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ANTIMICROBIAL ACTIVITY OF ESSENTIAL OILS OF *OCIMUM GRATISSIMUM* L. FROM DIFFERENT POPULATIONS OF KENYA

Lexa G. Matasyoh[a,*], Josphat C. Matasyoh [b], Francis N. Wachira [c], Miriam G. Kinyua [d], Anne W. Thairu Muigai [a] and Titus K. Mukiama [e]

[a]Department of Botany, Jomo Kenyatta University of Agriculture and Technology, P.O. Box 62000, Nairobi, Kenya.
[b]Department of Chemistry, Egerton University, P.O. Box 536, Njoro, Nakuru, Rift Valley, Kenya.
[c]Department of Biochemistry, Egerton University, P.O. Box 536, Njoro, Nakuru, Rift Valley, Kenya.
[d] Kenya Agricultural Research Institute, P.O. Njoro, Nakuru, Kenya.
[e] Department of Botany, Nairobi University, P.O. Box 29053, Nairobi, Kenya.
***E-mail:** lexa111@hotmail.com

Abstract

Hydro-distilled volatile oils from the leaves of *Ocimum gratissimum* L. (Lamiaceae) of 13 populations of different silvicultural zones were evaluated for antimicrobial activity against Gram positive (*Staphylococcus aereus, Bacillus* spp.) and Gram negative (*Escherichia coli, Pseudomonas aeruginosa, Samonella typhi, Klebisiella pneumoniae, Proteus mirabilis*) bacteria and a pathogenic fungus, *Candida albicans*. All the essential oils are active to the tested microbiles with different strength. The highest antimicrobial activity against Gram positive bacteria (*Staphylococcus aureus*) and Gram negative bacteria (*Pseudomonas aeruginosae* and *Proteus mirabilis*) was observed from the eastern Kenya (Meru) oil. Meru oil was the best and its effectiveness was consistent on nearly all the microbes tested. The oil from the plant growing in the coastal region of Kenya (Mombasa) showed the best effect only on Gram negative bacteria (*Escherichia coli* and *Proteus mirabilis*). Both oils (Meru and Mombasa) were dominated by monoterpenes accounting for 92.48 % and 81.37 % respectively. The monoterpene fraction was characterized by a high percentage of eugenol (68.8 %) for Meru oil and 74.10 % for Mombasa oil. The other major monoterpene was methyl eugenol (13.21 %). Camphor (0.95 %) was observed only in the Meru oil. (*Cis*)-Ocimene, (*trans*)-ocimene and β-pinene were present in both Meru and Mombasa oils. The sesquiterpenes present in fairly good amounts in both oils were germacrene D and (*trans*)-caryophyllene. The minor sesquiterpenes were α-farnesene (0.85 %) and β-bisabolene (0.74 %) which were present in the Meru oil only.

Key words: Antimicrobial activity, *Ocimum gratissimum* L., Eugenol, Essential oil

Introduction

Ocimum gratissimum L. (Lamiaceae) is an important herbal medicinal plant not only in Kenyan communities but also in the sub-Saharan Africa. The leaves are rubbed between the palms and sniffed as remedy for blocked nostrils (Kokwaro, 1993) and also used for abdominal pains, sore eyes, ear infections, coughs, barrenness, fever, convulsions, and tooth gargle, regulation of menstruation and prolapse of the rectum (Watt and Breyer-Brandwijk, 1962; Harjula, 1980; FAO, 1986 and Kokwaro, 1993).

Several species and varieties of plants belonging to the genus *Ocimum* have been reported to yield oil of diverse nature, commonly known as basilica oils. Lemos et al., (2005), Adebolu et al. (2005) and Matasyoh et al., (2007) reported some chemical compounds and active ingredients from these plants such as eugenol, linalol, methyl cinnamate, camphor and thymol. Various species of *Ocimum* have been reported for their numerous medical uses (Mshana et al., 2000).

The present work reports the antimicrobial activity of the essential oil of *Ocimum gratissimum* L., and the chemical composition of the essential oils of the most active antimicrobial populations growing in different parts of Kenya.

