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Chemical composition and antimicrobial activity of the essential oils of four *Ocimum* species growing in Tanzania

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ABSTRACT

As part of ongoing research on Tanzanian plants used as edibles or spices, six samples of essential oils from four *Ocimum* species (*O. basilicum, O. kilimandscharicum*, *O. lamiifolium, O. suave*) were analyzed by GC and GC–MS. Eighty-one compounds, corresponding to 81.1–98.2% of the chemical components of the oils, were identified. Major compounds were either phenyl propane derivatives or terpenoids, including methyl eugenol, 1,8-cineole, camphor, bornyl acetate, germacrene-D, E-myroxide, germacrene-B, caryophylene oxide and *p*-cymene. The oils were also evaluated for antimicrobial activity against eight bacterial strains and three fungi. The oil of *O. suave* (B) showed the strongest antibacterial activity; *O. suave* (A), O. *kilimandscharicum* and, *O. lamiifolium* were moderately active, while *O. basilicum* oil was weakly active. However, none of the oils was active against the fungi species. The study has shown that, *Ocimum* oils could potentially be used as anti-infective agents.

1. Introduction

In the third world and developing countries, and even in developed nations, food-borne diseases are a major dilemma. The consumption of foods contaminated with some microorganisms represents a serious health risk to humans. The subsistence and growth of microorganisms in foods may lead to spoilage, formation of toxins and quality deterioration of food products (Celiktas et al., 2007). Since ancient times, herbs and spices have been added to food to improve the flavour and organoleptic properties, but also as preservatives. In recent years, the essential oils and the herbal extracts from various species of edible and medicinal plants have attracted a great deal of scientific interest due to their potential as a source of natural agents to increase the safety and shelf life of foods and of natural biologically active compounds (Bozin, Mimica-Dukic, Simin, & Anackov, 2006). Especially, the antimicrobial activity of essential oils have formed the basis of many applications, including fresh and processed food preservation, pharmaceuticals, alternative medicine and natural therapies (Celiktas et al., 2007).

Guided by ethnobotanical literature and availability from natural sources, our main objective is to validate the use of selected African aromatic plants for their antimicrobial properties and to emphasise the need to promote their natural botanical resources in Africa as well as their uses worldwide. In the framework of our research on odoriferous Tanzanian plants and their biological activities, we report herein the analysis of essential oils from four *Ocimum* species.

The genus *Ocimum* (Lamiaceae) consists of about 50–150 species (Simon, Quinn, & Murray, 1990) with a large number of varieties containing both terpene and non-terpene constituents in their essential oils (Evans, 1995). People of the Haya tribe of North West Tanzania refer to plants of this genus, almost without exception, as "*Akashwagara*". Members of the genus find a number of uses in African traditional medicine (Chogo & Crank, 1981; Githinji & Kokwaro, 1993; Janssen, Scheffer, Ntezurubanza, & Baerheim-Svendsen, 1989). The Haya use the decoctions prepared from the leaves of the plants for relief of stomach upsets. Leaves from the plants in the genus are either, rubbed between the palms and inhaled, or are boiled and the hot vapour inhaled for treatment of blocked nostrils and bronchial catarrh (Kokwaro, 1993). Tanzanians, especially those living along the Indian Ocean coastal regions, use the plants to repel mosquitoes and as flavouring agents. Plants of the genus *Ocimum* are also reported for many biological activities, such as mosquito repellent and antimicrobial activity (Chogo & Crank, 1981; Githinji & Kokwaro, 1993; Kokwaro, 1993), insecticidal activity against crop pest insects (Bekele & Hassanali, 2001), antipyretic (Makonnen, Debella, Zerihun, Abebe, & Teka, 2003) and antioxidant activity (Javanmardi, Stushnoff, Locke, & Vivanco, 2003).

