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Composition of the Essential Oil of Leaves, Galls, and Ripe and Unripe Fruits of Jordanian *Pistacia palaestina* Boiss.

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Pistacia palaestina Boiss. (*Pistacia terebinthus* L. var. *palaestina* (Boiss.) Engl.) is a medicinal and foodstuff plant. The ripe fruits are used largely in the Middle East as a component of the so-called Zaatar, a mix of aromatic and food plants. Results of GC and GC−MS analyses of the essential oils of leaves, galls produced by *Baizongia pistaciae* (L.), and ripe and unripe fruits of *Pistacia palaestina* Boiss. collected in Jordan are reported. Both qualitative and quantitative differences between different parts of the plant were observed. The oil was rich in monoterpenes, and the main constituents were α-pinene (63.1%) and myrcene (13.3%) in the leaves and α-pinene (49.4%), sabinene (22.8%), and limonene (8.1%) in the galls. (*E*)-Ocimene (33.8−41.3%), sabinene (20.3−24.1%), and (*Z*)-ocimene (3.8−13.0%) were the main ones in both unripe and ripe fruits. Sesquiterpenes have been detected in small quantities in leaves and fruits and in trace amounts in galls.

KEYWORDS: ***Pistacia palaestina*; essential oil; Zaatar; GC−MS; fruits; leaves; gall; insect; *Baizongia pistaciae*; monoterpenes**

INTRODUCTION

The Anacardiaceae family includes 76 genera with over 600 species. This family includes some species with very important economic value such as the mango tree (*Mangifera indica*), the pistachio nut (*Pistacia vera*), and the cashew tree (*Anacardium occidentale*). Besides the edible plants, this family is well-known for its poisonous species, which cause contact dermatitis.

Pistacia palaestina Boiss. (*Pistacia terebinthus* L. var. *palaestina* (Boiss.) Engl.) is a deciduous tree or a shrub living in maquis and garigues, in association with *Quercus calliprinus*, mainly on hills and mountains of Palestine, Lebanon, Syria, Turkey, and adjacent islands (*1*).

The fruits of *P. palaestina* are known in Arabic as “Butom”. They are edible and sold in markets (*1*, *2*). In the Middle East, raw or toasted ripe fruits of *P. palaestina* are grinded and mixed with other aromatic plants to blend the so-called “Zaatar”, a food daily consumed with bread, olive oil, and tea. Zaatar itself can be classified according to its content in less or more expensive aromatic plants, affecting the taste and the quality. Zaatar containing *P. palaestina* fruits is considered of high quality. The plants used to prepare Zaatar are summarized in **Table 1**.

Table 1. Plants Used To Prepare Zaatar

plant name	used part	treatment
Majorana syriaca (L.) Rafin.	leaves	air-dried
Sesamum indicum L.[a]	seeds	toasted
Pistacia palaestina Boiss.[b]	fruits	raw or toasted
Foeniculum vulgare Mill.	fruits	raw
Coriandrum sativum L.	fruits	raw
Carum carvi L.	fruits	raw
Anethum graveolens L.	fruits	raw
Cuminum cyminum L.	fruits	raw
Cicer arietinum L.	seeds	toasted
Triticum vulgare L.	seeds	toasted
Rhus coriaria L.	fruits	raw

[a] All the constituents of Zaatar are grinded except *Sesamum indicum* seeds. [b] Can be substituted with *Pistacia atlantica*.

The galls of *P. palaestina* are induced by infection of a variety of galling aphids, which belong to the order Homoptera. These aphids live by sucking on the phloem of the host plant and excrete large amounts of sweet honeydew. This sweet material attracts a variety of insects, especially the ants, which frequent the aphid colonies. Very often an aphid colony is protected against all enemies by forming the galls (*3*). Galls from *P. palaestina* and other species of *Pistacia* are used as a source of tannins (*1*) employed for tanning goat skin (*4*).

