Available online at www.sciencedirect.com
ScienceDirect
Food Chemistry 105 (2007) 1711–1717
Food Chemistry
www.elsevier.com/locate/foodchem

Analytical, Nutritional and Clinical Methods

Chemical analysis of edible aromatic plants growing in Tanzania

K. Vagionas [a], O. Ngassapa [b], D. Runyoro [b], K. Graikou [a], O. Gortzi [c], I. Chinou [a,*]

[a] University of Athens, School of Pharmacy, Division of Pharmacognosy and Chemistry of Natural Products, Panepistimiopolis-Zografou, 15771 Athens, Greece
[b] Muhimbili University College of Health Sciences, Faculty of Pharmacy, Department of Pharmacognosy, P.O. Box 65 013, Dar Es Salaam, Tanzania
[c] Department of Food Technologies, Technological Educational Institution (T.E.I.) of Larissa, Temponera street, Karditsa, Greece

Received 18 January 2007; received in revised form 22 March 2007; accepted 8 May 2007

Abstract

The volatiles from the aerial parts of edible plants growing in Tanzania, *Leucas glabrata*, *Plectranthus laxiflorus*, *Salvia nilotica* and *Vernonia smithiana*, were investigated by GC and GC/MS. Thirty-five compounds were identified from *L. glabrata*, representing 80.4% of the total oil; forty-three from *P. laxiflorus* (86.7%); twenty-four from *S. nilotica* (94.3%); and thirty-nine compounds from *V. smithiana* (92.9%). Among the identified components, menthone, ($p + o$)-cymene, *trans*-caryophyllene and caryophyllene oxide were found as the main ones. Furthermore, the essential oils were investigated for their antimicrobial activity as well as for their antiradical activity, through the DPPH method. Upon antimicrobial assays, the oil of *V. smithiana* showed very strong antimicrobial activity against Gram-positive bacteria, oral pathogens and pathogenic fungi; the oil of *P. laxiflorus* also exhibited strong activity, mostly against Gram-positive bacteria and especially oral pathogens, while *L. glabrata* showed strong activity against all assayed bacteria. The essential oil of *S. nilotica* appeared to have the most antioxidant activity but was almost inactive against all tested microorganisms.

Keywords: *Leucas glabrata*; *Plectranthus laxiflorus*; *Salvia nilotica*; *Vernonia smithiana*; Asteraceae; Lamiaceae; Essential oil composition; Antimicrobial activity; Antiradical activity

1. Introduction

Essential oils are complex natural mixtures of volatile secondary metabolites, isolated from different parts of plants, and are responsible for the fragrant and the biological properties of aromatic and medicinal plants. Most of them are used as flavours in the food and beverage industry, as well as in perfumery, and they are also recognized as having several therapeutic applications. They demonstrate pharmacological effects, such as antiinflammatory, antioxidant, cytotoxic, and they are biocides against a broad range of organisms, such as bacteria, fungi, viruses, protozoa, as well as insects and plants. Due to these properties, since ancient times, spices and herbs have been added to food not only as flavouring agents, but also as preservatives. Recently, natural products have become of greater interest, due to their availability, few side effects or toxicity, as well as their biodegradation ability in comparison to available antibiotics and preservatives. In this regard, plant essential oils may offer great potential. Therefore, searches for their antimicrobial activities, in connection with their usefulness in medicine and the food industry, and analysis of their chemical composition have been systematically conducted (Kalemba & Kunicka, 2003).

Guided by ethnobotanical literature and availability from natural sources, our main object is to validate the use of selected African aromatic plants for their antioxidant and antimicrobial properties and to emphasize the need to promote natural botanical resources in Africa as well as worldwide (Nakatsu, Lupo, Chinn, & Kang, 2000).

In the framework of our research on the essential oils of aromatic plants growing in Tanzania and their biological activities (Bougatsos, Ngassapa, Runyoro, & Chinou,

* Corresponding author. Fax: +30 210 7274115.
E-mail address: ichinou@pharm.uoa.gr (I. Chinou).

