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Composition, enantiomeric distribution, and antimicrobial activity of *Tanacetum argenteum* subsp. *flabellifolium* essential oil

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Abstract

Tanacetum argenteum (Lam.) Willd. subsp. *flabellifolium* (Boiss. & Heldr.) Grierson of Asteraceae is an endemic species in Turkey. Hydrodistillation of aerial parts using a Clevenger apparatus yielded an essential oil, which was subsequently analyzed by gas chromatography–mass spectroscopy (GC–MS). α-Pinene (29%), (*E*)-sesquilavandulol (16%), and camphor (14%) were found as main constituents. Enantiomeric distribution of the monoterpenes α-pinene and camphor was determined on a fused silica Lipodex E capillary column using a multidimensional gas chromatography–mass spectroscopy (MDGC–MS) system, (−)-α-pinene (86%), (+)-α-pinene (14%), and (−)-camphor (100%) enantiomeric distributions were found in the oil. Furthermore, antimicrobial activity of the oil was carried out using a micro-dilution assay against human pathogenic bacteria and the yeast *Candida albicans* resulting in moderate inhibitory concentrations (MIC = 125 μg/mL).

Keywords: *Tanacetum argenteum* subsp. *flabellifolium*; Asteraceae; Enantiomeric distribution; GC–MS; MDGC–MS; Antimicrobial activity

1. Introduction

The genus *Tanacetum* L. (Asteraceae) has been used in traditional medicine, with *T. parthenium* (L.) Schultz Bip. (Feverfew) being the most prominent species. Various remedies containing *Tanacetum* species are used in the treatment of arthritis, fever, migraine, menstrual disorders, stomach-ache, toothache, and insect bites [1]. Parthenolide is considered the most important bioactive principle of Feverfew [1–3]. *Tanacetum vulgare* L. (tansy) has also traditionally been used as an antihelmintic, carminative, antispasmodic, diuretic, tonic, and antihypertensive [4,5]. A comprehensive review of the chemotypes of tansy oils were compiled by Lawrence and 23 chemotypes were described [6]. Lawrence reported that commercial tansy oils are mostly of the thujone type [6].

The genus *Tanacetum* is represented in Turkey by 44 species and altogether 59 taxa. *Tanacetum* species are rich in essential oils and sesquiterpene lactones [7]. Sesquiterpene lactones have proven to be valuable chemosystematic markers in the Asteraceae [8]. They are known for their allergenic, antihistaminic, antiinflammatory, antitumor, cytotoxic, antimicrobial, and insecticidal activities, as well as several other biological activities [8–11].

Tanacetum argenteum (Lam.) Willd. is classified into three subspecies: *T. argenteum* (Lam.) Willd. subsp. *argenteum*, *T. argenteum* (Lam.) Willd. subsp. *flabellifolium* (Boiss. & Heldr.) Grierson and *T. argenteum* (Lam.) Willd. subsp. *canum* (C. Koch) Grierson [12]. *T. argenteum* subsp. *canum* is further divided into two varieties: var. *canum* and var. *pumilum* Grierson [12]. Several new and known sesquiterpene lactones were isolated and identified from three subspecies of *T. argenteum* by Goren et al. [13–16]. Among the new sesquiterpene lactones, one new compound, 8α-angeloyloxycostunolide showed cytotoxic activity and antifeedant activity against neonate larvae of *Spodoptera littoralis* [13]. We have previously reported the chemical composition of essential oils of various *Tanacetum* species [7,17]. The main constituents of the investigated *Tanacetum* oils were identified as: caryophyllene oxide (13%) and α-thujone (12%) in *T. argenteum* subsp. *canum* var. *canum* [17]; 1,8-cineole (31% and 11%) and camphor (9% and 27%) in

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leaf and herb oils of *T. armenum*, respectively; *T. balsamita* contained carvone (52%); *T. chiliophyllum* var. *chiliophyllum* and *T. haradjani* contained camphor (17% and 16%), respectively [7].

