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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 ^{o}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 ^{o}C to 280 ^{o}C at a rate of 3 ^{o}C /min. The injector and detector temperatures were

programmed at 230 ^{o}C and 300 ^{o}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 ^{o}C and then it was heated to 280 ^{o}C at a rate of of 3 ^{o}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 CH_4 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 (C_8-C_{25}), 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 ^{o}C and then it was heated to 230 ^{o}C at a rate of of 2 ^{o}C /min, He (0.8 mL/min). The identification of the enantiomers was performed by co-injection with commercially available authentic samples

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

Compounds	RI	% Fruit	% Leaves	Methods[a]
2*E*-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-menthatriene	1111	-	Tr	RI, MS
endo-fenchol	1115	0.1		RI, MS
α-campholenal	1127	-	Tr	RI, MS

Table 1. Cont.

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

[a]RI: Retention index, MS: mass spectrum, Co: co-injection with authentic sample

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References

1. Kucukoner, E.; Yurt, B. Some chemical characteristics of *Pistacia vera* varieties produced in Turkey. *Eur. Food Res. Technol.* **2003**, *217*, 308-310.
2. Duke, J.A. *CRC Handbook of Nuts*: CRC Press: Boca Raton, FL., **1989**; pp. 240-243.
3. Walheim, R.; Walheim, L. *Western Fruit and Nuts*; HP Books, Inc.: Tucson, AZ, **1981**; p. 166.
4. Tutin, T. G.; Heywood, V. H.; Burgess, N. A.; Moore, D. M.; Valentine, D. H.; Walters, S. M.; Webb, D. A. *Flora Europaea;* Cambridge University Press: Cambridge, U.K., **1968**;Vol 2, p. 237.
5. Magiatis, P.; Melliou, E.; Skaltsounis, A.L.; Chinou, I.B.; Mitaku, S. Chemical composition and antimicrobial activity of the essential oils of *Pistacia lentiscus* var. *chia. Planta Med.* **1999**, *65*, 749-751.
6. Paraschos S.; Magiatis P.; Mitaku S.; Petraki K.; Kaliaropoulos A.; Maragoudakis P.; Mentis A.; Sgouras D.; Skaltsounis A.-L. In vitro and in vivo activity of chios mastic gum extracts and constituents against *Helicobacter pylori. Antimicrob. Agents Chemother.* **2007**, *51*, 551-559.
7. Kordali, S.; Cakir, A.; Zengin, H.; Duru, M.E. Antifungal activities of the leaves of three Pistacia species grown in Turkey. *Fitoterapia* **2003**, *74*,164-167.