0308-8146/$ - see front matter © 2007 Elsevier Ltd. All rights reserved.
doi:10.1016/j.foodchem.2007.05.029

2004; Ngassapa, Runyoro, Harvala, & Chinou, 2003), we report herein the analysis of the volatiles from the aerial parts of the edible plants, *Leucas glabrata* (Vahl) R. Br., *Plectranthus laxiflorus* Benth., *Salvia nilotica* Juss. ex Jacq. and *Vernonia smithiana* (DC) Less. from Tanzania.

L. glabrata (Lamiaceae) is known in the Ethiopian language by the vernacular name "umhlonyana" and its leaves are commonly eaten cooked. The genus has been used in the Uttara Kannada district in oral health care (Bhandary, Chandrashekhar, & Kaveriappa, 1995), while some species (*L. aspera, L. lavadulaefolia, L. inflata*) have been reported to have antimicrobial, analgesic (Saha, Mukherjee, Mandal, Pal, & Saha, 1995), anti-inflammatory (Saha et al., 1996), and antidiarrhoeal effects (Mukherjee et al., 1998), and wound-healing activity (Saha, Mukherjee, Das, Pal, & Saha, 1997). Labdane-type diterpenes, coumarins, triterpenes and long chain compounds have been previously isolated from the genus (Al-Yousuf, Bashir, Blunden, Yang, & Patel, 1999).

Plectranthus (Lamiaceae) is an important genus containing about 300 species found in Tropical Africa, Asia and Australia. Some species are difficult to identify botanically because of a lack of clear-cut morphological criteria, resulting in numerous taxonomic problems in the naming of species. The plant species *P. laxiflorus* Benth. (synonym is *P. albus* Gürke), studied in our report, is an endemic African plant, spread across Ethiopia through eastern to southern Africa. It is known by the vernacular name "uhlololwane" and in the Zulu dialect as "umsuthuza" and it has been used in the treatment of teeth disorders, abdominal pain rheumatisms and as a purgative (Lukhoba, Simmonds, & Paton, 2006). In South Africa and Kenya, herbal tea made from the leaves of the plant, is widely used for coughs and colds (Rabe & van Staden, 1998). More than one hundred and forty diterpenes have been isolated and identified from several *Plectranthus* species (Abdel-Mogib, Albar, & Batterjee, 2002).

Salvia (Lamiaceae) is an important genus, widely cultivated and used in flavouring and folk medicine. Some of the species have been used for the treatment of chronic bronchitis, as medication against perspiration, fever, rheumatism and in treating mental and nervous problems, as well as for their insecticidal activity (Baricevic & Bartol, 2000). They have also been studied, all over the world, and found to possess antibacterial (Ulubelen, Oksuz, Topcu, Goren, & Voelter, 2001), anti-oxidant, anti-inflammatory and anticholinesterase activities (Perry, Bollen, Perry, & Ballard, 2003). *S. nilotica* Juss. ex. Jacq. is known in the Galinya dialect by the vernacular name "shokoksa" and it is used, in traditional medicine of central Africa, in herbal teas.

Vernonia is a large genus (Asteraceae) with about 1000 species found in the southern hemisphere, among which 50 are in South Africa. *Vernonia smithiana* (DC) Less. [=*Hilliardiella smithiana* (Less.) H. Rob.] is known in the Kinyarwanda dialect by the vernacular name "umwanzuranya". Leaves of *V. smithiana* have been used in traditional African medicine for dysentery and gastrointestinal problems, eye diseases and as a cardio tonic. The roots have been used for urinary problems and the aerial parts of the plant are widely used as herbal tea (Adjanohoun et al., 1988).

To the best of our knowledge, there are no previous reports in the literature concerning the chemical composition and the antimicrobial activity of the volatile oils of all four studied plants.

2. Materials and methods

2.1. Plant material

The aerial parts of the plants were collected in Mbeya region (Rungwe district), Tanzania. The plants were identified by the staff of the Department of Botany, University of Dar es Salaam. Voucher specimens were deposited in the Herbarium of the Department of Pharmacognosy, Muhimbili University College of Health Sciences.

