Communication

Composition and Enantiomeric Analysis of the Essential Oil of the Fruits and the Leaves of *Pistacia vera* from Greece

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**Abstract:** The essential oils of the fruits and the leaves of pistachio (*Pistacia vera* L.) were analyzed by GC and GC/MS. Fresh unripe pistachio fruits were richer in essential oil (0.5 %, w/w) than the leaves (0.1 %, w/w). Twenty one compounds were identified in the essential oil of the fruits and the major components were (+)-α-pinene (54.6 %) and terpinolene (31.2 %). The enantiomeric ratio of the major constituents of the essential oil of the fruits was determined using chiral GC/MS and it was found that the (+)/(-)-α-pinene ratio was 99.5:0.5, (+)/(-)-limonene 80:20, (+)/(-)-β-pinene 96:4, and (+)/(-)-α-terpineol 0:100. Thirty three compounds were identified in the essential oil of the leaves and the major components were found to be α-pinene (30.0 %), terpinolene (17.6 %) and bornyl acetate (11.3 %).

**Keywords:** *Pistacia vera*, fruit, leaves, essential oil, enantiomeric analysis

**Introduction**

The fruits of *Pistacia vera* L. (pistachio nuts) are considered one of the prime edible nuts, along with almonds, macadamias and cashews. They are an excellent source of protein and they also contain about 55% of an oil very rich in oleic acid [1]. Unsalted pistachios have a high-potassium/low-sodium content and are also a good source of vitamin E. They have been used as a food as early as 7,000 BC.
Greece is one of the most important pistachio producing countries, along with Iran, Turkey, and India. Pistachios are commercially used as in-shell snacks, in confectionery, in ice creams, candies, bakery goods and as a flavouring [2,3]. In Greece, the unripe whole fruits are also used for the preparation of traditional spoon sweets.

From a botanical point of view, the genus *Pistacia* belonging to the family Anacardiaceae comprises 11 European species [4]. Many of these plants yield resin in some degree. Among them, *Pistacia lentiscus* L. var. *chia* is the major source of a resin known as mastic gum, which is highly reputed in the traditional medicine field as an antimicrobial agent [5,6] and has been an important article of commerce for centuries. *Pistacia* plants are known for their medicinal properties since antiquity. They have played important roles in folk medicine and are used in eczema treatment, throat infections, renal stones, asthma and stomach ache, and as a astringent, anti-inflammatory, antipyretic, antibacterial, antiviral, pectoral and stimulant [7].

Several members of the genus *Pistacia* have been chemically investigated. They are characterized mainly by the occurrence of flavonoids and flavonoid glycosides [8]. These plants have also been reported to contain phenolic compounds and triterpenoids [9,10]. Previous works on *Pistacia vera* concern mainly the resin of the plant [11-13], the hull [9,14] or the nutritional value of the nut [1]. Concerning the leaves of the plant there is one chemical study of the essential oil along with its antifungal activities from leaves of *Pistacia vera* grown in Turkey [15]. Interestingly, in spite of the commercial value of fruits, the contained essential oil has never been studied.

The commercial interest of pistachio fruit as well as its possible use for the adulteration of the highly prized mastic gum oil coming from *Pistacia lentiscus* L. var. *chia* stimulated our interest in the investigation of the essential oil of the fruits of *Pistacia vera* and perform the enantiomeric analysis of its major constituents. Additionally the essential oil of the leaves was studied as a possible alternative source of flavouring constituents.

