenes exist in leaf, branch and fruit essential oils but not in gum-resin oil. The essential oil of the leaves showed stronger antioxidant and radical scavenging activities than the other oils.

Keywords: *Juniperus excelsa;* Essential oil; GC-MS analysis; Monoterpenes; Antioxidant; DPPH radical scavenging.

مقارنة المحتوى الكيميائي ودراسة القدرة على تثبيط التأكسد لأربعة زيوت عطرية مستخلصة من أجزاء مختلفة من شجرة العلعلان

صالح البوصافي، سالم السعيدي، أماني الريامي، نوال المنذري

الملخص: تم دراسة المحتوى الكيميائي لأربعة زيوت عطرية مستخلصة من أوراق وأغصان وثمار ولبان شجرة العلعلان (*Juniperus excelsa*) المنتشرة في الجبل الأخضر في سلطنة عمان باستخدام طيف الأشعة تحت الحمراء و كروماتوجرافيا الغاز المرتبط بجهاز كشف الكتلة. لقد كشف لنا جهاز طيف الأشعة تحت الحمراء عن وجود الروابط الكيميائية التالية في مركبات الزيوت العطرية: C-H, O-H, C=C, C=O بنسب متفاوته. بينما كشف جهاز الكروماتوجرافيا الغازية عن وجود 35 مركب كيميائي مختلف من نوع المونوتيربينات في الزيوت العطرية الأربعة منها 11 مركّب (31.43%) مشترك. مركّب الفا- بينين يوجد في زيت الأغصان واللبان كجزء اساسي بكميات مختلفة: % 80.50 في زيت لبان العلعلان و 79.03% في زيت أغصان النبات بينما يوجد مركّب الليمونين بكثرة في زيت الأوراق بنسبة % 50.46 وفي زيت الثمار بنسبة 42.5%. يمتاز زيت لبان العلعلان بعدم وجود مركّبات السيسكويتربين بينما توجد هذه المركبات بنسب متفاوتة في الزيوت الثلاثة الأخرى. يظهر زيت أوراق شجرة العلعلان نشاط أكبر على كبح التأكسد مقارنة مع الزيوت الأخرى.

الكلمات المفتاحية: شجرة العلعلان، زيوت طيارة، الكروماتوجرافيا الغازية، المونوتيربينات، تثبيت التأكسد.

1. Introduction

The Juniper tree (*Juniperus excelsa*) grows at an altitude of about 2,300 m on Jabal Al-Akhdar (Sultanate of Oman) in an open dry mountain forest under suitable conditions [1]. In addition, *J. excelsa* can be found throughout the eastern Mediterranean from northeastern Greece and southern Bulgaria across Turkey to Syria [2], and in the mountains of Iran, Pakistan, Saudi Arabia, and Yemen [3]. Traditionally, *J. excelsa* is used in folk medicine to treat different ailments such as abdominal rigidity, asthma, fever, headache, diabetes and leucorrhoea [4,6]. In other parts of the world, the plant is considered useful as an antihypertensive, diuretic, appetizer, carminative, stimulant, anticonvulsant, flavoring agent, and as a remedy for tuberculosis [7,8].

Bioactivity studies performed on *J. excelsa* have revealed antibacterial [9], antifungal [10], and bronchodilatory activities [11]. Sandracopimaric acid, a diterpene isolated from *J. excelsa* was found to exhibit significant antibacterial activity against *Bacillus subtilis*, *Staphylococcus aureus* and *Streptococcus durans* [12].

Moreover, hexane extract of the berries of *J. excelsa* exhibited potent activity against a human colon cancer cell line [13].

Chemical compositions of essential oil of *J. excelsa* leaves and fruits have revealed diverse ratios of monoterpenes and sesquiterpenes from one region to another. In this regard, chemical analysis of the essential oil of the leaves of *J. excelsa* from the Crimea revealed the presence of α-pinene (56.2%), limonene (10.4%), δ-3-carene (3.1%), camphene (2.9%), β-pinene (1.2%), γ-terpinene (1.9%) and terpinolene (0.8%) [14]. The essential oil (EO) extracted from the leaves of *J. excelsa* from Jammu (India) was reported to contain sabinene (36.1%) and cedrol (26.8%) as the major components [15]. Chemical analysis of the leaf oil of *J. excelsa* from Greece revealed the presence of cedrol (28.1%), α-pinene (22.5%) and limonene (22.7%) as the major components [16]. The essential oil extracted from the *J. excelsa* fruits of Iran origin showed α-pinene (89.5%), myrcene (2.6%), germacrene (2.2%), and limonene (1.3%) with other minor components [17]. Essential oil of the leaves of *J. excelsa* from Oman has been found to possess limonene (49.6%), germacrene (9.6%), δ-3-carene (5.9%), α- pinene (4.81%) and γ-cadinene (3.76%) [18].