2.2. Isolation procedure

All materials were air-dried in the shade prior to distillation of essential oils. Plant materials, *L. glabrata* (200 g), *P. laxiflorus* (250 g), *S. nilotica* (350 g) and *V. smithiana* (280 g), were subjected to hydro-distillation for 3 h, in a modified Clevenger-type apparatus, with a water-cooled oil receiver, to reduce formation of artifacts due to overheating during hydro-distillation. The essential oils were collected over water, separated and dried over anhydrous sodium sulphate. They were stored in sealed vials at 4 °C–6 °C prior to chemical analysis and antimicrobial screening.

2.3. Gas chromatography

GC analyses were carried out on a Perkin–Elmer 8500 gas chromatograph with FID, fitted with a Supelcowax-10 fused silica capillary column (30 m × 0.32 mm i.d., 0.25 μm film thickness). The column temperature was programmed from 75 °C to 200 °C at a rate of 2.5 °C/min. The injector and detector temperatures were programmed at 230 °C and 300 °C, respectively. Helium was used as carrier gas, flow rate 1 ml/min.

2.4. Gas chromatography–mass spectrometry

The GC–MS analyses were carried out using a Hewlett Packard 5973-6890 GC–MS system operating on EI mode (equipped with a HP 5MS 30 m × 0.25 mm × 0.25 μm film thickness capillary column). He (2 ml/min) was used as carrier gas. The initial temperature of the column was 60 °C and then it was heated to 280 °C with a 3 °C/min rate. Split ratio, 1:10.

2.5. Identification of components

The components of the oils were identified by comparison of their mass spectra with those obtained from authen-

Table 1
Quantities (%) of components of the volatiles of *L. glabrata*, *P. laxiflorus*, *S. nilotica* and *V. smithiana*

Constituents	RI	*L. glabrata*	*P. laxiflorus*	*S. nilotica*	*V. smithiana*
α-Thujene	930	0.09			
α-Pinene	937	1.80	2.98	3.55	0.61
Camphene	951	0.59	1.23	0.95	0.21
Sabinene	975	tr			
β-Pinene	978	1.83	3.12		0.25
3-Octanone	984	tr			
Myrcene	992	0.44	0.49		
3-Octanol	995	0.64			
α-Phellandrene	1003		1.41		
p-Cymene	1026	0.30		5.53	0.59
(*p* + *o*)-Cymene	1026		18.0		
Limonene	1032	4.00			0.86
1,8-Cineole	1033	1.30			1.54
β-Phellandrene	1035		1.33		
cis-Ocimene	1040		0.29		
trans-β-Ocimene	1052	tr	1.70		
Artemisia ketone	1064	0.17	0.13		
Fenchone	1087		1.72		
cis-Linalool oxide	1088				0.26
Linalool	1099		0.99		1.38
α-Thujone	1102	0.76			0.86
β-Thujone	1103		0.36		
trans-Pinocarveol	1139				0.69
trans-Verbenol	1146				0.54
Camphor	1150		9.11	9.70	2.55
Enthone	1154		0.13		
iso-Menthone	1165	31.8			
Borneol	1166		0.44		
1,8-Menthadien-4-ol	1167			0.53	
4-Terpineol	1176		0.66		0.46
p-Cymen-8-ol	1183			0.95	
Cryptone	1184		0.72		
α-Terpineol	1189		0.21	0.68	0.71
Myrtenal	1193				0.66
trans-Carveol	1220				0.41
Pulegone	1235	11.4	0.17		
Carvone	1244				0.45
Piperitone	1257	10.6			
Bornyl acetate	1284				0.46
2-Undecanone	1291		0.43		
δ-Elemene	1335		0.76		
Piperitenone	1344	6.67			
α-Cubebene	1348		0.65		0.26
Eugenol	1358	0.66			
Piperitenone oxide	1366	1.10			
α-Ylangene	1370		0.24	5.19	
α-Copaene	1372	0.15	2.48		1.10
β-Bourbonene	1381	0.40	1.58		
β-Cubebene	1383		0.54		
β-Elemene	1388	0.14	1.83		5.41
Methyl eugenol	1404				3.12
α-Gurzunene	1408			2.07	
trans-Caryophyllene	1414	0.39	4.03	10.9	
Unknown	1423		1.46		
Unknown	1429		1.08		
β-Gurzunene	1434			0.74	1.08
3,7-Guaiadiene	1444			4.31	
α-Humulene	1451		0.52	1.50	
Allo-aromadendrene	1453		1.03		
Unknown	1454			0.85	
γ-Muurolene	1473				2.07
α-Amorphene	1479	0.40		4.77	
β-Selinene	1481				3.40

