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Full Length Research Paper

Chemical composition and antimicrobial activity of the essential oil of *Ocimum gratissimum* L. growing in Eastern Kenya

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Hydro-distilled volatile oils from the leaves of *Ocimum gratissimum* L. (Lamiaceae) from Meru district in Eastern Kenya were analysed by gas chromatography-mass spectrometry (GC-MS) and also evaluated for antimicrobial activity. The oil was dominated by monoterpens which accounted for 92.48%. This monoterpene fraction was characterized by a high percentage of eugenol (68.8%) . The other major monoterpenes were methyl eugenol (13.21%), cis-ocimene (7.47%), trans-ocimene (0.94%), -pinene (1.10%) and camphor (0.95%). The sesquiterpenes present in fairly good amounts were germacrene D (4.25%) and trans-caryophyllene (1.69%). The minor sesquiterpenes were -farnesene (0.85%) and -bisabolene (0.74%). The antimicrobial activities of the essential oils were evaluated against both Gram positive (*Staphylococcus aureus, Bacillus* spp.) and Gram negative (*Escherichia coli, Pseudomonas aeruginosae, Salmonella typhi, Klebisiella pneumoniae, Proteus mirabilis*) bacteria and a pathogenic fungus *Candida albicans*. The oil had pronounced antibacterial and antifungal activities on all the microbes.

Key words: Antimicrobial activity, *Ocimum gratissimum* L., eugenol, methyl eugenol.

INTRODUCTION

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

Several species and varieties of plants of the genus *Ocimum* have been reported to yield oil of diverse nature, commonly known as basilica oils. Craveiro et al. (1981) and Janine de Aquino Lemos et al. (2005) reported some chemical compounds and active ingredients found in 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 on the antimicrobial activity and chemical composition of the essential oils of *O. gratissimum* L. growing in Meru district of Eastern Kenya.

MATERIALS AND METHODS

Plant material

The leaves of *O. gratissimum* L. were collected from a wild population during the pre flowering season in August, 2005 in Meru district of Eastern Kenya, which is at an altitude of 0° 03 'N 37° 39'E. Avou-

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cher specimen was deposited at the Department of Botany, Egerton University, Kenya.

Essential oil distillation

Fresh leaf samples were subjected to hydro- distillation in a modified Clevenger -type apparatus for a minimum of 4 h in accordance with the British pharmacopoeia. The essential oil was obtained in a yield of 0.49% w/w after drying over anhydrous sodium sulphate (Na_2SO_4). The oil was stored in a sealed glass vial (bijoux bottle) in a refrigerator at 4°C until required.

GC, GC-MS Analyses

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 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°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 El 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 (Adams, 1995) and supplemented by Wiley and Quadlib 1607 GC-MS libraries.

Antimicrobial screening

The micro-organisms used were *S. aureus* ATCC 25923, *P. aeruginosae* ATCC 27853, *E. coli* ATCC 25922 and clinical isolates *S. typhi, K. pneumoniae*, *P. mirabilis, Bacillus* spp. and *Candida albicans*. The agar disc diffusion method was employed for the screening of antimicrobial activities of the essential oils 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 h of incubation at 37°C. Chloramphenical and Nystatin were used as antibiotic and antifungal reference products respectively. All tests were performed in duplicate.

Minimum inhibitory concentration (MIC)

Serial dilutions of the essential oil were done using 10% TWEEN 80 in distilled sterile water which was also 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 h of incubation at 37°C.

RESULTS

Chemical composition of the essential oils

Table 1 shows the constituents identified by GC-MS analysis, their Kovat's index and area percentages of the essential oils from *O. gratissimum* L. 100% of the volatile oils were identified in the sample. The oil was dominated by eugenol, which accounted for 68.81% of oil and methyl eugenol (13.21%). Minor components include cis-ocimene (7.47%), germacrene-D (4.25 %), trans-caryophyllene (1.69 %) and - pinene (1.10%).

