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Isolation and Identification of the Major Chemical Compounds Found in the Oleoresin Obtained from the *Pistachia atlantica* Tree (Persian Turpentine Tree, Desf. Subsp. Kurdica) Grown in Ilam Province of Iran

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Oleoresin collected after tapping the trunk of the *Pistachia atlantica* tree grown wild in Ilam province of Iran at the end of spring and the earlier months of summer at intervals was dried and powdered. Water-distilled/Soxhlet extraction of the powdered oleoresin gave an essential oil. TLC and column chromatography (silica gel as stationary phase and methylene chloride as mobile phase) resulted in the separation of three components with $R_f = 0.23$, 0.48 and 0.92 which were identified as β-caryophylline, spiro[4,5]decane-6-methylene and β-pinene, respectively. The essential oil was analyzed by GC-MS which showed the following nine major components: α-pinene, camphene, β-pinene, bicyclo[3.1.1]hepta-3-ene-2-ol-4,6,6-trimethyl; 1,4-cyclo- hexadiene-1-methanol-4-(1-methylethyl); bicyclo[3.1.1]hepta-3-ene-2-one-4,6,6-trimethyl; β-caryophylline and spiro[4,5]decane-6-methylene.

Key Words: *Pistachia atlantica*, Oleoresin, Essential oil, Persian turpentine.

INTRODUCTION

The *Pistachia* species is classified into the Anacardiaceae family. *Pistachia atlantica* contains three subspecies: Cabulica, Kurdica and Mutica. *Pistachia atlantica* and *Pistachia khinjuk* are two major species that grow in the Zagrossian region with various *Quercus* species. *Pistachia atlantica* is grown 600–3000 metres above sea level[1].

The term 'resin' is applied to more or less solid, amorphous products of complex chemical nature. On heating they soften and finally melt. They are insoluble in water and usually insoluble in petroleum spirit but dissolve more or less completely in alcohol, chloroform and ether. Resins are often associated with volatile oils (oleoresins), with gums (gum-resins) or with oil and gum (oleo-gum-resins). Therefore, oleoresins are homogeneous mixtures of resins and volatile oils. There is no sharp line of demarcation between these various types of resinous

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substances and classification is sometimes difficult. Small proportions of volatile oils are present in many resins. Depending on the relative amount of volatile oil in the mixture, oleoresins may be liquid, semisolid or solid. Usually there is a small amount of "natural" exudates from oleoresin containing trees owing to insect damage, broken branches and other injuries, but the commercial supplies are generally obtained by artificial incision through the bark and even into the wood.

An oleoresin is extracted naturally and through tapping from *Pistachia atlantica* tree. About 80% of the oleoresin is soluble in ethyl alcohol and is called α-resin and the rest of it which is insoluble in alcohol is labelled as β-resin. The fruit of the tree is small with the size of a pea and has a green colour hull and has a very hard seed. In Greece and the Middle East, the gum has been used as a protective agent for the stomach. Recent investigations have shown that it can be used for the treatment of stomach ulcer.

Pistachia atlantica is regarded by some as a source of turpentine, which was traded under a variety of names such as Chios, Chio or Chian turpentine or Cyprus Balsam[2, 3].

EXPERIMENTAL

The wild growing *Pistachia atlantica* tree grown in Ilam province of Iran was identified as *Pistachia atlantica* Desf. Subsp. Kurdica (zohary) Rech. f by Herbarium Department of Ahwaz Shahid Chamran University and Ilam Natural Products Research Centre.

Oleoresin was collected after tapping the trunk of the wild *Pistachia atlantica* tree grown in Ilam province of Iran at intervals which depended on the length and diameter of the tree by local large axes at the end of spring and the earlier months of summer. About 60 earthenware cups were attached to the trunk of each tree for the collection of the oleoresin. After 24–36 h the cups were emptied in early morning since the weather was cold enough to solidify the oleoresin. The oleoresin was crushed and powdered, then 30 g of it was steam distilled.

