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Research Article

Comparative Analysis of the Chemical Composition of *Juniperus excelsa* ssp. *polycarpos* Bark and Wood Extracts

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Abstract: In the present study, extracts from the bark and wood of *Juniperus excelsa* ssp. *polycarpos* were obtained with acetone solvent. Chemical composition were analyzed and compared by gas chromatography-mass spectrometry (GC-MS). The results showed that the major components identified in the bark acetone extract as trimethylsilyl (TMS) derivatives were β-d-glucofuranose, 1,2,3,5,6-pentakis-O-(TMS) (19.97%), followed by pimaric acid TMS (18.89%), d-mannopyranose, 1,2,3,4,6-pentakis-O-(TMS) (13.90%), d-fructose, 1,3,4,5,6-pentakis-O-(TMS) (12.37%). The major components identified in the wood acetone extract as trimethylsilyl (TMS) derivatives were pimaric acid TMS (24.56%), followed by α-d-glucopyranoside, 1,3,4,6-tetrakis-O-(TMS)-β-d-fructofuranosyl 2,3,4,6-tetrakis-O-(TMS) (21.39%), β-d-galactopyranose, 1,2,3,4,6-pentakis-O-(TMS) (12.10%), d-glucose, 2,3,4,5,6-pentakis-O-(TMS) (9.97%), trifluoromethyl-bis-(TMS)methyl ketone (9.32%). One of the more important components identified both in the bark and wood extracts was pimaric acid TMS. Cedrol as the essential oil was found in the acetone wood extract (0.72%).

Keywords: Bark and wood extracts, Chemical composition, *Juniperus excelsa* ssp. *polycarpos*, Pimaric acid.

1. Introduction

The extracted compounds of different types of *Juniperus* and their antibiotic activities have been expressed (Angioni *et al*., 2003; Filipowicz *et al*., 2003). The potential uses of the extracted compounds include aromatherapy, fragrance, soaps, candle, lotions, and cosmetics materials (Yesenofski, 1996).

Different types of *Juniperus* are also used to treat diseases such as diabetes, tuberculosis, bronchitis, pneumonia and intestinal ulcers as well as traditional treatment of liver diseases (Burits *et al*., 2001; Loizzo *et al*., 2007).

The other studies indicated that pentadecanoic acid, hexadecanoic acid, oleic acid, linoleic acid, and p-1-isopropyl phenyl are the main extracted compounds of the internal and the external wood, root and the stalk of *Juniperus foetidissima* in Turkey (Tunalier, 2003).

Recent studies also indicated that inhibitory effect of the extracted compounds on various types of pathogenic fungi and similar microorganisms (Soković *et al*., 2004). The largest compounds in the fruit cones of *Juniperus communis* was terpenes, 32.1%, which is used to treat indigestion, and also as disinfectant in dyspepsia as well as some other antibiotic effects (Lamparsky and Klimes, 1985).

Juniperus has high resistance against wood eating pests; humidity has no effect on it. The scent extracted from the tree also repels snakes and scorpions as well as other blood-sucking insects (Zargari, 1993).

Cedrol was found in the essential oil of *Conifers*, especially in the genera *Cupressus* and *Juniperus* (Connolly and Hill, 1991). Its main uses are in the chemistry of aroma compounds (Breitmaier, 2006). Result of Lindh *et al*., (2015) studies suggested that cedrol strongly attracts pregnant female mosquitoes after to create cedrol-baited traps.

Hosseinihashemi *et al*., (2017) were analyzed antioxidant activity and chemical composition of *Juniperus excelsa* ssp. *polycarpos* wood and the most active extracts using the DPPH and gas chromatography-mass spectrometry methods; and compared it's with ascorbic acid and butylated hydroxytoluene. Acetone extract was found to be moderately active as an antioxidant agent at 58.38%, which was lower than the value of vitamin C (98.56%) at the concentration of 14.20 mg/mL. The dissolved water: methanol (1:1 v/v) partitioned from acetone extract afforded 12 fractions; among them, the F9 fraction was found to have good antioxidant activity (88.49%) at the concentration of 14.20 mg/mL. The major compounds identified in F9 fraction were α-d-glucopyranoside, 1,3,4,6-tetrakis-O-(TMS) (20.22%) and trifluoromethyl-bis-(TMS)methyl ketone (5.10%).
In the present study, the aim was to analyze and compare the chemical composition of the bark and

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wood extracts from *J. excelsa* ssp. *polycarpos* with voucher specimen number 1893 by GC/MS.