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As part of ongoing chemical and biological studies of essential oils from odoriferous Tanzanian plants, which are used as spices, six oil samples from aerial parts of four *Ocimum* species were investigated. The plants included *O. basilicum* Linn. (2 samples), *O. kilimandscharicum* Baker ex Gurke, O. *lamiifolium* Hochst ex Benth. and *O. suave* Willd (2 samples).

Ocimum basilicum is a stout, bushy aromatic herb with white flowers in loose racemes (Hutchinson & Dalziel, 1963). It is mostly cultivated for culinary purposes (Grayer et al., 1996) and it is known as "*Lutatambwa lunywamu*" by the Nyakyusa of the Mbeya region (Tanzania), where it is used as a tick repellent in chickens. The plant is found in European and African countries as a major essential oil crop, producing 42.5 tonnes of oil worldwide (Grayer et al., 1996). The essential oil from the plant, which is known as "sweet basil", is widely employed in flavouring and is one of the most studied oils of the genus.

Ocimum kilimandscharicum is an aromatic perennial woody shrub up to 2 m tall. In Rwanda the plant is used in traditional medicine to cure eye infections (Ntezurubanza, Scheffer, Looman, & Baerheim-Svendsen, 1984) and in Kenya it is used as a grain protectant against insect pests (Jembere, Obeng-Ofori, Hassanali, & Nyamasyo, 1995).

Ocimum lamiifolium is an erect, hairy perennial, several feet in height; flowers are white in long lax racemes. The Nyakyusa refer to the plant as "*Lufisu lunyambala*". In Ethiopia fresh leaves of *O. lamiifolium* are squeezed and sniffed to treat colds and coughs; and as an eye rinse for eye infections, while crushed leaves are used to arrest nose bleeding (Demissew & Asfaw, 1994).

O. suave is a branched erect, pubescent, aromatic shrub, reaching one metre in height, with dense spikes of small greenish white flowers. In Africa it is used as a hemorrhoids remedy and to perfume chewing tobacco and snuffs. Smoke from the burning plant is used as a mosquito repellent, and Maasai girls and warriors use the leaves and flowers as perfume. An infusion of leaves is used as a disinfectant and an insecticide and in Kenya, leaves are used as an insect repellent and grain protectant against insect pests (Kokwaro, 1993). In the literature on Tanzanian *O. suave* (Arusha, Northeastern Tanzania), there is only one study by Chogo and Crank (1981) that reported eugenol as the major volatile constituent of its essential oil.

To our knowledge, there are no previous studies on *O. basilicum*, *O. kilimandscharicum* and *O. lamiifolium* growing in Tanzania, while several studies have been published on the volatiles from *O. basilicum* (Githinji & Kokwaro, 1993; Lachowicz et al., 1998; Lewinson et al., 2000; Wan, Wilcock, & Coventry, 1998), *O. kilimandscharicum* (Githinji & Kokwaro, 1993; Jembere et al., 1995; Ntezurubanza et al., 1984) and *O. lamiifolium* (Tchoumbougnang et al., 2006) from different geographical origins.

We report herein the chemical composition and antimicrobial activity of essential oils from *O. basilicum, O. kilimandscharicum*, *O. lamiifolium* and *O. suave* growing in the Mbeya region, in the southwestern part of Tanzania.

2. Materials and methods

2.1. Plant material

Leaves and flowering tops of various *Ocimum* species were collected from the wild, in the Mbeya region, Tanzania. Locations and dates of collection are as detailed in Table 1. The plants were authenticated by comparison with herbarium specimens, by the staff of the Department of Botany, University of Dar es Salaam. Voucher specimens are deposited in the herbarium of the Department of Pharmacognosy, School of Pharmacy, Muhimbili University of Health and Allied Sciences. The materials were air-dried indoors prior to the isolation of essential oils.