In addition, the juniper tree produces gum-resin that appears on the surface of the branches. The chemical compositions of the essential oil extracted from the gum-resin of *J. excelsa* have not yet been described in the literature. In this study we analyzed the chemical contents and compositions of the essential oil of the gum-resin of *J. excelsa* along with essential oils of the leaves, branches and fruits using IR spectroscopy and GC-MS spectrometry. Additionally, the four extracted materials were subjected to antioxidant and radical scavenging activity tests.

2. Materials and Methods

2.1 Plant materials

Gum resin, leaves, branches and fruits of *J. excelsa* were collected in October 2013 at Jabal Al-Akhdhar, Sultanate of Oman. The plant was botanically identified by Dr. Amina Al-Farsi at the Botanical section of the Life Science Unit, College of Science, Sultan Qaboos University.

2.2 Extraction of volatile oil

Fresh 70.2 g, 152.0 g, 212.0 g and 230.4 g samples of gum-resin, leaves, branches and fruits, respectively, of *J. excelsa* were subjected to hydro distillation using Clevenger's apparatus until complete exhaustion to yield 3.1 g (4.4%), 0.41 g (0.27%), 0.68 g (0.32%) and 1.2 g (0.52%) of essential oils respectively. The obtained oils were collected, dried over magnesium sulphate and kept at 4^0C until analysis. The isolation percentage was calculated based upon the weight of the fresh materials used. IR spectra were obtained using the neat liquid method with a Nicolet model Magna 560 spectrometer; absorption bands are recorded in wave number (cm^{-1}).

2.3 Gas chromatography-mass spectrometry

The GC-MS analyses were carried out with a Shimadzu GC-MS-QP/5050A apparatus equipped with a quadrupole mass spectrometer and a J&W Scientific DB-5MS (5% phenyl / 95% dimethylpolysiloxane) fused-silica capillary column (30 m x 0.25 mm I.D. x 0.25 mm film thickness). The oven temperature was programmed to increase from 31˚C to 271˚C at 3 ˚C /min. Injector and interface temperatures were kept at 275 ˚C and 300 ˚C respectively. Helium was used as carrier gas with a linear velocity of 44.6 cm/s, column flow rate of 1.5 mL/min and total flow rate of 36 ml/min. The split ratio was 1:21. Mass spectra were continuously recorded over the mass range 35 to 501 amu. The MS operating parameters were as follows: ionization voltage 70 eV and scan rate 500 amu/s. The oil constituents were identified by comparison of their Kovat's relative retention indices (RIs), determined relative to the retention times (tR) of a homologous series of n-alkanes (C8 – C30), with those reported in the literature. Additionally, the mass spectra obtained were compared to those recorded in the computer MS library (Wiley 229,000 database). The percentage composition was determined by using the single area percentage method without considering corrections for response factors.

2.4 Evaluation of total antioxidant activity by the phosphomolybdenum method

The antioxidant activity of the essential oils was evaluated by the phosphomolybdenum method according to the procedure of Prieto *et al*. [19]. An aliquot of 0.3 ml of sample solution (1 mM in methanol) was combined in a 4 ml vial with 3 ml of reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The vials were capped and incubated in a water bath at 95 °C for 90 min. After the samples had cooled to room temperature, the absorbance of the mixture was measured at 695 nm against a blank. Triplicate measurements were made and the antioxidant activity was expressed as ascorbic acid (AA) equivalent using the following linear equation established using ascorbic acid as standard: [$A = 0.0013\ C + 0.049$; $R^2 = 0.974$] where A is the absorbance at 695 nm and C the concentration of ascorbic acid (mg/L). The values are presented as the means of triplicate analysis.