Materials and Methods

Plant material

The leaves of *O. gratissimum* L. were collected from wild populations during the pre flowering season in August, 2005 from 13 districts of Kenya. Voucher specimens (voucher No. 805LG) were deposited at the Department of Botany, Egerton University.

Essential oil distillation

Fresh leaves were subjected to hydro-distillation in a modified Clevenger-type apparatus for at least 4 hrs according to the British pharmacopoeia. The essential oil (EO) was obtained in a yield of w/w after drying over anhydrous sodium sulphate (Na_2SO_4). The oil was stored in a sealed glass vial (Bijoux bottle) at 4 °C.

Antimicrobial screening

The micro-organisms used were *Staphylococcus aureus* ATCC25923, *Pseudomonas aeruginosaea* ATCC 27853, *Escherichia coli* ATCC 25922 and clinical isolates: *Salmonella typhi, Klebsiella pneumoniae*, *Proteus mirabilis, Bacillus* spp. and *Candida albicans*. The agar disc diffusion method was employed for the screening of antimicrobial activities of the EO according to the National Committee of Clinical Laboratory Standards (NCCLS, 1999). The test was performed in sterile Petri-dishes (90 mm diameter) containing solid and sterile Mueller-Hinton agar (MHA) medium for the growth of bacteria and Sabouraud dextrose agar (SDA) for the growth of fungi. The oils absorbed on sterile paper discs (10 µl per Whatman disc of 6 mm diameter) were placed on the surface of the media previously inoculated with 0.1 ml of microbial suspension (1 µg per Petri-dish). The microbial suspension, freshly grown in Nutrient Broth was standardized to a cell density of 1.5×10^8 (Mc Farland No. 0.5). The positive antibacterial and antifungal activities were established by the presence of measurable zones of inhibition after 24 hrs of incubation at 37°C. Chloramphenical and Nystatin were used as antibiotic and antifungal references respectively. All tests were performed in duplicate.

GC, GC-MS Analysis

Gas chromatographic (GC), and GC-Mass spectrometry (MS) was carried out on Mombasa and Meru oils only. Gas chromatographic (GC) analyses of essential oils diluted in methyl tert-butyl ether (MTBE) were performed on an Agilent GC-MSD apparatus equipped with an Rtx-5SIL MS ('Restek') (30 m x0.25 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 °C and the transfer line at 280 °C. The column was maintained at 50 °C for 2 min and then programmed to 260 °C at 5 °C / min and held for 10 min at 260 °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 reported (Adams, 1995) and supplemented by Wiley and Quadlib 1607 GC-MS libraries.

Minimum inhibitory concentration (MIC)

Serial dilutions of the EO were done using 10 % TWEEN 80 in distilled sterile water. Water was used as a control. The MIC was considered the lowest concentration of the sample that no visible growth was observed. Visible growth (the positive antibacterial and antifungal activities) was established by the presence of measurable zones of inhibition after 24 hrs of incubation at 37 °C.

Results

Essential oil distillation

The percentage yields (w/w) of the EOs obtained from the 13 populations is shown in Table 1

Table 1: Yields of essential oil from *Ocimum gratissimum* L.

Population		% yield weight/weight
1	Njoro I	0.78
2	Njoro II	0.12
3	Mill House II	0.79
4	Kericho	0.21
5	Mill House I	0.83
6	Kabarnet	0.16
7	Meru	0.49
8	Kakamega	0.40
9	Thika	0.52
10	Taita taveta	0.74
11	Mombasa	1.40
12	Kisumu	1.24
13	Nyeri	0.40

Antimicrobial activity

The EOs were evaluated for antimicrobial activity against pathogenic strains of Gram positive (*Staphylococcus aereus, Bacillus spp*.) and Gram negative (*Escherichia coli, Pseudomonas aeruginosa, Samonella typhi, Klebisiella pneumoniae, Proteus mirabilis*) bacteria and a pathogenic fungus *Candida albicans*. The EOs are active against all the bacterial strains (Table 2) but the effectiveness of the oils is different from one population to another (Table 3). The reference antibiotic showed no activity in the three Gram negative bacteria among the five tested. It showed significant activity only on *E. coli* and *K. pneumoniae* (Table 2).

