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Comparison of hydrodistillation and microdistillation methods for the analysis of fruit volatiles of *Prangos pabularia* Lindl., and evaluation of its antimicrobial activity

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Abstract

The volatile constituents of *Prangos pabularia* Lindl. fruits (Umbelliferae) were obtained by hydrodistillation (HD) and microdistillation (MD) techniques, and then analyzed by gas chromatography (GC) and gas chromatography–mass spectrometry (GC/MS) methods. One hundred and twenty-eight compounds have been characterized representing 90.5% of hydrodistilled oil (HDO). In the microdistilled oil (MDO), 115 compounds constituting 93.0% of the oil were detected. α-Humulene (16.6% and 15.5%), bicyclogermacrene (16.1% and 7.9%), spathulenol (10.6% and 5.7%), germacrene D (5.7% and 2.9%) and α-pinene (4.2% and 23.9%) were found to be the major constituents of HDO and MDO, respectively. Antimicrobial activity of the oil was tested via microdilution broth technique. *Escherichia coli*, *Pseudomonas aeruginosa*, *Proteus vulgaris, Salmonella typhimurium, Staphylococcus epidermidis*, Methicillin-resistant *Staphylococcus aureus*, and *Candida albicans* were used as the test microorganisms.

Keywords: Antimicrobial activity; Essential oil; Hydrodistillation; Microdistillation; *Prangos pabularia*; Umbelliferae

1. Introduction

The genus *Prangos* (Umbelliferae) encompasses 28 species worldwide, with a diversity centre in the Irano–Turanian region. Only *Prangos pabularia* Lindl. and *Prangos ferulacea* Lindl. have a wide geographic ranges among the other species of this genus. In the Flora of Turkey 13 species of the *Prangos* genus are known (Davis, 1972, 1988; Duman, 2000; Herrnstadt and Heyn, 1977).

In Turkey, representatives of the genus *Prangos* are used in folk medicine as tonic and to stop external bleeding, and to heal the scars (externally application) (Ulubelen et al., 1995). Roots of *Prangos* species are known as aphrodisiac like *Ferula* and *Ferulago* species (Akalin, 1999). In India, the roots of *P. pabularia* are known as emmenagogue while the entire plant is used to kill snails in water (Singh and Kohli, 1956; Kamboj, 1988). Fruits of *P. pabularia* are used as stimulant and antiflatulent (Baytop, 1999).

Recently, we have reported on the fruit oil composition of *Prangos turcica* A. Duran, M. Sagiroglu & H. Duman, a new described endemic from Turkey (Özek et al., 2006). As a continuation of our previous research on *Prangos* species, the volatiles of *P. pabularia* have been hydrodistilled and microdistilled from dried fruits and analyzed by GC and GC/MS methods, then tested for antimicrobial activity via microdilution broth technique.

A literature search revealed a few papers which described the presence of coumarins, furocoumarins, alkaloids, phenolic acids and lactonic constituents in *P. pabularia* (Zevarshoev et al., 1986; Mukhamedova et al., 1967; Chatterjee et al., 1972; Tsetlin et al., 1972; Yunusov et al., 1957). The antibacterial activity and inhibition of cytokine release by coumarins and γ-pyrone derivatives, the antioxidant activity of some constituents from this species were reported (Tada et al., 2002; Kogure et al., 2004). The oil compositions of *P. pabularia* of Russian and Indian origins have earlier been reported (Kuznetsova et al., 1973; Koul and Thakur, 1978). The present work is the first

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Table 1
Stepwise Heating Programme for essential oil microdistillaion

	Step1	Step 2	Step 3	Step 4
Heating rate	20 °C/min		20 °C/min	
Final temperature	100 °C	100 °C	112 °C	112 °C
Time	5 min	15 min	<1 min	35 min
Cooling temperature	−1 °C	−1 °C	−1 °C	−1 °C
Post-run	–	–	–	2 min

report on the fruits oil composition and antimicrobial activity of *P. pabularia* growing in Turkey. In order to achieve a thorough characterization of the volatile components present in the fruits, two techniques were employed for the isolation of volatiles, namely hydrodistillation and microdistillation.