2.2. Isolation procedure

The plant materials of *O. basilicum* sample A (420 g), *O. basilicum* sample B (205 g), *O. kilimandscharicum* (735 g), *O. lamiifolium* (107 g), *O. suave* sample A (940 g) and *O. suave* sample B (670 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 sulfate. They were stored at 4–6 °C prior to chemical analysis and antimicrobial studies. The colours of six samples of essential oils from *Ocimum* species varied from bright yellow (*O. basilicum*, *O. lamiifolium*) to almost colourless (*O. kilimandscharicum*). It is noteworthy that a considerable amount of essential oil from *O. kilimandscharicum* underwent crystallization, forming colourless fragrant crystals. The yields of oils ranged from about 0.5% (*O. basilicum* B) to about 4% (*O. basilicum* A); those of *O. basilicum* A, *O. lamiifolium* and *O. kilimandscharicum* were reasonably high (4.05%, 3.3% and 3.13%, respectively). Table 1 shows the yield of the various oils under investigation.

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, at a flow rate of 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). Helium (2 ml/min) was used as carrier gas. The temperature gradient was programmed from 60 °C to 280 °C, at 3 °C/min. Helium was used, at a flow rate of 1 ml/min. Split ratio, 1:10.

Table 1
Oil yield of investigated *Ocimum* species.

	Plant species	Voucher specimen number	Local name	Collection location (District)	Date of collection	Oil yield (% v/w)
I	*O. basilicum* A	EO-015	Lutatambwa lunywamu	Lema (Rungwe)	March, 2000	4.05
II	*O. basilicum* B	EO-063	Lutatambwa lunywamu	Utengule (Mbeya)	March, 2000	0.54
III	*O. kilimandscharicum*	EO-049	–	Kawetere (Mbeya)	July, 2000	3.13
IV	*O. lamiifolium*	EO-071	Lufisu lunyambala	Mwakaleli (Rungwe)	July, 2000	3.3
V	*O. suave* A	EO-025	–	Lwangwa-Manow (Rungwe)	March, 2000	1.15
VI	*O. suave* B	EO-045	–	Uyole (Mbeya)	March, 2000	1.01

Table 2
The chemical composition (%) of essential oils of various *Ocimum* species.