2. Materials and Methods

2.1 Plant materials

Fresh stem of *J. excelsa* ssp. *polycarpos* was collected from Sira, Chalous road, and Alborz, Iran in February of 2015. The plant material was identified by Department of Wood Science and Paper Technology, Karaj Branch, Islamic Azad University, Karaj, Iran, and a voucher specimen number 1893 was deposited in the Herbarium College of Agricultural and Natural Resources, Karaj Branch, Islamic Azad University, Karaj, Iran. The bark and wood was separated manually from the stems and air-dried to achieve 8.0% moisture content. Because, these species are not widely available, and their utilization is limited, therefore we prepared the wood samples with smaller diameters and the age is approximately 10 years.

2.2 Extraction

The bark and wood of stem was cut into small pieces and chopped using a laboratory electrical rotary mill to obtain bark and wood flour. The flour size was between 40 and 60 meshes. Approximately 100g of this flour were placed into the 10 extraction thimbles, separately and then 10 independent extracted using pure acetone (400 mL in a 500 mL balloon) and a Soxhlet-type apparatus for 8 h. The combined extracts from bark and wood was concentrated using a Heidolph Laborota 4001 rotary-evaporator apparatus (at 40 °C to reach total solvent evaporation) for approximately 15 min, separately. Then, the bark and wood extracts were collected, separately and dried over anhydrous sodium sulphate, and stored at 4°C until further analysis.

2.3 Analysis of extracts

Gas chromatography-mass spectrometry (GC/MS) analysis of the acetone bark and wood extracts were performed using split mode (50:1) injection. One microlitre of the silylated extract, 300 µL N,O-bis(trimethylsilyl)trifluoroacetamide (BSTFA), 30 µL 1% trimethylchlorosilane (TMCS) reagent, and approximately 60 µL of pyridine were run on a HP 7890 (Hewlett Packard, USA) gas chromatograph fitted with a cross-linked Polysiloxane HP-5 capillary column (dimensions: 30 m x 0.25 mm, 0.25 µm coating thickness) and coupled with a model 5975C mass detector. The GC/MS operation conditions were as follows: injector temperature 250 °C; transfer line 280 °C; oven temperature program 50 to 250 °C (5 °C/min); carrier gas: He at 1.4 mL/min; mass spectra: electron impact (EI+) mode 70 eV with a mass range of 40 to 450 m/z; and ion source temperature at 250 °C. Individual components were identified using Wiley 275L and NIST05 mass database matching and by comparing the retention times and mass spectra of constituents with published data (Julian and Konig, 1988; Adams, 1995, 2001).

3. Results and Discussion

3.1 Bark extract

Acetone extract of the fresh bark of *J. excelsa* ssp. *polycarpos* afforded 10.4% (v/w) in yield. Nine compounds of trimethylsilyl (TMS) derivatives were identified (Table-1 and Fig. 1).

Table 1. The suggestible chemical composition of bark and wood extracts of *J. excelsa* ssp. *polycarpos*.

Components	Retention time (min)	Area (%) for ABE*	Area (%) for AWE*
Trisiloxane, octamethyl	6.464	4.15	**-**
	6.608	-	**2.17**
Trifluoromethyl-bis-(TMS)methyl ketone	8.819	-	**9.32**
Cyclotetrasiloxane, octamethyl	10.676	1.57	**-**
Tetrasiloxane, decamethyl	12.445	7.80	**2.03**
TMS ether of glycerol	19.653	-	**0.45**
Hexasiloxane, tetradecamethyl	22.140	0.97	**0.30**
Trimethyl(2,6 ditert.-butylphenoxy)silane	26.242	1.38	**-**
Cedrol	27.513	-	**0.72**
Androstan-3-one,1,17-dimethyl-17-(TMS)oxy)-O-methyloxime,(1.α,5.α,17.β)	30.686	-	**0.63**
D-Fructose, 1,3,4,5,6-pentakis-O-(TMS)	32.587	12.73	**-**
	32.599	-	**9.97**
β-D-Galactofuranose, 1,2,3,5,6-pentakis-O-(TMS)	33.439	-	**1.57**
	34.312	19.97	**-**
β-D-Galactopyranose, 1,2,3,4,6-pentakis-O-(TMS)	34.323	-	**12.10**
D-Mannopyranose, 1,2,3,4,6-pentakis-O-(TMS)	36.125	13.90	**-**
D-Glucose, 2,3,4,5,6-Pentakis-O-(TMS)	36.136	-	**11.30**
Hexadecanoic acid, TMS ester	36.601	-	**0.78**
9,12-Octadecadienoic acid (Z,Z)-, TMS ester	39.541	-	**0.46**
Oleic acid, TMS ester	39.641	-	**0.34**
Octadecanoic acid, TMS ester	40.094	-	**0.27**
Pimaric acid TMS	41.085	-	**24.56**
	41.089	18.89	**-**
α-D-Glucopyranoside, 1,3,4,6-tetrakis-O-(TMS)-β-D-fructofuranosyl 2,3,4,6-tetrakis-O-(TMS)	45.555	-	**21.39**
Total	**-**	**81.36**	**98.36**