2.5 Free radical scavenging activity (DPPH, 2,2-diphenyl-1-picrylhydrazyl)

The free radical scavenging activity of the essential oils was determined by the DPPH free radical method [20]. A 2.0 ml methanolic solution of DPPH (0.1 mM) was mixed with 0.1 ml of oil solution (0.1 mg/ml) in methanol and, after standing for 60 min, the absorbance of the mixture was measured at 517 nm against methanol as the blank. Triplicate measurements were made and the radical scavenging activity was calculated by the percentage of DPPH that was scavenged using the following formula:
% Reduction = [(AB – AA)/AB] x 100, where AB is the absorption of blank sample and AA is the absorption of tested oil solution.

3. Results and Discussion

The essential oils of different parts of *J. excelsa* were obtained by conventional hydrodistillation of the crushed fresh materials. The essential oil of the gum-resin of *J. excelsa* (4.4%) was a colorless oil which solidified with time to a white solid. This behavior can be attributed to the high percentage of oxygenated monoterpenes compared with other essential oils (Table 2). The essential oil of the leaves of *J. excelsa* (0.27%) was colorless oil which stayed liquid on standing. The essential oils of the branches of *J. excelsa* (0.32%) and of the fruits (0.52%) were light yellow.

3.1 IR analysis

IR analysis of the essential oils of different parts of *J. excelsa* revealed the presence of four characteristic symmetrical stretching bands for O-H, C-H, C=O and C=C bonds (Table 1). The stretching frequency of the C-H bond ranged from 2916 cm^{-1} in fruits and leaves to 2965 cm^{-1} in gum-resin. The O-H stretching band varied from strong frequencies as in the essential oils of branches and fruits, at 3430 cm^{-1} and 3524 cm^{-1} respectively, to moderate and weak frequencies as in the essential oils of gum-resin and leaves. The C=O stretching frequency ranged from 1700 cm^{-1} in fruit oil to 1753 cm^{-1} in branch oil. Such frequency ranges indicate the presence of saturated carbonyl compounds.

Table 1: IR data of essential oils of different parts of *J. excels*a [s: strong; m: medium; w: weak]

Functional group	Wavenumber (cm^{-1})			
	Gum-resin	**Leaves**	**Branches**	**Fruits**
O-H	3439 (m)	3418 (w)	3430 (s)	3524 (s)
C-H	2965 (s)	2916 (s)	2941 (s)	2916 (s)
C=O	1731 (m)	1740 (w)	1753 (w)	1700 (w)
C=C	1685 (w)	1645 (s)	1654 (s)	1644 (s)

3.2 GC-MS analysis

Twenty-five components, comprising 97%, were detected in the essential oil of *J. excelsa* gum-resin using GC-MS (Table 2, Figures 1 & 2). The major compound in the *J. excelsa* gum oil was α-pinene (80.50%); other compounds that were identified in minute proportions were *E*-pinocarveol (2.85%), limonene (2.46%) and *E*-verbenol (2.09%). Among the identified components, twelve compounds, comprising 48%, were monoterpene hydrocarbons and thirteen compounds, comprising 52%, were oxygenated monoterpenes. The oil was characterized by the absence of sesquiterpenes. The essential oil of the branches of *J. excelsa* contained 39 compounds. The major component in the branch oil was α-pinene (79.03%), followed by limonene (2.20%), myrcene (1.53%), and β-pinene (1.20%). Amid the revealed compounds, twelve, comprising 31%, were monoterpene hydrocarbons and ten, comprising 26%, were oxygenated monoterpenes. The essential oil of the branches of *J. excelsa* was distinguished by the presence of seventeen sesquiterpenes (44%). Thirty-seven compounds, comprising 98%, were identified in the EO of the leaves of *J. excelsa*. Limonene (50.46%) and α-pinene (31.67%) were the major compounds. Other compounds found to exist in minor proportions were myrcene (2.53%) and δ–3-carene (1.87%). Twelve compounds, comprising 32%, were monoterpene hydrocarbons and ten compounds, comprising 27%, were oxygenated monoterpenes. Fifteen sesquiterpenes, comprising 41%, of the oil were identified. The EO of the fruits of *J. excelsa* contained thirty-four components, comprising 98% of the oil. The major compounds were limonene (42.51%) and α-pinene (35.09%) followed by myrcene (3.09%), β-eudesmol (2.16%), δ-3-carene (2.14%) and α-humulene (2.11%). Twelve compounds, comprising 35%, were identified as monoterpene hydrocarbons and five compounds comprising 15% were oxygenated monoterpenes. The oil was characterized by the presence of seventeen sesquiterpenes comprising 50% of the oil.

