Journal of Chromatography A, 1217 (2010) 741–748

Contents lists available at ScienceDirect

Journal of Chromatography A

journal homepage: www.elsevier.com/locate/chroma

Gas chromatographic–mass spectrometric analysis of volatiles obtained by four different techniques from *Salvia rosifolia* Sm., and evaluation for biological activity

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ARTICLE INFO

Article history:
Received 19 June 2009
Received in revised form 9 October 2009
Accepted 27 November 2009
Available online 2 December 2009

Keywords:
Salvia rosifolia
Essential oil
Hydrodistillation
Microwave
Microdistillation
Solid-phase microextraction
α-Pinene
1,8-Cineole
Biological activity

ABSTRACT

Four different isolation techniques, conventional hydrodistillation (HD), microwave-assisted hydrodistillation (MWHD), microdistillation (MD) and micro-steam distillation-solid-phase microextraction (MSD-SPME), have been used to analyze the volatile constituents from the aerial parts of *Salvia rosifolia* Sm. by gas chromatography and gas chromatography coupled to mass spectrometry. HD and MWHD techniques produced quantitatively (yield, 0.39% and 0.40%) and qualitatively (aromatic profile) similar essential oils. α-Pinene (15.7–34.8%), 1,8-cineole (16.6–25.1%), β-pinene (6.7–13.5%), β-caryophyllene (1.4–5.0%) and caryophyllene oxide (1.4–4.4%) were identified as major constituents of this Turkish endemic species. Besides, the hydrodistilled oil of *S. rosifolia* was evaluated for antibacterial, antifungal, anticancer, antioxidant and cytotoxic activities. The hydrodistilled oil of *S. rosifolia* showed antibacterial activity against Methicillin-resistant *Staphylococcus aureus* (MRSA) with a MIC value of 125 μg/mL. Other human pathogenic microorganisms (*Escherichia coli*, *Pseudomonas aeruginosa*, *Enterobacter aerogenes*, *Salmonella typhimurium*, *Staphylococcus epidermidis*, *Candida albicans*) were also inhibited within a moderate range (MIC = 125–1000 μg/mL). Antifungal activity of the oil was also observed against the strawberry anthracnose-causing fungal plant pathogens *Colletotrichum acutatum*, *C. fragariae* and *C. gloeosporioides*. No cytotoxicity was observed for *S. rosifolia* oil up to 25 mg/mL against malignant melanoma, epidermal, ductal and ovary carcinoma.

1. Introduction

The genus *Salvia* L. (Lamiaceae) encompasses over 900 species worldwide [1,2]. In Turkey *Salvia* is represented by 90 species, containing distinct 95 taxa, 47 of which are endemic. Since the last revision of the genus, four new species have been described from Turkey: *Salvia nydeggeri* Hub.-Mor. [3], *Salvia aytachii* Vural & Adiguzel [4], *Salvia hedgeana* Donmez [5], and *Salvia anatolica* Hamzaoglu & A. Duran [6].

Salvia is commonly known as "sage" in the world markets and represents one of the most diversified genera in Turkey with 52% endemic. Chemical diversity among *Salvia* taxa in Turkey has been reported by Başer [7]. Several *Salvia* species are known locally as "adacayi" where they grow in southern and western Turkey and are consumed as hot teas due to their unique flavor, pleasant aroma, and medicinal properties as well as are sources of essential oil important in cosmetics, perfumes, and medicine industries [8–11]. Antispasmodic, antibacterial, antifungal and antioxidant activities are reported for many *Salvia* species [12–15]. Several Turkish *Salvia* species have been reported earlier for the chemical constituents of their oils [16–21]. In terms of *S. rosifolia* there was published just one paper about its morphological and autecological properties [22].

Salvia plants and their essential oils are of economical importance worldwide in food, pharmacy, perfumery, and cosmetics. So, investigation for applicability of different techniques for the isolation of *Salvia* oils with high yield and quality is very desirable. The most common method for essential oil isolation is by hydrodistillation as described in various pharmacopoeias such as *European Pharmacopoeia* 2005 [23]. However, this technique has been controversial for subsequent determination of the oil chemical composition because of the possible transformation of

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doi:10.1016/j.chroma.2009.11.086

aroma-active compounds by heat, steam, and pH [24]. Conventional methods have several drawbacks because of large amounts of plant material, labor intensive, increased potential loss of volatile constituents, long extraction times, and high energy expense. Thus, developing an alternative rapid, sensitive, safe, and energy conserving extraction technique is highly desirable. There is an increased demand for new extraction techniques, amenable to automation, with shortened extraction times and reduced organic solvent consumption, preventing pollution and reducing sample preparation costs. Microwave-assisted procedures for essential oil isolation have become attractive for applications in laboratory as well as in industry. Also, microscale techniques which need a small amount of plant sample and give the same result for a short extraction time with low energy consumption are advantageous for isolation of volatiles.

Microwave-assisted extraction was extensively studied for application on a wide range of materials for medicine, food, and the environment [25]. Production of volatiles from plant materials exposed to microwave energy in an air stream was discussed by Craveiro et al. [26]. Pare patented a general extraction method for biological matter using microwave energy [25]. The advantages of using microwave energy for the oil extraction are more effective heating, fast energy transfer, reduced thermal gradients, selective heating, reduced equipment size, faster response to process heating control, faster start-up, increased production and elimination of process steps. Up to now, however, there are only a few articles in literature that have reported the acceleration of essential oil extraction by microwave irradiation [27–30]. So, investigation for application of microwave-assisted distillation of commercially important plant materials is desirable.

Micro-steam distillation-solid-phase microextraction (MSD-SPME) is a new sampling and concentration technique developed and introduced for the extraction of the volatiles from aromatic plant material [31]. MSD-SPME involved concurrent solid-phase microextraction combined with continuous hydrodistillation of the oil. This method offered important advantages in time and energy saving for the isolation of the volatiles. MSD-SPME combined with GC/FID and GC/MS has been proven to be simple, sensitive, rapid, solvent-less and non-toxic technique for volatile constituents analysis at the microscale level.

Microdistillation (MD) is another microscale capillary technique used for qualitative and quantitative determination of volatiles from small amounts of plant material for subsequent GC/FID and GC/MS analysis. This capillary technique was successfully proposed for essential oils' analysis [32]. The distillation product can be used for GC/FID and GC/MS analysis without further preparation [33].

The aim of the present study was to investigate an applicability of MWHD, MD and MSD-SPME techniques as an alternative to conventional HD for isolation of *S. rosifolia* volatiles. The applicability was appreciated by using the results of subsequent GC/FID and GC/MS analysis. Also, within scope of the present work, the hydrodistilled oil of *S. rosifolia* was further evaluated for antimicrobial, antifungal, anticandidal, anticancer, and cytotoxic properties. To the best of our knowledge, the present work is the first report about the composition and biological activity of *S. rosifolia* essential oil.