(*continued on next page*)

Table 1 (*continued*)

Constituents	RI	*L. glabrata*	*P. laxiflorus*	*S. nilotica*	*V. smithiana*
β-Ionone	1482	tr		0.71	
Germacrene-D	1484		10.2		
Valencene	1490			2.95	
α-Selinene	1495			2.21	1.39
Bicyclogermacrene	1497		3.71		
β-Bisabolene	1507		0.71		
γ-Cadinene	1508	0.27	0.62		
δ-Cadinene	1519	0.45	1.91	3.20	0.58
trans-Calamenene	1520	0.45			0.72
γ-Selinene	1532			0.59	
α-Cadinene	1535		0.13		
α-Calacorene	1542	0.11		2.43	0.45
Unknown	1549				1.48
Germacrene-B	1558		1.57		
Spathulenol	1574	2.06	4.28		
Caryophyllene oxide	1579	0.40		1.30	37.0
Unknown	1580			2.46	
Globulol	1582		0.25		
Viridiflorol	1589	0.12		7.91	
Salvial-4(14)-en-1-one	1592				0.89
Unknown	1605				2.04
Unknown	1634			4.47	
Unknown	1636			2.07	
Isospathulenol	1639		1.50		
Epi-α-muurolol	1645	0.63			1.94
Unknown	1653			2.17	
α-Cadinol	1656				5.54
Cedalene	1675				5.73
Unknown	1684				1.58
Unknown	1810				2.28
6,10,14,Trimethyl-2-pentadecanone	1847				1.29

tic samples and/or the NIST/NBS and Wiley mass spectral database. They were also confirmed by comparison of their retention indices (RI) (Van den Dool & Kratz, 1963) and retention times (RT), either with those of authentic compounds or with published data (Adams, 2001; Massada, 1976).

2.6. Antimicrobial activity

Antimicrobial activity of the essential oils against bacteria, oral pathogens and fungi was determined using the agar dilution technique (Bougatsos et al., 2004). The essential oils were individually tested against a panel of microorganisms, including two Gram-positive bacteria: *Staphylococcus aureus* (ATCC 25923) and *S. epidermidis* (ATCC 12228), four Gram-negative bacteria: *Escherichia coli* (ATCC 25922), *Enterobacter cloacae* (ATCC 13047), *Klebsiella pneumoniae* (ATCC 13883) and *Pseudomonas aeruginosa* (ATCC 227853), and the pathogenic fungi *Candida albicans* (ATCC 10231), *C. tropicalis* (ATCC 13801) and *C. glabrata* (ATCC 28838). The oils were also tested against the oral pathogens *Streptococcus mutans* and *S. viridans*, both sensitive strains, clinically isolated. Standard antibiotic (netilmicin) was used in order to control the sensitivity of the tested bacteria and 5-flucytocine and sanguinarine were used in order to control the tested fungi and the oral pathogens, respectively. Minimum inhibitory concentrations (MICs) were determined for the four oil samples and standard pure compounds (1,8-cineole, limonene, linalool, camphor, pulegone, piperitone, bornyl acetate, borneol, spathulenol, α-pinene, β-pinene, *trans*-caryophyllene, caryophyllene oxide), under identical conditions, for comparison purposes.

