

## PRELIMINARY STUDIES ON MONOTERPENE COMPOSITION OF JUNIPERUS POLYCARPOS

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Analysis of the essential oil of *Juniperus polycarpus* (Persian Juniper) by capillary GC and GC/MS revealed presence of monoterpene hydrocarbons (76.86%), oxygenated monoterpenes (2.95%) and sesquiterpene hydrocarbons and their alcohols (20.19%). The major component was found to be  $\alpha$ -pinene.

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مطالعات مقدماتی ترکیبات منوترپن در گونه ارس *Juniperus polycarpus*

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تجزیه روغنهای اسانسی گونه ارس (*Juniperus polycarpus*) از طریق جی سی و جی سی / ام اس وجود ۷۶/۸۶٪ هیدروکربنهای منوترپنی، ۲/۹۵٪ منوترپنهای اکسیژنه و ۲۰/۱۹٪ هیدروکربنهای سسکوترپنی و الکل‌های مربوطه را در ترکیب آنها نشان داد. آلفاینین از اجزای اصلی ترکیبات مذکور تشخیص داده شد.

## INTRODUCTION

*Juniperus polycarpos* C. Koch (Persian Juniper) is an important conifer distributed in Caucasus and Iran. This species sometimes has been mentioned as a synonym of *J. excelsa* Bieb. (Browicz 1982). However, further studies are needed to elucidate correct taxonomic position of the species.

For design of a long-term tree breeding program and decision making regarding conservation of gene resources, information on the genetic structure of natural populations from different geographical origins is essential. Botanical classifications rest largely on comparative studies of morphological and anatomical characters. Chemosystematics is widely recognized as an essential ingredient of the taxonomic process in plants (Bisby et al. 1980; Stace 1980). The most widely application of chemical methods has been in those instances where morphological criteria fail to provide a key to separation (Harborne 1984).

Secondary plant products, plant extracts, are most suitable for chemosystematic investigations. And group of chemically, or biosynthetically, related compounds give more useful data than do

single compounds which may serve in such studies. Monoterpene composition has been shown to be very informative in investigation of infrastructure and population variation for a wide variety of conifer species (von Rudloff 1969). Composition of the monoterpenes of conifers is strongly inherited and is often controlled by single genes with major effects (von Rudloff 1969; Squillace 1976). Selected references on monoterpene composition of *Juniperus* spp. and other conifers are given by Squillace (1976).

Composition of these compounds can be accurately and rapidly determined by gas-liquid chromatography and their chromatograms serve readily as fingerprints which facilitate comparison between related species and hybrids (von Rudloff 1969).

The purpose of this study is to determine, for the first time, the monoterpene composition concentrations of *Juniperus polycarpos* native to Iran as a prelude for a comprehensive chemosystematic studies.

## MATERIAL AND METHODS

### Plant Material

All initial experiments were made with

foliage collected from Karaj and the National Botanical Garden of Iran. The plant material for the main study was collected near Firouzkuh, NE. of Tehran, Iran, in 1987. A voucher specimen (Tehran: ca. 3 km to Firouzkuh on the road from Damavand, 2100 m, 16.11.1987, Assadi 61693) is deposited at the Herbarium of the Research Institute of Forests and Rangelands (TARI). Branch tips of 50 gr. weight in each batch, were used for steam distillation soon after collection.

### Isolation of the Volatile Oil

For isolation of the essential oils a circulatory apparatus of Kaizer and Lang (1951) as modified by Hefendehl (1962) was used. The plant material was steam distilled for 4 hours. The resultant oil was collected over diethyl ether (10 ml.) in the side arm of condenser assembly. Oil yield on the dry weight basis was 1.35 percent. The diethyl ether solution of the oil was fractionated by solvent extraction with Na<sub>2</sub>CO<sub>3</sub> (5 percent aqueous solution) into an acidic and a neutral fraction. The neutral fraction consisting of terpene hydrocarbons and oxygenated terpenes along with sesquiterpenes totaling 25 ml (in ether) was kept in a sealed bottle and

stored at -20°C until analyzed.

