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Composition and antimicrobial activity of the essential oils of three *Satureja* species growing in Tanzania

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Abstract

Hydro-distilled volatile oils from the aerial parts of three *Satureja* species were investigated, mainly by a combination of GC and GC/MS. One hundred and thirteen compounds were identified, representing 82.9–92.0% of the total oil. Among the identified components, spathulenol, *cis*-piperitone oxide, α-bisabolol oxide-B, terpinen-4-ol, linalool, bornyl acetate, β-bourbonene, isomenthone, thymol, neo-isomenthol and menthone were found as the main components. Furthermore, the essential oils were investigated for their antimicrobial activity, by the agar dilution technique. The antimicrobial test results showed that the oils had a high antimicrobial activity against two Gram-positive and four Gram-negative bacteria, two oral pathogens and three pathogenic fungi. Gram-positive bacteria were more sensitive to the investigated oils than were Gram-negative bacteria. These results could support the suggestion of *Satureja* species as a source of antimicrobial ingredients for the food industry.

Keywords: *Satureja biflora*; *S. masukensis*; *S. pseudosimensis*; Lamiaceae; Essential oil composition; Antimicrobial activity

1. Introduction

Satureja species are well-known aromatic and medicinal herbs. In our continuing research on the essential oils of aromatic plants from Tanzania and their biological activities, we report herein the analysis of three *Satureja* species from Tanzania. *Satureja biflora* (Buch-Ham ex D. Don) Briquet (Lamiaceae) (syn. *Satureja ovata* R. Brown and *Thymus biflorus* Buch-Ham ex D. Don) (Demissew, 1993) is known in the Ethiopian language (Geez dialect) by the vernacular name ''Etse libona'' and in the Nyakyusa dialect (Rungwe, Tanzania) as ''Luswambeba''. It is a perennial herb up to 35 cm high with several stems arising from a woody base. It is a highland plant, which mainly grows in the alpine zone and mountainsides at altitudes of 2560–4560 m in many regions of Ethiopia, as well as in tropical Africa and reaches to South Africa. The plant has an odour of wild thyme and yields a significant amount of volatile oil. As a condiment, it is used to flavour tea and different types of ''Wet'' (Gindaba, 1989). In east Africa, roots and leaves of *S. biflora* are used for the treatment of headache (Kokwaro, 1993). *Satureja masukensis* (Baker) Eyles is known as ''Luswambeba lunywamu'' in the Nyakyusa dialect. It is an erect herb with a height of 1–1.5 m, with a stem, which is almost unbranched, except for small branches that arise just before the flowers. To our knowledge, there are no reports in the literature concerning the chemical composition of their essential oils. *Satureja pseudosimensis* Brenan (syn. *Calamintha simensis* Benth.) (Troupin, 1985) is a procumbent herb, used to treat headaches and coughs and to scent public places (Ntezurubanza, Scheffer, & Baerheim-Svendsen, 1987). There are reports in the literature concerning the analysis of the essential oil of *S. pseudosimensis* growing in Rwanda (Muhayimana, Chalcat, & Garry, 1998; Ntezurubanza et al., 1987).

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2. Materials and methods

2.1. Plant material

All three *Satureja* species were collected in the Mbeya region, Tanzania. Aerial parts of *S. biflora* were collected during the flowering period, in March 2000, at Kasanga-Mwakaleli while those of *S. masukensis* were collected along the Katumba-Mwakaleli, in July 2000; all in the Rungwe district. Leaves and flowering tops of *S. pseudosimensis* were collected, in March 2000, on Kawetere Mountain, in the Mbeya district. The plants were identified by the staff of the Herbarium, 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, *S. biflora* (350 g), *S. masukensis* (100 g) and *S. pseudosimensis* (105 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 artefacts 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 4–6 °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 was heated to 280 °C with a 3 °C/min rate. Split ratio was 1:10.