2. Experimental

2.1. Plant material

Aerial parts of *S. rosifolia* were collected in June 2004 at an altitude of 1650 m in Turkey between Bayburt and Maden provinces, at western base of Kuz Mountain. Identification of the plant material was performed by A. Duran and voucher specimens (A. Duran 6626 & Hamzaoglu) were deposited at the Herbarium of the Faculty of Education, Department of Biology of Selcuk University in Konya, Turkey.

2.2. Chemicals

Anhydrous sodium sulfate (ACS-ISO, for analysis), *n*-hexane (ACS, for analysis) and dimethyl sulfoxide (DMSO) were purchased from Carlo Erba (Italy). Sodium chloride (extra pure) was obtained from Merck (Darmstadt, Germany). Technical grade commercial fungicides benomyl, cyprodinil, azoxystrobin, and captan (Chem Service, Inc., West Chester, PA) were used as fungicide standards at 2 mM in 2 μL of 95% ethanol. For antifungal assay, potato-dextrose broth (PDB, Difco, Detroit, MI), glass silica gel thin layer chromatography (TLC) plates with a fluorescent indicator (250 mm, Silica Gel GF Uniplate, Analtech, Inc., Newark, DE) and a moisture chamber (398-C, Pioneer Plastics, Inc., Dixon, KY) were used.

SPME fiber coated with PDMS-DVB (polydimethylsiloxane-divinylbenzene) 65 μm "blue type" was provided from Supelco (Supelco Park, Bellefonte, PA, USA). Before use, the fiber was reconditioned in accordance to manufacture recommendations.

2.3. Hydrodistillation (HD)

Distillation was performed according to the method described in the *European Pharmacopoeia* [23]. Air-dried aerial parts of *S. rosifolia* (50.0 g) were ground and then hydrodistilled for 3 h using a Clevenger-type apparatus. The oil yield was calculated on dry weight basis, dried over anhydrous sodium sulfate, and stored in sealed vials in the dark, at 4 °C. The oil was subsequently subjected to GC/FID and GC/MS analyses and biological activity screening. The oil was dissolved in *n*-hexane (10%, v/v) before the chromatographic determination of its composition.

2.4. Microwave-assisted hydrodistillation (MWHD)

In MWHD procedure, dried and ground aerial parts of *S. rosifolia* (50 g) were subjected to hydrodistillation for 45 min using a Clevenger-type apparatus placed in a modified microwave oven (Milestone ETHOS E Microwave Labstation, Sorisole (BG), Italy). Time, temperature, pressure and intensity (watts) were monitored during distillation and controlled with the "easy-CONTROL" software package of the system (Sorisole (BG), Italy). Microwave intensity applied to the plant material was controlled by a shielded thermocouple inserted directly into the flask [34]. The oven was operated according to stepwise microwave-assisted hydrodistillation programme (Table 1).

2.5. Micro-steam distillation-solid-phase microextraction (MSD-SPME)

The dried and ground plant material (1.0 g) was placed in 25 mL round bottom flask used as refluxing vessel along with 10 mL of water. The flask was fitted with a Claisen distillation head with plug and a condenser set up for refluxing rather than distillation. Heating was achieved using electric heater, and threaded plug was used for SPME fiber assembly. A manual SPME holder (57330-U, SUPELCO, Bellefonte, PA) and the PDMS-DVB 65 μm fiber "blue type" were used for SPME procedure of volatiles. Fiber was conditioned at 250 °C for 30 min before the experiment. After the SPME needle pierced the plug, the fiber was expressed through the needle and exposed to the headspace above a plant sample. MSD-SPME procedure was carried out at the boiling temperature of water used as solvent. The time of equilibrium was period between loading of SPME fiber into flask and starting of the extraction. The period 1.0 min was used as suitable time for equilibrium. The extraction

Table 1
Stepwise programmes for microwave-assisted hydrodistillation and microdistillation procedures.

Method	Parameter	Step 1	Step 2	Step 3	Step 4
Microwave-assisted hydrodistillation (MWHD)	Microwave power (Watts)	1000	700	500	–
	Final temperature (°C)	95	100	100	Ventilation
	Time (min)	5	5	30	–
	Post-run (min)	–	–	–	5
Microdistillation (MD)	Heating rate (°C/min)	20		20	
	Final temperature (°C)	100	100	112	112
	Time (min)	5	15	<1	35
	Cooling temperature (°C)	−1	−1	−1	−1
	Post-run (min)	–	–	–	2

times as 3 min, 1 min and 0.5 min were tested to ensure the complete composition of the volatiles in comparison with conventional HD. The minimum extraction time giving the most suitable results was estimated as 0.5 min. So, the fiber exposure time was 0.5 min after start of boiling. After the trapping of volatile, the loaded SPME fiber was withdrawn into the needle, and then the needle was removed from the plug and subsequently used for GC/FID and GC/MS analyses. Desorption of the analytes from the fiber coating was performed by heating the fiber in the injection port to 250 °C for 5 min. The analytes were then transferred directly into the chromatographic column for analysis. Afterwards, the SPME fiber was reconditioned at 250 °C for the next extraction experiment for 30 min. The fiber was subjected to a blank injection to ensure fiber integrity and the absence of any analytes after each reconditioning period.

2.6. Microdistillation (MD)

MicroDistiller device (Eppendorf-Netheler-Hinz, Hamburg, Germany) was used for isolation of the volatile metabolites from 0.5 g of the plant. The dried ground plant material was placed in a sample vial together with 10 mL of water. 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. Sodium chloride (2.0 g), water (1.0 mL) and *n*-hexane (0.3 mL) were placed in the collecting vial to trap volatile compounds. The microdistiller was operated according to stepwise heating programme (Table 1). After completing the distillation, the organic layer in the collection vial was separated from the water phase and injected directly into GC/FID and GC/MS.

2.7. Gas chromatography–mass spectrometry (GC/MS)

The GC/MS analysis was carried out with an Agilent 5975 GC-MSD system. HP-Innowax FSC column (60 m × 0.25 mm, 0.25 μm film thickness, Agilent, USA) was used with a helium carrier gas at 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, kept constant for 10 min at 220 °C, and then programmed to increase at a rate of 1 °C/min to 240 °C. The oils isolated by HD and MWHD were analyzed with a split ratio of 40:1. Volatiles obtained by MD and MSD-SPME techniques were analyzed in the splitless mode. For MSD-SPME/GC the fiber was desorbed as described above. The injector temperature was 250 °C. Mass spectrums were taken at 70 eV and the mass range was from *m*/*z* 35 to 450.

2.8. Gas chromatography (GC)

The volatiles were analyzed by capillary GC using an Agilent 6890N GC system (SEM Ltd., Istanbul, Turkey). Flame ionization detector (FID) temperature was set at 300 °C in order to obtain the same elution order with GC/MS. Simultaneous injection was performed using the same column and appropriate operational conditions, and in the MSD-SPME/GC/FID analysis the fiber was desorbed as described above.