Essential oils are important natural products used mainly to enhance flavors and fragrances. Terpenes are characteristic compounds in many essential oils and interest in their biological and pharmacological activities has gained momentum in recent years [18]. Their biological activities may be influenced from their stereochemical properties [18,19]. (+)-Limonene and (+)-carvone have demonstrated their effectiveness in the prevention of chemically induced tumors [19]. The pattern of distribution of enantiomers may serve as chemical fingerprints to indicate the authenticity or adulteration of certain essential oils [20]. In other cases, essential oils may be adulterated by the addition of substances to increase yield and profit as in the example of bergamot oil which is a valuable product where adulterations can be detected with enantiomeric purity. Only pure (−)-linalool and (−)-linalyl acetate are major compounds of natural bergamot oils [20]. Enantiomer separation of linalool and linalyl acetate can be use to detect this adulteration. Another important essential oil is balm oil obtained from herb *Melissa officinalis* [20]. (−)-Citronellal (100%) is a key compound in the balm oil. Adulterations of balm oil can be detected through the presence of minor amounts of (+)-citronellal [20]. Enantiomeric separations can be effectively applied in these cases to assess the quality of these products.

To the best of our knowledge, we report for the first time the essential oil composition, antimicrobial activity, and GC/MS fingerprinting for the endemic *T. argenteum* subsp. *flabellifolium* from central Turkey. The enantiomeric distribution of the major monoterpenoids α-pinene and camphor was also determined by multidimensional gas chromatography–mass spectroscopy (MDGC–MS) system using a chiral stationary phase.

2. Materials and methods

2.1. General

Enantiomeric compounds, (+)-α-pinene ≥97%, (−)-α-pinene (99%), (+)-camphor (98%), and (−)-camphor (99%) were purchased from Sigma–Aldrich (St. Louis, MO, USA). Antimicrobial standards chloramphenicol (Sigma–Aldrich, Taufkirchen, Germany) and ketoconazole (Sigma–Aldrich, Taufkirchen, Germany) were included as antimicrobial standards in each assay.

2.2. Plant material

Aerial portions of the plants, consisting of flowers, stems with leaves were collected while in full flower from Konya: Hadim-Beyreli road, 12 km, 1840 m altitude. Voucher specimens were deposited at the Herbarium of the Faculty of Pharmacy, Anadolu University in Eskisehir (ESSE: 12643).

2.3. Isolation of the essential oil

Dried aerial parts were subjected to hydrodistillation for 3 h using a Clevenger-type apparatus [21]. Essential oil was stored at 4 °C in an amber vial and then submitted to GC–MS, MDGC–MS analysis and antimicrobial activity assessment.

2.4. Gas chromatography/mass spectrometric (GC–MS) analysis

GC–MS analysis was performed with a Hewlett-Packard GCD, system (SEM Ltd., Istanbul, Turkey) and an Innowax FSC column (60 m × 0.25 mm, 0.25 μm film thickness) was used with helium as the carrier gas. Oven temperature program was set as follows: 60 °C for 10 min at 4–220 °C held 10 min, at 1 °C/min to 240 °C. Split flow was adjusted at 50 mL/min, the injector temperature was 250 °C, and mass spectra were recorded at 70 eV. Injection volume of the essential oil sample was 1 μL (in 10% *n*-hexane). Mass range was from *m/z* 35 to 425.

2.5. Identification of components

Essential oil components were identified by GC/MS *via* peak matching and by utilizing their retention indices on a Innowax FSC column. *n*-Alkanes (C9–C20) were used as reference points in the calculation of retention indices (RRI) [22–24]. Computer matching against commercial libraries (Wiley and MassFinder Ver. 2.1) [25,26], "Baser Library of Essential Oil Constituents," which was built from genuine compounds and components of known oils [18], and the reported MS literature library data [27–29] were utilized in the final characterization of oil components. Relative percentages of the characterized components are cited in Table 1.

2.6. Multidimensional gas chromatography/mass spectrometric (MDGC–MS) analysis

Two Hewlett-Packard GC 6890 systems with MSD and Gerstel Multi Column Switching (MCS) system were used for chiral separations. The cooled injection system (CIS) was kept at 40 °C for injection. Helium was used as a carrier gas (1 mL/min).

