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FATTY ACID PATTERNS OF WASTE PARTS OF TURKISH *PISTACIA VERA* L. TREE

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The fatty acid composition of waste products of *Pistacia vera* (pistachio tree) grown in Turkey was assayed. The waste products and various parts of the tree were classified as fresh leaves (FL), dried leaves (DL), stem (ST), branches, fresh skin of natural woody shell (unprocessed) (FSN), fresh kernel, and skin of processed woody shell. Gas chromatography-mass spectrometry data showed FSN, FL, DL and ST to be rich sources of fatty acids. In particular, FL contains a remarkable amount of linolenic acid (30.4±3.28%).

Key words: *Pistacia vera*, Anacardiaceae, pistachio, fatty acid, GC-MS.

INTRODUCTION

Pistacia vera L. (Anacardiaceae), the only one of the 11 species of the genus *Pistacia* that produces edible nuts, is a small tree grown in Southern Europe and Asia Minor. Pistachio is the nut of the tree, having an edible green kernel enclosed in a woody shell. *P. vera* is widely cultivated in southern Anatolia for its nuts, making a significant contribution to the major agricultural exports of Turkey. Extensive research has shown pistachio nuts to be a rich source of fatty acids (Garcia et al., 1992; Agar et al., 1995a,b; Aslan et al., 2002; Satil et al., 2003; Kucukoner and Yurt, 2003).

In our continuing research on Turkish *P. vera*, we have so far investigated the antibacterial, antifungal, antiviral and antiprotozoal activity of lipophilic extracts obtained from diverse parts of the plant, as well as the anti-inflammatory and antinociceptive activity of ethanolic and aqueous extracts of fruits, leaves, branches, peduncles, and oleoresin obtained from it (Ozcelik et al., 2005; Orhan et al, 2006a,b). In surveying the literature we have not encountered any reports on the fatty acid content of waste plant parts of *P. vera*. In Turkey, after pistachio nuts are processed the skins of the shells are discarded, and the rest of the tree, including leaves, stem and branches, is not used.

In the present study we determined the fatty acid composition of the waste plant parts of *P. vera*. by capillary gas chromatography-mass spectrometry (GC-MS).

MATERIALS AND METHODS

PLANT MATERIAL

Plant materials were collected in September 2003 from several pistachio trees cultivated in the town of Nizip in Gaziantep province, southeast Turkey, and were classified as fresh leaves (FL), dried leaves (DL), stem (ST), branches (BR), fresh skin of natural woody shell (unprocessed) (FSN), fresh kernel (FK) and skin of processed woody shell (SP). Voucher specimens are preserved at the Herbarium of the Department of Pharmacognosy, Faculty of Pharmacy, Gazi University, Ankara, Turkey (GUE 2381).

OIL EXTRACTION AND METHYL ESTERIFICATION

The plant parts were dried at room temperature and ground mechanically. Each part was extracted in *n*-hexane (Merck) for 8 h in a Soxhlet apparatus in the presence of anhydrous Na_2SO_4. The *n*-hexane extracts were filtrated and evaporated in a vacuum at 40°C. The extract yields (w/w) were 3.44% for SP, 8.71% for FSN, 5.78% for FL, 3.79% for DL, 1.98% for ST, 1.03% for BR, and 3.44% for FK. Fatty acids in the oily extracts were converted to their methyl esters by *trans*-esterification according to Morrison's method using boron trifluoride (BF_3) in methanol (Sigma) prior to GC-MS analysis (Morrison and Smith, 1964).

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TABLE 1. Percentages of the fatty acids identified in oily extracts of waste parts of *Pistacia vera* identified by GC-MS

Fatty acids detected	Retention time (min)	Part of plant						
		Skin of processed woody shell	Fresh skin of natural woody shell*	Fresh leaves	Dried leaves	Stem	Branches	Fresh kernel
Myristic (C14:0)	17.15	–	0.2 ± 0.01	1.6 ± 0.19	2.8 ± 0.80	–	–	–
Palmitoleic (C16:1, *cis*-9)	19.19	9.3 ± 1.24	0.9 ± 0.01	–	–	1.2 ± 0.06	–	–
Palmitic (C16:0)	19.37	2.8 ± 0.05	11.2 ± 0.28	7.8 ± 0.76	5.6 ± 1.64	10.0 ± 0.55	3.3 ± 0.15	4.7 ± 0.28
Linoleic (C18:2, *cis*-9,12)	20.90	–	Trace	12.3 ± 1.13	–	–	8.2 ± 0.72	6.6 ± 0.62
Linolenic (C18:3, *cis*-9,12,15)	20.96	–	–	30.4 ± 3.28	–	–	–	–
Oleic (Cis18:1, *cis*-9)	21.07	–	78.3 ± 0.61	2.8 ± 0.32	10.3 ± 1.33	35.0 ± 1.79	–	19.3 ± 3.21
Stearic (C18:0)	21.27	–	3.2 ± 0.11	–	2.8 ± 0.59	2.1 ± 0.11	–	1.8 ± 0.30
Elaidic (*Cis*18:1, *trans*-9)	22.67	0.5 ± 0.01	2.4 ± 0.08	–	2.2 ± 0.90	–	–	15.8 ± 0.90
Gondoic (C20:1, *cis*-11)	22.77	–	0.5 ± 0.04	–	–	–	–	–
Arachidic (C20:0)	23.00	–	0.3 ± 0.06	Trace	Trace	1.3 ± 0.25	–	–
Behenic (C22:0)	24.60	–	–	2.0±0.21	1.0 ± 0.37	1.6 ± 0.36	–	–
Melissic (C30:0)	24.88	–	–	–	4.2 ± 0.46	–	–	–
Lignoceric (C24:0)	26.10	–	–	3.3±0.15	2.5 ± 0.38	0.6 ± 0.04	–	–
Cerotic (C26:0)	27.75	–	–	–	–	1.4 ± 0.29	–	–
Heptacosanoic (C27:0)	29.43	–	–	–	–	–	1.5±0.21	–
Montanic (C28:0)	29.71	–	2.7 ± 0.34	–	2.8 ± 0.51	–	–	–
Total	–	12.6	99.7	60.2	34.2	53.2	13.0	48.2