8. Kawashty, A.; Mosharrata S.A.M.; El-Gibali M.; Saleh N.A.M. The flavonoids of four Pistacia species in Egypt. *Biochem. Syst. Ecol.* **2000,** *28*, 915-917.
9. Yalpani, M.; Tyman, J.H.P. Long-chain phenols.24. The phenolic acids of *Pistacia ver*a. *Phytochemisrty* **1983**, *22*, 2263-2266.
10. Marner, F.J.; Freyer, A.; Lex, J. Triterpenoids from gum mastic, the resin of *Pistacia lentiscu*s. *Phytochemistry* **1991,** *30*, 3709-3712.
11. Alma, M.H.; Nitz, S.; Kollmannsberger, H.; Digrak, M.; Efe F.T., Yilmaz, N. Chemical composition and antimicrobial activity of the essential oils from the gum of Turkish Pistachio (*Pistacia vera*). *J. Agr. Food Chem*. **2004**, *52*, 3911-3914.
12. Caputo, R.; Mangoni, L.; Monaco, P.; Palumbo, G.; Aynehchi, Y.; Bagheri, M. Triterpenes from bled resin of *Pistacia ver*a. *Phytochemistry* **1978**, *17*, 815-817.
13. Ramezani, M.; Khaje-Karamoddin, M.; Karimi-Fard. Chemical composition and anti-*Helicobacter pylori* activity of the essential oil of *Pistacia vera*. *Pharm. Biol.* **2004**, *42*, 488-490.
14. Ozel, M.Z.; Gogus, F.; Hamilton, J.F.; Lewis, A.C. The essential oil of *Pistacia vera* L. at various temperatures of direct thermal desorption using comprehensive gas chromatography coupled with time-of-flight mass spectrometry. *Chromatographia* **2004,** *60*, 79-83.
15. Duru, M.E., Cakir, A.; Kordali, S.; Zengin, Harmandar, H.M.; Izumi, H.; Hirata, T.Chemical composition and antifungal properties of essential oils of three Pistacia species. *Fitoterapia* **2003,** *74*,170-176.
16. Raman, A.; Weir, U.; Bloomfield, S.F. Antimicrobial effects of tea-tree oil and its major components on *Staphylococcus aureus*, *Staphylococcus epidermidis* and *Propionibacterium acne*s. *Lett. Appl. Microbiol.* **1995,** *21*, 242-245.
17. Carson, C.F.; Riley, T.V. Antimicrobial activity of the major components of the essential oil of *Melaleuca alternifoli*a. *J. Appl. Bacteriol.* **1995,** *78*, 264-269.
18. Farooq, A.; Choudhary, M.I.; Atta-ur-Rahman, Tahara, S.; Baser, K.H.C.; Demirci, F.Z. Detoxification of terpinolene by plant pathogenic fungus *Botrytis cinere*a. *Z. Naturforsch. C* **2002,** *57*, 863-866.
19. Kim, H-J.; Chen, F.; Wu, C.; Wang, X.; Chung, H.Y.; Jin, Z. Evaluation of Antioxidant Activity of Australian Tea Tree (*Melaleuca alternifolia*) Oil and Its Components. *J. Agr. Food Chem*. **2004,** *52*, 2849-2854.
20. Graßmann, J.; Hippeli, S.; Spitzenberger, R.; Elstner, E.F. The monoterpene terpinolene from the oil of *Pinus mugo* L. in concert with alpha-tocopherol and beta-carotene effectively prevents oxidation of LDL. *Phytomedicine* **2005,** *12*, 416-423.
21. Park, I-K., Lee, S-G.; Choi, D-H.; Park, J-D.; Ahn, Y-J. Insecticidal activities of constituents identified in the essential oil from leaves of *Chamaecyparis obtusa* against *Callosobruchus chinensis* (L.) and *Sitophilus oryzae* (L.). *J. Stored Prod. Res.* **2003**, *39*, 375-384.
22. Couladis, M.; Oʒcan, M.; Tzakou, O.; Akgül, A. Comparative essential oil composition of various parts of the turpentine tree (*Pistacia terebinthus* L) growing wild in Turkey. *J. Sci. Food Agric.* **2003**, *83*, 136-138.
23. Barrero, A.F.; Herrador, M.M.; Arteaga, J.R.; Akssira, M.; Mellouki, F.; Belgarrabe, A.; Blázquez, M.A. Chemical composition of the essential oils of *Pistacia atlantica* Desf. *J. Essent. Oil Res***. 2005,** *17*, 52-54.

24. Vidrich, V.; Fusi, P.; Graziano, A.; Silvestrini, E.; Michelozzi, M.; Marco, F. Chemical composition of the essential oil of *Pistacia lentiscus* L. *J. Essent. Oil Res.* **2004**, *16*, 223-226.
25. Flamini, G.; Bader, A.; Cioni, P.L.; Katbeh-Bader, A.; Morelli, I. Composition of the Essential Oil of Leaves, Galls, and Ripe and Unripe Fruits of Jordanian *Pistacia palaestina* Boiss. *J. Agr. Food Chem.* **2004**, *52*, 572-576.
26. Adams, R.P. *Identification of Essential Oil Components by Gas Chromatography/Mass Spectrometry*. Allured Publishing Corp.: Carol Stream, IL, **2001**.

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