2.9. Identification and quantification of compounds

Identification of the volatile constituents was achieved by parallel comparison of their retention indices and mass spectra with data stored in the Wiley GC/MS Library (Wiley, New York, NY, USA), MassFinder software 3.0 (Dr. Hochmuth Scientific Consulting, Hamburg), Adams Library, NIST Library and the in-house "Başer Library of Essential Oil Constituents". *n*-Alkanes (C9–C20) were used as reference points in calculating retention indices (RI).

All the experiments were performed simultaneously three times under the same conditions for each isolation technique. Percent composition was obtained for each constituent on the basis of GC/FID analyses of the all oils were used for calculation of mean values and standard deviation values. The results were evaluated statistically and presented as percentage means ± standard deviation.

2.10. Biological activity of the oil

2.10.1. Microorganisms

Microorganisms were obtained from American Type Culture Collection or the U.S. Department of Agriculture, Agricultural Research Service Culture Collection (NRRL) or from clinical isolates obtained by the Faculty of Medicine, Eskisehir Osmangazi University, Turkey and were stored until use at −86 °C in Eppendorf micro-test tubes containing 10% glycerol. Pathogen preparation prior to each experiments was conducted so that *Candida albicans* was freshly inoculated on Sabouraud Dextrose Agar (SDA), whereas *Escherichia coli*, *Pseudomonas aeruginosa*, *Enterobacter aerogenes*, *Salmonella typhimurium*, *Staphylococcus epidermidis*, *Staphylococcus aureus* were inoculated on Mueller Hinton Agar (MHA) at 37 °C, as previously described [35].

2.10.2. Antimicrobial assay

Use of essential oils as functional ingredients in foods, drinks, toiletries and cosmetics is gaining momentum, both for the growing interest of consumers in ingredients from alternative natural sources and also because of increasing concern about potentially harmful synthetic additives [36,37]. Most essential oils and their components are generally regarded as safe (GRAS), and their wide acceptance by consumers and their exploitation for potential multipurpose functional use is expanding [38,39]. In fact many authors have earlier reported about antimicrobial properties of a number *Salvia* oils [18,40,41]. Generally, their overall action is a result of the combined effect of both their active and inactive compounds [42].

Antibacterial and anticandidal activities of the essential oil (HD) were evaluated using the broth micro-dilution methods [35]. The oil, pure compounds (α-pinene, 1,8-cineole) and antibiotic stan-

dards were dissolved in 20% DMSO solution to obtain a stock solution of 2000 μg/mL. Dilution series for each test compound and standard was prepared in sterilized distilled water as 1.95 μg/mL in Eppendorf micro-test tubes (2 mL) and 100 μL aliquots were then transferred to 96-well microtiter plates. The last column of wells in the 96-well microtiter plate filled with distilled water served as a positive growth control. Microorganism suspensions were allowed to grow overnight in liquid medium and subsequently diluted again in double strength liquid medium and standardized to McFarland No.: 0.5 (1×10^8 colony forming units/mL). Aliquots (100 μL) of the appropriate microorganism suspension were then added to their respective wells in the 96-well plate and incubated at 37 °C for 24 h (dark conditions). Chloramphenicol and ampicillin were standards for bacteria whereas ketoconazole was used as standard for *C. albicans*. After incubation, the first well in the dilution sequence for each compound without turbidity was determined as the minimal inhibitory concentration (MIC, μg/mL) for that compound or standard. Average results of separately performed three experiments are given in Table 3.

2.10.3. Antifungal activity test against plant pathogens

Conidia of *Colletotrichum fragariae*, *C. acutatum* and *C. gloeosporioides* suspensions were adjusted to 3.0× 105 conidia/mL with liquid potato-dextrose broth (PDB) and 0.1% Tween-80. Using a 50 mL chromatographic sprayer, each glass silica gel thin layer chromatography (TLC) plates with a fluorescent indicator was sprayed lightly (to a dampness) three times with the conidial suspension. Inoculated plates were placed in a 30 cm × 13 cm × 7.5 cm moisture chamber and incubated in a growth chamber at 24 ± 1 °C and 12-h photoperiod under 60 ± 5 mmols m^{-2} s^{-1} light. Inhibition of fungal growth was measured 4 days after treatment. Bioautography experiments were performed multiple times using both dose– and non-dose–response formats. Technical grade commercial fungicides benomyl, cyprodinil, azoxystrobin, and captan (Chem Service, Inc., West Chester, PA) were used as fungicide standards at 2 mM in 2 μL of 95% ethanol. *Salvia rosifolia* essential oil was applied as 20 mg/mL in 8 μL of *n*-hexane on to TLC plates. Mean inhibitory zone size and standard deviations were used to evaluate antifungal activity of essential oils (Table 4).

2.10.4. Cytotoxicity assay

Essential oil of *S. rosifolia* was also tested for its *in vitro* cytotoxicity against a panel of four human cancer cell lines (SK-MEL: malignant melanoma; KB: epidermal carcinoma, oral; BT-549: ductal carcinoma, breast and SK-OV3: ovary carcinoma) as well as noncancerous VERO cells (monkey kidney fibroblast). The assay was performed in 96-well tissue culture-treated microplates. Cells (25,000 cells/well) were seeded to the wells of the plate and incubated for 24 h. Samples were added and again incubated for 48 h. Cell viability was determined using the supravital dye neutral red according to a modification of the previous procedure [43]. Briefly, the cells were washed with saline followed by incubation for 1.5 h with a solution of neutral red. The cells were washed to remove extracellular dye. A solution of acidified isopropanol was added to liberate the incorporated dye from viable cells and the absorbance was read at 540 nm.

2.10.5. Antioxidant activity

Myelomonocytic HL-60 cells (ATCC) were grown in RPMI 1640 medium supplemented with 10% fetal bovine serum (Hyclone) and 60 μg/mL amikacin at 37 °C in an environment of 95% humidity and 5% CO_2. For the assay, 125 μL of the cell suspension (1×10^6 cells/mL) was added to the wells of a 96-well plate. After treating with different concentrations of the test samples for 30 min, the cells were stimulated with 100 ng/mL phorbol 12-myristate 13-acetate (PMA, Sigma) for 30 min. DCFH-DA (Molecular Probe, 5 μg/mL) was added and the cells were incubated for 15 min. The levels of DCF produced were measured on a Spectramax plate reader with an excitation wavelength of 485 nm and an emission of 530 nm. The ability of the test materials to inhibit exogenous cytoplasmic ROS-catalyzed oxidation of DCFH to fluorescent DCF in HL-60 cells was measured in comparison to the controls without the test material. The IC50 values were calculated from dose curves of the % DCF production versus test concentrations. Vitamin C (Sigma) was included as a positive control [44].

3. Results and discussion

3.1. Composition of the volatiles

This is the first report on the oil composition of *S. rosifolia*, endemic to Turkey. GC/FID and GC/MS analysis performed simultaneously resulted, that HD, MWHD, MD and MSD-SPME concluded with isolation of the volatiles from *S. rosifolia* with similar composition, although the percentages of some components depended on the technique applied. The list of detected compounds with their relative percentages, retention indices [45–58] and percentages of compound classes are given in Table 2 in order of their elution on the HP-Innowax FSC column.