Pre-column: HP-Innowax Fused Silica Capillary Column (60 m × Ø0.25 mm, with 0.25 μm film thickness). GC oven temperature was kept at 60 °C for 10 min and programmed to 220 °C at a rate of 4 °C/min and then kept constant at 220 °C for 10 min, after which it was programmed to increase to 240 °C at a rate of 1 °C/min and kept constant at 240 °C for 40 min. The FID detector temperature was set at 250 °C. *Main column*: Lipodex E [Octakis (3-*O*-butyryl-2,6-di-*O*-pentyl)-γ-cyclodextrin] (70% in OV 1701), (25 m × Ø0.25 mm). The temperature program for the camphor sample was 40 °C for 34 min followed by an increase to 120 °C at a rate of 1 °C/min, the system was then kept constant at 120 °C for 6 min; whereas for α-pinene the temperature was kept at 40 °C for 14 min and then raised at a rate of 1 °C/min to 120 °C, held 26 min. Mass spectra (MS) were recorded at 70 eV, and the mass range was from *m/z* 35 to 425. The enantiomers of α-pinene and camphor purchased from Sigma–Aldrich were used to confirm identities. Chiral compounds were diluted 1:10 (v/v) in *n*-hexane prior to analyses, which utilized an injection volume of 1 μL.

Table 1
Essential oil composition of *Tanacetum argenteum* subsp. *flabellifolium*

Compounds	RRI[a]	(%)[b]	Method of identification
α-Pinene	1032	29.1	GC–MS
Camphene	1076	1.1	GC–MS
β-Pinene	1118	1.3	GC–MS
Sabinene	1132	0.6	GC–MS
α-Terpinene	1188	0.2	GC–MS
Limonene	1203	0.5	GC–MS
Isoamyl alcohol	1213	0.2	GC–MS
γ-Terpinene	1255	0.5	GC–MS
2-Methyl butyrate	1276	0.1	MS
p-Cymene	1280	0.1	GC–MS
Isoamylisovalerate	1285	0.1	GC–MS
Octanal	1296	0.1	GC–MS
6-Methyl-5-hepten-2-one	1348	0.1	MS
trans-Sabinenehydrate	1474	0.4	MS
α-Campholene aldehyde	1500	0.2	MS
Camphor	1532	14.0	GC–MS
Benzaldehyde	1541	0.2	GC–MS
cis-Sabinenehydrate	1556	0.6	MS
1-Methyl-4-acetylcyclohex-1-ene	1568	0.3	MS
trans-*p*-Menth-2-en-1-ol	1571	tr	MS
Pinocarvone	1586	tr	GC–MS
Bornylacetate	1597	tr	GC–MS
Terpinen-4-ol	1611	3.1	GC–MS
β-Caryophyllene	1612	3.1	GC–MS
cis-Verbenol	1663	0.2	GC–MS
trans-Pinocarveol	1664	2.2	GC–MS
p-Mentha-1,5-dien-8-ol	1674	tr	MS
trans-Verbenol	1691	2.0	GC–MS
α-Humulene	1687	0.4	GC–MS
α-Terpineol	1706	0.4	GC–MS
Borneol	1719	0.3	GC–MS
Germacrene-D	1726	1.3	GC–MS
δ-Cadinene	1773	tr	MS
γ-Cadinene	1776	tr	MS
ar-Curcumene	1786	tr	MS
cis-Sabinol	1800	0.1	MS
Myrtenol	1804	0.2	GC–MS
cis-Jasmone	1969	0.4	MS
Isocaryophyllene oxide	2001	0.8	MS
Caryophyllene oxide	2008	2.7	GC–MS
Humulene epoxide-I	2071	0.3	MS
(*E*)-Lavandulyl acetate	2100	4.9	MS
Spathulenol	2144	0.4	GC–MS
(*E*)-Sesquilavandulol	2148	15.9	MS
Thymol	2198	0.5	GC–MS
Carvacrol	2239	1.8	GC–MS
α-Cadinol	2255	0.7	MS
cis-α-*trans*-Bergamotol acetate	2296	1.5	MS
Decanoic acid	2300	1.1	GC–MS
Caryophylla-2(12),6(13)-dien-5α-ol (=caryophylladienol-II)	2324	0.3	MS
Pentacosane	2500	0.3	GC–MS
Heptacosane	2700	0.2	GC–MS
Hexadecanoic acid	2931	0.7	GC–MS
Monoterpene hydrocarbons		33.4	
Oxygenated monoterpenes		31.0	
Sesquiterpene hydrocarbons		4.8	
Oxygenated sesquiterpenes		22.5	
Others		3.9	
Total:		95.7	