The EOs showed significant activity on all the Gram negative bacteria including those (*Pseudomonas aeruginosa, Samonella typhi,* and *Proteus mirabilis*) with resistance to reference antibiotic.

Chemical composition of the essential oils

As shown in Table 4, constituents in the sample from Eastern Kenya (Meru) and the coastal region of Kenya (Mombasa) were identified by GC-MS analysis. The oil was dominated by eugenol, which accounted for 68.81 % (Meru) and 74.10 % (Mombasa) respectively. Methyl eugenol (13.21%) was found only in the sample from Meru.

Minimum inhibitory concentration (MIC)

The minimum inhibition concentration tested on the Eastern Kenya oil (Meru) showed that dilution of the EO affected its activity on some microbes (Matasyoh et al., 2007). That is, the activity of the oil varied with its concentration and kind of bacteria being treated. There was also a marked antifungal activity against *Candida albicans.* This antifungal activity seems not to be affected by the dilution of the oil. Among the Gram negative bacteria, the oil was very active against *E. coli*. The activity response to *E. coli* was more or less the same at (75 $\times 10^2$ µg) as that of chloramphenicol (30 µg). The minimum inhibition concentration (MIC) for the oil was greater than that of reference antibiotic.

The MIC of oil ranged from 107 to 750 mg/ml for Gram negative bacteria and 93.7 to 150 mg/ml for Gram positive bacteria. The MIC of oil for the fungus *C. albicans* was 50 mg/ml. The MIC of chloramphenical ranged from 22.5 to 31.3 mg/ml for both Gram negative and Gram positive microbes.

Discussion

The EO percentage yield of leaves from different population ranged from 0.16 -1.40 % weight/weight (w/w) Table 1. It was observed that the thicker and more velvet the leaves felt, the less percentage oil yield.

Table 2: Antimicrobial activity of the essential oil of *Ocimum gratissimum* L. from 13 different ecological zones (populations) of Kenya

Micro organism	**INHIBITION ZONE**													
	A	**B**	**C**	**D**	**E**	**F**	**G**	**H**	**I**	**J**	**K**	**L**	**M**	**N**
Gram positive														
Staphylococcus aureus ATCC25923	20.0±0	20.0±0	13.5±0.7	20.0±0	15.0±0	21.0±1.4	26.6±5.7	22.0±4.0	25.3±1.2	19.3±1.2	25.3±4.6	15.0±1.4	15.0±1.4	24.5±0.7
Bacillus spp. (Clinical isolate)	21.0±1.4	20.5±0.7	16.0±1.4	22.0±0	16.5±0.7	25.0±1.4	22.3±1.5	17.3±2.5	21.7±1.5	16.7±1.2	21.7±1.5	18.3±2.5	19.0±1.7	30.0±0
Gram negative														
Escherichia coli ATCC25922	14.0±0	14.0±0	10.5±0.7	18.5±0.7	10.5±0.7	18.5±0.7	21.7±2.1	14.3±0.6	21.7±5.0	16.3±4.9	19.3±3.8	24.0±0	25.0±1.4	32.5±2.5
Pseudomonas aeruginosae ATCC27853	R	8.0±0	R	7.5±0.7	R	8.0±1.4	9.0±2.6	9.0±0	8.0±1.7	7.0±0	9.3±3.2	7.6±0.6	7.3±0.6	R
Klebsiella pneumoniae (Clinical isolate)	12.0±0	11.5±0.7	9.0±1.4	13.5±0.7	9.0±1.4	14.5±2.1	18.0±2.8	12.0±1.4	15.5±0.7	13.5±2.1	25.0±4.2	14.0±3.5	15.0±2.6	27.3±1.2
Proteus mirabilis (Clinical isolate)	11.5±0.7	11.5±0.7	8.0±0	15.0±0	8.5±0.7	16.0±2.8	16.0±1.7	11.3±0.6	12.7±2.1	10.3±1.2	13.0±1.7	13.3±1.2	15.3±2.5	R
Salmonella typhi (Clinical isolate)	13.5±0.7	13.5±0.7	10.5±0.7	18.0±2.8	10.0±0	18.5±2.1	20.5±0.7	13.5±0.7	17.5±0.7	10.5±0.7	18.5±0.7	20.0±1.7	18.6±2.3	R
Fungi														
Candida albicans (Clinical isolate)	*	*	*	*	*	*	*	*	*	*	*	*	*	R