Hydrodistillation of the dried aerial parts of *S. rosifolia* performed for 180 min gave the light yellowish oil with a pleasant smell in 0.4% yield. Seventy compounds were characterized, representing 93.73 ± 1.59% of the oil obtained by HD.

MWHD technique allowed the isolation of the oil in 0.39% yield for 45 min. In this technique, microwave heating was originally combined with hydrodistillation at atmospheric pressure. In this application, microwave irradiation highly accelerated the extraction process, but without causing considerable changes in the volatile oil composition, phenomenon which was already described by Pare and Belanger [59]. In the present report, the potential of MWHD technique was compared with a conventional HD method used as the reference. As can be seen, in Table 2 and Fig. 1 the extraction time of 45 min with MWHD provided oil composition comparable to that obtained after 180 min by means of conventional HD (reference method). Nearly the same number (70 and 68) of constituents was detected in both of the oils.

It is interesting to note that distillation time of 45 min with MWHD provided oil yield comparable to that obtained after 180 min by means of HD (0.39% and 0.40%, respectively). These results indicated a substantial saving of time and energy in the extraction procedure.

MD procedure performed with MicroDistiller device enabled the distillation of the volatiles from very small quantities of aromatic material (0.5 g) about 55 min. Profile of the volatiles is presented in Fig. 1. Among the four techniques employed, HD yielded a higher amount (20.8%) of heavier compounds (i.e. sesquiterpenes), while with MD method only 9.4% of these compounds were isolated (Table 2). At the same time, the relative percentage of the most volatile compounds (i.e. monoterpene hydrocarbons) was very high (87%). MD was shown to be particularly effective in the isolation of the most volatile metabolites and is an excellent technique that compliments existing methodology in the investigation of volatile compounds from a small amount of plant material.

This technique provided rapid attainment (0.5 min) of volatiles with the same composition as that obtained by hydrodistillation (in Clevenger apparatus) from 1.0 g of plant material (Fig. 1). MSD-SPME was therefore well suitable for the extraction of aroma-active compounds from minute amounts of aromatic plants. Furthermore, the isolated product can be directly used for GC and GC/MS analysis without further preparation. MSD-SPME was only useful for the analytical determination of the volatiles and not for the preparation of essential oils.

Table 2
The composition of *Salvia rosifolia* volatiles obtained by hydrodistillation, microwave-assisted hydrodistillation, microdistillation and micro-steam distillation-solid-phase microextraction techniques.