*ABE: Acetone bark extract; AWE: Acetone wood extract.

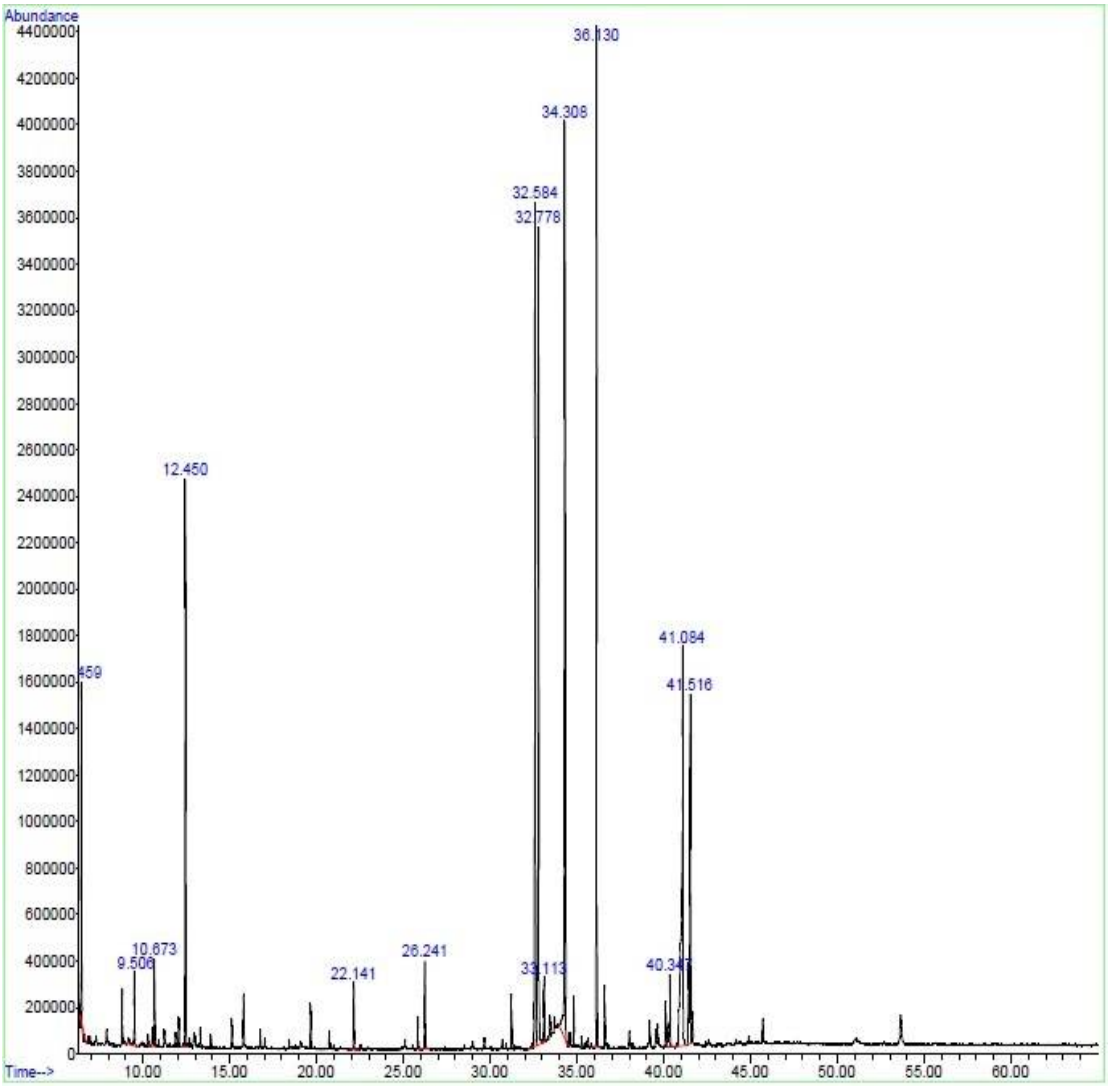

Fig. 1. GC-MS chromatogram of acetone bark extract of *J. excelsa* ssp. *polycarpos*.