Table 2. Chemical composition of essential oils of different parts of *Juniperus excelsa*

Compounds	RI	Percentage in			
		Gum-resin	Branches	Leaves	Fruits
Tricyclene	928	0.77	0.36	0.29	0.09
α-Thujiene	932	0.19	0.22	_	_
α-Pinene	942	80.50	79.03	31.67	35.09
α-Fenchene	951	0.36	0.31	0.23	0.20
Camphene	955	0.57	0.45	0.32	0.22
Thuja-2,4(10)-diene	960	0.96	0.27	0.12	_
Sabinene	977	0.22	0.41	0.05	0.04
β-pinene	982	1.03	1.20	0.96	1.73
Myrcene	994	0.14	1.53	2.53	3.09
α-Phellandrene	1005	_	_	0.13	_
δ-3-Carene	1011	_	0.15	1.87	2.14
α-Terpinene	1019	_	_	_	0.05
p-Cymene	1027	0.46	0.26	_	0.42
Limonene	1033	2.46	2.20	50.46	42.51
Z-β-Ocimene	1040	0.10	_	_	_
γ-Terpinene	1062	_	_	0.11	0.25
α-Terpinolene	1088	0.25	0.79	0.24	0.94
α-Pinene oxide	1097	0.33	_	_	_
Linalool	1098	0.13	_	_	_
α-Campholenal	1127	1.31	0.30	0.15	_
trans-Pinocarveol	1141	2.85	0.92	0.36	_
Camphor	1143	_	_	_	0.09
trans-Verbenol	1144	2.09	0.34	_	_
trans-Pinocamphone	1161	0.38	_	_	_
Pinocarvone	1168	_	0.13	_	_
α-Phellandren-8-ol	1170	_	0.28	_	_
Terpinene-4-ol	1179	0.11	0.31	0.25	0.08
p-Cymen-8-ol	1188	0.55	0.39	_	_
cis-Piperitol	1194	_	0.86	0.28	0.15
Myrtenal	1197	0.47	_	_	_
Verbenone	1204	0.44	_	0.13	_
trans-Carveol	1229	0.26	_	0.50	_
cis-Carveol	1230	_	_	0.14	_
Carvone	1242	_	_	0.35	_
Bornyl acetate	1285	0.49	1.20	0.56	0.08
δ-Elemene	1339	_	0.11	_	_
α-Copaene	1376	_	_	0.49	_
β-Bourbonene	1384	_	_	0.16	_
β-Elemene	1393	_	0.18	0.29	0.12
β-Caroyophyllene	1418	_	0.78	0.61	1.79
γ-Elemene	1430	_	0.87	0.18	0.51
α-Humulene	1452	_	0.10	0.50	2.11
γ -Gurjunene	1473	_	_	0.17	_
Germacrene D	1480	_	0.26	_	0.79
β-Selinene	1485	_	0.39	0.73	0.11
α-Selinene	1493	_	0.33	_	0.23
α-Muurolene	1499	_	0.16	0.30	0.08
α-Amorphene	1506	_	_	0.32	0.08
γ-Cadinene	1512	_	0.19	_	0.34
δ-Cadinene	1516	_	0.78	0.85	0.53
cis-Calamenene	1521	_	0.26	0.94	0.21
Elemol	1547	_	0.28	0.15	0.22
Germacrene B	1556	_	1.19	0.23	0.68
Caryophylene oxide	1573	_	0.53	0.33	0.50
α-Cedrol	1604	_	0.84	_	_

δ-Cadinol	1636	_	_	_	0.09
β-Eudesmol	1651	_	_	_	2.16
α-Cadinol	1652	_	0.19	_	_
Total identified (%)		**97%**	**99%**	**98%**	**98%**