2.5. Identification of components

The components of the oil were identified by comparison of their mass spectra with those obtained from authentic 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

2.6.1. Microbial strains

Antimicrobial activity of the essential oils against bacteria, oral pathogens and fungi was determined using the agar dilution technique (Janssen, Scheffer, & Baerheim Svedsen, 1987). 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.

For all assays, stock solutions of the tested essential oils and pure compounds in sterile distilled water with 10% Tween 80 were prepared at 10 and 1 mg/ml, respectively. Serial dilutions of the stock solutions in broth medium (100 μl of Müller–Hinton broth, Sabouraud broth for fungi and blood agar 10% for oral pathogens) were prepared in a microtitre plate (96 wells). Then 1 μl of the microbial suspension (the inoculum, in sterile distilled water) was added to each well. For each strain, the growth conditions and the sterility of the medium were checked and the plates were incubated at 37 °C and the MICs were determined as the lowest concentrations preventing visible growth.

Standard antibiotics (netilmicin, and amoxicillin with clavulanic acid) were used in order to control the sensitivity of the tested bacteria and 5-flucytocine, amphotericin B and sanguinarine were used in order to control the tested fungi and the oral pathogens. Technical data have been described previously (Bougatsos, Ngassapa, Runyoro, & Chinou, 2004). Minimum inhibitory concentrations (MICs) were determined for the three oil samples and the standard pure compounds, under identical conditions, for comparison purposes.

3. Results and discussion

The results obtained in the qualitative and quantitative analyses are shown in Table 1. From the oil of *S. biflora*, 80 constituents were identified, representing 92.0% (area percent) of the total oil, among which spathulenol (11.9%), α-bisabolol oxide-B (8.77%), terpinen-4-ol (7.12%), linalool (6.03%), bornyl acetate (4.75%) and β-bourbonene (4.19%) were the major components. From the essential oil of *S. masukensis,* 54 constituents were iden-

Table 1
Chemical composition of the essential oils of three studied species of *Satureja*