No	Compounds[a]	Essential oils[b]							
		I	II	III	IV	V	VI	RI	Identification[c]
1	Tricyclene	–	–	0.2	0.3	–	–	927	1,2,3
2	α-Thujene	0.33	–	–	0.46	0.34	–	930	1,2,3
3	α-Pinene	4.39	1.39	1.07	5.7	2.17	tr	939	1,2,3
4	Camphene	–	0.54	5.36	5.91	–	tr	954	1,2,3
5.	Sabinene	–	–	tr	0.5	0.18	–	975	1,2,3
6.	β-Pinene	8.15	–	0.87	4.2	–	tr	979	1,2,3
7	1-Octen-3-ol	–	1.00	–	0.2	0.14	tr	979	1,2,3
8	Myrcene	1.06	–	1.09	0.2	–	–	991	1,2,3
9	3-Octanol	–	tr	–	–	0.09	–	–	1,2,3
10	α-Phellandrene	–	–	0.41	–	–	–	1003	1,2,3
11	α-Terpinene	–	–	0.49	–	–	–	1017	1,2,3
12	*p*-Cymene	–	1.24	–	11.4	0.76	tr	1025	1,2,3
13	Limonene	2.30	1.86	7.13	–	0.41	tr	1029	1,2,3
14	β-Phellandrene	–	–	–	2.94	–	–	1030	1,2,3
15	1,8-Cineole	54.3	3.10	14.3	–	0.19	tr	1031	1,2,3
16	*cis*-ocimene	–	–	0.15	–	0.24	–	1037	1,2,3
17	*trans*-β-ocimene	–	–	1.39	–	0.11	–	1050	1,2,3
18	γ-Terpinene	–	–	0.87	–	0.35	–	1060	1,2,3
19	*cis*-sabinene hydrate	1.91	–	0.24	–	–	–	1070	1,2,3
20.	trans-linalool oxide	–	–	–	0.10	–	0.17	1073	1,2,3
21	*cis*-linalool oxide	–	–	–	0.15	–	0.15	1087	1,2,3
22	α-Terpinolene	–	–	2.23	–	0.15	–	1089	1,2,3
23	Rosefuran	–	–	–	–	–	0.18	–	3
24	Linalool	–	–	1.6	2.99	–	1.21	1097	1,2,3
25	*trans*-sabinene hydrate	tr	–	–	–	–	–	1098	1,2,3
26	1-Octen-3-yl acetate	–	–	–	1.35	–	–	1113	1,2,3
27	α-Camphonelal	–	–	0.35	–	–	–	1126	1,2,3
28	*cis*-Limonene oxide	0.16	–	–	–	–	–	1137	1,2,3
29	*cis*-Verbenol	–	–	–	–	0.23	–	1141	1,2,3
30	E-myroxide	–	19.6	–	–	–	–	1145	1,2,3
31	Camphor	–	–	52.4	0.53	–	–	1146	1,2,3
32	Borneol	–	–	0.67	3.68	–	–	1169	1,2,3
33	Rosefuran epoxide	–	6.03	–	–	–	–	1177	1,2,3
34	Terpinen-4-ol	1.56	–	3.24	0.71	0.14	–	1177	1,2,3
35	*p*-Cymen-8-ol	–	–	tr	–	0.16	–	1183	1,2,3
36	Cryptone	–	–	–	1.67	–	–	1186	1,2,3
37	α-Terpineol	6.6	–	1.01	0.81	0.18	–	1189	1,2,3
38	Myrtenol	–	–	0.66	–	–	–	1196	1,2,3
39	Mytenal	–	–	–	0.16	–	–	1196	1,2,3
40	Verbenone	–	–	–	–	0.1	–	1205	1,2,3
41	*trans*-carveol	0.36	–	–	–	0.08	–	1217	1,2,3
42	Cuminal	–	–	–	0.39	–	–	1242	1,2,3
42	Carvone	0.24	–	–	–	–	–	1243	1,2,3
43	Bornyl acetate	–	–	–	30.3	–	–	1289	1,2,3
45	*p*-Cymen-7-ol	–	–	–	0.72	–	–	1291	1,2,3
46	δ-Elemene	–		–	–	0.70	–	1338	1,2,3
47	α-Cubebene	–	0.82	–	–	0.08	0.08	1351	1,2,3
48	Eugenol	–	–	–	–	0.12	0.43	1359	1,2,3
49	α-Copaene	3.01	7.50	–	–	0.58	0.85	1377	1,2,3
50	β-Bourbonene	0.23	–	–	0.65	0.36	0.46	1388	1,2,3
51	β-Cubebene	–	1.79	–	–	0.15	–	1388	1,2,3
52	β-Elemene	–	–	–	–	0.49	–	1391	1,2,3
53	Methyl eugenol	–	–	–	–	–	82.7	1404	1,2,3
54	β-Caryophyllene	1.34	2.53	1.12	0.52	5.13	–	1419	1,2,3
55	β-Gurjunene	0.26	–	–	–	–	–	1434	1,2,3
56	γ-Elemene	–	–	–	–	0.51	–	1437	1,2,3
57	α-Humulene	3.55	6.28	–	–	2.62	–	1455	1,2,3
58	*trans*-β-farnesene	–	–	0.82	–	–	–	1457	1,2,3
59	*cis*-muurola-4(14)5-diene	–	–	–	–	0.09	–	1467	1,2,3
60	Germacrene-D	0.21	–	0.46	–	29.2	–	1485	1,2,3
61	β-Selinene	0.24	1.71	–	–	2.76	–	1490	1,2,3
62	α-Selinene	–	3.57	–	–	1.44	–	1498	1,2,3
63	Bicyclogermacrene	–	–	–	–	1.60	–	1500	1,2,3
64	α-Muurolene	–	–	–	–	1.2	–	1500	1,2,3
65	β-Bisabolene	–	–	–	–	–	0.64	1506	1,2,3
66	Germacrene-A	–	–	–	–	0.22	–	1509	1,2,3
67	γ-Cadinene	–	–	–	–	1.81	–	1514	1,2,3
68	7-Epi-α-selinene	–	–	–	–	1.01	–	1522	1,2,3
69	δ-Cadinene	0.81	0.61	–	–	3.04	0.09	1523	1,2,3
70	α-Cadinene	–	–	–	–	0.16	–	1539	1,2,3
71	Germacrene-B	–	–	–	–	14.0	–	1561	1,2,3
72	Palustrol	0.26	–	1.16	–	–	–	–	3
73	Germacrene-D-4-ol	–	–		–	1.28	–	1576	1,2,3