tr: trace (<0.1%). GC, identification was based on retention times of genuine compounds on HP Innowax column. MS, were tentatively identified on the basis of computer matching of the mass spectra of peaks with the Wiley and MassFinder libraries [25,26].

[a] Relative retention indices calculated against *n*-alkanes on the HP Innowax column.

[b] Relative percentages were calculated from TIC by computer.

2.7. Antimicrobial bioassay

A micro-dilution broth susceptibility assay was used to evaluate antimicrobial compounds [30,31]. All microorganisms were obtained from the American Type Culture Collection (ATCC) or Northern Regional Research Laboratory (NRRL, currently National Center for Agricultural Utilization Research, US Department of Agriculture, Agricultural Research Service) culture collection or clinical isolates from Osmangazi University, (OGU, Eskisehir, Turkey) and stored at −85 °C. Microorganisms were inoculated on Mueller-Hinton agar (MHA, Merck, Germany) in Petri dishes for purity evaluations prior to use in the bioassay. Stock solutions of the essential oil and the antimicrobial agents were prepared in dimethylsulfoxide (DMSO, Carlo Erba, Italy). Serial dilutions up to 1.94 μg/mL were prepared using sterile distilled water in a 96-well microtiter plate. Microbial suspensions grown overnight in double strength Mueller-Hinton broth (MHB, Merck, Germany) were standardized to 10^8 CFU mL^{-1} (corresponding to McFarland no.: 0.5). One hundred microliters of each microbial suspension was then added to the appropriate well. DMSO was run as solvent control to eliminate or account for solvent effects. The last row of wells contained only the essential oil serial dilutions without microorganism were used as a negative controls as a purity check. After incubation at 37 °C for 24 h the first well without turbidity was determined as the minimal inhibition concentration (MIC, μg/mL). Chloramphenicol and ketoconazole were used as antimicrobial internal standards. MIC means are given in Table 3.

3. Results and discussion

3.1. Chemical analysis

Hydrodistilled essential oil from the aerial parts of *T. argenteum* subsp. *flabellifolium* was analyzed by GC–MS. The compounds identified are given in Table 1 with their relative percentages. The essential oil yield obtained was 0.36% (v/w). Fifty-three compounds representing 95.7% of the oil were characterized, of these 53 α-pinene (29.1%), (*E*)-sesquilavandulol (15.9%), and camphor (14.0%) were the main components. The percentage of monoterpene hydrocarbons and oxygenated monoterpenes are present in about equal amounts in the oil: 33.4% and 31.0%, respectively. The percentage of the oxygenated sesquiterpenes (22.5%) in the oil was higher than the percentage of the sesquiterpene hydrocarbons (4.8%).

In this study, the enantiomeric distributions of α-pinene and camphor in the oil of *T. argenteum* subsp. *flabellifolium* were determined by (MDGC–MS), employing a chiral capillary column coated with γ-cyclodextrin, which was previously reported to resolve monoterpenoids efficiently [7,32,33]. The MDGC–MS system allows the separation of essential oil components on an achiral normal phase column and through heart-cutting techniques, the separated components are subsequently sent to a second chiral column for enantiomeric separation [18]. Mass detector ensures the correct identification of separated compounds [18]. Table 2 shows the enantiomeric distribution and enantiomeric purity of the oil. The enantiomeric excess was calculated using peak area and excess of predominant enantiomer reported as a percentage according to Ruiz Del Castillo et al. [34]. We detected enantiomerically pure (1*S*)(−)-camphor (100%) in the oil, and also a distribution of (−)-α-pinene (86%), and (+)-α-pinene (14%). The enantiomeric excess of α-pinene was observed as 72% in *T. argenteum* subsp. *flabellifolium* oil.