	Compounds[A]	*S. biflora*	*S. masukensis*	*S. pseudosimensis*	RI[B]	Method of identification
	Monoterpene hydrocarbons					
1	Tricyclene	–	0.02	0.01	927	a,b,d
2	α-Thujene	0.03	0.02	0.01	930	a,b,d
3	α-Pinene	0.33	0.21	0.31	939	a,b,c,d
4	Camphene	0.22	0.23	0.34	954	a,b,d
5	Thuja-2,4(10)-diene	–	–	0.02	960	a,d
6	Sabinene	0.05	0.06	0.10	975	a,b,d
7	β-Pinene	0.14	0.07	0.30	979	a,b,c,d
8	β-Myrcene	0.06	0.14	0.29	991	a,b,d
9	α-Phellandrene	0.03	–	0.03	1003	a,b,d
10	*para*-Menthan-1(7),8-diene	–	0.04	–	1004	a,d
11	α-Terpinene	0.05	–	–	1017	a,b,d
12	*para*-Cymene	1.98	0.36	0.02	1025	a,b,d
13	Limonene	0.55	0.45	*2.62*	1029	a,b,c,d
14	*cis*-β-Ocimene	0.14	0.38	0.19	1037	a,b,d
15	*trans*-β-Ocimene	0.15	–	0.20	1050	a,b,d
16	γ-Terpinene	0.34	0.02	–	1060	a,b,d
	Oxygenated monoterpenes					
17	1,8-Cineole	1.92	–	–	1031	a,b,c,d
18	Artemisia ketone	0.06	–	–	1062	a,b,d
19	*cis*-Sabinene hydrate	0.61	–	0.02	1070	a,b,d
20	*trans*-Linalool oxide	*3.15*	0.69	0.16	1073	a,b,d
21	Camphenilone	0.13	–	–	1082	a,b,d
22	*cis*-Linalool oxide	2.27	–	0.15	1087	a,b,d
23	Linalool	*6.03*	*4.44*	0.25	1097	a,b,c,d
24	β-Thujone	0.29	–	–	1114	a,b,d
25	*cis*-*para*-Menth-2-en-1-ol	0.87	–	–	1122	a,d
26	α-Campholenal	0.25	–	–	1126	a,b,d
27	*trans*-Pinocarveol	1.50	–	–	1139	a,b,d
28	Camphor	*3.89*	0.49	0.12	1146	a,b,c,d
29	Menthone	0.27	0.35	*2.49*	1153	a,b,d
30	Sabina ketone	0.38	–	–	1159	a,b,d
31	Isomenthone	–	–	*8.47*	1163	a,b,d
32	Pinocarvone	0.93	–	–	1165	a,b,d
33	Borneol	2.97	2.44	–	1169	a,b,d
34	Menthol	–	–	1.77	1172	a,b,c,d
35	Terpinen-4-ol	*7.12*	0.09	–	1177	a,b,c,d
36	*para*-Cymen-8-ol	1.49	–	–	1183	a,b,d
37	Neoisomenthol	–	–	*2.89*	1187	a,d
38	α-Terpineol	1.32	–	–	1189	a,b,d
39	Myrtenal	0.88	0.28	–	1196	a,b,d
40	Myrtenol	0.66	–	–	1196	a,b,d
41	Verbenone	1.53	–	–	1205	a,b,d
42	*trans*-Carveol	1.54	–	–	1217	a,b,d
43	*cis*-Carveol	0.02	–	–	1229	a,b,d
44	Pulegone	–	–	0.08	1237	a,b,d
45	Cuminal	0.88	–	–	1242	a,b,d
46	Carvone	0.84	–	–	1243	a,b,d
47	Piperitone	0.40	–	–	1253	a,b,d
48	*cis*-Piperitone oxide	–	*27.04*	*25.00*	1254	a,b,d
49	Carvenone	0.06	–	–	1258	a,b,d
50	Isopiperitenone	–	–	0.34	1272	a,b,d
51	Neo-menthyl acetate	–	–	0.20	1274	a,b,d
52	Iso-bornyl acetate	–	–	0.13	1286	a,b,d
53	Bornyl acetate	4.75	1.84	–	1289	a,b,d
54	Thymol	–	3.13	*6.67*	1290	a,b,c,d
55	*para*-Cymen-7-ol	0.47	–	–	1291	a,b,d
56	Carvacrol	0.14	–	–	1299	a,b,c,d
57	*trans*-Carvyl acetate	–	0.03	0.06	1342	a,b,d
58	Piperitenone	0.09	0.09	0.51	1343	a,b,d
59	*cis*-Carvyl acetate	–	–	0.08	1368	a,b,d
60	Piperitenone oxide	–	0.07	0.24	1369	a,b,d
61	Geranyl acetone	0.24	–	–	1455	a,b,d

(*continued on next page*)

Table 1 (*continued*)