**Results and Discussion**

The chemical analysis of the essential oil of the whole unripe fruits showed that it was very rich in α-pinene (54.6 %) and terpinolene (31.2 %). In total, twenty one constituents were identified (98.3%), with only three other compounds exceeding 1% (limonene, 3-carene and β-pinene). The enantiomeric analysis showed that the (+)/(−)-α-pinene ratio was 99.5:0.5, (+)/(−)-limonene 80:20, (+)/(−)-β-pinene 96:4 and (+)/(−)-α-terpineol 0:100. Additionally, both (+) and (−)-camphene were identified, but the relative ratio could not be determined due to the overlap of the latter with myrcene. It is noteworthy that the highly valuable mastic gum oil, coming from the distillation of *Pistacia lentiscus* var. *chia* resin is also very rich in α-pinene (>65 %) and contains the majority of the constituents found in *P. vera* oil. For this reason, the oil coming from *P. vera* could potentially be used for the adulteration of mastic gum oil. Interestingly, the terpinolene content of mastic gum oil is less than 0.5% [5] and consequently the adulteration by *P. vera* oil could be easily identified by the presence of the increased percentage of terpinolene. It should be noted that the two major volatile constituents, α-pinene and terpinolene, are compounds with interesting antibacterial [16,17] and antifungal [18] properties. Additionally, terpinolene has been identified as an antioxidant agent [19] that can prevent LDL oxidation [20] and also as an insecticide agent useful in food storage [21].
It is also very interesting that essential oil can be found only in the unripe whole pistachio fruit in the maturation stage that it is used for the preparation of sweets. In contrast, the distillation performed on a sample of unroasted ripe nuts without hull and shell, even directly after their collection, failed to give a measurable quantity of essential oil.

Comparison with previously studied essential oils of the fruits from related species (P. terebinthus, P. atlantica, P. lentiscus and P. palaestina) showed that both α-pinene and terpinolene, which are the major constituents of P. vera, are also present, but in much lower concentrations. All four species contain α-pinene, ranging from 3.8 % to 15.6 %, while terpinolene is found in concentrations ranging from 0.1 % to 6.9 %. It should be noted that Pistacia terebinthus is characterized by an elevated concentration of limonene (34.2 %) [22], Pistacia atlantica is characterized by an elevated concentration of bornyl acetate (21.5 %) [23], Pistacia lentiscus contained as major components myrcene (68.2-71.0 %) and limonene (9.6-19.7 %) [24] and Pistacia palaestina is characterized by an elevated concentration of (E)-ocimene (41.3 %) and sabinene (20.3 %) [25]. All the major components of the related species are found in P. vera in concentrations amounting to less than 2.5 %.

Concerning the essential oil of the leaves, the major components were found to be α-pinene (30.0 %), terpinolene (17.6 %) and bornyl acetate (11.3 %). Thirty three compounds were identified, corresponding to 87.5 % of the total. Interestingly, terpinolene had not been identified as a constituent of the essential oil of the leaves in a previous report from Turkey [12]. Additionally, major quantitative differences in camphene, limonene, α-terpineol and bornyl acetate were also observed. Only thirteen among the identified constituents in this work – and among them α-pinene as the major one – had also been previously reported as constituents of the essential oil of P. vera leaves [12].

Experimental

Plant material

The unripe fruits and the leaves were collected in Salamina (Attiki region, Greece) in May 2005 and the ripe fruits in September 2005. A voucher specimen is deposited in the herbarium of the Laboratory of Pharmacognosy, University of Athens, Greece.

Oil distillation

Fresh unripe whole pistachio fruits, leaves and fresh ripe pistachio nuts (after removal of the hull and the hard shell, 100 g each) were subjected to hydrodistillation for 3 h using a modified Clevenger-type apparatus to give 0.5 %, 0.1 % and an insignificant quantity of essential oil, respectively. The distilled oil was collected, dried over anhydrous sodium sulphate and stored at 4 °C.

Analysis

The GC-FID analysis was carried out on a Perkin-Elmer Clarus 500 gas chromatograph, fitted with a HP 5MS 30 m x 0.25 mm, 0.25 μm film thickness capillary column. The column temperature was programmed from 60 °C to 280 °C at a rate of 3 °C/min. The injector and detector temperatures were
programmed at 230 °C and 300 °C, respectively. Helium was used as the carrier gas at a flow rate 1 mL/min.

The GC-MS analyses were carried out using a Hewlett Packard 6890-5973 GC-MS system operating on EI mode (equipped with a HP 5MS 30 m x 0.25 mm, 0.25 μm film thickness capillary column). He (1 mL/min) was used as carrier gas. The initial temperature of the column was 60 °C and then it was heated to 280 °C at a rate of of 3 °C /min. GC-MS analyses were also performed on a Finnigan Trace GC-MS with an external ion source in both the EI and chemical ionization (CI) modes at a flow rate of 1.0 mL/min, using CH₄ as the CI ionization reagent.