Table 2
Enantiomeric excess (ee) and distribution (ed) of α-pinene and camphor enantiomers identified by MDGC–MS using γ-cyclodextrin column

Compounds	Relative (%)	ee (%)	ed (%)
α-Pinene	29.1		
(−)-α-Pinene		72.0	86.0
(+)-α-Pinene			14.0
Camphor	14.0		
(1*S*)(−)-Camphor		100.0	100.0
(1*R*)(+)-Camphor			–

Camphor is most commonly used externally to relieve arthritic and rheumatic pains. It is often used in steam vaporizers to help control coughs by producing a local anesthetic action to the throat and to loosen congestion due to colds [7,35]. Both enantiomers of camphor are found in nature, but the (−)-form is less common compared to the (+)-form. Although these two enantiomers have a similar camphoraceous odour [35], little is known about their biological activity. According to Ravid et al. [36], enantiomerically pure (−)-camphor (100%) was found in *Tanacetum parthenium*, while *T. vulgare* was rich in (+)-camphor (75%). Enantioselective analysis can easily differentiate these two common *Tanacetum* oils. Enantiomerically pure (−)-camphor (100%) was also detected in *T. armenum* and *T. haradjani* leaf oils [7].

α-Pinene enantiomers can be widely found in nature. α-Pinene is used by the fragrance industry as a starting material in the syntheses of terpineols, borneol, and camphor [35]. (+)-α-Pinene has a slight minty-terpene odor while (−)-α-pinene has a coniferous odor [35]. Yassaa and William recently reported that (+)-α-pinene was the major enantiomer in the *Pinus sylvestris* chemotypes [37].

Shinde et al. reported [18,38] that enantiomers have attracted worldwide attention and that drug regulatory authorities are insisting that enantiomers should be studied separately for their biological activities. Enantiomers in essential oils may exhibit different biological activities. Viljoen et al. [39] demonstrated that 1,8-cineole and (−)-camphor have higher antimicrobial activity against *Candida albicans* in combination than when used independently. Tabanca et al. [31]. reported that (+)-borneol exhibited twice the activity with the MIC value of 125 μg/mL compared to (−)-borneol, but it showed the same level of activity when compared to the chloramphenicol standard against *Pseudomonas aeruginosa*. Both enantiomers displayed the same level of antimicrobial activity as chloramphenicol with a MIC value of 125 μg/mL against *Enterobacter aerogenes*. (−)-Borneol demonstrated twice the antimicrobial activity as (+)-borneol with a MIC value of 125 μg/mL and comparable

Table 3
Antimicrobial activity of *T. argenteum* subsp. *flabellifolium* essential oil (MIC values in μg/mL)

Microorganism	Source	EO	ST
Escherichia coli	ATCC 25922	250	62.5
Staphylococcus aureus	ATCC 6538	125	7.81
Pseudomonas aeruginosa	ATCC 27853	125	250
Enterobacter aerogenes	NRRL 3567	125	125
Proteus vulgaris	NRRL 123	125	31.25
Salmonella typhimurium	NRRL 4420	250	62.5
Candida albicans	O.G.Ü.	125	125[a]

EO: *T. argenteum* subsp. *flabellifolium* essential oil; ST: chloramphenicol.
[a] Ketoconazole.

inhibitory activity against *C. albicans* with ketoconazole [31]. We recently tested both camphor [40] and α-pinene enantiomers (unpublished experimental results) against strawberry anthracnose-causing fungal plant pathogens *Colletotrichum acutatum*, *C. fragariae*, and *C. gloeosporioides* and they showed no significant activity against the three *Colletotrichum* species at 8 μL of 2 mM concentration.