GC-MS analysis of the four oils of *J. excelsa* show some similarities and differences in their monoterpene and sesquiterpenes chemical composition. The essential oil of the gum-resin contains the lowest number of components (25 compounds) whilst the essential oil of the branches contains the highest number of components (39). Each one of the four oils contains twelve monoterpene hydrocarbons but they differ in the number of oxygenated monoterpenes. The four essential oils share 11 monoterpenes and differ in 24 monoterpenes. The identity of the major component also differs among the four oils; while the essential oils of the gum and branches contain α-pinene as the major compound (80.50% and 79.03% respectively) the oils of leaves and fruits contain limonene as the major component (50.46% and 42.51% respectively). In terms of composition of sesquiterpenes, the essential oils of leaves, branches and fruits contain varied proportions of sesquiterpenes while that of gum-resin contains no sesquiterpenes.

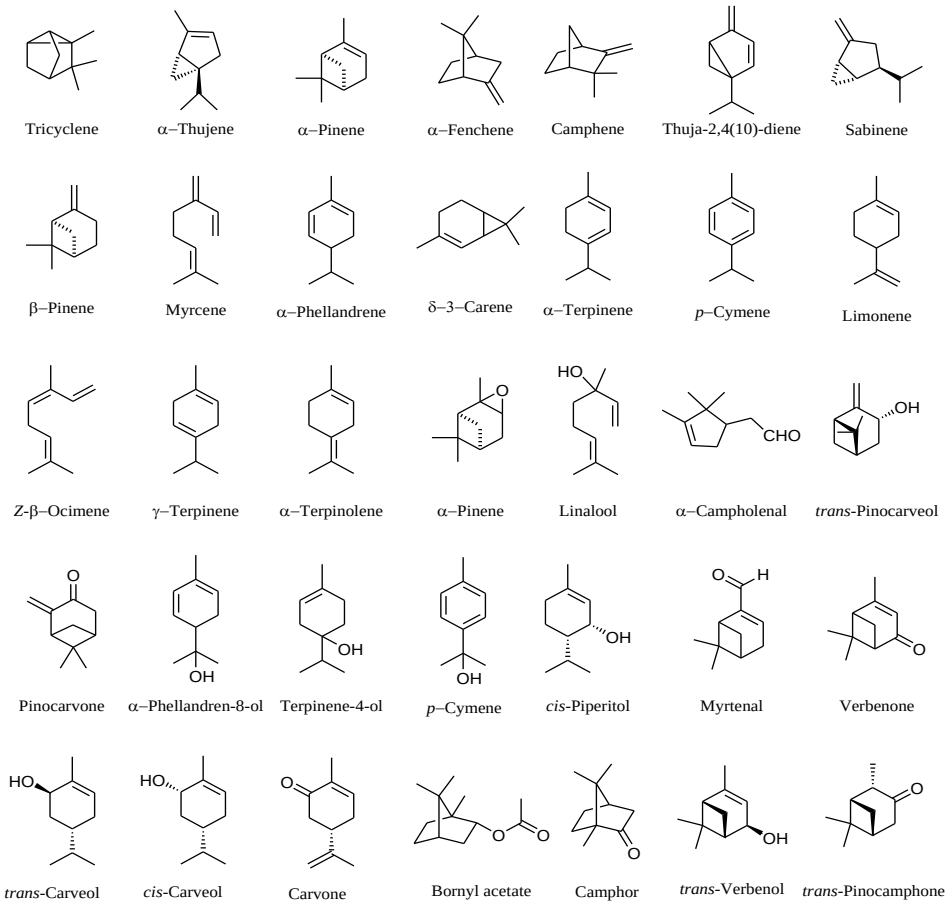

Figure 1. Monoterpenes from the essential oils of the *J. excelsa*.