Another batch of 30 g of the powder was extracted by Soxhlet extraction method using 300 mL toluene. The distillate/extract was dried over anhydrous sodium sulphate, then filtered. 2.5 mL bright yellow essential oil with a pleasant odour was obtained. The essential oil was soluble in ethyl alcohol but insoluble in water. It had a refractive index of $\eta = 1.4740$. TLC and column chromatography of the essential oil on silica gel with methylene chloride as mobile phase were carried out which resulted in the separation of three components with $R_f = 0.23$, 0.48 and 0.92. Infrared (IR) and UV-Vis spectra of the crude essential oil revealed the presence of ketonic, olefinic or aromatic groups and $\lambda_{max} = 225$, 252.5, 260 and 267.5 nm.

Analysis

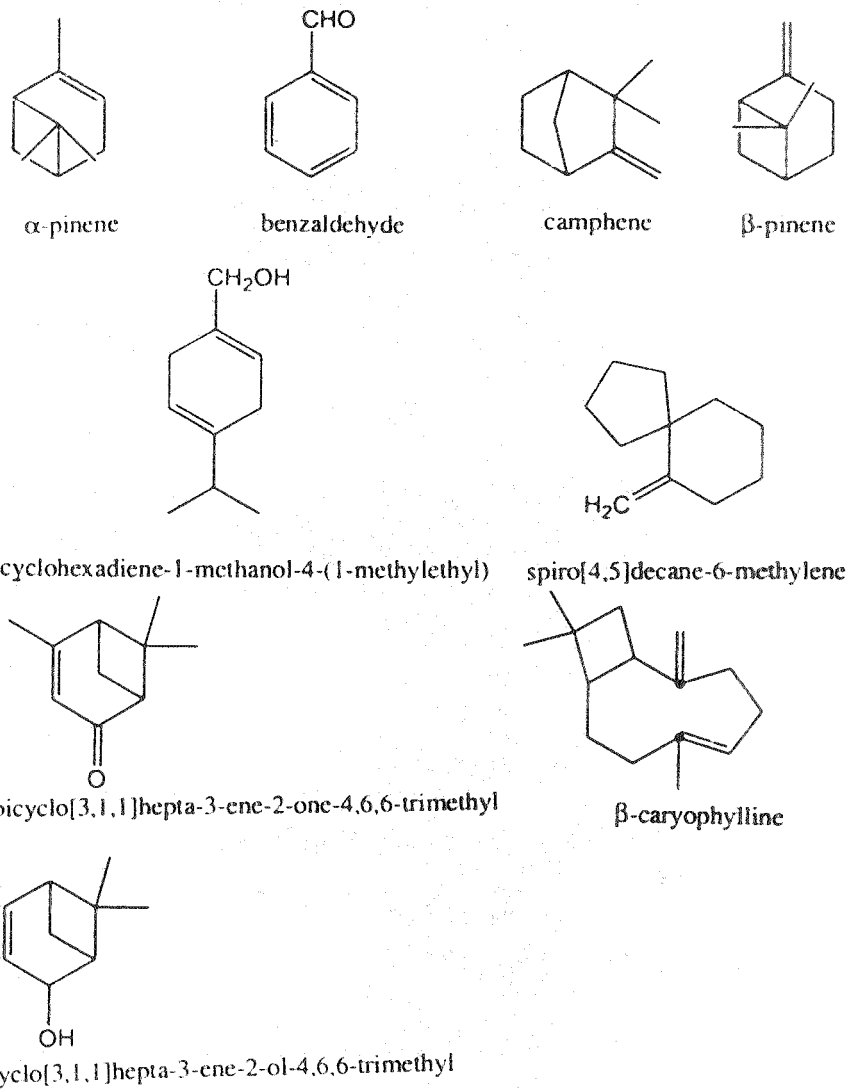
A sample of the essential oil was analyzed by GC-MS using a QP-1000 Shimadzu gas chromatograph-mass spectrometer, equipped with a CPB5 nonpolar bonded phase fused silica capillary column (25 m × 0.25 mm). Detector: MS.

Operating conditions were as follows: carrier gas, helium with a flow rate of 2mL/min, column temperature was programmed at 80–180°C (Table-1); injector temperature, 200°C; detector temperature, 250°C.