3.2. Antimicrobial assay

Antimicrobial activity of the *T. argenteum* subsp. *flabellifolium* oil was evaluated against human pathogens *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *E. aerogenes*, *Proteus vulgaris*, *Salmonella typhimurium*, and *C. albicans* chloramphenicol and ketoconazole were employed as positive standards. The essential oil showed good growth inhibitory effects against *P. aeruginosa*, *E. aerogenes*, and *C. albicans* with MIC values of 125 μg/mL. *T. argenteum* subsp. *flabellifolium* essential oil appeared as active as the chloramphenicol and ketoconazole standards. The *T. argenteum* subsp. *flabellifolium* oil demonstrated weak to moderate growth inhibition against the pathogenic bacteria *E. coli*, *S. aureus*, *P. vulgaris*, and *S. typhimurium* (Table 3).

Antimicrobial activity of many essential oils has been previously attributed to the monoterpene camphor [41]. α-Pinene showed antibacterial activity with MIC values >900 μg/mL against various food borne bacteria and yeast [42].

4. Conclusions

To the best of our knowledge, this is the first report on the essential oil composition of *T. argenteum* subsp. *flabellifolium* and antimicrobial activity of this species. The enantiomeric distribution of α-pinene and camphor was determined by MDGC–MS using a γ-cyclodextrin (Lipodex E) capillary column. Enantiomeric separation can be effectively used to assess the quality of essential oils, recognize stereochemical constituents, and evaluate phytochemical differences between closely related species. *T. argenteum* subsp. *flabellifolium* oil demonstrated broad spectrum activity against gram negative bacteria, *S. aureus*, and the yeast *C. albicans*.

Antimicrobial activity of *T. argenteum* subsp. *flabellifolium* essential oil may be directly associated with its major constituents or the presence of synergy between the major and minor constituents within the oil. As a result of this study, we believe that it would be worthwhile to test the individual enantiomers for their possible antimicrobial effects and subsequently evaluate active compounds for potential synergistic effects.

References

[1] J.Z. Zhou, X. Kou, D. Stevenson, J. Agric. Food Chem. 47 (1999) 1018–1022.
[2] N.K. Jain, S.K. Kulkarni, J. Ethnopharmacol. 68 (1999) 251–259.
[3] H. Hendriks, R. Bos, H.J. Woerdenbag, Flavour Fragr. J. 11 (1996) 367–371.
[4] J. Rohloff, R. Mordal, S. Dragland, J. Agric. Food Chem. 52 (2004) 1742–1748.
[5] S. Dragland, J. Rohloff, R. Mordal, T.H. Iversen, J. Agric. Food Chem. 53 (2005) 4946–4953.
[6] B.M. Lawrence, Perfum. Flavor. 25 (2000) 34–40.
[7] K.H.C. Baser, B. Demirci, N. Tabanca, T. Ozek, N. Goren, Flavour Fragr. J. 16 (2001) 195–200.
[8] J. Cis, G. Nowak, W. Kisiel, Biochem. Syst. Ecol. 34 (2006) 862–867.
[9] M. Milbrodt, F. Schröder, W.A. Koenig, Phytochemistry 44 (1997) 471–474.
[10] A. Kery, E. Ronyai, B. Simandi, E. Lemberkovics, T. Keve, A. Deak, S. Kemeny, Chromatography 49 (1999) 503–508.
[11] N. Goren, H.J. Woerdenbag, C. Bozok-Johansson, Planta Med. 62 (1996) 419–422.
[12] P.H. Davis, Flora of Turkey and the East Aegean Islands, vol. 5, University Press, Edinburgh, 1982, pp. 256–292.
[13] N. Goren, E. Tahtasakal, J.M. Pezzuto, G.A. Cordell, B. Schwarz, P. Proksch, Phytochemistry 36 (1994) 389–392.
[14] N. Goren, E. Tahtasakal, M. Krawiec, W.H. Watson, Phytochemistry 42 (1996) 757–760.
[15] N. Goren, E. Tahtasakal, Phytochemistry 45 (1997) 107–109.
[16] N. Goren, E. Tahtasakal, M. Krawice, W.H. Watson, J. Nat. Prod. 61 (1998) 560–563.
[17] N. Goren, B. Demirci, K.H.C. Baser, Flavour Fragr. J. 16 (2001) 191–194.
[18] K.H.C. Baser, F. Demirci, Essential oil chemistry, in: R.G. Berger (Ed.), Flavours and Fragrance: Chemistry, Bioprocessing and Sustainability, Springer, Berlin, 2007, pp. 42–86.
[19] M.L. Ruiz Del Castillo, M.M. Caja, M. Herraiz, J. Agric. Food Chem. 51 (2003) 1284–1288.
[20] W.A. Koenig, D.H. Hochmuth, J. Chromatogr. Sci. 42 (2004) 423–439.
[21] European Pharmacopoeia, Council of Europe, 2004, vol. 1, 5th ed., European Pharmacopoeia, Strasbourg, 2005, pp. 217–218.
[22] T. Wang, Y. Sun, J. Chromatogr. 390 (1987) 261–267.
[23] J. Curvers, J. Rijks, C. Cramers, K. Knauss, P. Larson, J. High Res. Chromatogr. 8 (1985) 607–610.
[24] R.P. Adams, Identification of Essential Oils Components by Gas Chromatography/Mass Spectroscopy, Allured Publ., IL, 1995.
[25] F.W. McLafferty, D.B. Stauffer, The Wiley/NBS Registry of Mass Spectral Data, J. Wiley and Sons, New York, 1989.
[26] D. Joulain, W.A. Koenig, D.H. Hochmuth, Terpenoids and Related Constituents of Essential Oils, Library of MassFinder 2.1, Hamburg, 2001.
[27] D. Joulain, W.A. Koenig, The Atlas of Spectra Data of Sesquiterpene Hydrocarbons, E.B.-Verlag, Hamburg, 1998.
[28] E.S.O. 2000, The Complete Database of Essential Oils, Boelens Aroma Chemical Information Service, The Netherlands, 1999.
[29] W.G. Jennings, T. Shibamoto, Quantitative Analysis of Flavor and Fragrance Volatiles by Glass Capillary GC, Academic Press, New York, 1980.
[30] E.W. Koneman, S.D. Allen, W.M. Janda, P.C. Schreckenberger, W.C. Winn, Color Atlas and Textbook of Diagnostic Microbiology, Lippincott-Raven Publ., Philadelphia, 1997, pp. 785–856.