Key: A=Njoro I; B=Njoro II; C=Mill House II; D=Kericho; E=Mill House I; F=Kabarnet; G=Meru; H=Nyeri; I=Kisumu; J=Kakamega; K=Thika; L=Taita taveta; M=Mombasa; N=Chloramphenical *The organism was highly susceptible to the plant extract. MIC to be done. Clinical isolate from Kenya Medical Research Institute (KEMRI).

Table 3: Variation in anti microbial activity among locally collected populations of *O. gratissimum* L. in Kenya

	Inhibition Zone (mm)							
Population	*E. coli* (Gram –ve)	*K. pneumoniae* (Gram –ve)	*S.typhi* (Gram –ve)	*P. aeruginosae* (Gram –ve)	*P. mirabilis* (Gram –ve)		*S. aureus* (Gram +ve)	*Bacillus spp* (Gram +ve)
Chloramphenical	32.50a *	28.00a	6.00h	6.00c	6.00f		24.50a	30.00a
Kabarnet	18.50cb	14.50cb	18.50bdc	8.00bac	16.00a		21.00ba	25.00b
Kisumu	21.00cb	15.50cb	17.50ed	8.50bac	13.00bac		25.00a	22.50cb
Thika	18.50cb	25.00a	18.50bdc	10.50a	13.50bac		24.00a	22.50cb
Meru	22.00b	18.00b	20.50ba	10.00ba	16.50a		25.00a	22.50cb
Kericho	18.50cb	13.50cbd	18.00dc	7.50bac	15.00ba		20.00bac	22.00cb
Njoro I	14.00cd	12.00cd	13.50f	6.00c	11.50bcde		20.00bac	21.00cd
Njoro II	14.00cd	11.50cd	13.50f	8.00bac	11.50bcde		20.00bac	20.50cd
Mombasa	25.00b	16.00cb	20.00bac	8.00bac	16.50a		15.00bc	19.50cde
Taita taveta	24.00b	15.00cb	21.00a	8.00bac	13.00bac		15.00bc	19.50cde
Nyeri	14.00cd	12.00cd	13.50f	7.50bac	11.50bcde		22.00ab	18.50fde
Kakamega	18.00cb	13.5cbd	10.50g	6.50bc	11.00cde		19.00bac	17.00fe

*Values in the same column (inhibition zone in mm) followed by the same letter do not differ significantly based on Duncan's multiple Range test ($P<0.05$)

Table 4: Chemical composition of *Ocimum gratissimum* L. leaf oil

Compound	KI	% Concentration		Method of identification
Monoterpenes		*MERU*	*MOMBASA*	
β- Pinene	978	1.10	1.27	RI, GC-MS
cis-Ocimene	1037	7.47	6.00	RI, GC-MS
trans- Ocimene	1050	0.94	0.00	RI, GC-MS
Camphor	1143	0.95	0.00	RI, GC-MS
Eugenol	1356	68.81	74.10	RI, GC-MS
Methyl eugenol	1401	13.21	0.00	RI, GC-MS
Total		92.48	81.37	
Sesquiterpenes				
trans-Caryophyllene	1430	1.69	3.70	RI, GC-MS
Germacrene-D	1487	4.25	8.74	RI, GC-MS
α- Farnese	1504	0.85	0.00	RI, GC-MS
β- Bisabolene	1508	0.73	0.00	RI, GC-MS
Total		100.00	93.81	