### Gas Chromatographic Analysis

The samples were analyzed by a Shimadzu gas chromatograph (model GC-9A) equipped with a split sample injection capillary unit system (split ratio-100:1) and a flame ionization detector (FID). The capillary column used was a Shimadzu Hicap-CBPIO (cyano-propyl, equivalent to OV-1701, stationary phase; 0.50 μm film thickness; 0.3 mm I. D. x 25 m) with the following conditions. The injection port temperature and the FID both were 250°C. The oven was programmed from 70 to 190°C at 4°C/min. Nitrogen was the carrier gas at 0.5 ml/min.

Retention times, peak areas and component names were processed with a Shimadzu Data Processor model C-R3A terminal. The peaks were identified by comparison with the retention times of standard hydrocarbons and MS. The commonly occurring terpenes were obtained from commercial sources. The relative percentage concentration were calculated from summation of all recorded peaks (over 2000 unit response) excluding the air and solvent peaks.

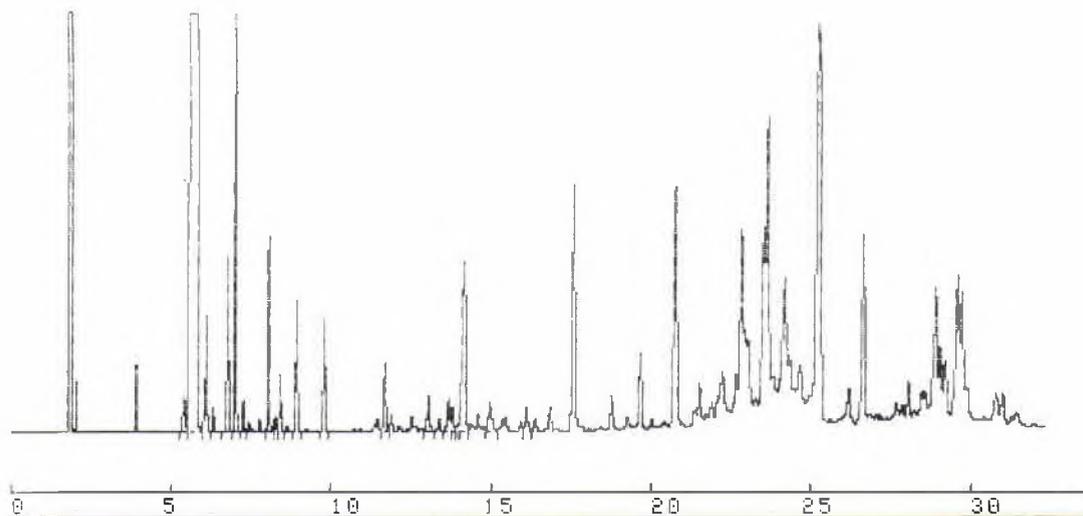


Fig. 1. Gas chromatographic separation of volatil fraction (neutral compounds) of *Juniperus polycarpus* essential oil. Conditions: Shimadzu Gas Chromatograph-GC-9A with CBP10 Column. Temperature programmed from 70 to 190°C at a rate of 4°C/min. Injection port and detector at 250°C. Detector: FID.

### GC/MASS SPECTROMETRIC ANALYSES

GC/MS analyses were performed on a 30 m x 0.22 mm DB-5 column installed in a Varian 3400 GC interfaced to a Varian Saturn II ion trap mass spectrometer equipped with a microcomputer and an automated library spectra search (NIST library) for comparing spectra with those

taken from pure substances. Compounds were identified by their mass spectrum and their retention index (the Kovats index in Table 1, determined on DB-5 column by co-injection of the homologous hydrocarbon series C6-C20 in a temperature programmed run).

### RESULTS AND DISCUSSION

Capillary GC and GC/MS analyses of the

neutral fraction of the essential oil obtained from branch tips of *Juniperus polycarpus* by steam distillation revealed presence of more than 60 components in the mixture as shown in the GC chromatogram (Fig.1). Many of the constituents were present in trace amounts. There was no significant difference in the gas chromatographic pattern of foliage samples collected in different areas.

The results of analyses of the hydrocarbon fraction, which accounted for about 1.35 percent of the volatile oil, showed that the fraction consisted almost exclusively of monoterpene hydrocarbons (76.86) percent, oxygenated monoterpenes (2.95) percent and sesquiterpene hydrocarbons and their alcohols (20.19) percent.