In Jordan, the resin of *P. palaestina* is used in the local folk medicine as a diuretic, laxative, stimulant, and aphrodisiac (*5*), while the decoction of the leaves is employed as a diuretic and

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antihypertensive and for jaundice (*2*). In Turkey, the decoction of the leaves *P. palaestina* is used as an antidiabetic remedy (*6*). Other species of *Pistacia* are used in various folk medicine systems, and some biological activities have been confirmed scientifically. The decoction of the leaves of *P. lentiscus* is used in Jordan and Palestine to treat liver intoxications. This property has been demonstrated in vivo on rats treated with CCl_4 (*7*) probably as a result of the presence of galloylquinic derivatives, which have antioxidant activity (*8*). The essential oil obtained from the resin of *P. lentiscus* and its variety *chia* showed a powerful antimicrobial activity (*9, 10*). In addition, the essential oil of *P. lentiscus* has been evaluated for insecticide activity against *Mayetiola destructor*; the vapors of the oil of *P. lentiscus* caused 100% mortality on the eggs of this insect within 24 h (*11*).

The essential oil from galls of *P. integerrima* was studied for its depressive activity on the mouse central nervous system (*12*). Previous studies on *P. palaestina* deal with triterpenoid derivatives from galls (*13*). Herein, the composition of the essential oil of leaves, galls, and ripe and unripe fruits of Jordanian *P. palaestina* Boiss. is reported using GC and GC−MS analyses.

MATERIALS AND METHODS

Plant Material. Leaves, galls, unripe fruits (red), and ripe fruits (green) from 10 adult plants of *P. palaestina* Boiss. were collected in As-Salt, Jordan, at the end of September 2002, in a Mediterranean climate. The plant material was identified by Dr. Ammar Bader, and a voucher specimen was deposited in the herbarium of Laboratory of Pharmacognosy at Al-Zaytoonah Private University of Jordan. The galling insect was identified by Professor Ahmad Katbeh-Bader as *Baizongia pistaciae* (L.) (Homoptera: Aphidoidea, Pemphigidae, Fordinae), and a voucher specimen was deposited in UJIM (University of Jordan Insect Museum).

An amount of 100 g of the air-dried and bulked material of each sample was hydrodistilled in a Clevenger-like apparatus for 2 h.

The GC analyses were accomplished with an HP-5890 series II instrument equipped with HP-WAX and HP-5 capillary columns (30 m × 0.25 mm, 0.25 μm film thickness) with the following parameters: 60 °C for 10 min, ramp of 5 °C/min up to 220 °C; injector and detector temperatures of 250 °C; carrier gas, helium (2 mL/min); detector, dual FID; split ratio of 1:30; injection of 0.5 μL. The identification of the components was performed for both the columns by comparison of their retention times with those of pure authentic samples and by means of their linear retention indices (lri) relative to the series of *n*-hydrocarbons. The relative proportions of the essential oil constituents were percentages obtained by FID peak-area normalization.

GC−EIMS analyses were performed with a Varian CP-3800 gas chromatograph equipped with a DB-5 capillary column (30 m × 0.25 mm; coating thickness of 0.25 μm) and a Varian Saturn 2000 ion trap mass detector. Analytical conditions were the following: injector and transfer line temperatures of 220 and 240 °C, respectively; oven temperature programmed from 60 to 240 °C at 3 °C/min; carrier gas helium at 1 mL/min; injection of 0.2 μL (10% hexane solution); split ratio of 1:30. Identification of the constituents was based on comparison of the retention times with those of authentic samples, comparing their linear retention indices relative to the series of *n*-hydrocarbons, and on computer matching against commercial (NIST 98 and ADAMS) and homemade library mass spectra built up from pure substances and components of known oils and MS literature data (*14*−*19*).

All the reference compounds were obtained from Sigma-Aldrich Italia (either normal or flavor and fragrances catalogues) except ocimene (mixture of isomers) and (*E*)-β-farnesene (mixture of isomers) (ChromaDex), while germacrene D was confirmed by NMR analyses of other essential oils (*20*). The only missing reference compounds were *p*-cymenene, β-bourbonene, γ-muurolene, bicyclogermacrene, α-muurolene, *cis*-γ-cadinene, and β-sesquiphellandrene, which were labeled as tentative identification in **Table 2** (however, their retention indexes and MS data were in good agreement with the literature). Moreover, the molecular weights of all the identified substances were confirmed by GC−CIMS, using MeOH as CI ionizing gas.

RESULTS AND DISCUSSION

A total of 41 compounds were identified, accounting for 98.1−99.6% of the whole essential oils. The compositions are reported in **Table 2**. The essential oil yields were very variable, ranging from 0.02% (w/w, calculated on dry plant material) in leaves to 2.71% in galls. Intermediate values were found for fruits; unripe fruits yielded 0.16% essential oil, while in ripe fruits the yield dropped to 0.06%.