The identification of the compounds (Table 1) was based on comparison of their retention indices (RI), obtained using n-alkanes (C₈-C₂₅), and comparison of their EI-mass spectra with the NIST/NBS-Wiley library spectra and literature data [26]. Additionally, the identity of all compounds was confirmed by comparison of the expected molecular weights with the results obtained from the CI spectra and in several cases by co-injection with available authentic samples.

Enantiomeric GC-MS analysis was performed using a b-Dex sm (30m x 0.25mm, 0.25 μm) column on a Finnigan Trace spectrometer. The initial temperature of the column was 40 °C and then it was heated to 230 °C at a rate of of 2 °C /min, He (0.8 mL/min). The identification of the enantiomers was performed by co-injection with commercially available authentic samples.

| Compounds          | RI    | % Fruit | % Leaves | Methodsa |
|--------------------|-------|---------|----------|----------|
| 2E-hexenal         | 855   | 0.1     | -        | RI, MS   |
| 1-hexanol          | 871   | 0.1     | -        | RI, MS   |
| tricyclene         | 925   | 0.1     | 1.1      | RI, MS   |
| α-pinene           | 941   | 54.6    | 30.0     | RI, MS, Co |
| camphene           | 953   | 0.8     | 4.7      | RI, MS, Co |
| β-pinene           | 979   | 1.6     | 2.0      | RI, MS, Co |
| β-myrcene          | 993   | 1.0     | 0.8      | RI, MS, Co |
| 2-pentyl furan     | 1002  | -       | 0.9      | RI, MS   |
| α-phellandrene     | 1005  | 0.3     | -        | RI, MS   |
| 3-carene           | 1011  | 2.7     | 2.4      | RI, MS   |
| α-terpinene        | 1018  | 1.0     | 0.4      | RI, MS, Co |
| limonene           | 1032  | 2.5     | 3.0      | RI, MS, Co |
| (Z)-β-ocimene      | 1040  | 0.1     | Tr       | RI, MS   |
| (E)-β-ocimene      | 1053  | 0.1     | -        | RI, MS   |
| γ-terpinene        | 1062  | 0.2     | 0.4      | RI, MS, Co |
| terpinolene        | 1092  | 31.2    | 17.6     | RI, MS, Co |
| linalool           | 1100  | 0.2     | 1.8      | RI, MS   |
| nonanal            | 1105  | -       | 1.8      | RI, MS, Co |
| 1,3,8-p-menthadiene| 1111  | -       | Tr       | RI, MS   |
| endo-fenchol       | 1115  | 0.1     | -        | RI, MS   |
| α-campholenal      | 1127  | -       | Tr       | RI, MS   |

Table 1. Percentage composition of the essential oils of whole unripe fruits and leaves of *Pistacia vera*
Table 1. Cont.

| Compound                  | RI     | MS   | Co     |
|---------------------------|--------|------|--------|
| trans-pinocarveol         | 1140   | -    | Tr     |
| camphor                   | 1145   | -    | Tr     |
| borneol                   | 1168   | 0.8  | 0.3    |
| p-cymen-8-ol              | 1183   | 0.1  | -      |
| α-terpineol               | 1193   | 0.2  | 0.7    |
| bornyl acetate            | 1287   | 0.5  | 11.3   |
| isodene                   | 1372   | -    | 0.1    |
| α-gurjenene               | 1406   | -    | 0.1    |
| aromadendrene             | 1439   | -    | 1.1    |
| alloaromadendrene         | 1460   | -    | 0.5    |
| β-ionone                  | 1487   | -    | 0.3    |
| ledene                    | 1494   | -    | 1.5    |
| α-farnesene               | 1509   | -    | 0.2    |
| (E)-nerolidol             | 1566   | -    | 0.4    |
| spathulenol               | 1578   | -    | 1.1    |
| globulol                  | 1584   | -    | 1.0    |
| viridiflorol              | 1592   | -    | 0.3    |
| (E, E)-farnesol           | 1726   | -    | 1.7    |
| Total                     | 98.3%  | 87.5%|        |

*RI: Retention index, MS: mass spectrum, Co: co-injection with authentic sample

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*Sample Availability:* Samples of the essential oils are available from the authors.

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