No.	RRI[a]	RRI[b]	Compound	Composition (%)[c]				ID method
				HD	MWHD	MSD-SPME	MD	
1	966		2-Ethyl furan	t	–	–	–	d, e
2	1014	1014 [45]	Tricyclene	0.2 ± 0.05	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	d, e
3	1032	1032 [46]	α-Pinene	24.3 ± 1.7	21.4 ± 0.2	15.7 ± 0.7	34.8 ± 0.3	d, e
4	1035	1035 [46]	α-Thujene	0.5 ± 0.1	0.7 ± 0.1	0.8 ± 0.1	1.3 ± 0.1	d, e
5	1076	1085 [46]	Camphene	3.1 ± 0.4	2.5 ± 0.0	1.5 ± 0.1	2.7 ± 0.0	d, e
6	1118	1118 [46]	β-Pinene	6.9 ± 0.9	8.8 ± 0.1	6.8 ± 1.1	13.6 ± 0.00	d, e
7	1132	1132 [46]	Sabinene	1.5 ± 0.3	2.1 ± 0.0	1.5 ± 0.3	2.0 ± 0.0	d, e
8	1136	1117 [47]	Thuja-2,4(10)-diene	0.1 ± 0.0	t	t	0.1 ± 0.0	d, e
9	1174	1156 [46]	Myrcene	1.1 ± 0.0	1.3 ± 0.0	1.6 ± 0.1	1.1 ± 0.0	d, e
10	1183	1183	*p*-Mentha-1,7(8)-diene (=*Pseudolimonene*)	t	t	t	t	d, e
11	1188	1179 [46]	α-Terpinene	t	0.1 ± 0.0	0.1 ± 0.0	0.2 ± 0.1	d, e
12	1203	1205 [46]	Limonene	2.7 ± 0.6	3.9 ± 0.1	4.5 ± 0.7	2.9 ± 0.0	d, e
13	1213	1210 [45]	1,8-Cineole	16.6 ± 2.0	19.2 ± 0.0	25.1 ± 0.6	18.4 ± 0.1	d, e
14	1225		(*Z*)-3-Hexenal	t	0.1 ± 0.0	0.8 ± 0.2	0.4 ± 0.0	d, e
15	1244	1237 [48]	2-Pentyl furan	t	t	-	-	d, e
16	1246	1230 [46]	(*Z*)-β-Ocimene	t	t	t	t	d, e
17	1255	1256 [46]	γ-Terpinene	2.3 ± 0.3	2.6 ± 0.0	3.3 ± 0.3	1.7 ± 0.0	d, e
18	1266	1252 [49]	(*E*)-β-Ocimene	t	0.1 ± 0.0	0.2 ± 0.0	0.1 ± 0.0	d, e
19	1269		5-Methyl-3-heptanone	t	0.1 ± 0.0	0.2 ± 0.0	0.1 ± 0.0	d, e
20	1280	1279 [46]	*p*-Cymene	6.7 ± 0.2	3.1 ± 0.1	4.3 ± 0.4	1.8 ± 0.0	d, e
21	1290	1283 [46]	Terpinolene	t	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	d, e
22	1304	1305 [50]	1-Octen-3-one	t	t	t	0.1 ± 0.0	d, e
23	1391	1393 [51]	(*Z*)-3-Hexenol	t	-	0.1 ± 0.0	0.1 ± 0.0	d, e
24	1393	1368 [52]	3-Octanol	0.1 ± 0.0	0.1 ± 0.0	0.2 ± 0.0	0.1 ± 0.0	d, e
25	1439		γ-Campholene aldehyde	t	t	t	0.1 ± 0.0	d, e
26	1452	1459 [46]	α,*p*-Dimethylstyrene	0.2 ± 0.0	0.2 ± 0.0	0.2 ± 0.0	0.1 ± 0.0	d, e
27	1453	1445 [53]	1-Octen-3-ol	0.2 ± 0.0	0.1 ± 0.0	0.9 ± 0.1	0.3 ± 0.0	d, e
28	1466	1458 [46]	α-Cubebene	0.3 ± 0.0	0.4 ± 0.0	1.0 ± 0.0	0.2 ± 0.0	d, e
29	1474	1474 [54]	*trans*-Sabinene hydrate	0.1 ± 0.0	0.4 ± 0.0	0.1 ± 0.0	0.2 ± 0.0	d, e
30	1477		4,8-Epoxyterpinolene	t	t	0.1 ± 0.0	t	d, e
31	1497	1497 [49]	α-Copaene	0.9 ± 0.1	0.5 ± 0.0	t	0.1 ± 0.0	d, e
32	1528		α-Bourbonene	t	t	t	t	d, e
33	1532	1532 [46]	Camphor	3.9 ± 0.6	4.7 ± 0.2	6.5 ± 0.1	3.0 ± 0.0	d, e
34	1535	1535 [41]	β-Bourbonene	0.6 ± 0.1	t	1.6 ± 0.2	0.4 ± 0.0	d, e
35	1542		4(15),5-Muuroladiene	t	t	1.4 ± 0.4	0.3 ± 0.0	f
36	1553	1556 [46]	Linalool	0.2 ± 0.0	0.2 ± 0.0	0.3 ± 0.0	0.2 ± 0.0	d, e
37	1556	1556 [41]	*cis*-Sabinene hydrate	0.1 ± 0.0	0.1 ± 0.0	0.2 ± 0.0	0.1 ± 0.0	d, e
38	1562	1565 [55]	Octanol	0.2 ± 0.0	0.8 ± 0.0	0.8 ± 0.0	0.2 ± 0.0	d, e
39	1586	1585 [56]	Pinocarvone	0.9 ± 0.5	0.2 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	d, e
40	1589		Aristolene	0.3 ± 0.1	0.1 ± 0.0	0.2 ± 0.0	0.1 ± 0.0	d, e
41	1590	1571 [46]	Bornyl acetate	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	d, e
42	1600	1594 [49]	β-Elemene	t	t	t	t	d, e
43	1610		Calarene (=β-*Gurjunene*)	0.8 ± 0.3	0.5 ± 0.0	t	0.3 ± 0.0	d, e
44	1611	1616 [46]	Terpinen-4-ol	t	0.1 ± 0.0	1.7 ± 0.2	0.4 ± 0.1	d, e
45	1612	1604 [49]	β-Caryophyllene	5.1 ± 0.1	2.1 ± 0.0	4.9 ± 0.2	1.4 ± 0.0	d, e
46	1628	1653 [46]	Aromadendrene	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	t	d, e
47	1648	1645 [55]	Myrtenal	t	01 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	d, e
48	1668	1632 [47]	(*Z*)-β-Farnesene	0.9 ± 0.4	0.5 ± 0.0	0.4 ± 0.2	0.4 ± 0.0	d, e
49	1683	1680 [56]	*trans*-Verbenol	0.1 ± 0.1	t	0.4 ± 0.1	0.2 ± 0.0	d, e
50	1687	1687 [54]	α-Humulene	1.3 ± 0.4	0.7 ± 0.0	0.9 ± 0.2	0.7 ± 0.1	d, e
51	1704	1695 [49]	γ-Muurolene	0.1 ± 0.0	t	0.1 ± 0.0	t	d, e
52	1706	1706 [41]	α-Terpineol	t	0.2 ± 0.0	0.2 ± 0.0	0.2 ± 0.0	d, e
53	1719	1705 [46]	Borneol	1.8 ± 0.0	2.9 ± 0.1	2.4 ± 0.2	1.4 ± 0.0	d, e
54	1722		Verbenone	t	t	0.1 ± 0.0	0.2 ± 0.0	d, e
55	1726	1716 [49]	Germacrene D	1.2 ± 0.5	0.9 ± 0.0	1.3 ± 0.3	0.7 ± 0.0	d, e
56	1737	1728 [49]	(*Z*,*E*)-α-Farnesene	0.1 ± 0.1	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	d, e
57	1755	1742 [49]	Bicyclogermacrene	0.1 ± 0.0	0.2 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	d, e
58	1758	1749 [49]	(*E*,*E*)-α-Farnesene	0.2 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	t	d, e
59	1773	1764 [49]	δ-Cadinene	0.2 ± 0.1	0.2 ± 0.0	0.4 ± 0.0	0.2 ± 0.0	d, e
60	1776	1766 [49]	γ-Cadinene	0.1 ± 0.0	0.1 ± 0.0	0.2 ± 0.0	0.1 ± 0.0	d, e
61	1786	1781 [56]	*ar*-Curcumene	0.1 ± 0.0	t	0.1 ± 0.0	0.1 ± 0.0	d, e
62	1853	1827 [57]	*cis*-Calamenene	t	0.1 ± 0.0	0.3 ± 0.0	0.4 ± 0.0	d, e
63	1900		*epi*-Cubebol	t	t	0.1 ± 0.1	0.1 ± 0.0	d, e
64	2008	2008 [54]	Caryophyllene oxide	4.3 ± 0.2	4.5 ± 0.12	1.4 ± 0.1	1.5 ± 0.1	d, e
65	2037	2016 [56]	Salvial-4(14)-en-1-one	0.2 ± 0.0	0.2 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	d, e
66	2071	2071 [54]	Humulene epoxide-II	0.5 ± 0.1	0.5 ± 0.0	0.1 ± 0.0	0.5 ± 0.0	d, e
67	2144	2150 [41]	Spathulenol	1.1 ± 0.1	2.3 ± 0.1	0.2 ± 0.1	0.7 ± 0.0	d, e
68	2324	2324 [58]	Caryophylla-2(12),6(13)-dien-5α-ol (=*Caryophylladienol II*)	0.3 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.4 ± 0.0	d, e
69	2366	2396 [51]	Eudesma-4(15),7-dien-1β-ol	1.7 ± 0.6	0.3 ± 0.0	t	0.2 ± 0.0	d, e
70	2392	2392 [58]	Caryophylla-2(12),6-dien-5β-ol (=*Caryophyllenol II*)	0.2 ± 0.1	0.8 ± 0.0	t	0.30 ± 0.0	d, e
Total				93.7 ± 1.6	91.8 ± 0.5	95.7 ± 0.1	97.6 ± 0.3	

Table 2 (*Continued*)

No.	RRI[a]	RRI[b]	Compound	Composition (%)[c]				ID method
				HD	MWHD	MSD-SPME	MD	
Monoterpene hydrocarbons				48	46.5	40.5	63	
Oxygenated monoterpenes				24	28.5	37.5	24	
Sesquiterpene hydrocarbons				12.4	6.2	12	5.6	
Oxygenated sesquiterpenes				8.4	9	1.5	3.8	

RRI[a]: Relative retention indices calculated against *n*-alkanes (C9–C20) on HP-Innowax column; RRI[b]: Relative retention indices on polar column reported in literature; %[c]: Calculated from flame ionization detector data; t: trace (<0.1%); d: identification based on retention index of genuine compounds on the HP-Innowax column; e: identification on the basis of computer matching of the mass spectra from Başer, Adams, MassFinder, Wiley and NIST libraries; f: correct isomer not identified; HD: hydrodistillation; MWHD: microwave-assisted hydrodistillation; MD: microdistillation; MSD-SPME: microsteam distillation-solid-phase microextraction.