The essential oil of the leaves consisted mainly of monoterpenes (92.6%, 31 constituents). Among these derivatives, hydrocarbons predominated (89.1%, 16 compounds); the remaining constituents were sesquiterpene hydrocarbons (3.7%, 7 compounds) and other non-terpene substances (1.0%, 4 compounds). The main monoterpenes were α-pinene (63.1%), myrcene (13.3%), and limonene (4.3%), while the principal sesquiterpene was β-caryophyllene (2.9%).

Galls produced an oil composed almost exclusively of monoterpenes (99.2%, 21 compounds); only three sesquiterpenes were detected in trace amounts. Again, hydrocarbons are the main derivatives (94.3%, 16 substances) while oxygenated compounds represented 4.9% (5 substances). The main constituents of this oil were α-pinene (49.4%), sabinene (22.8%), limonene (8.1%), 4-terpineol (4.4%), and myrcene (4.2%). With respect to the oil from leaves, this oil contained considerably smaller amounts of α-pinene and myrcene but greater concentrations of sabinene, limonene, and terpinen-4-ol.

In unripe fruits, monoterpenes accounted for 95.3% (15 constituents) of the whole essential oil, while sesquiterpenes (5 hydrocarbon derivatives) amounted to 3.8%. Monoterpene hydrocarbons summed to 87.6%, while oxygenated compounds summed to 7.7%. The constituents characterizing this essential oil were (*E*)-ocimene (41.3%), sabinene (20.3%), terpinen-4-ol (6.4%), α-pinene (5.3%), and (*Z*)-ocimene (3.8%).

Ripeness caused a slight decrease in monoterpene percentage (92.2%, 17 compounds) and a halving in the concentration of oxygenated compounds (3.0%), although their number doubled (4 vs 2). In contrast, sesquiterpenes showed a small increase in both percentage and number (5.7%, 6 derivatives). The main compounds identified in ripe fruits were (*E*)-ocimene (33.8%), sabinene (24.1%), (*Z*)-ocimene (13.0%), and α-pinene (6.5%).

All the essential oils were characterized by high percentages of monoterpenes (always higher than 90%), mainly hydrocarbon derivatives. The differences are principally quantitative. In the essential oil of unripe and ripe fruits, (*E*)-ocimene and (*Z*)-ocimene accounted for more than 40% of the whole oils. In ripe fruits, the percentage of the (*Z*)-isomer increased and the percentage of the (*E*)-isomer decreased. Ocimene isomers are monoterpenes found in a number of culinary herbs such as *Ocimum basilicum* L., *Thymus pulegioides* L., and *Origanum vulgare* L. (*21*−*23*), and they contribute to the pleasant and characteristic odor of the aromatic plants. The essential oils from leaves and galls contained considerably smaller amounts of ocimenes, while α-pinene represented more than half of the total composition.

Many species of *Pistacia* have been investigated for the essential oil composition of their leaves (*P. lentiscus*, *P. khinjuk*, *P. chinensis*, *P. terebinthus*), fruits (*P. lentiscus*, *P. vera*), resin (*P. lentiscus*) and galls (*P. lentiscus*, *P. integerrima*). Comparison of previous studies shows a remarkable variability that seems to depend on plants species and plant organ. In the essential oil

Table 2. Composition[a] of the Essential Oils Obtained from Leaves, Galls, and Unripe and Ripe Fruits of *Pistacia palaestina*

