Bull. Chem. Soc. Ethiop. **2007**, 21(2), 249-254. ISSN 1011-3924
Printed in Ethiopia

CHEMICAL COMPOSITION AND ANTIMICROBIAL ACTIVITY OF THE ESSENTIAL OIL OF *SATUREJA BIFLORA* (LAMIACEAE)

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(Received June 8, 2006; revised July 26, 2006)

ABSTRACT. Hydro-distilled essential oil from *Satureja biflora* (Lamiaceae) growing in Kenya was analysed by gas chromatography mass spectrometry (GC-MS) and also evaluated for antimicrobial activity. Twenty two compounds which constitute 99.29 % of the total oil were identified. The oil was dominated by monoterpenes, which accounted for 62.02 % of the oil. This monoterpene fraction was characterized by a high percentage of linalool (50.60 %) such that this *Satureja* species can be classified as the linalool chemotype. The other major monoterpenes were α-terpineol (2.80 %), β-ocimene (2.25 %), β-pinene (1.96 %) and *cis*-linalool oxide (1.91 %). Sesquiterpenes present in fairly good amounts are germacrene D (10.63 %), α-cadinol (4.53 %), β-bourbonene (2.33 %), δ-cadinene (2.19 %), τ-cadinol (2.17 %), endo-1-bourbonanol (2.14 %) and β-caryophyllene (1.98 %). Aliphatic alcohols and acids accounted for 7.23 % of the oil, of which the major one was linoleic acid (4.48 %). The oil was screened for antimicrobial activity against both gram-positive (S*taphylococcus aureus*, *Bacillus ssp*.) and gram-negative (*Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella typhi*, *Klebsiella pheumoniae, Proteus mirabilis*) bacteria and a pathogenic fungus (*Candida albicans)*. To the best of our knowledge nothing concerning the chemical composition and biological activity of the essential oil of *S. biflora* has been reported.

KEY WORDS: *Satureja biflora*, Essential oil, Antimicrobial activity

INTRODUCTION

The genus *Satureja* is in the family of Lamiaceae and has about 30 species distributed in tropical Africa, Europe and North America. Oils obtained from the leaves and flowers of *Satureja* spp. have varied industrial applications as flavouring materials, medicine and perfumes [1].

Literature review showed variation between chemical compositions of different *Satureja* species oils. The main component of the oil of *S. icarica*, *S. boissieri* and *S. pilosa* from Turkey was carvacrol (59.2 %, 44.8 %, 42.1 %), respectively [2]. The main constituent of *S. brownei* [3] oil from Venezuela was found to be *pulegone* (64.3 %), while that of *S. parvifolia* [4] oil from Argentina is piperitone oxide. Germacrene D has been detected to be the main compound of *S. coerulea* [5]. The main components of *S. mutica*, *S. macrantha* and *S. intermedia* growing in Iran were found to be carvacrol (30.9 %), *p*-cymene (25.8 %) and thymol (32.3 %), respectively [6]. Essential oil from *S. khuzistinica* Jamzad growing widely in Iran contained 93.9 % carvacrol [7].

Different *Satureja* species have been used in traditional medicine as antimicrobial, spasmolytic, analgesic, cicatrizing and diuretic agents. The antibacterial properties of several essential oils of *S. montana* [8] and *S. thymbra* [9] have been studied. The essential oils of *S. obovata* [10, 11], *S. cuncifolia* [5] and *S. hortensis* [12] have been evaluated as spasmolytic agents. *S. biflora* is a perennial shrub with white flowers, and is used to relieve headache in some communities in Kenya [13]. Little is known of the medicinal value of this particular species of *Satureja*. To the best of our knowledge nothing has been reported concerning the chemical composition and biological activity of the essential oil of *S. biflora*.

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The purpose of this research was to study the chemical composition and biological effects of the oil of *S. biflora*. The research findings will contribute towards the use of this plant in herbal medicine and aromatherapy.

EXPERIMENTAL

Plant material

The leaves of *S. biflora* were collected from the Botanical-garden of Egerton University in Kenya which is at an altitude of 2127 m. A voucher specimen (EU-1262) was deposited at the Department of Botany, Egerton University.