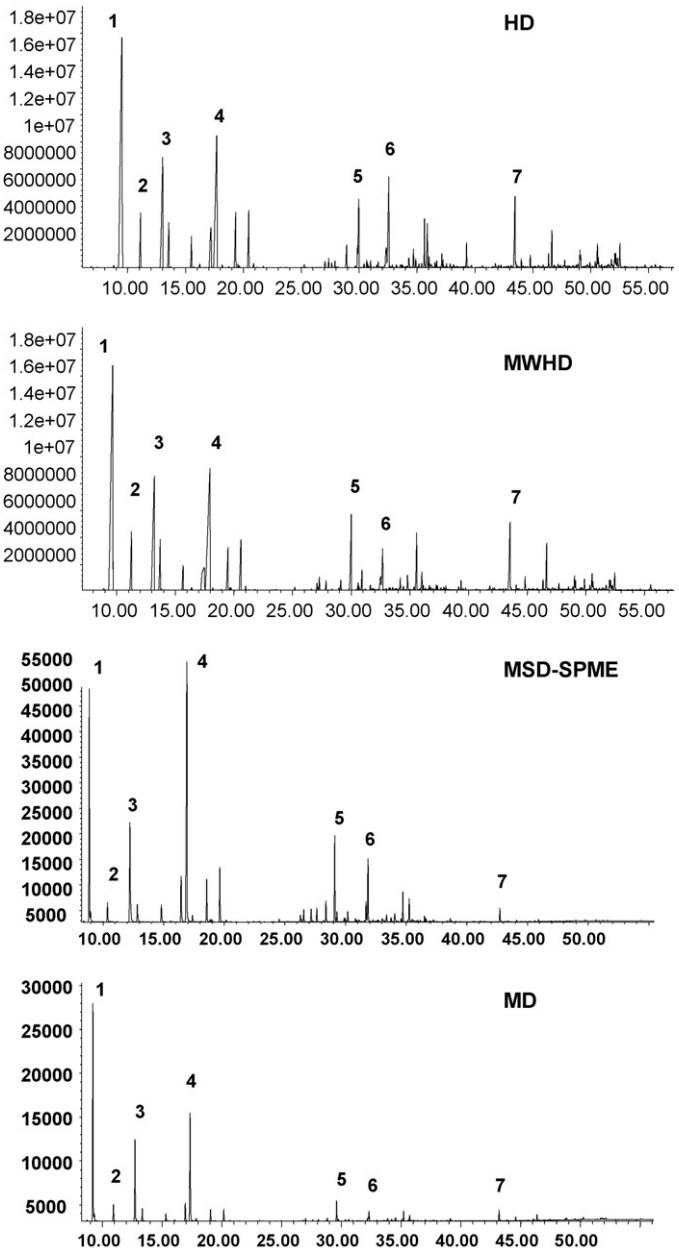

Fig. 1. Typical chromatographic profiles of the oils of *Salvia rosifolia* obtained by different isolation techniques. HD: hydrodistillation, MWHD: microwave-assisted hydrodistillation, MSD-SPME: micro-steam distillation-solid-phase microextraction, MD: microdistillation, (1) α-pinene, (2) camphene, (3) β-pinene, (4) 1,8-cineole, (5) camphor, (6) β-caryophyllene, (7) caryophyllene oxide.

All the techniques applied to *S. rosifolia* resulted in the volatiles richer monoterpenes (Table 2). We reported on the monoterpene-rich oil (from flowering tops) of *S. anatolica* with α-pinene (10.9%), β-pinene (6.7%) and limonene (3.2%) as the major monoterpene hydrocarbons [60]. Earlier, several Turkish *Salvia* species were found to be rich in monoterpene hydrocarbons [61,62] and oxygenated monoterpenes [16].

3.2. Antimicrobial activity of the essential oil

In the present study, antimicrobial activity of *S. rosifolia* oil and its main constituents, 1,8-cineole and α-pinene were evaluated in comparison to chloramphenicol, ampicillin and ketoconazole as standards. *S. rosifolia essential* oil showed activity against two Gram (+) and four Gram (−) bacterial strains and one fungal strain (*C. albicans*) (Table 3). It appeared as non-selective antibacterial agent. *S. rosifolia* essential oil was found to be the most active against *S. aureus* (MRSA) with a MIC of 125 μg/mL while *S. epidermidis* was less sensitive (MIC 250 μg/mL). Among the Gram (−) bacteria, the oil was more effective towards *E. coli* and *P. aeruginosa* (MIC, 250 μg/mL) than *E. aerogenes* and *S. typhimurium* (MIC, 500 μg/mL). In addition, the oil also showed weak antifungal activity against *C. albicans* with MIC of 500 μg/mL.

Antimicrobial activity of the oil was probably associated with the presence of terpenes and particularly a high percentage of α-pinene (24.3%), 1,8-cineole (16.6%), and β-pinene (7.0%). These compounds are known to display antibacterial activity [63,64]. In literature, the oils of *S. fruticosa* and *S. tomentosa* (1,8-cineole rich), *S. ringens* and *S. officinalis* (1,8-cineole and α-pinene rich) were reported to have strong antimicrobial activity [65–68]. In a recent study, it was suggested that complex oil can form a greater barrier towards pathogen adaptation than a simple mixture of monoterpenes as in the study of *Myrica gale* volatile oil and its inhibitory properties against a broad spectrum of bacterial species [69]. Also, complicated mixtures of monoterpenes and sesquiterpenes in the whole oil can represent a stronger barrier to microbial infections [42,63]. As in our study, comparison of MIC values of the oil of *S. rosifolia*, with its pure components 1,8-cineole and α-pinene, revealed that towards some strains, the total oil was somewhat more active than pure compounds.

3.3. Activity of the oil against plant pathogens

Antifungal activity of *S. rosifolia* essential oil against *C. acutatum*, *C. fragariae*, and *C. gloeosporioides* was investigated in comparison with commercial fungicide standards.

Bioautography (Table 4) demonstrated activity of the oil to *Colletotrichum acutatum*, *C. fragariae* and *C. gloesporioides* in a dose-dependent manner with inhibitory zones from 8.5 mm to 11.30 mm. The data on essential oil activity were evaluated at 20 mg/mL, using an 8 μL test volume, against all three *Colletotrichum* species. The essential oil of *S. rosifolia* was less active than the systemic azoxystrobin and the protectant fungicide

Table 3
Minimal inhibitory concentration (MIC) (μg/mL) values for *Salvia rosifolia* oil.

Pathogen	Source	A	B	C	St1	St2	St3
Escherichia coli (G−)	NRRL B-3008	250	500	1000	3.9	15.6	–
Pseudomonas aeruginosa (G−)	NRRL B-23	250	250	500	7.8	15.6	–
Enterobacter aerogenes (G−)	NRRL 3567	500	500	1000	1.9	7.8	–
Salmonella typhimurium (G−)	NRRL B-4420	500	250	1000	7.8	–	–
Staphylococcus epidermidis (G+)	ATCC 12228	250	125	1000	0.9	–	–
Staphylococcus aureus (MR) (G+)	OGU	125	250	1000	31.2	250	–
Candida albicans, yeast	OGU	500	500	500	–	–	62.5

A: *S. rosifolia* essential oil; B: 1,8-cineole; C: α-pinene; St1: chloramphenicol (antibacterial); St2: ampicillin (antibacterial); St3: ketoconazole (antifungal); MRSA: methycillin resistant *Staphylococcus aureus*.