The microdistillation is known as a capillary technique used for distillation processes at the microscale level (Bicchi and Sandra, 1987; Briechle et al., 1997; Başer et al., 2000a). Our previous experience demonstrates convincingly that microdistillation and the subsequent GC/MS analysis can safely be used to investigate the composition of volatiles in minute samples of aromatic plants even using herbarium specimens. The distillation product can be used for GC and GC/MS analysis without further preparation (Başer et al., 2001, 2002, 2006a,b,c; Kürkçüoglu et al., 2003; Özek et al., 2007, in press).

2. Materials and methods

2.1. Plant material

The plant material was collected on 28 July 2004 in Sivas: space between Imranli and Refahiye, on stony slope of a hill at an altitude of 1800 m of the west foot of a Kizildag mountain, in Turkey. The identification of the plant material was performed by one of us (A.D.). Voucher specimens (A.Duran 6784 et Hamzaoglu (KNYA)) are kept at the Herbarium of Selcuk University.

2.2. Isolation of essential oils

2.2.1. Hydrodistillation

Air-dried fruits of the plant material (50 g) were crushed and subjected to hydrodistillation for 180 min using a Clevenger type apparatus. The oil yield (v/w) on moisture free basis was 0.2%. The oil was dried over anhydrous sodium sulphate and stored in sealed vial in the dark, at 4 °C, ready for GC and GC/MS analyses and antimicrobial test.

2.2.2. Microdistillation

The essential oil was extracted from 0.5 g plant material using the MicroDistiller benchtop distillation device (Eppendorf-Netheler-Hinz, Hamburg, Germany). The vials (20 mL for the sample vials, 10 mL for the collection vials), capillary columns, crimp caps and septa were original accessories from the manufacturer. The dried fruits were crushed and placed in a sample vial together with 10 mL of water. Sodium chloride (2.0 g) and water (1.0 mL) were placed in the collecting vial. *n*-Hexane (0.3 mL) was added to the collecting vial to trap volatile compounds. The apparatus was operated according to Stepwise Heating Programme for Essential Oils (Table 1). After completing the distillation, the organic layer in the collection vial was separated from the water phase, concentrated under nitrogen gas and injected into GC and GC/MS. Data on the microdistilled volatiles are statistically calculated and presented as mean value ± standard deviation (σ) for three replicates.

2.3. Microorganisms and preparation of inoculum

Microorganisms used for antimicrobial test and sources are given in Table 2. The microorganisms were refreshed in Mueller Hinton Broth (Merck) at 35–37 °C, and inoculated on Mueller Hinton Agar (Mast Diagnostics, Merseyside, U.K.) media for preparation of inoculum.

2.4. Antimicrobial assay

Antibacterial and anticandidal activity of HDO was evaluated using the microdilution broth technique (Koneman et al., 1997; Amsterdam, 1997). Stock solution of the fruit oil was prepared in dimethylsulfoxide (DMSO, Carlo-Erba, France). In sterile distilled water, dilution series were prepared from 1 mg/mL to 0.003 mg/mL in micro-test tubes (Eppendorf) which were transferred to 96-well microtiter plates. Overnight grown microorganism suspensions in Mueller–Hinton broth were standardized to (for bacteria and *C. albicans* app. 10^8 and 10^6 cfu/mL respectively) McFarland No.: 0.5 standard. Each microorganism suspension was then added into the wells. The last well-column with medium served as a positive growth control. After incubation at 37 °C for 18–24 h the first well without turbidity was determined as the minimal inhibitory concentration (MIC). Chloramphenicol was used as standard antibacterial agent whereas ketoconazole was used as antifungal.

2.5. Gas chromatography–mass spectrometry (GC/MS) conditions

The GC/MS analysis was carried out with an Agilent 5975 GC-MSD system. HP-Innowax FSC column (60 m × 0.25 mm,

Table 2
Strain numbers and sources of the microorganisms used for antimicrobial assay (microdilution broth technique)

Microorganism	Strain number	Comments
Escherichia coli	NRRL B-3008	Gram (−), Pathogenic
Pseudomonas aeruginosa	ATCC 27853	Gram (−)
Proteus vulgaris	NRRL B-123	Gram (−)
Salmonella typhimurium	NRRL B-4420	Gram (−)
Staphylococcus epidermidis	ATCC 12228	Gram (+)
MRSA	Clinical isolate, Osmangazi University, Faculty of Medicine, Department of Microbiology, Eskişehir, Turkey	Gram (+)
Candida albicans	Clinical Isolate, Osmangazi University, Faculty of Medicine, Department of Microbiology, Eskişehir, Turkey	