Isolation of volatile components

Fresh leaves of *S. biflora* were subjected to hydro-distillation in a Clevenger-type apparatus for a minimum of 4 h. The essential oil was obtained in a yield of 0.2 % w/w after drying over anhydrous Na_2SO_4.

GC, GC-MS analysis

Samples of essential oils were diluted in methyl-*t*-butylether (MTBE) (1:100) and analysed on an Agilent GC-MSD apparatus equipped with an Rtx-5SIL MS ('Restek') (30 m x 0.25 mm i.d., 0.25 μm film thickness) fused-silica capillary column. Helium (at 0.8 mL/min) was used as a carrier gas. Samples were injected in the split mode at a ratio of 1:10 – 1: 100. The injector was kept at 250 ^{o}C and the transfer line at 280 ^{o}C. The column was maintained at 50 ^{o}C for 2 min and then programmed to 260 ^{o}C at 5 ^{o}C/min and held for 10 min at 260 ^{o}C. The MS was operated in the EI mode at 70 eV, in *m/z* range 42-350. The identification of the compounds was performed by comparing their retention indices and mass spectra with those found in literature [14] and supplemented by Wiley and QuadLib 1607 GC-MS libraries. The relative proportions of the essential oil constituents are expressed as percentages obtained by peak area normalization, all relative response factors being taken as one.

Pharmacological screening

The antimicrobial activity of the essential oil was tested according to the National Committee of Clinical Laboratory Standards [15] against the following microorganisms: *Staphylococcus aureus ATCC 25923*, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* 27853, and clinical isolates *Bacillus ssp*., *Salmonella typhi*, *Klebsiella pneumoniae*, *Proteus mirabilis*, and *candida albicans.* Freshly grown microbial suspensions in Mueller Hinton Broth were standardized to a cell density of 1.5 x 10^8 (McFarland No. 0.5). Serial dilutions of the essential oil were done using 10 % TWEEN 80 in distilled sterile water which was also used as the control. The essential oil was diluted to 11.1 %, 12.5 %, 14.3 %, 16.7 %, 20 %, 25 %, 33.3 %, and 50 %. Neat oil was also used giving a corresponding concentration of 75 x 10^2 μg per sensitivity disc. The positive antibacterial and antifungal activities were established by the presence of measurable zones of inhibition after 24 hours of incubation at 37 ^{o}C. Minimum inhibition concentration (MIC) was defined as the lowest concentration that inhibited growth of the microorganism detected visually. Chloramphenicol and nyastatin were used as standard antibiotics.

RESULTS AND DISCUSSION

The chemical composition and antimicrobial activity of the essential oil of *S. biflora* is reported here for the first time. Table 1 lists twenty-two compounds identified by GC and GC-MS, which constitute 99.29 % of the total oil. The oil was dominated by monoterpene hydrocarbons which accounted for 62.02 % of the oil. This monoterpene fraction was characterized by a high percentage of linalool (50.60 %) such that this *Satureja* species can be classified as the linalool chemotype. Considering components with concentrations of about 2 % and above, the other major monoterpenes were α-terpineol (2.80 %), β-ocimene (2.25 %), β-pinene (1.96 %) and cis-linalool oxide (1.91 %). The main sesquiterpene component was germacrene D (10.63 %). Other sesquiterpenes present in appreciable amounts were α-cadinol (4.53 %), β-bourbenene (2.33 %), δ-cadinene (2.19 %), τ-cadinol (2.17 %), endo-1-bourbonanol (2.14 %) and β-caryophyllene (1.98 %). The aliphatic hydrocarbons represent only 7.23 % of the oil, of which 4.48 % was linoleic acid.