	Compounds[A]	*S. biflora*	*S. masukensis*	*S. pseudosimensis*	RI[B]	Method of identification
62	β-E-Ionone	0.42	0.57	0.32	1489	a,b,d
	Sesquiterpene hydrocarbons					
63	α-Copaene	0.39	0.71	0.25	1377	a,b,d
64	β-Bourbonene	*4.19*	*5.53*	2.93	1388	a,b,d
65	β-Elemene	0.10	0.55	0.21	1391	a,b,d
66	α-Gurjunene	–	0.75	–	1410	a,b,d
67	*trans*-Caryophyllene	0.64	1.76	0.74	1419	a,b,d
68	β-Gurjunene	0.15	1.02	0.38	1434	a,b,d
69	Aromadendrene	–	0.11	0.16	1441	a,b,d
70	α-Amorphene	0.25	0.53	0.26	1441	a,b,d
71	α-Humulene	–	0.16	0.34	1455	a,b,d
72	Alloaromadendrene	0.52	–	0.20	1460	a,b,d
73	*cis*-Muurola-(4,14),5-diene	–	–	0.06	1467	a,b,d
74	γ-Muurolene	0.22	–	1.05	1480	a,b,d
75	*ar*-Curcumene	0.04	–	–	1481	a,b,d
76	Germacrene-D	0.13	0.30	0.07	1485	a,b,d
77	Valencene	–	0.21	0.15	1496	a,b,d
78	α-Selinene	0.03	–	–	1498	a,b,d
79	α-Muurolene	–	–	0.22	1500	a,b,d
80	Cuparene	0.22	–	–	1505	a,b,d
81	*E,E*-α-Farnesene	–	0.23	–	1506	a,b,d
82	γ-Cadinene	0.11	0.25	0.29	1514	a,b,d
83	δ-Cadinene	0.21	0.97	0.77	1523	a,b,d
84	α-Cadinene	0.05	–	0.11	1539	a,b,d
85	α-Calacorene	–	0.29	0.12	1546	a,b,d
	Oxygenated sesquiterpenes					
86	*endo*-Bourbanol	–	–	0.11	1520	a,b,d
87	Spathulenol	*11.88*	*22.36*	*13.26*	1578	a,b,d
88	Caryophyllene oxide	2.26	–	–	1583	a,b,c,d
89	Salvial-4(14)-en-1-one	0.90	–	0.23	1595	a,d
90	nor-Copaanone	1.24	0.52	0.06	1623	a,b,d
91	Isospathulenol	–	2.49	0.23	1640	a,d
92	epi-α-Muurolol	–	0.40	0.30	1642	a,b,d
93	α-Muurolol	–	–	0.21	1646	a,b,d
94	α-Cadinol	–	3.54	0.63	1654	a,b,d
95	α-Bisabolol oxide-B	*8.77*	–	–	1658	a,b,d
96	α-Bisabolol	1.78	–	–	1686	a,b,d
97	Aristolone	0.52	–	–	1763	a,b,d
	Diterpene hydrocarbons					
98	Isophytol	0.05	–	–	1948	a,b,d
	Others					
99	Hexanal	0.03	–	–	802	a,d
100	*cis*-3-Hexenol	0.04	–	0.13	859	a,b,d
101	1-Hexanol	0.03	–	–	871	a,b,d
102	Benzaldehyde	0.09	0.03	0.04	960	a,b,d
103	1-Octen-3-ol	2.82	0.02	0.14	979	a,b,d
104	3-Octanone	0.66	–	0.13	984	a,b,d
105	6-Methyl-5-hepten-2-one	–	0.06	–	986	a,d
106	3-Octanol	0.98	0.37	–	991	a,b,d
107	1-Octen-3-yl acetate	–	0.06	1.36	1113	a,d
108	3-Octanol acetate	–	0.64	2.79	1123	a,b,d
109	*p*-Methoxy-acetophenone	–	–	0.05	1350	a,d
110	*p*-Methyl-acetophenone	0.28	–	–	1183	a,d
111	Eugenol	0.09	0.12	0.12	1359	a,b,c,d
112	*cis*-Jasmone	–	0.24	–	1393	a,b,d
113	Methyl-eugenol	0.64	–	0.45	1404	a,d
	Total	*92.01*	*87.76*	*82.94*		

a = Retention time; b = Retention index; c = Peak enrichment; d = Mass spectra.

[A] Compounds listed in order of elution from a HP-5 MS column.

[B] Retention indices (RI) on HP-5 MS capillary column.

tified (87.8% of the total oil), *cis*-piperitone oxide (27.0%), spathulenol (22.4%) and β-bourbonene (5.53%) being the major components. The oil of *S. pseudosimensis* yielded 68 compounds, which represented 82.9% (area percent) of the total oil, with *cis*-piperitone oxide (25.0%), spathulenol (13.3%), isomenthone (8.47%), thymol (6.67%), β-bourbonene (2.93%), neoisomenthol (2.89%) and menthone (2.49%) being the most abundant. In all cases, the most abundant were the oxygenated monoterpenes (41.6–50.0%), followed by the oxygenated sesquiterpenes (15.0–29.3%) and the sesquiterpene hydrocarbons (7.25–13.4%).