(*continued on next page*)

Table 2 (*continued*)

No	Compounds[a]	Essential oils[b]							
		I	II	III	IV	V	VI	RI	Identification[c]
74	Spathulenol	–	–		4.08	0.50	–	1578	1,2,3
75	Caryophyllene oxide	0.76	11.4	0.13	1.87	1.43	4.13	1583	1,2,3
76	Humulene epoxide II	–	11.0	–	0.65	–	0.37	1608	1,2,3
77	10-Epi-γ-eudesmol	–	–		–	0.39	–	1624	1,2,3
78	γ-Eudesmol	–	–	–	–	2.91	–	1632	1,2,3
79	τ-Cadinol	–	–	–	–	0.1	–	1640	1,2,3
80	β-Eudesmol	–	–	–	–	3.11	–	1651	1,2,3
81	α-Cadinol + α-eudesmol	–	–	–	–	8.11	–	1654	1,2,3
	Total	91.99	82.11	98.2	83.14	92.01	91.41		

[a] Compounds listed in order of elution.
[b] I = *O. basilicum* A; II = *O. basilicum* B; III = *O. kilimandscharicum*; IV = *O. lamiifolium*; V = *O. suave* A; VI = *O. suave* B.
[c] 1 = Retention time; 2 = Kovat's retention indices; 3 = mass spectra.

2.5. Identification of components

The compounds were identified by comparison of their retention indices (KI) (Van den Dool & Kratz, 1963), retention times (RT) and mass spectra with those of authentic samples and/or the NIST/NBS, Wiley libraries spectra and the literature (Adams, 1995; Massada, 1976). The percentage composition of the essential oils is based on computer-calculated peak areas without correction for FID response factor.

2.6. Antimicrobial activity

Antimicrobial activity of the essential oils against bacteria and fungi was determined by using the agar dilution technique (Janssen, Scheffer, & Baerheim Svedsen, 1987). The microorganisms included four Gram-positive bacteria: *Staphylococcus aureus* (ATCC 25923), *Staphylococcus epidermidis* (ATCC 12228), *Streptococcus mutans* (clinical isolates) and *Streptococcus viridans* (clinical isolates); the last two are oral pathogens. Also, four Gram-negative bacteria: *Escherichia coli* (ATCC 25922), *Enterobacter cloacae* (ATCC 13047), *Klebsiella pneumoniae* (ATCC 13883) and *Pseudomonas aeruginosa* (ATCC 227853) and three species of *Candida*: *C. albicans* (ATCC 10231), *C. tropicalis* (ATCC 13801) and *C. glabrata* (ATCC 28838). Standard antibiotics (netilmicin, and amoxicillin with clavulanic acid) were used as positive controls for bacteria, 5-flucytocine and amphotericin B as a positive control for *Candida* and sanguinarine, an alkaloid, as a positive control for oral pathogens. Technical data have been described previously (Vagionas et al., 2007). Minimum inhibitory concentrations (MICs) were determined for all the samples and the standard pure compounds, under identical conditions, for comparison purposes.

3. Results and discussion

The chemical composition analysis of all studied samples is shown in Table 2. In total, 81 components were identified, representing 82–98% of all components in the oils. The components varied between the oils and only α-pinene and caryophyllene oxide were identified in varied amounts in all the studied samples of *Ocimum* oils.