0.25 μm film thickness) was used with helium as carrier gas (0.8 mL/min). GC oven temperature was kept at 60 °C for 10 min and programmed to 220 °C at a rate of 4 °C/min, and kept constant at 220 °C for 10 min and then programmed to 240 °C at a rate of 1 °C/min. Split ratio was adjusted at 40:1. The microdistilled sample was analyzed in the splitless mode. The injector temperature was at 250 °C. MS were taken at 70 eV. Mass range was from *m*/*z* 35 to 450.

2.6. Gas chromatography (GC) conditions

The oils were analyzed by capillary GC using an Agilent 6890N GC system. The same column and analysis conditions were used for both GC and GC/MS. The microdistilled sample was analyzed in the splitless mode. FID detector temperature was 300 °C. In order to obtain same elution order with GC/MS, simultaneous injection was done by using the same column and appropriate operational conditions.

2.7. Identification of compounds

Quantification of volatile components was performed on the basis of their GC peak areas on the Innowax column and percentages of the characterized components were as listed in Table 3. The identification of the volatile constituents was achieved through retention indices and mass spectrometry by the comparing mass spectra of the unknown peaks with those stored in the Wiley GC/MS Library, MassFinder, Adams Library, NIST Library and the in-house "Başer Library of Essential Oil Constituents", which includes more than 3300 genuine compounds with MS and retention data. *n*-Alkanes (C9–C20) were used as reference points in the calculation of retention indices (RI).

3. Results and discussion

3.1. Essential oils composition

This is the first report on the composition of the fruit oil of *P. pabularia* growing in Turkey. Hydrodistillation (180 min) of the dried fruits of *P. pabularia* gave the yellowish oil with a specific odour in 0.2% yield. The microdistillation procedure performed with MicroDistiller device has enabled the distillation of aromatic material in very small quantities (0.5 g) for shorter period (55 min). Both of the volatile samples (HDO and MDO) were analyzed by GC and GC/MS methods simultaneously. The list of detected compounds with their relative percentages, and retention indices are given in Table 3 in order of their elution on a HP-Innowax FSC column. Generally, the overall compositions of the volatiles, isolated using HD and MD techniques, appeared to be the same. There was no significant qualitative difference detected, although the percentage compositions for some components varied significantly.

One hundred and twenty-eight compounds have been characterized representing 90.5% of HDO. MD procedure resulted in 115 compounds constituting 93.0% of volatiles. Fig. 1 presents two typical chromatographic profiles of *P. pabularia* fruit volatiles obtained by HD and MD techniques. α-Humulene,

Table 3
Compositions of *P. pabularia* fruit volatiles obtained by hydrodistillation and microdistillation