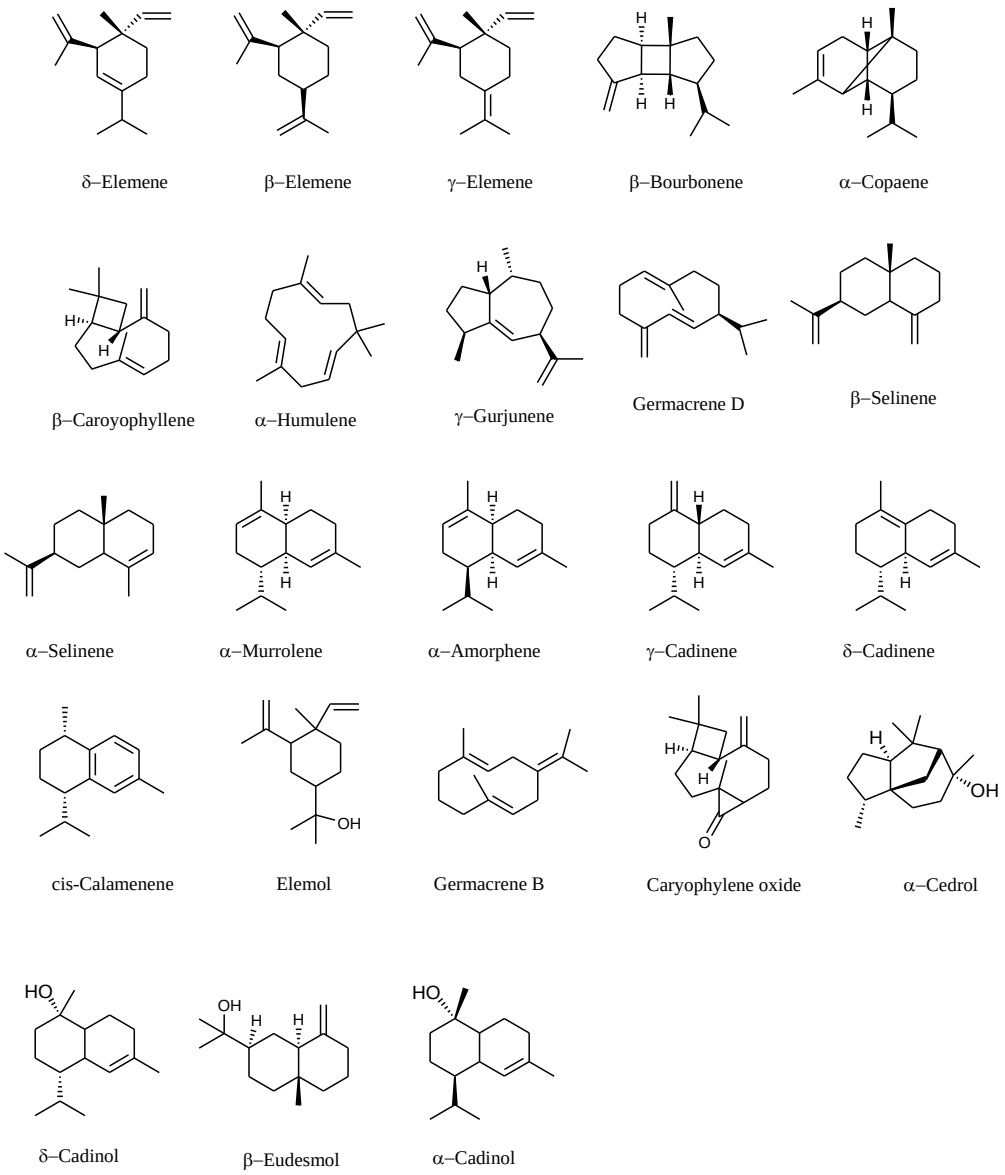

Figure 2. Sesquiterpenes from the essential oils of the *J. excelsa*.

3.3 Total antioxidant activity

The antioxidant activity of the essential oils of different parts of *J. excelsa* was evaluated by using the phosphomolybdenum method which is based on the reduction of Mo(VI) by the sample analyte and the subsequent formation of green phosphate Mo(V) complex with a maximum absorption at 695 nm. Total antioxidant activity of the four essential oils, expressed as ascorbic acid equivalent (mg of AA per g of the oil), was obtained from the calibration curve of ascorbic acid (AA) as shown in Figure 1. The essential oil of *J. excelsa* leaves showed higher total antioxidant activity compared to the other three oils scoring 385.0 mg ascorbic acid (AA) equivalents followed by the EO of gum-resin with total antioxidant activity of 277.0 mg (Figure 3). The EO of the fruits showed the lowest activity with 108.0 mg AA.