Table 4
Antifungal activity of *Salvia rosifolia* oil using direct bioautography against three *Colletotrichum* species.

Sample	Mean fungal growth inhibition[a] (mm) ± SD		
	C. acutatum	*C. fragariae*	*C. gloeosporioides*
S. rosifolia oil[b]	8·5 ± 0.71	11·30 ± 0.71	8·5 ± 0.71
Benomyl[c]	Dz	21.3 ± 0.35	Dz
Captan[c]	11.5 ± 0.71	15.0 ± 0.49	18.9 ± 1.41
Cyprodinil[c]	Dz	Dz	Dz
Azoxistobin[c]	Dz	26.0 ± 1.41	Dz

[a] Mean inhibitory zones and standard deviations (SD) were used to determine the level of antifungal activity against each fungal species.
[b] *S. rosifolia* oil was applied as a 20 mg/mL in 8 μL sample onto a silica TLC plate.
[c] Technical grade agrochemical fungicides (without formulation) with different modes of action were used as internal standards; Dz: diffuse inhibitory zone.

captan. The oil was active in benomyl resistant strains and showed non-selective activity against *C. acutatum* and *C. gloeosporioides* with 8.5 mm zones. Earlier studies of *S. macrochlamys* and *S. recognita* showed that *Salvia* essential oils from these species had antifungal activity against *C. acutatum*, *C. fragariae*, and *C. gloeosporioides* [70].

3.4. Cytotoxicity

No cytotoxicity was observed towards mammalian kidney fibroblasts (Vero cells) or cancer (SK-MEL, SK-OV3, BT-549 and KB) cells up to 25 μg/mL.

3.5. Antioxidant activity of the oil

The antioxidant activity of *S. rosifolia* oil was evaluated in HL-60 cells using DCFH-DA. This cell-based method examines directly the ability of test material to penetrate living cells and inhibit ROS-catalyzed oxidation of DCFH to DCF. DCFH-DA is a non-fluorescent probe that diffuses into cells. Cytoplasmic esterases hydrolyze DCFH-DA to DCFH which is oxidized to DCF (22,72-dichlorofluorescin) that fluoresces. The antioxidant activity of test sample was determined by measuring the inhibition of ROS-catalyzed generation of DCF in treated cells compared with untreated controls [44]. *S. rosifolia* essential oil did not inhibit the generation of DCF and thus did not show any antioxidant activity.

The results on the application of MWHD technique to *S. rosifolia* volatiles provided a scientific support for the use this technique in the oil production. MD and MSD-SPME techniques allowed reliable and rapid analytical determination of *S. rosifolia* volatiles. Antimicrobial and antifungal properties of *S. rosifolia* oil may be considered as a contribution into development and utilization of new natural products with a pleasant taste or fragrance with a combined preservative action that may increase the shelf life of certain foods.

Acknowledgments

The authors are grateful to NAPRALERT for the use of database. We also thank Ms. Linda Robertson, Ms. Ramona Pace, Ms. Xiaoning Wang, John Trott and Ms. Katherine Martin for performing biological assays.

References

[1] H. Duman, L. Salvia, in: A. Güner, N. Özhatay, T. Ekim, K.H.C. Baser (Eds.), Flora of Turkey and the East Aegean Islands (Supplement 2), vol. 11, University Press, Edinburgh, 2000.
[2] I.C. Hedge, in: R.M. Harley, T. Reynolds (Eds.), A Global Survey of the Biogeography of Labiatae, Royal Botanical Gardens, Kew, 1992.
[3] P.H. Davis, R.R. Mill, T. Kit, Flora of Turkey and East Aegean Islands, vol. 10, Edinburgh University Press, Edinburgh, 1988.
[4] M. Vural, N. Adiguzel, Turk. J. Bot. 20 (1996) 531.
[5] A.A. Donmez, Bot. J. Linnean Soc. 137 (2001) 413.
[6] E. Hamzaoglu, A. Duran, N.M. Pinar, Ann. Bot. Fenn. 42 (2005) 215.
[7] K.H.C. Başer, Pure Appl. Chem. 74 (2002) 527.
[8] T. Baytop, Therapy with Medicinal Plants in Turkey (Past and Present), vol. 2, Nobel Tip Kitabevleri, Istanbul, 1999, p. 142.
[9] J.C. Chalchat, A. Michet, B. Pasquier, Flavour Fragr. J. 13 (1998) 68.
[10] H. Gali-Muhtasib, C. Hilan, C. Khater, J. Ethnopharm. 71 (2000) 513.
[11] J. Pokorny, Trends Food Sci. Technol. 2 (1991) 223.
[12] M.C. Garcia Vallejo, L. Moujir, J. Burillo, L. Leon Guerra, M. Gonzalez, R. Diaz Renate, L. San Andres, J. Gutierrez Luis, F. Lopez Blanco, C.M. Ruiz de Galarreta, Flavour Fragr. J. 21 (2006) 72.
[13] J. Bruneton, Pharmacognosy, Phytochemistry, Medicinal Plants, Lavoisier Publishing Inc., Paris, 1995.
[14] M. Wang, J. Li, M. Rangarajan, Y. Shao, E. Lavoie, J. Agric. Food Chem. 46 (1998) 4869.
[15] B. Bozan, N. Ozturk, M. Koşar, Z. Tunalier, K.H.C. Başer, Chem. Nat. Comp. 38 (2002) 198.
[16] G. Ozkan, O. Sagdic, R.S. Gokturk, O. Unal, S. Albayrak, LWT-Food Sci. Technol. 43 (2010) 186.
[17] M. Kelen, B. Tepe, Biores. Technol. 99 (2008) 4096.
[18] B. Tepe, D. Daferera, A. Sokmen, M. Sokmen, M. Polissiou, Food Chem. 90 (2005) 333.
[19] A. Akgül, M. Ozcan, F. Chialva, F. Monguzzi, J. Essent. Oil Res. 11 (1999) 209.
[20] G. Özek, T. Özek, K.H.C. Başer, E. Hamzaoglu, A. Duran, Chem. Nat. Comp. 43 (2007) 667.
[21] R. Kotan, S. Kordali, A. Cakir, M. Kesdek, Y. Kaya, H. Kilic, Biochem. Syst. Ecol. 36 (2008) 360.
[22] Y. Kaya, O. Aksakal, Pak. J. Bot. 10 (2007) 2178.
[23] European Pharmacopeia (Ph. Eur.), 5th Ed., Council of Europe, Strasbourg, France, 2005.
[24] M.M. Jiménez-Carmona, J.L. Ubera, M.D. Luque de Castro, J. Chromatogr. A 855 (1999) 625.
[25] J.R.J. Pare, US Patent 5338557, 1994.
[26] A.A. Craveiro, F.J.A. Matos, J.W. Alencar, Flavour Fragr. J. 4 (1989) 43.
[27] K. Ganzler, A. Salgo, K. Valko, J. Chromatogr. 371A (1986) 299.
[28] M.E. Lucchesi, F. Chemat, J. Smadja, Flavour Fragr. J. 19 (2004) 134.
[29] E. Stashenko, B.E. Jaramillo, J.R. Martinez, J. Chromatogr. A 1025 (2004) 93.
[30] D. Chunhui, X. Xiuqin, Y. Ning, L. Ning, Z. Xiangmin, Anal. Chim. Acta 556 (2006) 289.
[31] M.R. Tellez, I.A. Khan, B.T. Schaneberg, S.L. Crockett, A.M. Rimando, M. Kobaisu, J. Chromatogr. A 1025 (2004) 51.
[32] C. Bicchi, P. Sandra, in: P. Sandra, C. Bicchi (Eds.), Capillary Gas Chromatography in Essential Oil Analysis, A. Huethig Verlag, Heidelberg, 1987.
[33] R. Briechle, W. Dammertz, R. Guth, W. Volmer, GIT Labor-Fachzeitschrift 41 (1997) 749.
[34] T. Özek, G. Özek, K.H.C. Başer, A. Duran, M. Sagiroglu, J. Essent. Oil Res. 20 (2008) 408.
[35] G. Iscan, F. Demirci, N. Kirimer, M. Kürkcüoglu, K.H.C. Baser, J. Agric. Food Chem. 50 (2002) 3943.