constituents	lri[b]	lri[c]	% from leaves	% from galls	% from unripe fruits	% from ripe fruits
(*E*)-2-hexenal	856	1220	0.5	*e*	*e*	*e*
n-nonane	900	900	*e*	*e*	*e*	tr[f]
α-thujene	933	1031	tr[f]	1.4	1.2	0.8
α-pinene	940	1029	63.1	49.4	5.3	6.5
camphene	955		0.3	0.3	*e*	0.4
sabinene	978	1126	1.5	22.8	20.3	24.1
β-pinene	981	1112	1.5	3.7	2.8	3.6
6-methyl-5-hepten-2-one	986	1333	tr[f]	*e*	*e*	*e*
myrcene	992	1170	13.3	4.2	1.3	1.2
α-phellandrene	1007	1152	1.1	0.3	0.4	*e*
3-carene	1013	1136	*e*	tr[f]	*e*	*e*
α-terpinene	1020	1165	tr[f]	1.4	2.7	0.5
p-cymene	1028	1273	tr[f]	tr[f]	0.3	0.5
limonene	1032	1198	4.3	8.1	2.7	4.1
β-phellandrene	1033	1190	0.4	0.1	*e*	*e*
(*Z*)-ocimene	1041	1199	2.7	0.2	3.8	13.0
(*E*)-ocimene	1051	1242	0.5	0.1	41.3	33.8
γ-terpinene	1064	1231	0.4	1.8	4.4	0.7
cis-sabinene hydrate	1070		*e*	tr[f]	*e*	*e*
terpinolene	1089	1284	tr[f]	0.5	1.1	tr[f]
p-cymenene[d]	1090		tr[f]	*e*	*e*	*e*
n-undecane	1100	1100	*e*	*e*	*e*	tr[f]
linalool	1102	1547	*e*	tr[f]	*e*	*e*
nonanal	1104	1385	tr[f]	*e*	*e*	1.1
borneol	1175	1707	1.1	*e*	*e*	*e*
terpinen-4-ol	1182	1607	1.1	4.4	6.4	1.5
α-terpineol	1192	1698	0.9	0.5	1.3	0.4
isobornyl acetate	1286		0.4	tr[f]	*e*	0.5
α-terpinyl acetate	1351		*e*	*e*	*e*	0.6
β-bourbonene[d]	1385		*e*	*e*	0.3	*e*
β-caryophyllene	1420	1605	2.9	tr[f]	0.5	1.3
α-humulene	1456	1676	2.0	*e*	0.4	*e*
(*E*)-β-farnesene	1459	1668	*e*	tr[f]	*e*	*e*
γ-muurolene[d]	1478	1682	0.1	*e*	*e*	0.5
germacrene D	1482	1722	0.5	*e*	2.3	3.0
bicyclogermacrene[d]	1496		*e*	*e*	*e*	0.3
α-muurolene[d]	1499	1720	tr[f]	*e*	*e*	*e*
cis-γ-cadinene[d]	1511		tr[f]	*e*	*e*	tr[f]
δ-cadinene	1524	1764	0.5	*e*	0.3	0.6
β-sesquiphellandrene[d]	1526	1773	*e*	tr[f]	*e*	*e*
(*Z*)-3-hexenyl benzoate	1572	1555	0.5	*e*	*e*	*e*
monoterpenes			92.6	99.2	95.3	92.2
sesquiterpenes			6.0	tr[f]	3.8	5.7
fatty acid derivatives			1.0	*e*	*e*	1.1
total identified			99.6	99.2	99.1	99.0
essential oil yield (% w/w)[g]			0.02	2.71	0.16	0.06

[a] Percentages obtained by FID peak-area normalization. [b] Linear retention indices (HP-5 column). [c] Linear retention indexes (DB0WAX column). [d] Tentatively identified. [e] Not detected. [f] Less than 0.1%. [g] Calculated on dry plant material.

obtained from the leaves, some authors found a dominance of monoterpene hydrocarbons (*24*−*30*). In particular, the main constituents of the most studied species, *P. lentiscus*, were α-pinene (Greece and France) (*29*−*31*), myrcene (Spain and Sicily (*27, 32*), 3-carene (Egypt) (*26*), and terpinen-4-ol/α-pinene, limonene, and myrcene (Corsica) (*30*). According to their intraspecific variability, the essential oil of *P. lentiscus* can be classified into three groups on the basis of their content of terpinen-4-ol/α-pinene, limonene, and myrcene. In the essential oil of the galls of *P. integerrima*, monoterpenes dominated, with α-pinene, sabinene, β-pinene, and limonene as the main components, whereas the oil of the galls from *P. lentiscus* was rich in sesquiterpene hydrocarbons such as β-caryophyllene, δ-cadinene, and germacrene D (*33*, *34*). In the resin of *P. lentiscus*, monoterpene hydrocarbons are the principal constituents, with α-pinene as the main compound (*27*). The same authors identified in the oil of ripe and unripe fruits of *P. lentiscus* more than 90% of monoterpene hydrocarbons, but in this case, myrcene was the main component. Other researchers (*24*) found as main constituents of the essential oil of the fruits of *P. lentiscus* terpinen-4-ol, α-terpineol, and sesquiterpene alcohols. The essential oil of the fruits of *P. vera* contained α-pinene as the main component (*35*).