No.	RRI	Compound	HD, %	MD, % (mean±σ)	Method of identification
1	1032	α-Pinene	4.2	23.96±2.05	RI, MS
2	1035	α-Thujene	t	t	RI, MS
3	1048	2-Methyl-3-buten-2-ol	t	–	RI, MS
4	1076	Camphene	t	0.10±0.00	RI, MS
5	1118	β-Pinene	0.2	1.00±0.10	RI, MS
6	1132	Sabinene	0.1	0.10±0.05	RI, MS
7	1137	Thuja-2,4(10)-diene	t	–	RI, MS
8	1146	δ-2-Carene	t	–	RI, MS
9	1159	δ-3-Carene	0.1	0.36±0.05	RI, MS
10	1174	Myrcene	t	t	RI, MS
11	1176	α-Phellandrene	0.7	2.70±0.47	RI, MS
12	1183	*p*-Mentha-1,7(8)-diene (=*Pseudolimonene*)	t	t	RI, MS
13	1197	Sylvestrene	t	t	RI, MS
14	1203	Limonene	0.6	1.36±0.12	RI, MS
15	1206	2-Methyl-2-butenal	t	–	RI, MS
16	1218	β-Phellandrene	1.5	3.90±0.49	RI, MS
17	1220	*cis*-Anhydrolinalool oxide	t	-	RI, MS
18	1244	2-Pentyl furan	t	t	RI, MS
19	1246	(*Z*)-β-Ocimene	0.2	0.50±0.00	RI, MS
20	1253	*trans*-Anhydrolinalool oxide	t	–	RI, MS
21	1255	γ-Terpinene	t	t	RI, MS
22	1266	(*E*)-β-Ocimene	0.1	0.2±0.00	RI, MS
23	1280	*p*-Cymene	0.5	1.13±0.05	RI, MS
24	1285	Isoamyl isovalerate	t	–	RI, MS
25	1286	Isoterpinolene	t	t	RI, MS
26	1286	2-Methyl butyl 2-methyl butyrate	t	–	RI, MS
27	1290	Terpinolene	0.1	0.20±0.00	RI, MS
28	1296	Octanal	t	t	RI, MS
29	1327	3-Methyl-2-butenol	t	0.10±0.00	RI, MS
30	1348	6-Methyl-5-hepten-2-one	–	0.16±0.05	RI, MS
31	1382	Alloocimene	–	0.30±0.00	RI, MS
32	1400	Nonanal	0.2	0.66±0.12	RI, MS
33	1424	Hexyl butyrate	–	t	RI, MS
34	1433	Tetradec-1-ene	t	–	RI, MS
35	1438	Hexyl 2-methylbutyrate	–	t	RI, MS
36	1452	α,*p*-Dimethylstyrene	t	t	RI, MS
37	1457	Hexyl-3-methyl butyrate (=*Hexyl isovalerate*)	t	–	RI, MS
38	1466	α-Cubebene	t	t	RI, MS
39	1468	*trans*-1,2-Limonene epoxide	t	–	RI, MS
40	1483	Octyl acetate	0.1	0.30±0.00	RI, MS
41	1492	Bicycloelemene	0.2	5.83±0.18	RI, MS
42	1495	α-Ylangene	t	t	RI, MS
43	1497	α-Copaene	0.5	0.56±0.05	RI, MS
44	1505	Italicene	t	t	RI, MS
45	1528	α-Bourbonene	0.1	0.30±0.08	RI, MS
46	1535	β-Bourbonene	0.6	0.43±0.05	RI, MS
47	1540	α-Funebrene (=*1,7-diepi-α-Cedrene*)	t	–	RI, MS
48	1544	α-Gurjunene	t	t	RI, MS
49	1548	(*E*)-2-Nonenal	t	–	RI, MS
50	1549	β-Cubebene	0.2	0.23±0.05	RI, MS
51	1552	Isoitalicene	0.1	t	RI, MS
52	1562	Octanol	0.1	1.30±0.02	RI, MS
53	1577	α-Cedrene	0.3	0.10±0.00	RI, MS
54	1587	β-Funebrene	0.3	–	RI, MS
55	1589	β-Ylangene	t	0.73±0.05	RI, MS
56	1594	*trans*-β-Bergamotene	t	–	RI, MS
57	1597	β-Copaene	0.1	0.23±0.05	RI, MS

(continued on next page)

Table 3 (*continued*)