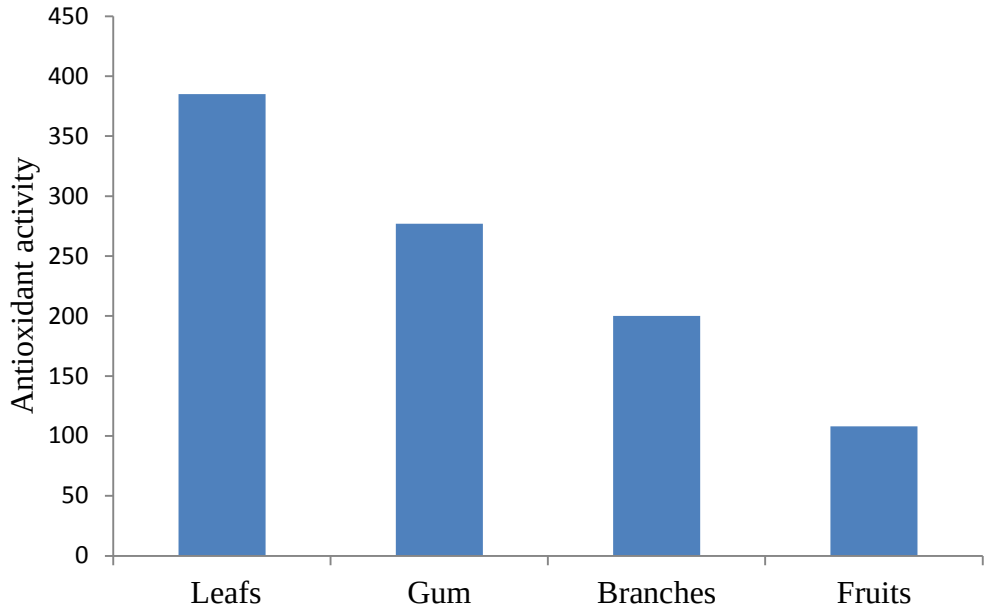

Figure 3. Antioxidant activity of *J. excelsa* essential oil.

3.4 DPPH radical scavenging activities

Another standard method to quantify antioxidant activity of essential oils is to measure their hydrogen donating ability to a DPPH radical. In this study, essential oils of different parts of *J. excelsa* were investigated for their radical scavenging activity and compared with that of ascorbic acid (AA) (Figure 4). The scavenging activity of the four essential oils, as well as that of ascorbic acid, increases as the concentration increases. The four oils, however, showed lower activity than ascorbic acid. Figure 5 exhibits the IC_{50} values which describe the concentration of antioxidant required to inhibit 50% of DPPH radicals. The smaller the IC_{50} value, the stronger the antioxidant activity obtained. The data obtained showed that the radical scavenging of the four essential oils decreased in the following order: leaves (IC_{50} = 20.47) > fruits (IC_{50} = 30.27) > resin-gum (IC_{50} = 36.77) > branches (IC_{50} = 40.46).

