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Characterization of some *Pistacia* spp. through phenolic content

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SUMMARY – Phenolic compounds are widely distributed in almost all higher plants and they occur in different tissues. The quality and quantity of phenolics is used as criteria for variety identification. The present study has been planned to determine the phenolic composition of *Pistacia* spp. and pistachio cultivars. Phenolic compounds were investigated by Thin Layer Chromatography (TLC). Some differences were observed in relation to spots from phenolics in pistachio and *Pistacia* spp.

Key words: *Pistacia* spp., pistachio, phenolic compounds, leaf.

RESUME – "Caractérisation de quelques espèces de *Pistacia* à travers la teneur en composés phénoliques". Les composés phénoliques sont largement distribués dans presque toutes les plantes supérieures et ils se trouvent dans différents tissus. La qualité et la quantité de composés phénoliques sont utilisées comme critères pour l'identification variétale. La présente étude vise à déterminer la composition phénolique des espèces de *Pistacia* et des cultivars de pistachier. Les composés phénoliques ont été étudiés par chromatographie en couche fine (TLC). Quelques différences ont été observées concernant des taches liées aux composés phénoliques chez les pistachiers et les espèces de *Pistacia*.

Mots-clés : Espèces de *Pistacia*, pistachier, composés phénoliques, feuille.

Introduction

In order to distinguish varieties, male and female plants can be used morphological markers such as leaf shape and size, length and thickness of petiole, etc. (Jindal and Singh, 1975). However, it is of great importance correct and definite identification of varieties, since they are often morphologically very similar. Additionally, some varieties are genetically not uniform, but are a mixture of different types. This has led to many local varieties characters are able to detect by biochemical methods. Phenolic compounds may be used in biochemical investigations due to the easy analytical methods in determinations and suitability for chemical examinations. Plant produce a great variety of secondary products of which the most important group is called phenolic compounds. These substances which are determined in different organs of the plants (Mısırlı *et al.*, 1995) play important roles in many physiological events. After the importance of phenolic compounds was revealed, to separate and identify them, some techniques were developed and Thin Layer Chromatography (TLC) was used (Janguard, 1970).

The quantity and quality of phenolic compounds have been used as criteria for variety identification since 1980 (Swain, 1980). In this context, some differences in relation to phenolics were determined between *P. avium* and *P. mahaleb* by using paper chromatography (Yu and Carlson, 1975). On the chromatograms of almond cultivars partially differed from each other (Mısırlı *et al.*, 1994). Similarly, some spots determined in female figs did not appear in caprifigs (Mısırlı *et al.*, 1998).

The current study was performed to determine the phenolic composition of pistachio and different *Pistacia* spp.

Materials and methods

This study was carried out on female types such as Çatlayan and Alyanak (Kırmızı), Topan and Söbü (Uzun); on male species such as male Siirt, *P. vera*, *P. atlantica* and *P. terebinthus*.

Leaf samples were collected in June. The phenolic substances were investigated TLC method.

10 x 10 cm sized Merck 5577 plates were used in qualification 30 µl of samples were spotted on the right corner of each plate and developed in two dimensions. The first development was carried in a solution of butan-1-ol: acetic acid: water (4:1:5 V/V) and the second dimension in acetic acid: water (5:95 V/V). Chromatographic plates were sprayed with Naturstoff (NS) reagent and examined under ultraviolet light (366 nm). Rf value of each spot after the second development was determined. The colour intensity of spots were investigated, as well (Mısırlı *et al.*, 1994).

Results and discussion

The investigation of chromatograms with NS reagent under UV light indicated some differences among *Pistacia* spp. and pistachios. Ethanolic extracts of male and female trees showed the presence of 20 different spots on the plates (Table 1) (Fig. 1).

Table 1. Distribution of spots[†]

Spot no.	Topan	Söbü	Çatlayan kırmızı	Alyanak	Siirt	<i>P. vera</i>	<i>P. atlantica</i>	<i>P. terebinthus</i>
1	+++++	+++++	+++++	+++	+++++	+++	+++	++++
2	+++++	+++++	+++++	++++	+++++	++++	+++	++++
3	—	—	—	—	+++++	++++	++++	++++
4	+++	+++	++	++	+++	+++	+++	++++
5	—	—	+	+	+	+++	++	++
6	+++	+++	++	++	++++	++	++++	++
7	++	++	+	+	+	+++	+	++
8	+++++	+++++	++++	++++	+++++	++++	+++	++++
9	++	++	+++	+++	+	+	+	+
10	—	—	+++	+++	—	+++	+++	++++
11	—	—	+++	+++	++	++	++	—
12	++	++	++	++	+	+	+	+
13	+	+	—	—	+	++	+++	—
14	++	++	+	+	+	+++	+++	+
15	+	+	+	+	+	+	+	+
16	+	+	—	—	—	+	—	—
17	—	—	—	—	+	—	—	—
18	—	—	—	—	+	—	—	—
19	++	++	++	++	+++	+++	++++	++++
20	+	+	—	—	—	—	—	—

[†]+ = relative amount of phenolic compounds: from +++++ = very dense to + = very scarce; — = not detected.