No.	RRI	Compound	HD, %	MD, % (mean±σ)	Method of identification
58	1598	β-Cubebene isomer	–	0.30±0.00	RI, MS
59	1612	β-Caryophyllene	1.7	1.06±0.05	RI, MS
60	1613	β-Cedrene	t	t	RI, MS
61	1614	Carvacrol methyl ether (=*Methyl carvacrol*)	t	–	RI, MS
62	1628	Aromadendrene	0.1	0.13±0.05	RI, MS
63	1641	3,5-Acoradiene	0.1	0.10±0.00	MS*
64	1664	Isogermacrene D	0.1	0.16±0.05	MS*
65	1668	(*Z*)-β-Farnesene	0.4	0.23±0.05	RI, MS
66	1670	γ-Gurjunene	–	0.10±0.00	RI, MS
67	1687	α-Humulene	16.6	15.46±1.20	RI, MS
68	1691	α-Acoradiene (=*4,11-Acoradiene*)	0.1	0.10±0.00	RI, MS
69	1693	β-Acoradiene (=*3,11-Acoradiene*)	0.1	t	RI, MS
70	1704	γ-Curcumene	2.4	1.93±0.26	RI, MS
71	1708	Ledene	0.2	–	RI, MS
72	1726	Germacrene D	5.7	2.93±0.18	RI, MS
73	1727	7-*epi*-Dehydrosesquicineole	1.3	1.03±0.05	RI, MS
74	1740	β-Bisabolene	0.1	0.10±0.00	RI, MS
75	1741	α-Muurolene	t	–	RI, MS
76	1755	Bicyclogermacrene	16.1	7.93±0.20	RI, MS
77	1763	Naphthalene	t	t	RI, MS
78	1771	γ-Bisabolene	0.1	t	RI, MS
79	1773	δ-Cadinene	0.6	0.40±0.08	RI, MS
80	1776	γ-Cadinene	t	0.10±0.00	RI, MS
81	1783	β-Sesquiphellandrene	0.1	t	RI, MS
82	1784	(*E*)-α-Bisabolene	0.2	0.30±0.00	RI, MS
83	1786	*ar*-Curcumene	2.1	1.00±0.00	RI, MS
84	1787	Aromadendra-4(10),14-diene	0.2	0.23±0.09	MS*
85	1798	Methyl salicylate	0.1	t	RI, MS
86	1802	Cumin aldehyde	t	t	RI, MS
87	1823	*p*-Mentha-1(7),5-dien-2-ol	t	0.08±0.03	RI, MS
88	1827	(*E,E*)-2,4-Decadienal	t	t	RI, MS
89	1839	Cuparene	t	–	RI, MS
90	1845	(*E*)-Anethole	0.1	0.16±0.05	RI, MS
91	1853	10-epi-Italicene ether	t	t	RI, MS
92	1864	*p*-Cymen-8-ol	–	0.10±0.00	RI, MS
93	1868	(*E*)-Geranyl acetone	t	t	RI, MS
94	1872	*trans*-Calamenene	t	t	RI, MS
95	1877	Italicene ether	0.1	t	RI, MS
96	1902	Benzyl isovalerate	–	t	RI, MS
97	1921	α-Phellandrene epoxide	–	t	RI, MS
98	1900	Epicubebol	0.1	–	RI, MS
99	1933	Tetradecanal	t	–	RI, MS
100	1941	α-Calacorene	t	t	RI, MS
101	1943	4-Hydroxy-2-methyl acetophenone	0.3	0.33±0.05	MS*
102	1953	Palustrol	–	t	RI, MS
103	1957	Cubebol	0.1	t	RI, MS
104	1984	γ-Calacorene	t	t	RI, MS
105	2001	Isocaryophyllene oxide	0.1	t	RI, MS
106	2008	Caryophyllene oxide	0.7	0.26±0.05	RI, MS
107	2022	Isoitalicene epoxide	0.1	–	MS*
108	2033	Salvial-4(14)-en-1-one	0.2	t	RI, MS
109	2045	Humulene epoxide-I	0.2	0.10±0.00	RI, MS
110	2071	Humulene epoxide-II	2.3	1.00±0.20	RI, MS
111	2081	Humulene epoxide-III	0.3	0.20±0.00	RI, MS
112	2098	Globulol	0.3	t	RI, MS
113	2100	Heneicosane	t	–	RI, MS
114	2104	Viridiflorol	0.3	0.40±0.08	RI, MS
115	2144	Rosifoliol	0.1	t	RI, MS
116	2144	Spathulenol	10.6	5.70±0.37	RI, MS
117	2187	T-Cadinol	0.2	0.10±0.00	RI, MS
118	2209	T-Muurolol	0.1	0.10±0.00	RI, MS
119	2214	ar-Turmerol	0.1	t	RI, MS
120	2232	α-Bisabolol	0.5	0.23±0.05	RI, MS
121	2240	*epi*-α-Bisabolol	2.2	1.53±0.12	RI, MS
122	2250	α-Eudesmol	0.1	t	RI, MS
123	2253	*trans*-α-Bergamotol	0.9	0.40±0.00	RI, MS
124	2256	Torilenol	–	t	RI, MS
125	2257	β-Eudesmol	0.1	0.10±0.00	RI, MS
126	2283	Guaia-6,10(14)-dien-4β-ol	–	t	RI, MS
127	2296	Myristicin	1.8	1.00±0.20	RI, MS
128	2300	Tricosane	0.4	–	RI, MS
129	2316	Decanoic acid	0.4	–	RI, MS
130	2332	Khusinol	–	0.10±0.00	RI, MS
131	2378	Eudesma-4(15),7-dien-1β-ol	0.3	0.16±0.05	RI, MS
132	2392	Caryophylla-2(12),6-dien-5β-ol (=*Caryophyllenol II*)	0.2	–	RI, MS
133	2500	Pentacosane	0.3	t	RI, MS
134	2510	Apiole	0.1	0.26±0.05	RI, MS
135	2503	Dodecanoic acid	0.1	–	RI, MS
136	2607	14-Hydroxy-δ-cadinene	t	t	RI, MS
137	2622	Phytol	0.1	t	RI, MS
138	2655	Benzyl benzoate	0.1	t	RI, MS
139	2700	Heptacosane	0.1	t	RI, MS
140	2724	Tetradecanoic acid (=*Myristic acid*)	0.5	–	RI, MS
141	2775	Epoxy-*trans*-pseudoisoeugenyl angelate	–	0.08±0.02	RI, MS
142	2800	Octacosane	–	–	RI, MS
143	2831	Pentadecanoic acid	0.9	–	RI, MS
144	2850	γ-Palmitolactone	–	–	RI, MS
145	2900	Nonacosane	–	t	RI, MS
146	2931	Hexadecanoic acid (=Palmitic acid)	5.6	t	RI, MS
		Total	90.5	93.0	