Table 1 gives the composition of this fraction and the others. The major constituents of the monoterpene hydrocarbons were  $\alpha$ -pinene (71), myrcene (1.6) and  $\alpha$ -terpineol (1.3) percent, while camphene, sabinene,  $\beta$ -pinene, 1-phellandrene, limonene,  $\alpha$ -terpinolene, cis-ocimene and  $\alpha$ -campholene aldehyde occurred in minor amounts. Lesser amounts of tricyclene,  $\gamma$ -terpinene were also found and one unidentified peak in the C12 region (1.32) percent occurred.

Among the sesquiterpene hydrocarbons,

again, the dominating constituents were  $\alpha$ -gurjunene, gamma and delta cadinenes with its isomers and their corresponding alcohols. Sesquiterpene hydrocarbons of less than 0.30 percent were not reported. It seems that sesquiterpenes, in conifers, have little chemosystematic value (Hunt and von Rudloff 1977) and are difficult to resolve into individual components; therefore, here they are reported as combined sesquiterpene hydrocarbons and alcohols in the table 1.

Studies by Squillace (1976) revealed that monoterpene fraction of oleoresin of Slash Pine consists mostly of  $\alpha$ -pinene,  $\beta$ -pinene, and  $\beta$ -phellandrene, and frequently camphene, myrcene,  $\alpha$ -phellandrene and limonene occur as minor constituents, while traces of delta-3-carene and  $\gamma$ -terpinene occur occasionally. This pattern more or less concurs with our findings also. Gansel and Squillace (1976) found that relative amounts of most of the monoterpenes are usually either high or low. That is, frequency distributions for oleoresin from a large number of trees are usually bimodal. While clear evidence of bimodality for  $\alpha$ -pinene was lacking, this constituent was excluded from their criteria used for classifying trees as having high or low amounts of each monoterpene.

Table 1. Composition (in%) of the volatile fraction (Neutral compounds) of *Juniperus polycarpus*.

Type	Compounds	Kovats index	Content (%)	Type	Compounds	Kovats index	Content (%)
Hydrocarbons & Oxygenated Compounds - Monoterpenes Total: 79.81%	Tricyclene	920	0.22	Hydrocarbons & Oxygenated Compounds - Sesquiterpenes Total: 20.19%	$\delta$ -Elemene	1340	0.35
	$\alpha$ -Pinene	932	71.36		trans-Carophyllene	1422	1.55
	Camphene	950	0.44		$\alpha$ -Humulene	1435	0.47
	Sabinene	976	0.35		EPI-Bicyclos-		
	$\beta$ -Pinene	980	0.75		quiphellandrene	1152	0.38
	Myrcene	986	1.57		cadinene-isomer	1474	1.26
	1-Phellandrene	1021	0.30		$\alpha$ -Copaene	1483	0.44
	dl-Limonene	1024	0.71		Muurolene	1501	0.60
	$\gamma$ -Terpinene	1036	0.20		$\gamma$ -Cadinene	1516	1.27
	$\alpha$ -Terpinolene	1085	0.49		$\delta$ -Cadinene	1521	1.86
	Cis-Ocimene	1095	0.47		Cadinene-isomers	1538	1.50
	$\alpha$ -Campholene				Calarene	1540	0.70
	Aldehyde	1144	0.33		$\beta$ -Seiinene	1544	0.58
	$\alpha$ -Terpineol	1189	1.30		$\alpha$ -Gurjunene	1560	5.15
C12-Unkown	1287	1.32	Cadinene-isomer	1580	0.74		
			Cadinol-isomer	1620	1.17		
			Cadinol-isomer	1632	1.20		
			Cadinol-isomer	1636	0.97		

Constituents included were 4 of the major compounds,  $\beta$ -pinene, myrcene, limonene and  $\beta$ -phellandrene.

Detection of monoterpenes present in samples (qualitative determination) along

with relative amounts, or yields of monoterpenes percent (quantitative determinations) will enable us to plan research programs on chemosystematic studies in the genus *Juniperus* or other

genera.

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