Twenty-three and nineteen compounds were identified in oils of the two samples of *O. basilicum*, corresponding to 91.99% and 82.11% of the chemical components for samples A and B, respectively. The major components for sample A were, 1,8-cineole (54.3%), β-pinene (8.15%) and α-terpineol (6.6%). Major components for sample B were E-myroxide (19.6%), caryophyllene oxide (11.4%), humulene epoxide II (11.0%), α-copaene (7.5%), v-humulene (6.28%), and rosefuran epoxide (6.03%). Components identified in appreciable amounts in both samples included 1,8-cineole, α-pinene, limonene, α-copaene, β-caryophyllene and α-humulene. However, the two oils differed in that some of the components present in one of the oils in appreciable amounts were absent in the other oil. For example E-myroxide, a major component of sample B, was absent in sample A while β-pinene and and α-terpineol, present in sample A, in appreciable amounts, were absent in sample B. In addition humulene epoxide II, rosefuran epoxide, and selinene, which were identified in sample B in appreciable amounts, were absent in sample A. The two samples of *O. basilicum* could actually be two different chemical races. This is in line with previous studies which have described various chemotypes of *O. basilicum* and other *Ocimum* species, such as methyl cinnamate, linalool, methyl chavicol and methyl eugenol chemotypes (Simon et al., 1990; Vina & Murillo, 2003).

Phenyl propane derivatives previously reported in the essential oils of *O. basilicum*, including methyl chavicol (Lachowicz et al., 1998; Wan et al., 1998), methyl eugenol (Lachowicz et al., 1998; Lewinson et al., 2000) methyl cinnamate (Simon et al., 1990), and eugenol (Lachowicz et al., 1998; Lewinson et al., 2000), were absent in both oil samples of *O. basilicum*. Linalool, a monoterpene alcohol, previously reported in *O. basilicum* oils (Grayer et al., 1996; Keita, Vincent, Schmit, & Belanger, 2000; Simon et al., 1990; Wan et al., 1998), was not detected in either oil sample. The compound 1,8-cineole, previously reported (Grayer et al., 1996), was the major component of sample A and was found in appreciable amounts, in sample B. Another compound, limonene, also previously identified in the oil (Grayer et al., 1996), was present to the extent of 2.30% and 1.86% in samples A and B, respectively.

Twenty-eight compounds, corresponding to 98.2% of the chemical components in the oil from *O. kilimandscharicum*, were identified. The major components of the oil were mainly monoterpenoids and included camphor (52.4%), 1,8-cineole (14.3%), limonene (7.13%) and camphene (5.36%). Camphor and eugenol chemotypes have been described for this plant species (Ntezurubanza et al., 1984). Studies on experimental plants in the USA showed that the fresh flowering herb yielded 0.5–1% of essential oil, including camphor, that could be separated easily from the oil (Ntezurubanza et al., 1984). As has already been noted, in the Section 2.2, characteristic colourless crystals were observed in the oil of *O. kilimandscharicum*. These crystals have been subjected to GC–MS analysis and were determined to be pure camphor, which has been the major constituent (52.4%) of the oil. According to this finding, the oil could be classified as a camphor chemotype, as it has been described by Ntezurubanza et al. (1984). The oil also contained 1,8-cineole in appreciable amounts, a compound, which was previously identified in *O. kilimandscharicum* growing in Rwanda (Ntezurubanza et al., 1984). Limonene, a third major compound in this oil, was also reported in appreciable amounts in the oil from Rwanda.

In the case of *O. lamiifolium*, twenty-eight compounds corresponding to 83.1% of all the components, were identified and were, mainly monoterpenoids. The major compounds included bornyl

acetate (30.3%), *p*-cymene, (11.4%), camphene (5.91%) and α-pinene (5.7%). A previous study by Tchoumbougnang et al. (2006) reported sabinene as the major compound identified in the essential oils of *O. lamiifolium* growing in tropical Africa.