Although *Pistacia palaestina* is a variety of *Pistacia terebinthus*, the composition of the essential oil of the leaves was very different (*36*). In *Pistacia terebinthus*, the monoterpenes terpinen-4-ol, γ-terpinene, limonene, and α-terpinene have been detected as the main compounds, while in *P. palaestina* the main compounds were α -pinene and myrcene (63.1% and 13.3%, respectively).

In **Table 3** the main constituents of some *Pistacia* species are summarized to show the relationship between the composition of the leaves, the galls, and the fruits in the same species. Fruit repining of *p. terebinthus* causes racemization of α-pinene to β-pinene, with the same total percentage being maintained after repining (α-pinene (15.6%) and β-pinene (11.5%)) compared with the percentages before repining (α-pinene (5.3%) and β-pinene (22.5%)). The sum of both is 27.1% and 27.8%,

Table 3. Composition of the Essential Oil of Some *Pistacia Species*

plant name	analyzed parts	country	main constituent of the essential oil	reference
P. terebinthus	leaves	Turkey	terpinen-4-ol (33.7%) γ-terpinene (9.3%) α-terpineol (8.1%)	*36*
P. terebinthus	young shoots	Turkey	limonene (3.0%) α-pinene (5.3%)	*28*
P. terebinthus	unripe fruits	Turkey	limonene (34.2%) α-pinene (15.6%) β-pinene (11.5%) germacrene D (3.5%)	*28*
P. terebinthus	ripe fruits	Turkey	limonene (32.8%) β-pinene (22.5%) α-pinene (5.3%) germacrene D (4.6%)	*28*
P. lentiscus	leaves	Turkey	terpenin-4-ol (29.9%) α-terpineol (10.6%) limonene (10.6%)	*36*
P. lentiscus	leaves	Spain (sample 1)	α-pinene(11.0%) β-myrcene (19.0%)	*27*
P. lentiscus	unripe fruits	Spain (sample 1)	α-pinene (22.0%) β-myrcene (54.0%)	*27*
P. lentiscus	ripe fruits	Spain (sample 1)	α-pinene (11.0%) β-myrcene (72.0%)	*27*
P. lentiscus	galls	Spain (sample 2)	β-caryophyllene (13.1%) δ-cadinene (8.1%) germacrene D (6.8%) α–pinene (4.4%)	*34*
P. lentiscus	aerial parts	Spain (sample 2)	β-caryophyllene (6.9%) δ-cadinene (4.1%) α–pinene (13.0%) β -pinene (4.9%) p-cymene (4.7%)	*34*

respectively. This observation is also noted in the essential oils of the fruits of *P. palaestina* but now with (*E*)-ocimene and (*Z*)-ocimene. In addition, the formation of galls in *P. lentiscus* causes only quantitative variation of the oil component. The same thing is observed in *P. palaestina*. This observation could justify the adaptation of the insect to the plant and its constituent from the beginning bite and oviposition till the development of the winged insects.

The life cycle of *Baizongia pistaciae* (L.) is well-known (*3*). In February, the fundatrices of *B. pistaciae* appear on the young leaves, which grow on the extremities of the stems of *P. palaestina* and produce multicolored galls that have the form of carob pods. From June onward, these galls dry and spilt at their distal ends. A winged generation appears and migrates to the secondary host. A winged generation leaves that secondary host at the beginning of winter and oviposits on *Pistacia* again (*3*). Besides gall formation, the plant reacts to the insect bite, producing a great amount of essential oil; the yield reaches 2.7%, whereas in the other parts it was much smaller. Furthermore, the composition of the oil is quite different quantitatively because of the simultaneous high percentages of α-pinene, sabinene, and limonene. However, the essential oil components in galls could have deterrent properties against parasites, including herbivorous and omnivorous organisms, but not against the gall maker species. In addition, when galls are opened spontaneously, an oleoresinous exudate is produced abundantly. The exposure of this exudate to the air transforms it into a solidified material (resin) that could have a cicatrizant property for the damaged part of the plant, preventing the rotting and microbial infection of the plant.

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