In contrast with the reports concerning the analysis of the essential oils of *S. pseudosimensis* growing in Rwanda (Muhayimana et al., 1998; Ntezurubanza et al., 1987), our study (Tanzanian species) showed different qualitative and quantitative profiles. So, in the oil of *S. pseudosimensis* from Tanzania, *cis*-piperitone oxide represents 25.0% of the total oil while, in Rwanda species, it appears to represent 28.4–71.7% in two different collections. Spathulenol and isomenthone, which appear in our study as abundant constituents (13.3% and 8.47%, respectively), together with thymol (6.67%), seem to be in traces (spathulenol, isomenthone) or absent in Rwanda species.

A large number of studies have reported that the essential oils of *Satureja* species are among the most potent essential oils regarding their antimicrobial properties (Sahin et al., 2003; Skočibušić, Bezić, & Dunkić, 2006), which has been confirmed and extended in this study. The oils were tested against two Gram-positive and four Gram-negative bacteria, three pathogenic fungi and two oral pathogens. The oils of many species of the genus *Satureja* are known to possess antibacterial and fungicidal properties, such as *S. brownie*, *S. montana*, *S. hortensis* and *S. thymbra* (Caceres, Alvarez, Ovando, & Samayoa, 1991; Ciani et al., 2000; Melegari et al., 1985; Ozcan & Boyraz, 2000; Ozcan & Erkmen, 2001). The results of the bioassays (Table 2), showed that all the tested essential oils of *Satureja*, exhibited strong antimicrobial activity against Gram-positive bacteria and oral pathogens (MIC values 0.37–0.98 mg/ml), activity which could be attributed to the high content of the oils, of compounds with known antimicrobial activity, such as linalool (Koutsoudaki, Krsek, & Rodger, 2005), camphor (Kim et al., 2003), spathulenol (Bougatsos et al., 2004), *cis*-piperitone oxide (Oumzil et al., 2002), menthone (Duru, Öztürk, Uğur, & Ceylan, 2004) and thymol (Ultee, Bennik, & Moezelaar, 2002). All the three oils showed moderate activities against the tested pathogenic fungi, while Gram-negative bacteria appeared as the most resistant ones. To the best of our knowledge, there are no reports in the literature concerning the chemical composition and the antimicrobial activity of the essential oils *S. masukensis* and *S. biflora* and therefore our results could be evaluated.

The results of the bioassays, together with the very interesting chemical profile of the oil of *S. pseudosimensis*, which appears almost different from the previous studied species, support the possibility of using the *Satureja* essential oils or

Table 2
Antimicrobial activities (MIC mg/ml) of the studied *Satureja* species 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*
Satureja biflora	0.75	0.98	>20	1.98	>20	1.77	0.64	0.85	0.95	0.88	0.79
Satureja masukensis	0.58	0.37	0.92	4.55	2.35	5.70	0.57	0.48	1.19	0.77	0.65
Satureja pseudosimensis	0.80	0.75	1.04	3.95	2.30	6.14	0.92	1.17	0.57	0.45	0.52
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	12.00	16.00	>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}					
Amoxicillin with clavulanic acid	3×10^{-3}	3×10^{-3}	3.1×10^{-3}	4.2×10^{-3}	4.8×10^{-3}	5×10^{-3}					
Sanguinarine							0.015	0.015			
5-Flucytocine									0.1×10^{-3}	1×10^{-3}	10×10^{-3}
Amphotericin B									1×10^{-3}	0.5×10^{-3}	0.4×10^{-3}

their major constituents as potent natural preservatives. In the framework of the considerable research interest in the use of extracts or of essential oils of aromatic, edible or medicinal plants, herbs, and spices for the development of alternative food additives for preventing the growth of pathogens or to delay the onset of food spoilage (Chorianopoulos et al., 2004), plants such as *Satureja*, commonly used as spices, or ethnopharmacologically as hot drink, after boiling in water, are feasible as they are considered safe. The production of such essential oils and their exploitation as potential natural food preservatives could be of economic benefit. However, further investigation should be carried out on their activities against foodborne pathogens (e.g. *Listeria* sp., *Salmonella* sp.).

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