RRI: Relative retention indices calculated against *n*-alkanes; %: calculated from FID data; t: Trace (<0.1%); RI: Identification based on retention index of genuine compounds on the HP-Innowax column; MS: Identification on the basis of computer matching of the mass spectra from Başer, Adams, MassFinder, Wiley and NIST libraries; MS*: Tentative mass spectrum similarity; HD: hydrodistillation technique; MD: microdistillation technique; σ: standard deviation for three replicates.

bicyclogermacrene, spathulenol, germacrene D and α-pinene were found to be the major constituents of HDO and MDO. Peak identification and relative amounts of the compounds detected in HDO and MDO, appear in Table 3.

Sesquiterpenes (70.9% and 52.4%) comprised the most abundant class of the compounds detected in HDO and MDO of *P. pabularia*. The sesquiterpene hydrocarbons (49.4% and 41.0%) were found as the predominant class with α-humulene (16.6% and 15.5%) and bicyclogermacrene (16.1% and 7.9%) as major constituents. Notably, in MDO the relative amount of bicycloelemene significantly rose (up to 5.8%). Germacrene D (5.7% and 2.9%), γ-curcumene (2.4% and 1.9%) and *ar*-curcumene (2.1% and 1.0%) were the next important sesquiterpenes of HDO and MDO, respectively. However, these sesquiterpenes had not been detected in the oils of *P. pabularia* from Russia and India at all. Caryophyllene and β-selinene were reported from the oil of Russian origin, while β- and γ-elemene, β-caryophyllene