2.7. Determination of hydrogen donation ability (DPPH· test)

The method used was a modification of that described by Tsaknis and Lalas (2005). The antiradical activity of a sample was expressed as % disappearance of DPPH· (1,1-diphenyl-2-picryl-hydrazyl from Sigma Chemicals Company Ltd., St. Louis, USA). One millilitre of 0.1 mm methanolic DPPH· solution was added to 4 ml methanolic sample solution (0.02 mM final concentration of DPPH·). The mixture was shaken vigorously and left to stand for 30 min in a water bath at 25.0 °C (±0.2 °C) in the dark. The absorbance of the resulting solution ($DPPH^{\cdot}_{Sample}$) was measured at 517 nm against a control (5 ml methanol). Similarly, the absorbance of DPPH· without sample ($DPPH^{\cdot}_{Blank}$) was determined by measuring the absorbance at 517 nm of 4 ml methanol + 1 ml of 0.1 mm methanolic DPPH· solution. A correction was applied should the

Table 2
Antimicrobial activities (MIC mg/ml) of the studied essential oils and their main components

Species–essential Oils	*S. aureus*	*S. epidermidis*	*P. aeruginosa*	*E. cloacae*	*K. pneumoniae*	*E. coli*	*S. mutans*	*S. viridans*	*C. albicans*	*C. tropicalis*	*C. glabrata*
L. glabrata	0.85	0.67	0.90	0.57	0.45	0.70	1.14	0.78	–	–	–
P. laxiflorus	0.80	0.55	1.25	4.30	2.40	3.50	0.90	0.67	1.19	0.77	0.65
S. nilotica	5.40	5.50	7.80	–	–	3.35	–	–	–	–	–
V. smithiana	0.25	0.55	2.53	5.50	3.25	>20	0.37	0.98	1.25	0.84	0.88
1,8-Cineole	9.5	9.5	2.75	3.00	2.35	2.00	–	–	–	–	–
Limonene	>20	>20	>25	>25	>25	>20	–	–	–	–	–
Linalool	0.25	0.25	>20	1.75	>20	1.25	0.37	0.45	–	–	–
Camphor	2.70	1.95	2.80	2.75	3.24	1.33	–	–	4.85	3.76	3.56
Pulegone	1.20	0.95	1.45	1.37	1.76	1.45	1.75	1.26	–	–	–
Piperitone	1.50	2.25	0.60	1.10	0.80	0.95	–	–	–	–	–
Bornyl acetate	1.95	1.75	2.30	3.75	3.25	4.88	–	–	–	–	–
Borneol	1.25	1.57	2.50	4.20	3.75	4.50	–	–	–	–	–
Spathulenol	1.35	1.50	>20	>20	>20	8.50	–	–	–	–	–
α-Pinene	7.50	9.50	6.00	8.00	15.00	2.00	–	–	4.00	4.00	2.00
β-Pinene	12.0	16.0	> 20	> 20	> 20	9.75	–	–	–	–	–
trans-Caryophyllene	> 20	> 20	> 20	> 20	> 20	> 20	–	–	–	–	–
Caryophyllene oxide	0.073	0.90	0.87	2.43	1.23	> 6.40	0.25	0.75	–	–	–
Netilmicin	4×10^{-3}	4×10^{-3}	8.8×10^{-3}	8×10^{-3}	8×10^{-3}	10×10^{-3}					
Sanguinarine							0.015	0.015			
5-Flucytocine									0.1×10^{-3}	1×10^{-3}	10×10^{-3}

sample absorb at 517 nm. This was performed by measuring the absorbance of 4 ml of methanolic sample + 1 ml methanol against the control ($Control_{Sample}$) after applying the same treatment as described above. The time taken for the reaction between the samples and DPPH$^{\cdot}$ to reach a steady state was established to be 30 min incubation at 25 °C. The antiradical activity of samples (100 ppm) was compared to that of the commonly used antioxidants BHT and BHA (both from Sigma Chemicals Company Ltd.).