The major components of acetone bark extract were β-d-galactofuranose, 1,2,3,5,6-pentakis-O-(TMS) (19.97%), pimaric acid TMS (18.89%), d-mannopyranose, 1,2,3,4,6-pentakis-O-(TMS) (13.90%), d-fructose, 1,3,4,5,6-pentakis-O-(TMS) (12.73%), tetrasiloxane, decamethyl (7.80%), trisiloxane, octamethyl (4.15%).

The other minor identified compounds were TMS derivatives of alkyl compounds such as cyclotetrasiloxane, octamethyl (1.57%), trimethyl(2,6 ditert.-butylphenoxy)silane (1.38%), and Hexasiloxane, tetradecamethyl (0.97%).

Among the identified components, five components (7.80%, 4.15%, 1.57%, 1.38%, and 0.97%) were alkyl, and three components (19.97%, 13.90%, and 7.80%) were sugar compounds. Pimaric acid TMS as conifer oleoresin (*i.e.*, resin acids) and diterpenoid compound has been also reported in the extracts of *J. polycarpus* var. *seravschanica* (Lewisohn *et al*., 1993; Flores, 2013).

3.2 Wood extract

Acetone extract of the fresh wood of *J. excelsa* ssp. *polycarpos* afforded 12% (v/w) in yield. Seventeen compounds of trimethylsilyl (TMS) derivatives were identified (Table-1 and Fig. 2).

The major components of acetone wood extract were pimaric acid TMS (24.56%), α-d-glucopyranoside, 1,3,4,6-tetrakis-O-(TMS)-β-d-fructofuranosyl 2,3,4,6-tetrakis-O-(TMS) (21.39%), galactopyranose, 1,2,3,4,6-pentakis-O-(TMS)-β-d-(12.10%), d-glucose, 2,3,4,5,6-pentakis-O-(TMS) (11.30%), d-fructose, 1,3,4,5,6-pentakis-O-(TMS) (9.97%), trifluoromethyl-bis-(TMS)methyl ketone (9.32%), and β-d-galactofuranose, 1,2,3,5,6-pentakis-O-(TMS) (1.57%).

The other minor identified compounds were TMS derivatives of fatty acids such as hexadecanoic acid, TMS ester (0.78%), 9,12-octadecadienoic acid (Z,Z)-, TMS ester (0.46%), oleic acid, TMS ester (0.34%), Hexasiloxane, tetradecamethyl (0.30%), and octadecanoic acid, TMS ester (0.27%).