*non-processed

GC-MS ANALYSIS

The methyl esters of fatty acids were dissolved in *n*-hexane (analytical grade, Merck) and analyzed by GC-MS (Hewlett Packard, HP 6890 series, equipped with a mass selective detector) at 0.2 µl volume. The injections for each sample were done in triplicate. The analytical conditions were as follows: carrier gas, helium; flow rate, 1.0 ml min^{-1}; detector temperature, 250°C; mode, split; split ratio, 10/1; split flow, 10 ml min^{-1}; run time, 31 min; column, HP-5MS (5% phenyl methylsiloxane, 30.1 m × 250 µm × 0.25 µm); average velocity, 37 cm/sec; pressure, 9.4 psi. MS parameters were as follows: ionization voltage, 70 eV; mass range (m/z), 20–440; ion source temperature, 250°C. Column temperature was initially

held at 80°C for 5 min, followed by a 10°C/min increase to a final temperature of 290°C (5 min).

Individual components in the chromatograms obtained were identified and quantified by comparison of their mass spectra and retention times (R_t) with those of reference fatty acids (Sigma Co.) and also a Wiley data bank search. The data were expressed as means±SD using Instat statistics software (Tab. 1).

RESULTS AND DISCUSSION

The greatest yields were obtained from the extracts of FSN (8.71%) and FL (5.78%). As seen in Table 1, 99.7% of fresh skin of natural woody shell, 60.2% of fresh leaves, 53.2% of stem, 48.2% of fresh kernel, and 34.2% of dried leaf extracts were identified as fatty acids. Branch extract had low fatty acid content (13%), but possessed many peaks belonging to terpene-type compounds and was the only plant part yielding heptacosanoic acid. BR also contained a substantial amount of linoleic acid (8.2%). FL had the most linoleic acid (12.3%), followed by BR. The most striking result was the high amount of linolenic acid, one of the polyunsaturated fatty acids important for health, found in FL (30.4%). Interestingly, linolenic acid was not found in a rest of the oils analyzed in this study. Another important finding was that FSN had the highest amount of oleic acid (78.3%), a monounsaturated fatty acid, followed by ST (35.0%). Except for SP and BR oils, the rest contained oleic acid in amounts ranging from 2.7% to 35.0%. Elaidic acid, a monounsaturated fatty acid that has the same structure as oleic acid except that it is a *trans* fatty acid, was found in FK in a considerable amount (15.8%), and also in SP, FSN and DL extracts. Elaidic acid has been reported to have several important pharmacological actions, including antineoplastic, antiviral, cytotoxic and antiproliferative activity (Breistol et al., 1999; Andrei et al., 2000; Lima et al., 2002; Bergman et al., 2004) Arachidic acid (also known as eicosanoic acid), a saturated 20-carbon fatty acid found in vegetable (e.g., peanut) oils and fish oils, was detected in very low amounts in FSN, FL, DL and ST oils. Among the oils, only FSN had gondoic acid (also known as (*Z*)-11-eicosenoic acid) at a very low concentration. Lignoceric acid (tetracosanoic acid), one of the major components of soldier frontal gland secretions of the Formosan subterranean termite (*Coptotermes formosanus*), was detected in FL, DL, and ST extracts of *P. vera*. Only ST extract possessed cerotic acid (hexacosanoic acid), a white solid fatty acid occurring in waxes such as beeswax or carnauba wax. FSN and DL were the only extracts that contained montanic acid (octacosanoic acid), a long-chain fatty acid mainly found in waxes. Melissic acid, a crystalline fatty acid found free or in the form of its ester with myricyl alcohol in beeswax and other waxes, was found only in DL. The extracts of FL, DL, and ST had behenic acid (docosanoic acid), a fatty acid found mainly in groundnut oil.

A noteworthy outcome of this study was the finding that fresh leaves apparently are very rich in linolenic acid, one of the body's essential fatty acids that is rarely found in natural sources. Most of the waste plant parts had considerable quantities of oleic acid. Fresh skin of unprocessed woody shells, fresh leaves, dried leaves and stems should be considered potential substitute sources for fatty acids. Note that the amounts of most of the fatty acids detected in fresh leaves were drastically lower or even absent in extract of dried leaves.

To the best of our knowledge, this is the first report on the fatty acid composition of waste plant parts of *Pistacia vera*, and as such is a practical contribution to the chemotaxonomic study of this genus.

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