Table 1. Chemical composition of *Satureja biflora* leaf oil

No.	Compound	KI	Concentration %	Method of identification
	Monoterpenes			
1.	Sabinene	976	0.49	RI, GC-MS
2.	β-Pinene	978	1.96	RI, GC-MS
3.	**β- Ocimene**	**1050**	**2.25**	**RI, GC-MS**
4.	*cis*-Linalool Oxide	1072	1.91	RI, GC-MS
5.	*trans*-Linalool oxide	1089	1.49	RI, GC-MS
6.	**Linalool**	**1098**	**50.60**	**RI, GC-MS**
7.	Terpinen-4-ol	1177	0.52	RI, GC-MS
8.	**α-Terpineol**	**1191**	**2.80**	RI, GC-MS
	Total		**62.02**	
	Sesquiterpenes			
9.	**β- Bourbonene**	**1384**	**2.33**	**RI, GC-MS**
10.	β- Elemene	1393	0.72	RI, GC-MS
11.	β-Caryophyllene	1430	1.98	RI, GC-MS
12.	β- Farnesene	1458	0.59	RI, GC-MS
13	**Germacrene D**	**1487**	**10.63**	**RI, GC-MS**
14.	**δ-Cadinene**	**1524**	**2.19**	**RI, GC-MS**
15.	Nerolidol	1566	1.02	RI, GC-MS
16.	**Endo-1-bourbonanol**	**1570**	**2.14**	**RI, GC-MS**
17.	Caryophyllene oxide	1582	1.74	RI, GC-MS
18.	**τ-Cadinol**	**1641**	**2.17**	**RI, GC-MS**
19.	**α-Cadinol**	**1653**	**4.53**	**RI, GC-MS**
	Total		**30.04**	
	Aliphatic alcohols and acids			
20.	1-Octen-3-ol	979	1.13	RI, GC-MS
21.	Palmitic acid	1970	1.62	RI, GC-MS
22.	**Linoleic acid**	**2030**	**4.48**	**RI, GC-MS**
	Total		**7.23**	
	Total percentages		**99.29**	

The essential oil was evaluated for antimicrobial activity against pathogenic strains of gram-positive (*S. aureus*, *Bacillus ssp*) and gram-negative (*E. coli*, *P. aeruginosa*, *S. typhi*, *K. pneumoniae*, *P.* m*irabilis*) bacteria. It was found to be active against all the bacteria strains

except for *P. aeruginosa*. It also showed a marked anti-fungal activity against *C. albicans*. *P. aeruginosa* is less susceptible to the antimicrobial properties of essential oils than many bacteria and its tolerance is considered to be due to its outer membrane [16]. The activity of the oil varies with its concentration and kind of bacteria. These differences in the susceptibility of the test organisms to essential oil could be attributed to variation in the rate of monoterpene penetration through cell wall and cell membrane structures. The ability of essential oil to disrupt the permeability barrier of cell membrane structures and the accompanying loss of chemiosmotic control is the mostly likely source of its lethal action [17].

Although the concentrations of the oil were generally in the range of 100 times more than the standard antibiotic (Chloramphenicol), they showed marked antibacterial and antifungal activities as evidenced by their zones of inhibition (Table 2). This difference in concentrations of the essential oil and the standard antibiotic can be explained in terms of the fact that the active components in the oil comprise of only a fraction of the oil used. Therefore, the concentration of the active components could be much lower than the standard antibiotics used. Among the gram-negative bacteria, the oil was much active against *S. typhi*. The activity response to *S. typhi* was three times more at 75 x 10^2 μg as that of chloramphenicol (30 μg). The inhibition zones of the neat oil against gram-positive bacteria compared very well with those of the standard antibiotic. The oil showed relatively similar activity across the concentration range to *E. coli* and *P. mirabilis*.

Table 2A. Antimicrobial activity of the oil of *Satureja biflora*.