TABLE-1
TEMPERATURE PROGRAMS FOR COLUMN

	Step I	Step II	Step III	Step IV	Step V
Initial temperature (°C)	80				
Initial time (min)	0				
Program rate (°C/min)	4	1	3	5	10
Final temperature (°C)	115	120	130	150	180
Final time (min)	0	0	0	0	20

GC-MS of the essential oil showed nine major chemical components (Fig.1); therefore, GC-MS analysis of each component was carried out as well. Identification of the components in the oil was based on retention indices relative to



α-pinene benzaldehyde camphene β-pinene

1,4-cyclohexadiene-1-methanol-4-(1-methylethyl) spiro[4,5]decane-6-methylene

bicyclo[3,1,1]hepta-3-ene-2-one-4,6,6-trimethyl β-caryophylline

bicyclo[3,1,1]hepta-3-ene-2-ol-4,6,6-trimethyl

Fig. 1

n-alkanes and computer matching with NBSR BASE, as well as by comparison of the fragmentation patterns of the mass spectra with those reported in literature[4, 5].

RESULTS AND DISCUSSION

The oleoresin obtained by tapping the trunk of several *Pistachia atlantica* trees grown wildy on the Zaggrosian mountains of Ilam Province of Iran was steam distilled by different methods. Soxhlet extraction with different solvents was carried out as well. The Soxhlet extraction results have shown that extraction with toluene gave the optimum yield. Both steam distillation and Soxhlet extraction gave the same amount of a bright yellow colour essential oil (2.5 mL out of 30 g of powdered oleoresin). GC-MS analysis of the essential oil resulted in the recognition and characterization of nine major chemical components (Fig. 2).

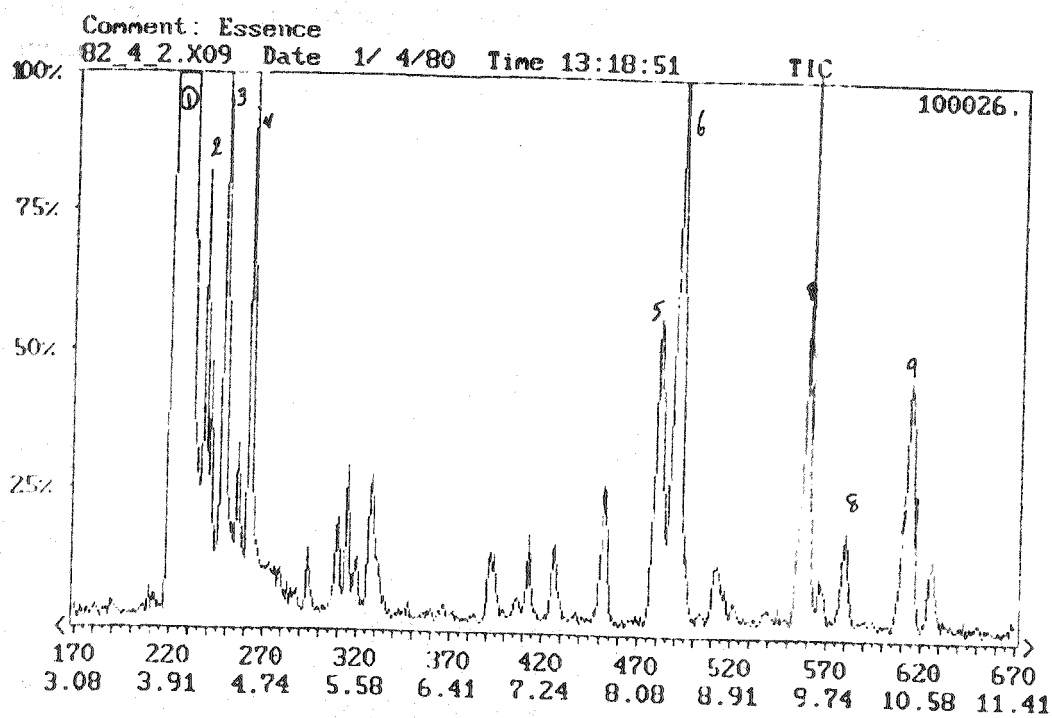


Fig. 2

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