[31] N. Tabanca, N. Kirimer, B. Demirci, F. Demirci, K.H.C. Baser, J. Agric. Food Chem. 49 (2001) 4300–4303.
[32] B. Demirci, N. Tabanca, K.H.C. Baser, Flavour Fragr. J. 17 (2002) 54–58.
[33] M. Kurkcuoglu, B. Demirci, N. Tabanca, T. Ozek, K.H.C. Baser, Flavour Fragr. J. 18 (2003) 192–194.
[34] M.L. Ruiz Del Castillo, G. Flores, M. Herraiz, G.P. Blanch, J. Agric. Food Chem. 51 (2003) 2496–2500.
[35] K. Bauer, D. Garbe, H. Surburg, Common Fragrance and Flavor Materials, VCH, Weinheim, 1997, p. 49, 60.
[36] U. Ravid, E. Putievsky, I. Katzir, Flavour Fragr. J. 8 (1993) 225–228.
[37] N. Yassaa, J. William, J. Chromatogr. A 1141 (2007) 138–144.
[38] P.D. Shinde, V.H. Jadhav, H.B. Borate, S.R. Bhide, K.B. Sonawane, R.D. Wakharkar, J. Chromatogr. A 1138 (2007) 184–189.
[39] A. Viljoen, S. van Vuuren, E. Ernst, M. Klepser, B. Demirci, K.H.C. Baser, B.E. van Wyk, J. Ethnopharmacol. 88 (2003) 137–143.
[40] N. Tabanca, B. Demirci, K.H.C. Baser, Z. Aytac, M. Ekici, S.I. Khan, M.R. Jacob, D.E. Wedge, J. Agric. Food Chem. 54 (2006) 6593–6597.
[41] J.J. Filippi, D.A. Lanfranchi, S. Prado, N. Baldovini, J. Agric. Food Chem. 54 (2006) 6308–6313.
[42] S. Cosentino, C.I.G. Tuberoso, B. Pisano, M. Satta, V. Mascia, E. Arzedi, F. Palmas, Lett. Appl. Microbiol. 29 (1999) 130–135.