3. Results and discussion

The results obtained in the qualitative and quantitative analyses are shown in Table 1. From the oil of *L. glabrata*, thirty-five constituents were identified, representing 80.4% (area percent) of the total oil, among which menthone (31.8%), pulegone (11.4%), piperitone (10.6%) and piperitenone (6.67%) were the major compounds. The most abundant chemical category was the oxygenated monoterpenes (64.4%), followed by monoterpene hydrocarbons (8.75%), oxygenated sesquiterpenes (3.60%) and sesquiterpene hydrocarbons (2.76%). Forty-three constituents have been identified from the oil of *P. laxiflorus*, representing 86.7% of the total oil, among which ($p + o$)-cymene (18.0%), germacrene-D (10.2%), and camphor (9.11%) were found as the most abundant components. Sesquiterpene hydrocarbons (32.5%) were the most abundant chemical category, followed by aromatic monoterpenes (18.0%), oxygenated monoterpenes (14.6%) and monoterpene hydrocarbons (12.6%). Twenty-nine constituents were detected from the oil of *S. nilotica*, representing 94.3% of the total oil, among which β-caryophyllene (10.9%), camphor and valeranone (9.70% and 9.57%, respectively) were the major ones. The most abundant chemical category was sesquiterpene hydrocarbons (41.6%), followed by oxygenated sesquiterpenes (18.8%), oxygenated monoterpenes (10.9%), monoterpene hydrocarbons (10.0%) and aromatic monoterpenes (0.95%), while from the oil of *V. smithiana*, thirty-nine compounds were identified, representing 92.9%, among which caryophyllene oxide (37.0%), cedalene (5.73%), α-cadinol (5.54%) and β-elemene (5.41%) were the main constituents. The oil was rich in regard to oxygenated compounds (60.6%), followed by the oxygenated sesquiterpenes (45.4%), the sesquiterpene hydrocarbons (16.5%) and the oxygenated monoterpenes (11.0%).

All four oils were tested against two Gram-positive and four Gram-negative bacteria, three pathogenic fungi and two oral pathogens. The results of the bioassays (Table 2), showed that the tested essential oil of *L. glabrata* has a strong antimicrobial activity against all tested bacteria (MIC values 0.45–1.14 mg/ml). This activity could be attributed to the high content of the oil in compounds with known antimicrobial activity, such as menthone (Duru, Öztürk, Uğur, & Ceylan, 2004), pulegone and piperitone (Oumzil et al., 2002). *P. laxiflorus* exhibited a very interesting antimicrobial profile with higher activity against

Table 3
The antiradical activities of BHT, BHA and samples with DPPH·

Sample (100 ppm)	% Disappearance level
BHT	95.7 (0.9)
BHA	94.8 (0.7)
Leucas glabrata	10.4 (0.1)
Plectranthus laxiflorus	3.8 (0.1)
Salvia nilotica	76.2 (0.1)
Vernonia smithiana	6.6 (0.1)

Values are means of triplicate determinations and standard deviation is given in parenthesis.

Gram-positive tested bacteria and oral pathogens (MIC values 0.55–0.90 mg/ml), as well as against the human pathogenic fungi (0.65–1.19 mg/ml), activity which could be attributed due to the high percentage in the oil of germacrene D (Jovanovic, Kitic, Palic, Stojanovic, & Ristic, 2005), camphor (Kim et al., 2003), α-pinene and spathulenol (Bougatsos et al., 2004), while it showed a weaker activity against the tested Gram-negative bacteria (MIC values 1.25–4.30 mg/ml). *S. nilotica* appeared to be almost inactive against all tested microorganisms, while the oil of *V. smithiana* exhibited a broad spectrum of strong antimicrobial activities. It showed the strongest activity against Gram-positive bacteria (MIC values 0.25–0.55 mg/ml), as well as against all the assayed oral pathogens (MIC values 0.37–0.98 mg/ml) and human pathogenic fungi (MIC values 0.84–1.25 mg/ml) and a weaker activity against Gram-negative bacteria. This profile could be attributed to the high concentration in the oil of caryophyllene oxide with known antimicrobial activity (Couladis, Chinou, Tzakou, & Loukis, 2002), while the existence of linalool (Koutsoudaki, Krsek, & Rodger, 2005) and camphor (Kim et al., 2003) in lower percentages could have a synergistic effect in the exhibited activity.