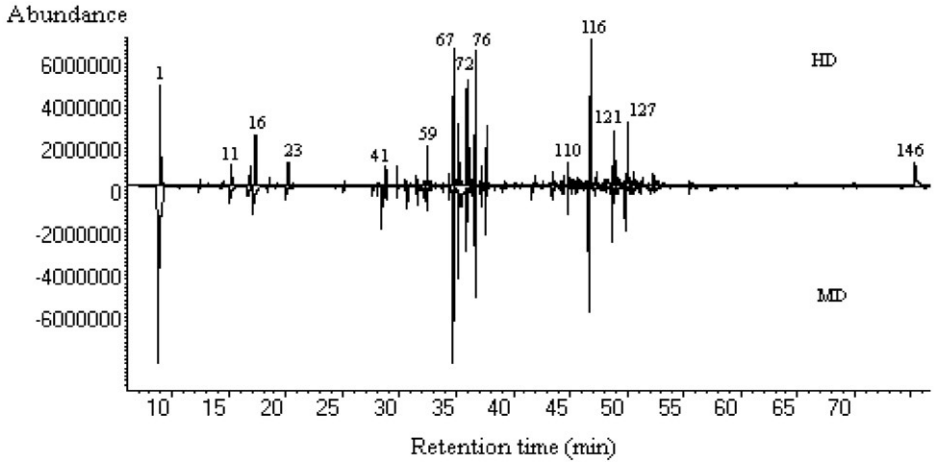

Fig. 1. Typical chromatograms of *P. pabularia* fruit volatiles obtained by hydrodistillation (HD) and microdistillation (MD) techniques. Peak assignments and identification appear in Table 3.

and β-selinene were reported as main sesquiterpenes for the oil of *P. pabularia* of Indian origin (Kuznetsova et al., 1973; Koul and Thakur, 1978).

Oxygenated sesquiterpenes comprised 21.5% of HDO with spathulenol (10.6%), humulene epoxide II (2.3%) and *epi*-α-bisabolol (2.2%) as major constituents. However, in MDO remarkable variation in the total amount of the oxygenated sesquiterpenes (11.4%) was observed. The relative percentages of the main components, spathulenol, humulene epoxide II, and *epi*-α-bisabolol decreased down to 5.7%, 1.0%, and 1.5%, respectively compared to HDO.

In the oil of Indian origin, eudesmol, elemol and α-caryophyllene oxide were mentioned as the major oxygenated sesquiterpenes (Koul and Thakur, 1978). Sesquiterpenes have previously been reported as predominant constituents in the oils of several *Prangos* species: *P. turcica* A. Duran, M. Sagiroglu & H. Duman (fruits) (Özek et al., 2006), *P. heyniae* H. Duman & M.F. Watson (fruits) (Başer et al., 2000b), *P. uloptera* DC. (aerial parts) (Sefidkon and Navaii, 2001), *P. uechtritzii* Boiss. et Hausskn. (fruits) (Başer et al., 2000a), *P. ferulacea* (L.) Lindl. (Başer et al., 1996).

Total percentage of monoterpenes in HDO was 8.3%, while in MDO a remarkable increase (up to 36.0%) was observed. Mainly, monoterpene hydrocarbons (8.3%) with α-pinene (4.2%) and β-phellandrene (1.5%) were the major representatives of this group in HDO. However, in MDO, the relative amount of the mentioned compounds significantly rose (up to 23.9% and 3.9%). α-Phellandrene (2.7%) and limonene (1.4%) were the next major monoterpenes in MDO.

In the oil of *P. pabularia* of Indian origin, common 7 monoterpene hydrocarbons were identified (Koul and Thakur, 1978), while in the oil from Russia, only sabinene, α-phellandrene and α-terpinene were reported as major monoterpenes (Kuznetsova et al., 1973). In the previous reports on *Prangos* species, high percentage of α-pinene was detected in the oils of *P. uloptera* (42%, seeds) (Sefidkon and Navaii, 2001), *P. uechtritzii* (7.9–11.2%, fruits) (Başer et al., 2000a), *P. ferulacea* (10.1–16.7%, fruits) (Başer et al., 1996; Sefidkon et al., 1998).

Oxygenated monoterpenes were present in minor percentages (=0.1% and 0.2%) in both samples. Notably, the Russian oil was also poor in oxygenated monoterpenes (Kuznetsova et al., 1973). However, in the Indian oil, the occurrence of camphor, carvone, citronellyl acetate and geranyl acetate was reported (Koul and Thakur, 1978).

Aliphatic compounds (8.7%) like hexadecanoic acid (5.6%) constituted one of the important compound groups in HDO, while the oil produced with MD technique had significantly lower abundance of this class (2.5%) and trace amount of fatty acid. Probably, it was connected with the diminishing of distillation time (down to 55 min). The composition of volatile fractions depended strongly on the distillation time. The longer this time, the higher the relative amounts of sesquiterpenoids and other less volatile oxygenated compounds could be isolated.

The slight decrease in phenylpropanoids (from 2.0% to 1.3%) corresponding mostly to the variation of myristicin (1.8% and 1.0%) was also detected in HDO and MDO, respectively.