KI – Kovat index

The essential oils from 13 populations of Kenya were found to be active against all the bacteria strains including Gram positive (*Staphylococcus aureus* and *Bacillus spp*.) and Gram negative (*Escherichia coli, Pseudomonas aeruginosae*, *Salmonella typhi*, *Klebsiella pneumoniae* and *Proteus mirabilis*) bacteria (Tables 2 and 3). It also showed a marked antifungal activity against *Candida albicans*. The essential oils were more effective on all the microbes tested simlar to chloramphenical (Table 2), which showed resistance to the Gram negative bacteria (*Pseudomonas aeruginosae, Proteus mirabilis* and *Salmonella typhi).* It was also observed that the oils from some populations were more effective on some microbes than from the others. For instance, Meru oil and Thika oil showed greater inhibition zones on *Staphylococcus aureus* than those from the other populations (Table 2). Similarly, Kabarnet EO was more effective against *Bacillus spp* than the oils of the other remaining populations (Tables 2 and 3). Meru, Taita Taveta and Mombasa oils presented good activity against Gram negative bacteria (*Escherichia coli* and *Salmonella typhi)* and moderate activity on *Pseudomonas aeruginosae, Klebsiella pneumoniae* and *Proteus mirabilis* (Tables 2 and 3). The essential oils from Njoro I, and Njoro II, (all these are areas in one geographical region) showed less activity on almost all the microbes (Table 3).

The concentration of the oil were generally in the range of 100 times or more than the standard antibiotic (Chloramphenical), the essential oils were more effective than thestandard antibiotic in view of the content of the active ingredient in the mixture of the oils. The essential oils from all the populations showed activity on both Gram negative and Gram positive bacteria in addition to the fungi (*Candida albicans*) as shown by their inhibition zones (Table 2). The difference in activity of the essential oils from the different populations could be attributed to variation of the chemical composition of the essential oil of *O. gratissimum* according to geographical distribution (Lemos et al., 2005).

The analysis of the essential oils from the Eastern region (Meru) and coastal region (Mombasa) of Kenya by GC-MS revealed a major compound (68.8 %) and (74.1 %) respectively with a Kovat's index of 1356 (Table 4). Eugenol was the major compound present in the essential oil of this plant. The compound which was identified as eugenol has been reported to present antimicrobial (Nakamura et al. 1999; Iwalokun et al. 2003; Lemos et al. 2005; Matasyoh et al., 2007), insecticidal (Chavan and Nikam, 1982), antihelminthic (Pessoa *et al.* 2002) and nematicidal (Chatterje et al. 1982) properties. Thus, eugenol is responsible for the activity of the essential oil of this plant.

Different geographical locations have shown different chemical percentages and chemical compositions of this plant. Other reports have shown chemical composition percentages similar or higher than ours (Lemos et al., 2005) with eugenol (57.82 %) followed by α-bisabolene (17.19 %) and thymol (9.8 %); (Iwalokun et al., 2001) with essential oil obtained from the seeds of *O. gratissimum* containing thymol and eugenol in amounts ranging from 32 % to 65 %; (Nakamura et al., 1999) reported eugenol (67 %) as a major component ; (Keita et al., 2000) reported thymol (46 %) p-cymeme (12 %) and γ-terpene + trans-sabiene hydrate (17 %) for *O. gratissimum* in the Republic of Guinea.

The MIC (Matasyoh et al., 2007) which was done on the EO from Eastern Kenya (Meru) only, showed very good activity and the results were comparable or sometimes better than standard antibiotic.

Meru EO gave the best consistent results in their effectiveness as compared to the other 12 remaining populations (Table 3). Even though the MIC for the Meru oil is greater than that of the standard reference antibiotic,

it should be realized that the EO comprises of many other compounds and not only eugenol (Table 4). This implies that pure eugenol from *O. gratissimum* could show higher inhibition than the crude essential oils (Table 2). This was also observed by Lemos et al. (2005).

Conclusion

This antimicrobial activity of *Ocimum gratissimum* L. varied from different geographic conditions. The sample from Eastern region (Meru) of Kenya showed very remarkable activity against Gram negative bacteria (*E. coli, K. pneumoniae, S. typhi, P. mirabilis)* and Gram positive bacteria (*S. aureus* and *Bacillus spp)*. This indicates that this plant can be used as herbal medicine in the management of ailments caused by these microbes.

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