In *O. suave*, forty eight and twenty compounds were identified, corresponding to 92% and 91.4% of the total chemical components in samples A and B, respectively. Sample A consisted mainly of sesquiterpenoids, which included germacrene-D (29.2%), germacrene-B (14.0%), α-cadinol and α-eudesmol (8.11%) and β-caryophyllene (5.13%). The only monoterpenoid found in sample A, in an appreciable amount, was α-pinene (2.17%). The oil of sample B was made up mainly of a phenyl propane compound, methyl eugenol (82.7%). This compound has also been reported as the main component in oils of other Lamiaceae species, such as *Melaleuca ericifolia* and *O. sanctum* (Farag et al., 2004). Other components in sample B included caryophylene oxide (4.13%) and linalool (1.21%). The two oil samples from *O. suave* were similar in that both contained very little amounts of monoterpenoids. Eugenol, which was previously reported as the major component of the oil from *O. suave* growing in Arusha (Chogo & Crank, 1981), was not identified in the present samples, while, instead of eugenol, methyl eugenol was determined as the major component of sample B in our study.

Generally, the observed differences in chemical composition of the various oils, when compared with those reported in previous studies, could be due to a number of factors. Such factors may include differences in climatic conditions, geographical locations, season at the time of collection, stage of development, processing of plant materials before extraction of the oils and occurrence of chemotypes.

The oils were also evaluated for antimicrobial activity against four Gram-positive bacteria (*S. aureus*, *S. epidermidis*, *S. mutans* and *S. viridans*), four Gram-negative bacteria (*P. aeruginosa*, *E. cloacae*, *K. pneumoniae* and *E. coli*) and three species of the yeast *Candida* (*C. albicans, C. tropicalis*, and *C. glabrata*). Oils from the two samples of *O. suave* were the most active and those of *O. basilicum* samples showed weaker activities (Table 3). However, none of the oils exhibited activity against the tested human pathogenic fungi.

Oils from *O. basilicum* showed a weak activity (MIC 3.14–12.5 mg/ml) against six tested bacteria, but were inactive on *Streptococcus viridans* and *S. mutans*. Between the two samples of *O. basilicum*, sample B was relatively more active than was sample A. The essential oil of *O. basilicum* which is commonly known as basil oil, has been reported previously to have antimicrobial activity on a number of Gram-negative and positive bacteria and fungi, some of which are food spoilage microorganisms (Koga, Hirota, & Takumi, 1999; Lachowicz et al., 1998; Wan et al., 1998; Wannissorn, Jarikasem, Siriwangchai, & Thubthimthed, 2005). However, the two samples of *O. basilicum* used in this study were the least active. *Ocimum basilicum* exists in a number of chemotypes including linalool, methylchavicol, geraniol, methyl eugenol and eugenol (Grayer et al., 1996) and these particular oils did not belong to any of these chemotypes, and this may be the reason for the observed weak activity. The major compound in the oil from *O. basilicum* A, 1,8-cineole, also exhibited a weak activity on the test microorganisms (MIC 2.0–9.5 mg/ml).

The oil of *O. kilimandscharicum* showed a moderate activity on six tested bacteria (MIC 1.55–3.35 mg/ml), while *O. lamiifolium* showed a moderate activity against *S. aureus, S. epidermidis,* and *P. aeruginosa* (MIC 1.75–2.75 mg/ml) and a weak activity against *E. cloacae, K. pneumoniae* and *E. coli*. The level of activity of *O. lamiifolium* oil was comparable with that of its major constituent, bornyl acetate. Hence, the observed activity could mainly be due to this component.