Almost the same number of components at concentrations above 0.1% was found in HDO (47 compounds) and in MDO (45 compounds).

Table 4 contains the relative amounts of different compound families, found in HDO and MDO of *P. pabularia*, and dropped as monoterpene and sesquiterpene hydrocarbons, their oxygenated derivatives, aliphatic compounds and phenylpropanoids. Among

Table 4
Composition according to compound families of the volatiles obtained by hydrodistillation and microdistillation from *P. pabularia* fruits

Compound family	Relative amount, %	
	HDO	MDO
Monoterpene hydrocarbons	8.3	35.8
Oxygenated monoterpenes	t	0.2
Sesquiterpene hydrocarbons	49.4	41.0
Oxygenated sesquiterpenes	21.5	11.4
Aliphatic compounds	8.7	2.5
Phenylpropanoids	2.0	1.3
Others	0.6	0.9

HDO: hydrodistilled oil; MDO: microdistilled oil.

Table 5
Antimicrobial activity of the fruit oil of *P. pabularia* (μg/mL)

Microorganism	Oil	Standard
Escherichia coli	2.5	0.015*
Pseudomonas aeruginosa	2.5	0.062*
Proteus vulgaris	2.5	0.007*
Salmonella typhimurium	5.0	0.007*
Staphylococcus epidermidis	5.0	0.007*
MRSA	1.25	0.062*
Candida albicans	2.5	0.062**

Standard: *Chloramphenicol, **Ketoconazole.

the two techniques employed, HD isolated a larger amount (72.9%) of heavier compounds (sesquiterpenoids, phenylpropanoids), while with MD method only 53.7% of these compounds were extracted. Basically, the same trend was observed in our previous studies on volatiles of *P. turcica* (Özek et al., 2006), *Angelica sylvestris* L. var. *sylvestris* (Özek et al., in press), *Rhabdosciadium oligocarpum* (Post ex Boiss.) Hedge & Lamond and *Rhabdosciadium microcalycinum* Hand.-Mazz. (Özek et al., 2006), where different extraction methods were compared, and it was shown that MD was particularly effective in the isolation of the most volatile metabolites, *i.e.* monoterpene hydrocarbons.

3.2. Antimicrobial activity

The antimicrobial activity of the fruit oil of *P. pabularia* from Turkey has not been previously reported. The oil was evaluated *in vitro* for antimicrobial properties using a microdilution broth technique on two strains of Gram positive bacteria (Methicillin-Resistant *Staphylococcus aureus*, *S. epidermis*), four strains of Gram negative bacteria (*Escherichia coli, Salmonella typhimurium*, *Proteus vulgaris* and *Pseudomonas aeruginosa*), and one strain of fungus (*Candida albicans*). Test results of the antimicrobial activity are given in Table 5.

Minimal Inhibitory Concentration (MIC) values of the fruit oil towards the selected human pathogenic bacteria and the fungus were determined as 5.0–1.25 μg/mL. No significant antimicrobial activity was detected towards the microorganisms tested except for MRSA (1.25 μg/mL).

4. Conclusion

As a result, in the oil of *P. pabularia* from Turkey 146 constituents (total amount for HDO and MDO) were detected indicating a much more complex composition than the oils from India and Russia (18 and 5 constituents detected, respectively) reported earlier. The reason why more components were identified in the Turkish material can be due to the fact that the work on Russian and Indian oils were carried out 30 years ago when GC and GC/MS were in their infancy. In the present study much improved techniques were employed. Comparative study of the main volatiles identified in *P. pabularia* of different origins showed that the oil of *P. pabularia* growing in Turkey had a unique composition with high percentages of α-humulene, bicyclogermacrene, germacrene D, and spathulenol. These compounds have never been reported for this species before. Antimicrobial assessment showed MRSA strains to be sensitive to *P. pabularia* fruit oil.

The comparative analysis of the oils isolated by HD and MD has not shown significant qualitative differences in the oil compositions. However, some quantitative differences were observed due to the different isolation techniques used. The microdistillation technique was found as a useful preparative tool for qualitative and quantitative determination of volatiles on microscale level from the small amount plant material in a short period. This technique offers fast and efficient handling, and good qualitative and quantitative results, making it valuable in comparison to conventional method.

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