The antiradical activity of the four essential oils was assayed and compared to that of the commonly used antioxidants BHT and BHA, using the stable radical, 1,1-diphenyl-2-picrylhydrasyl (DPPH·) (Table 3). The greatest antiradical activity was observed with 100 ppm of BHT, followed by that of 100 ppm of BHA (however, not significant at $P < 0.05$). Such a result was anticipated, since BHT is a chain-breaking antioxidant and a concentration of 100 ppm of BHT corresponds to 0.45 mM BHT, which is a large molar excess compared to that of DPPH· (0.02 mM). Furthermore, one molecule of BHT was reported to have the ability to reduce two or more of DPPH· (Brand-Williams, Cuvelier, & Berset, 1995). The antiradical activity of the examined samples was significantly lower ($P < 0.05$) than that of BHT and BHA. The essential oil of *S. nilotica* appeared to be the most active (76.2%), while the other samples showed much less (3.8–10.4%) antiradical activity (significant at $P < 0.05$). *S. nilotica* shows ability to reduce DPPH·, possibly by donating hydrogen atoms and this could potentially represent a mechanism of antioxidant activity.

The results support the potential use of plant essential oils in food and herbal teas as conjunctive therapy, alternatively to antibiotics, against microbial diseases, in African countries.

References

Abdel-Mogib, M., Albar, H. A., & Batterjee, S. M. (2002). Chemistry of the genus Plectranthus. *Molecules, 7*, 271–301.

Adams, R. P. (2001). *Identification of essential oil components by gas chromatography/mass spectroscopy*. Carol Stream, USA, IL: Allured Publishing Corp.

Adjanohoun, E., Ahyi, A. M. R., Aké Assi, L., Baniakina, J., Chibon, P., & Cusset, G., et al. (1988). *Contribution aux études ethnobotaniques et floristiques en République populaire du Congo*. Collection Médecine Traditionnelle et Pharmacopée, Agence de Coopération Culturelle et Technique (ACCT), Paris, France, p. 605.

Al-Yousuf, M. H., Bashir, A. K., Blunden, G., Yang, M., & Patel, A. V. (1999). Phytochemical analysis of *Lecuas inflata* L. *Phytochemistry, 51*, 95–98.

Baricevic, D., & Bartol, T. (2000). The biological/pharmacological activity of the Salvia genus. In E. S. Kintzios (Ed.), *Sage: the Genus Salvia* (pp. 143–184). The Netherlands: Harwood Academic Publishers.

Bhandary, M. J., Chandrashekhar, K. R., & Kaveriappa, K. M. (1995). Medical ethnobotany of the Siddis of Uttara Kannada district, Karnataka, India. *Journal of Ethnopharmacology, 47*, 149–158.

Bougatsos, C., Ngassapa, O., Runyoro, D. K. B., & Chinou, I. B. (2004). Chemical composition and in vitro antimicrobial activity of the essential oils of two Helichrysum species from Tanzania. *Zeitschrift für Natürforschung, 59*(5–6), 368–372.

Brand-Williams, W., Cuvelier, M. E., & Berset, C. (1995). Use of free radical method to evaluate antioxidant activity. *Lebensmittel-Wissenschaft und Technologie, 28*(1), 25–30.

Couladis, M., Chinou, I. B., Tzakou, O., & Loukis, A. (2002). Composition and antimicrobial activity of the essential oil of *Ballota pseudodictamnus* L. Bentham. *Phytotherapy Research, 16*, 723–726.

Duru, E. M., Öztürk, M., Uğur, A., & Ceylan, Ö. (2004). The constituents of essential oil and in vitro antimicrobial activity of *Micromeria cilicica* from Turkey. *Journal of Ethnopharmacology, 94*(1), 43–48.

Jovanovic, T., Kitic, D., Palic, R., Stojanovic, G., & Ristic, M. (2005). Chemical composition and antimicrobial activity of the essential oil of *Acinos arvensis* (Lam.) Dandy from Serbia. *Flavour and Fragrance Journal, 20*, 288–290.