The oil of *O. suave* (B) showed activity on all tested bacteria (MIC 0.05–1.45 mg/ml); this could be attributed to its major component, methyl eugenol (82.7%). A previous study by Farag et al. (2004) on essential oils of four *Melaleuca* species revealed that the oil from *M. ericifolia*, which had methyl eugenol as the major component (96.8%) exhibited the highest antimicrobial activity when compared with other *Melaleuca* species investigated. This suggests that the antimicrobial effect is most likely due to methyl eugenol. *Ocimum suave* (A), though devoid of methyl eugenol, was found to be moderately active on all the tested bacteria (MIC 1.19–3.10 mg/ml). *O. suave* sample B was several times more active on *S. aureus* when compared to the Arusha sample previously studied by Chogo and Crank (1981). However, the oil from sample B was less active on *E. coli* when compared to the oil from Arusha plants. A previous study also showed that, the essential oil from Rwandese

Table 3
Antimicrobial activity (MIC mg/ml) of the studied *Ocimum* species essential oils and their main components.

Tested samples	Microorganisms										
	S. aureus	*S. epidermidis*	*P. aeruginosa*	*E. cloacae*	*K. pneumoniae*	*E. coli*	*S. mutans*	*S. viridans*	*C. albicans*	*C. tropicalis*	*C. glabrata*
O. basilicum (A)	12.5	11.5	6.84	6.85	5.30	4.25	–	–	–	–	–
O. basilicum (B)	10.7	9.45	5.37	4.21	4.79	3.14	–	–	–	–	–
O. kilimandscharicum	2.85	3.35	2.50	2.97	2.70	1.55	–	–	–	–	–
O. lamiifolium	1.95	1.75	2.75	4.10	3.50	4.90	–	–	–	–	–
O. suave (A)	1.35	1.28	2.37	3.10	2.75	1.95	1.65	1.19	–	–	–
O. suave (B)	0.05	0.90	1.20	1.37	1.18	1.45	0.19	0.92	–	–	–
Borneol	1.25	1.57	2.50	4.20	3.75	4.50	–	–	–	–	–
Bornyl acetate	1.95	1.75	2.30	3.75	3.25	4.88	–	–	–	–	–
Camphor	2.70	1.95	2.80	2.75	3.24	1.33	–	–	–	–	–
Caryophyllene oxide	0.073	0.90	0.87	2.43	1.23	>6.40	0.25	0.75	–	–	–
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	–	–	–
α-Pinene	7.50	9.50	6.00	8.00	15.0	2.00	–	–	4.00	4.00	2.00
β-Pinene	12.00	16.00	>20	>20	>20	9.75	–	–	–	–	–
Spathulenol	1.35	1.50	>20	>20	>20	8.50	–	–	–	–	–
trans-caryophyllene	>20	>20	>20	>20	>20	>20	–	–	–	–	–
Amoxycillin + clavulanic acid	$3X10^{-3}$	$3X10^{-3}$	$3.1X10^{-3}$	$4.2X10^{-3}$	$4.8X10^{-3}$	$5X10^{-3}$	–	–	–	–	–
Amphotericin B	–	–	–	–	–	–	–	–	$1X10^{-3}$	$0.5X10^{-3}$	$0.4X10^{-3}$
5-Flucytocine	–	–	–	–	–	–	–	–	$0.1X10^{-3}$	$1X10^{-3}$	$10X10^{-3}$
Netilmicin	$4X10^{-3}$	$4X10^{-3}$	$8.8X10^{-3}$	$8X10^{-3}$	$8X10^{-3}$	$10X10^{-3}$	–	–	–	–	–
Sanguinarine	–	–	–	–	–	–	0.015	0.015	–	–	–

plants, evaluated by bioautography agar overlay, possessed antimicrobial activity (Janssen et al., 1989).

4. Conclusions

This study has shown that essential oils from all four studied *Ocimum* species could be used as potential antimicrobial agents, as well as accordingly, as food preservatives against food spoilage microorganisms.

Besides, as the yields of the oils of *O. basilicum* (Sample A), *O. kilimandscharicum* and *O. lamiifolium* are reasonably high, the plants have the potential for a further large-scale cultivation and possible source of income for farmers, especially in a developing African country, like Tanzania.

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