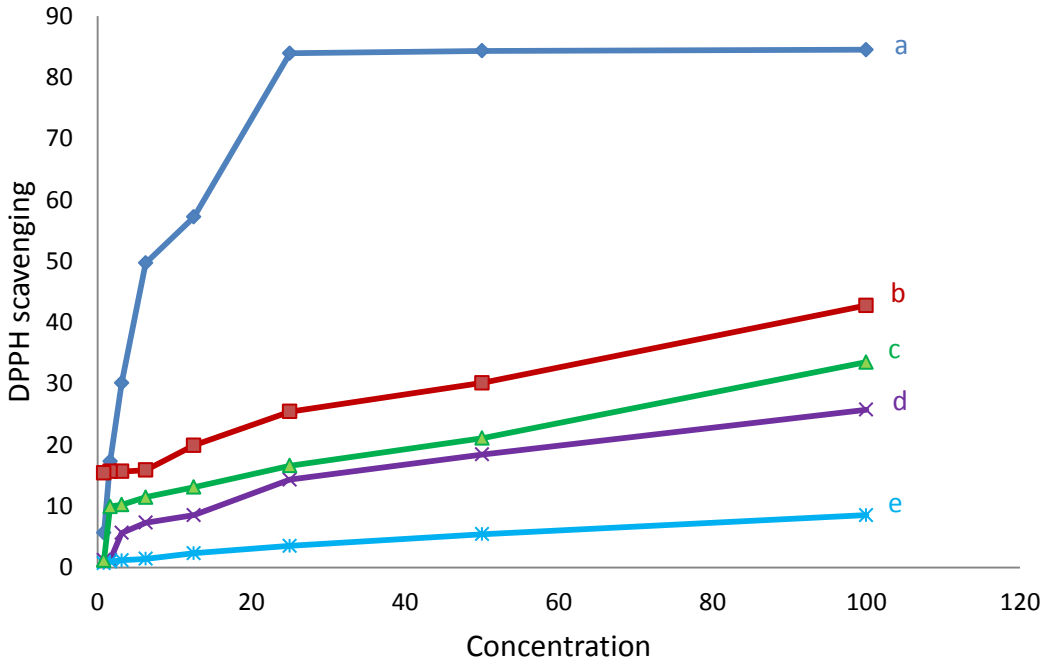

Figure 4. DPPH radical inhibition activity of *J. excelsa* essential oils compared with ascorbic acid (AA) activity. a: Ascrorbic acid; b: *J.excelsa* leaves oil; c: *J. excelsa* branches oil; d: *J. excelsa* fruits oil; e: *J.excelsa* gum oil.

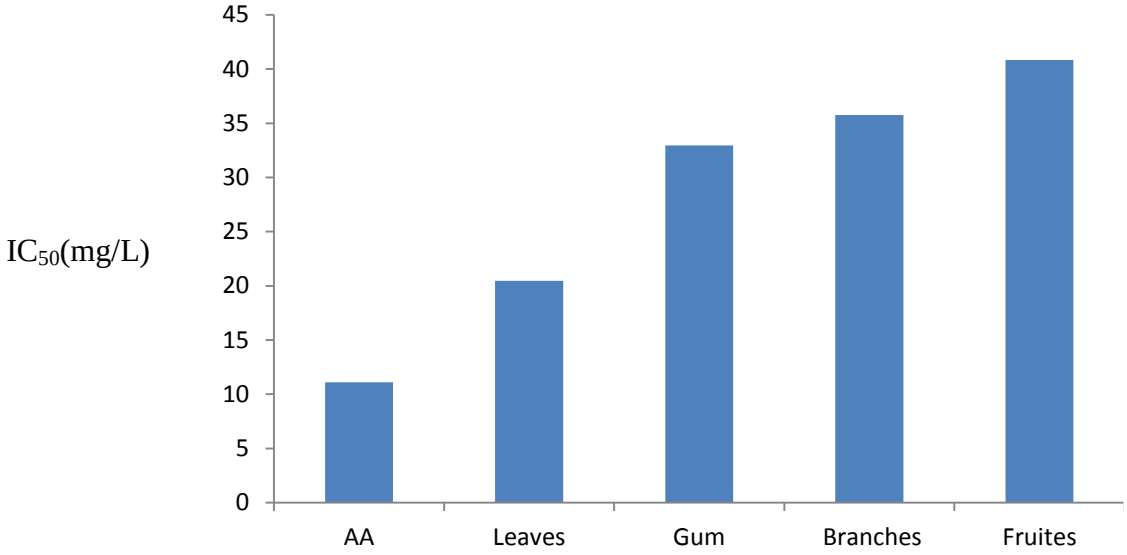

Figure 5. IC_{50} values of ascorbic acid (AA) and *J. excelsa* essential oils.

4. Conclusion

The chemical content and compositions of four essential oils extracted from resin-gum, leaves, branches and fruits of *J. excelsa* from Jabal Al-Akhdhar (Sultanate of Oman) were analyzed by the GC-MS method. The analysis showed some variations as well as similarities in their chemical contents and compositions. Out of a total of 35 different monoterpenes existing in the four essential oils, 11 monoterpenes are shared by the four oils. The essential oil of the resin-gum of *J. excelsa* is characterized by the absence of sesquiterpenes. α–pinene was found to be the major compound in the essential oil of resin-gum and branches with 80.50% and 79.03%, respectively, while limonene is the major compound in the essential oil of leaves and fruits with 50.46% and 42.51%, respectively. The essential oil of the leaves of *J. excelsa* shows stronger antioxidant activity and radical scavenging activity than other essential oils, but all four oils show lower antioxidant and radical inhibition activities when compared to ascorbic acid (AA).

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