Kalemba, D., & Kunicka, A. (2003). Antibacterial and antifungal properties of essential oils. *Current Medicinal Chemistry, 10*(10), 813–829.

Kim, K. J., Kim, Y. H., Yu, H. H., Jeong, S. I., Cha, J. D., Kil, B. S., et al. (2003). Antibacterial activity and chemical composition of essential oil of Chrysanthemum boreale. *Planta Medica, 69*(3), 274–277.

Koutsoudaki, C., Krsek, M., & Rodger, A. (2005). Chemical composition and antibacterial activity of the essential oil and the gum of Pistacia lentiscus var. chia. *Journal of Agricultural and Food Chemistry, 53*(20), 7681–7685.

Lukhoba, C. W., Simmonds, M. S. J., & Paton, A. J. (2006). *Plectranthus*: A review of ethnobotanical uses. *Journal of Ethnopharmacology, 103*(1), 1–24.

Massada, Y. (1976). *Analysis of essential oil by gas chromatography and spectrometry*. New York: John Wiley & Sons.

Mukherjee, P. K., Saha, K., Murugesam, T., Mandal, S. C., Pal, M., & Saha, B. P. (1998). Screening of anti-diarrhoeal profile of some plant extracts of a specific region of West Bengal, India. *Journal of Ethnopharmacology, 60*(1), 85–89.

Nakatsu, T., Lupo, A. T., Chinn, J. W., & Kang, R. K. L. (2000). Biological activity of essential oils and constituents. *Studies in Natural Products Chemistry, Atta-ur-Rahman, 21*, 571–631.

Ngassapa, O., Runyoro, D., Harvala, E., & Chinou, I. (2003). Composition and antimicrobial activity of the essential oils of two different

populations of *Lippia javanica*, growing in Tanzania. *Flavour and Fragrance Journal, 18*(3), 221–224.

Oumzil, H., Ghoulami, S., Rhajaoui, M., Ilidrissi, A., Fkih-Tetouani, S., Faid, M., et al. (2002). Antibacterial and antifungal activity of essential oils of *Mentha suaveolens*. *Phytotherapy Research, 16*(8), 727–731.

Perry, N. S., Bollen, C., Perry, E. K., & Ballard, C. (2003). *Salvia* for dementia therapy: review of pharmacological activity and pilot tolerability clinical trial. *Pharmacology Biochemistry Behaviour, 75*, 651–659.

Rabe, T., & van Staden, J. (1998). Screening of *Plectranthus* species for antibacterial activity. *South African Journal of Botany, 64*, 62–65.

Saha, K., Mukherjee, P. K., Das, J., Mandal, S. C., Saha, B. P., & Pal, M. (1996). Anti-inflammatory evaluation of *Leucas lavadulaefolia* Rees extracts. *Natural Product Sciences, 2*, 119–122.

Saha, K., Mukherjee, P. K., Das, J., Pal, M., & Saha, B. P. (1997). Wound healing of *Leucas lavadulaefolia* Rees. *Journal of Ethnopharmacology, 56*, 139–144.

Saha, K., Mukherjee, P. K., Mandal, S. C., Pal, M., & Saha, B. P. (1995). Antibacterial activity of *Leucas lavadulaefolia* Rees (Labiatae). *Indian Drugs, 32*, 402–404.

Tsaknis, J., & Lalas, S. (2005). Extraction and identification of natural antioxidants from *Sideritis euboea* (mountain tea). *Journal of Agricultural and Food Chemistry, 53*(16), 6375–6381.

Ulubelen, A., Oksuz, S., Topcu, G., Goren, A. C., & Voelter, W. (2001). Antibacterial diterpenes from the roots of *Salvia blepharochlaena*. *Journal of Natural Products, 64*, 549–551.

Van den Dool, H., & Kratz, P. D. (1963). A generalization of the retention index system including linear temperature programmed gas-liquid partition chromatography. *Journal of Chromatography, 11*, 463–471.