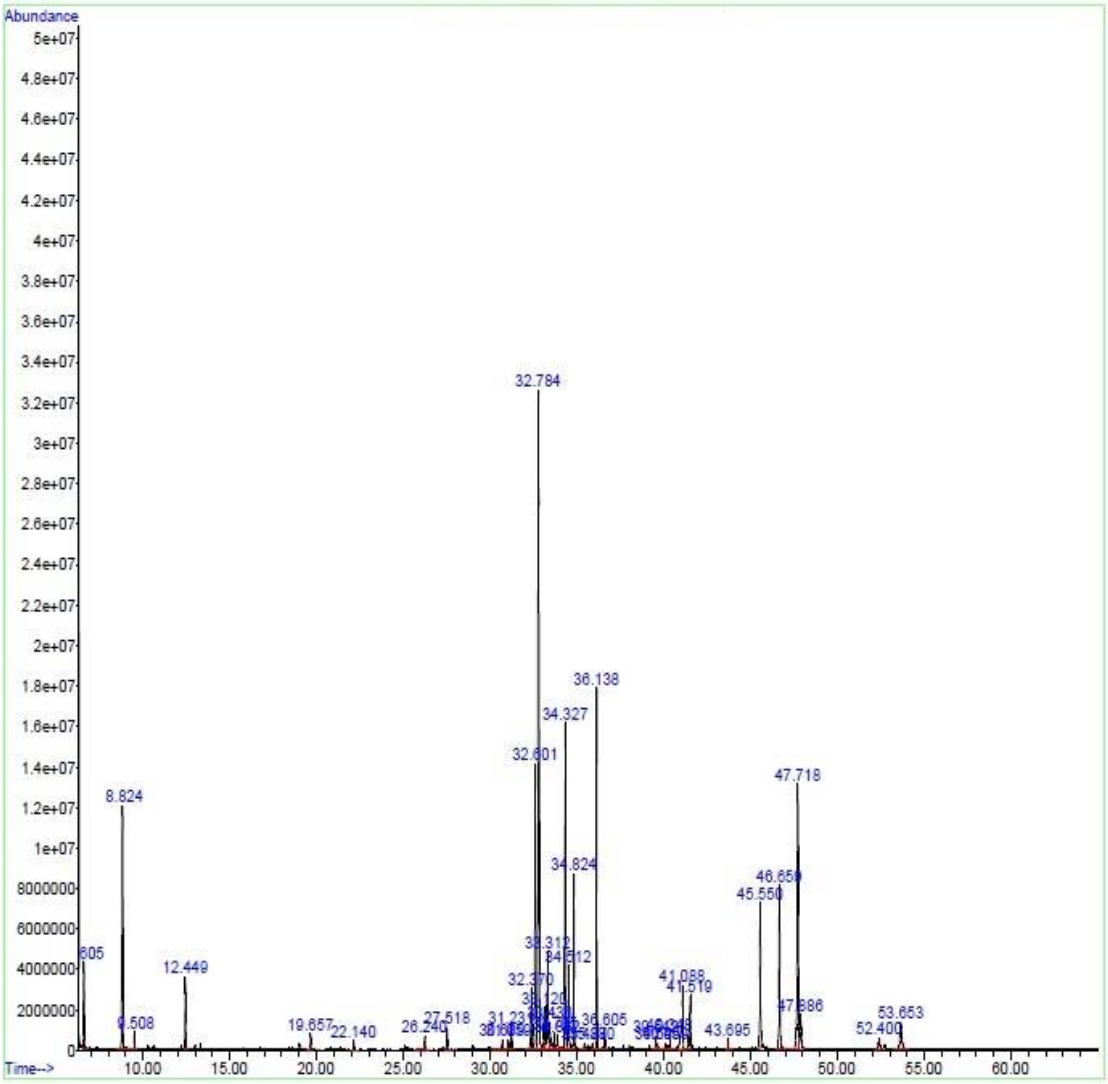

Fig. 2. GC-MS chromatogram of acetone wood extract of *J. excelsa* ssp. *polycarpos*.

Among the identified components, two components (9.32%, 0.63%) were ketone, three components (0.78%, 0.46%, and 0.18%) were saturated fatty acid ester, one component (0.72%) was alcohol sesquiterpene, one component (0.45%) was glycerol, one component (24.56%) was diterpenoid, two components (0.34%, 0.27%) were unsaturated fatty acid ester, three components (2.17%, 2.03%, and 0.30%) were alkyl, and five components (21.39%, 12.10%, 11.30%, 9.27%, and 1.57%) were sugar compounds. Cedrol has been reported in the extracts of *J. polycarpus* var. *seravschanica* (Joseph-Nathan *et al*., 1984; Okasaka *et al*., 2006).

4. Conclusions

Comparative analysis of the chemical composition of extracts from the bark and wood of *J. excelsa* ssp. *polycarpos* were reported for the first time. The major components of acetone bark extract were β-d-galactofuranose, 1,2,3,5,6-pentakis-O-(TMS), pimaric acid TMS, d-mannopyranose, 1,2,3,4,6-pentakis-O-(TMS), d-fructose, 1,3,4,5,6-pentakis-O-(TMS), tetrasiloxane, decamethyl and trisiloxane, octamethyl. The major compounds from the wood extract of *J. excelsa* ssp. *polycarpos* were pimaric acid TMS, α-d-glucopyranoside, 1,3,4,6-tetrakis-O-(TMS)-β-d-fructofuranosyl 2,3,4,6-tetrakis-O-(TMS), galactopyranose, 1,2,3,4,6-pentakis-O-(TMS)-β-d-, d-glucose, 2,3,4,5,6-pentakis-O-(TMS), d-fructose, 1,3,4,5,6-pentakis-O-(TMS), and trifluoromethyl-bis-(TMS)methyl ketone. Among the identified components pimaric acid TMS as one of the most important diterpenoid component was found both in the acetone extracts of bark and wood of *J. excelsa* ssp. *polycarpos*.

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