Micro-organism		Inhibition zone (mm)								
		Essential oil μg x 10^2								
Gram negative bacteria	Source	75.0	37.5	25.0	18.8	15.0	12.5	10.7	9.4	8.3
E. coli	ATCC 25922	15 ± 0	14 ± 0	13 ± 0.5	12 ± 2.0	11 ± 1.0	10 ±1.0	9 ± 0.5	8 ± 1.0	7 ± 0
S. typhi	[a]KEMRI	31 ± 0.5	15 ± 2.5	14 ± 1.0	12 ± 0.5	10 ± 0	9 ± 0.5	0	0	0
K. pneumoniae	[a]KEMRI	12 ± 0.5	11 ± 0.5	9 ± 0.5	0	0	0	0	0	0
P. mirabilis	[a]KEMRI	15 ± 0	14 ± 0.5	13 ± 0.5	12 ± 0	11 ± 0.5	10 ± 0	9 ± 1.0	8 ± 1.0	7 ± 0.5
P. aeruginosa	ATCC 27853	0	0	0	0	0	0	0	0	0
Gram positive bacteria										
S. aureus	ATCC 25923	24 ± 0	20 ± 0	14 ± 0.5	12 ± 1.0	11 ± 2.0	10 ± 0.5	10 ± 0.0	8 ± 0.5	0
Bacillus spp.	[a]KEMRI	34 ± 2	32 ± 1.0	22 ± 1.0	17 ± 1.5	12 ± 1.0	10 ± 1.0	9 ± 1.0	0	0
Fungus										
Candida albicans	[a]KEMRI	15 ± 0	12 ± 0	11 ± 0.5	10 ± 0	9 ± 0.5	7 ± 1.0	0	0	0

Table 2B. Antimicrobial activity and MIC for the essential oil and standard.

Micro-organism		Inhibition zone (mm)			
		STD[b]		MIC mg/mL	
Gram negative bacteria		30 μg	Control	EO[c]	STD[b]
E. coli	ATCC 25922	28 ± 1.5	0	83.3	25
S. typhi	[a]KEMRI	10 ± 1.0	0	125.0	25
K. pneumoniae	[a]KEMRI	25 ± 0	0	250.0	22.5
P. mirabilis	[a]KEMRI	8 ± 0	0	83.3	0
P. aeruginosa	ATCC 27853	0	0	0	0
Gram positive bacteria					
S. aureus	ATCC 25923	24 ± 1.0	0	93.8	31.3
Bacillus spp.	[a]KEMRI	26 ± 2.0	0	107.0	26.3
Fungus		Nyastatin 30 μg			
Candida albicans	[a]KEMRI	10 ± 0.5	0	125	0

a – Kenya Medical Research Institute, b – Chloramphenicol, c – Essential oil.

The minimum inhibition concentration (MIC) of oil for gram-negative bacteria ranged from 83.3 to 250 mg/mL and 93.8 to 107 mg/mL for gram-positive bacteria. The MIC for the fungus *C. albicans* is 125 mg/mL. The MIC values for chloramphenicol range from 22.5 to 31.3 mg/mL. In general, the oil showed greater antibacterial activity than antifungal activity (see Table 2).

Linalool, the major component in this study, has been found to have antimicrobial activity against various microbes except for *P. aeruginosa* [18]. Linalool is also known to inhibit spore germination and fungal growth. The inhibition of sporelation appeared to arise from respiratory suppression of aerial mycelia [19].

α-Terpineol, which occurs in appreciable amounts in this oil, has been reported to inhibit the growth of quite a number of bacteria and fungi [18, 20]. Other compounds present in the oil though in minor concentration have previously been known to possess antimicrobial properties. These include β-caryophyllene, a common sesquiterpene widely distributed in plants, which possesses anti-inflammatory and anticarcinogenic activities [21]. Its oxygenated form caryophyllene oxide is known to possess antimicrobial properties against a wide range of bacteria and fungi [22]. Terpinen-4-ol is present in a minor quantity of 0.52 %, but it is known to have a broad spectrum activity against micro-organisms [16].

Linoleic acid, which is one of the major constituents of the oil under study, is known as an essential fatty acid in human health. As a polyunsaturated fatty acid, linoleic acid helps lower the ratio of low density lipoproteins (LDLs) to high density lipoproteins (HDLs). It is also known to have a high antimicrobial activity [23].

AKNOWLEDGEMENTS

The authors are thankful to Prof. Uzi Ravid of Newe Yaár Centre in Israel for making available the GC-MS apparatus for this work.

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