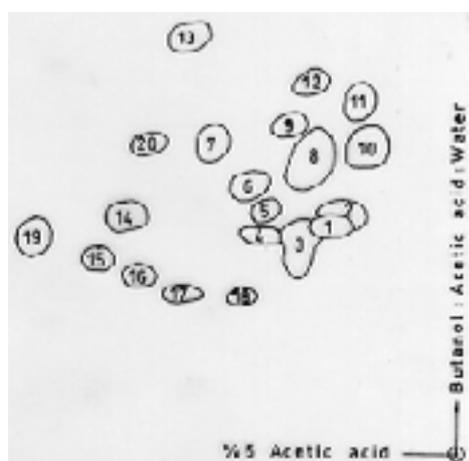


Fig. 1. The main chromatogram of spots.

Spots 1, 2, 4, 6, 7, 8, 9, 12, 14, 15 and 19 were identical in all of the examined materials (Table 1). Spot 3 which was not determined in female types was peculiar in male species. Spot 5 occurred in all female types and *Pistacia* spp., except Topan and Söbü (Table 1). Spot 10 was not observed in Topan, Söbü and male Siirt, similarly, spot 11, in Topan, Söbü and *P. terebinthus*. Spot 13 did not occur in Çatlayan and Alyanak. Spot 16 appeared in Topan, Söbü and *P. vera*. Spot 17 and 18 were typical in male Siirt and could not be detected in the others. Spot 20 was characteristic for only in Topan and Söbü.

In the comparison of female types, spots 5, 10 and 11 appeared in Çatlayan and Alyanak (Kırmızı types) but was not found in Topan and Söbü (Uzun types). In contrary, spots 13, 16 and 20 were detected in Uzun types and they did not occur in Kırmızı types. When the male species were compared, male Siirt did not have spot 10 but it was the only variety displaying spots 17 and 18. Spots 11 and 13 were absent in *P. terebinthus*. Spot 16 was present in *P. vera*. In the comparison of male and female species and types, spots 3, 17 and 18 were found to be common in male species. Similarly, spot 20 was determined in Uzun varieties. Generally, male species had more spots than the female ones (Table 1).

The investigation of spots on the chromatograms showed that the concentrations of the separated compounds vary markedly in applied concentration (30 µl). Spots 1, 2, 3 and 8 were denser in the most of the plates. Spots 15, 16, 17, 18 and 20 were observed as faint. The other spots differed based on the types and species as dense or light.

The Rf values, colour reaction and identification of spots are given in Table 2. Orange and blue coloured spots were accepted to represent flavonoid and phenylpropane compounds, respectively (Tanrısever, 1982).

Table 2. The Rf values, colour reaction and identification of spots

Spot no.	Rf value [†]		Colour ^{††} NS + UV	Identification
	BAW	AA		
1	0.45	0.11	Dark orange	Flavonoid
2	0.49	0.09	Dark orange	Flavonoid
3	0.38	0.19	Dark blue	Phenylpropane
4	0.43	0.25	Orange	Flavonoid
5	0.55	0.22	Light orange	Flavonoid
6	0.59	0.32	Orange	Flavonoid
7	0.67	0.37	Dark blue	Phenylpropane
8	0.62	0.16	Dark orange	Flavonoid
9	0.71	0.18	Light orange	Flavonoid
10	0.69	0.07	Light orange	Flavonoid
11	0.75	0.10	Light orange	Flavonoid
12	0.77	0.17	Light orange	Flavonoid
13	0.85	0.45	Blue	Phenylpropane
14	0.45	0.53	Dark blue	Phenylpropane
15	0.32	0.58	Blue	Phenylpropane
16	0.28	0.50	Blue	Phenylpropane
17	0.26	0.47	Light orange	Flavonoid
18	0.30	0.35	Light orange	Flavonoid
19	0.43	0.88	Bright blue	Phenylpropane
20	0.67	0.46	Blue	Phenylpropane

[†]BAW = Butan-1-ol: acetic acid: water (4:1:5); AA = 5% acetic acid.

^{††}NS = Naturstoff, reagent; UV = Ultraviolet lights.

In the relative evaluation in relation to the size and colour intensity of the spots, it was seen that flavonoid content was higher than phenylpropane compounds in all female types and male species (Table 1).

Among the females, in Alyanak and Çatlayan, flavonoid and phenylpropane content were higher and lower than Topan and Söbü, respectively. In the comparison of male species, male Siirt contained much more flavonoid than the others. It was found that phenylpropane content of *P. vera* was the highest. Flavonoid content was seen to be approximately same in female types and male species. On the other hand, phenylpropane content of male species was higher than the others (Table 1).

As a result of this study, some spots were typical for male (3, 17, 18) or female (20). Confirming this, some spots determined in female figs did not appear in caprifigs (Mısırlı *et al.*, 1998). Some differences in relation to the distribution of spots appeared both female cultivars and different *Pistacia* spp. This result paralleled to identifying mandarin varieties (Ulubelde and Lester, 1982).

According to the relative evaluation, flavonoid content was determined to be high in all female and male. About this matter, amount of flavonoid was reported to be higher than the other phenolics in all studied mahaleb types (Mısırlı and Özeke, 1999).

Conclusions

There were some differences between and in male species and female types. Detailed investigations may be carried out by using HPLC procedure.

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