[36] D.W. Reische, D.A. Lillard, R.R. Eitenmiller, in: C.C. Ahoh, D.B. Min (Eds.), Chemistry, Nutrition and Biotechnology, Marcel Dekker, New York, 1998.
[37] M.M. Cowan, Clin. Microbiol. Rev. 12 (1999) 564.
[38] X. Ormancey, S. Sisalli, P. Coutiere, Parfums, Cosmetiques, Actualites 157 (2001) 30.
[39] G. Sacchetti, S. Maietti, M. Muzzoli, M. Scaglianti, S. Manfredini, M. Radice, R. Bruni, Food Chem. 91 (2005) 621.
[40] M.Z. Haznedaroglu, N.U. Karabay, U. Zeybek, Fitoterapia 72 (2001) 829.
[41] V. Cardile, A. Russo, C. Formisano, D. Rigano, F. Senatore, N.A. Arnold, F. Piozzi, J. Ethnopharmacol. 126 (2009) 265.
[42] K.P. Svoboda, J. Hampson, Speciality Chemicals for the 21st Century, ADEME/IENICA Seminar, ADEME, Paris, 1999, pp. 43–49.
[43] E. Borenfraund, H. Babich, N. Martin-Alguacil, In Vitro Cell Dev. Biol. 26 (1990) 1030.
[44] R. Stanikunaite, Sh.I. Khan, J.M. Trappe, S.A. Ross, Phytother. Res. 23 (2009) 575.
[45] M.C. Garcia Vallejo, L. Moujir, J. Burillo, L.L. Guerra, M. Gonzalez, R.D. Penate, L.S. Andres, J.G. Luis, F.L. Blanco, C.M.R. Galarreta, Flavour Fragr. J. 21 (2006) 72.
[46] A. Abdelwahed, N. Hayder, S. Kilani, A. Mahmoud, J. Chibani, M. Hammami, L. Chekir-Ghedira, K. Ghedira, Flavour Fragr. J. 21 (2006) 129.
[47] D. Lopez-Lutz, D.S. Alviano, C.S. Alviano, P.P. Kolodziejczyk, Phytochemistry 69 (2008) 1732.
[48] G. Chen, H. Song, C. Ma, Flavour Fragr. J. 24 (2009) 186.
[49] Y. Shimizu, Y. Imayoshi, M. Kato, K. Maeda, H. Iwabuchi, K. Shimomura, Flavour Fragr. J. 24 (2009) 251.
[50] M.F. Valim, R.L. Rouseff, J. Lin, J. Agric. Food Chem. 51 (2003) 1010.
[51] Y. Frum, In vitro 5-lipoxygenase and anti-oxidant activities of South African medicinal plants commonly used topically for skin diseases, Dissertation MS, 2006.
[52] T.Y. Chung, J.P. Eiserich, T. Shibamoto, J. Agric. Food Chem. 41 (1993) 1693.
[53] P.K.C. Ong, T.E. Acree, J. Agric. Food Chem. 47 (1999) 665.
[54] M.B. Taarit, K. Msaada, K. Hosni, B. Marzouk, Food Chem. 119 (2010) 951.
[55] A. Hognadottir, R.L. Rouseff, J. Chromatogr. A 998 (2003) 201.
[56] P. Weyerstahl, H. Marschall, K. Thefeld, G.C. Subba, Flavour Fragr. J. 13 (1998) 377.
[57] F. Narzallah-Skhiri, I. Cheraif, H.B. Jannet, M. Hammami, Pak. J. Biol. Sci. 8 (2005) 249.
[58] K.H.C. Baser, H.R. Nuriddinov, T. Özek, B. Demirci, N. Azcan, A.M. Nigmatullaev, Chem. Nat. Comp. 38 (2002) 51.
[59] J.R.J. Pare, J.M.R. Belanger, Instrumental Methods in Food Analysis, Elsevier, Amsterdam, 1997.
[60] G. Özek, T. Özek, K.H.C. Başer, E. Hamzaoğlu, A. Duran, Chem. Nat. Comp. 43 (2007) 667.
[61] B. Demirci, N. Tabanca, K.H.C. Başer, Flavour Fragr. J. 17 (2002) 54.
[62] M. Kürkçüoglu, B. Demirci, K.H.C. Başer, T. Dirmenci, G. Tümen, G.U. Ozgen, J. Essent. Oil Res. 17 (2005) 192.
[63] J.C. Chalchat, F. Chiron, R.Ph. Garry, J. Lacoste, J. Essent. Oil Res. 12 (2000) 125.
[64] S.G. Griffin, S.G. Wyllie, J.L. Markham, D.N. Leach, Flavour Fragr. J. 14 (1999) 322.
[65] H. Norouzi-Arasi, I. Yavari, F. Chalabian, P. Baghaii, V. Kiarostami, M. Nasrabadi, A. Aminkhani, Flavour Fragr. J. 20 (2005) 633.
[66] A. Sivropoulou, C. Nikolaou, E. Papanikolaou, S. Kokkini, T. Lanaras, M. Arsenakis, J. Agric. Food Chem. 45 (1997) 3197.
[67] O. Tzakou, D. Pitarokili, I.B. Chinou, C. Harvala, Planta Med. 67 (2001) 81.
[68] D.T. Velickovic, M.S. Ristic, N.V. Randjelovic, A.A. Smelcerovic, J. Essent. Oil Res. 14 (2002) 453.
[69] R.R. Carlton, P.G. Waterman, A.I. Gray, S.G. Deans, Chemoecology 3 (1992) 55.
[70] N. Tabanca, B. Demirci, K.H.C. Başer, Z. Aytac, M. Ekici, S.I. Khan, M.R. Jacob, D.E. Wedge, J. Agric. Food Chem. 54 (2006) 6593.