Antimicrobial activity

The essential oil was evaluated for antimicrobial activity against pathogenic strains of Gram positive (*S. aureus, Bacillus spp*.) and Gram negative (*E. coli, P. aeruginosae, S. typhi, K. pneumoniae, P. mirabilis*) bacteria and a pathogenic fungus *C. albicans*. It was found to be active against all the bacterial strains. Dilution of the essential oil affected the effectiveness in some cases. That is, the activity of the oil varies with its concentration and kind of bacteria. The fungus, *C. albicans,* was highly susceptible to the essential oil. Although the concentrations of the oil were generally higher than the standard antibiotic (chloramphenicol), they showed marked antibacterial and antifungal activities as evidenced by their zones of inhibition (Table 2). Among the Gram negative bacteria, the oil was very active against *E. coli*. The activity response to *E. coli* was more or else the same at (75×10^2 µg) as that of chloramphenicol (30 µg). The refe-rence antibiotic showed no activity in the three Gram negative bacteria out of the five tested. It showed significant activity only on *E. coli* and *K. pneumoniae*.

This activity was higher than that exhibited by the essential oil on the same microbes. On the other hand, the essential oil showed significant activity on all the Gram negative bacteria including those which were resistant (*P. aeruginosae, S. typhi,* and *P. mirabilis*) to reference antibiotic. Therefore, the oil was superior to the reference antibiotic in this particular instance. The activity of the oil on the Gram positive bacteria tested showed higher or similar activity with the reference antibiotic. The activity of the oil on fungi was also very significant as compared to Nystatin. The minimum inhibition concentration (MIC) for the oil was greater than that of reference antibiotic, however, it is important to note that the active ingredient against the test microbe from the essential oil constitute a small percentage of the oil.

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

DISCUSSION

Fresh leaves of *O. gratissimum* yielded 0.49% w/w of the essential oil and its density was 0.75 g/ ml. The analysis of the oil by GC-MS revealed a major compound (68.8%) with a Kovat's index of 1356. The compound was identified as eugenol as also reported previously (Nakamura

Table 1. Chemical composition of the oil from *Ocimum gratissimum* L. leaves.

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

KI – Kovat index

et al., 1999; Janine de Aquino Lemos et al., 2005). The compound (eugenol) has been demonstrated to have both antibacterial (Nakamura et al., 1999; Adebolu and Oladimeji, 2005) and antifungal (Janine de Aquino Lemos et al., 2005) activities.

Other reports have shown chemical composition percentages similar or higher than ours (Janine de Aquino Lemos et al., 2005) with eugenol (57.82%) followed by - bisabolene (17.19%) and thymol (9.8%). Iwalokun et al. (2001) reported essential oil obtained from the seeds of *O. gratissimum* contain 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 genus *Ocimum* has been reported to yield oil of diverse nature commonly known as basilic oils. Janine de Aquino Lemos et al. (2005) reported some chemical compounds and active ingredients found in these plants such as eugenol, linalool, methyl cinnamate, camphor and thymol. Guenther, (1948) observed that *O. gratissimum* oils could be divided into two groups: thymol and eugenol-rich chemotypes. Since that time, review by Lawrence (1997) indicated other chemotypes characterized by high contents of linalool/methyl chavicol (Lawrence, 1992), eugenol/l,8-cineole/sesquiterpenes (Lawrence, 1992; De Medici et al., 1992; Fun et al., 1992) and methyleugenol / eugenol (Vostrowsksy, 1990). Other chemotypes are characterized by high contents of geraniol (Charles and Simon, 1992), methyl cinnamate (Fun et al., 1990), ethyl cinnamate (Ali and Shamsuzzaman, 1968) and citral (Hegnauer, 1967). According to the eugenol, methyl eugenol content, the extracted oil from this study could be classified as the eugenol/methyl eugenol chemotype which to the best of our knowledge has not been reported.

The essential oils showed variable activities against tested bacteria. The highest antimicrobial activity was observed on Gram positive bacteria as opposed to Gram negative bacteria although the oil was effective on all microbes tested. The oil inhibited *S. aureus* at an MIC of 93.7 mg/ml. In contrast to the relatively low MIC of the oil for Gram positive bacteria, Gram negative bacteria belonging to the genera *Escherichia, Salmonella, Klebsiella, Proteus* and *Pseudomonas* were inhibited by the oil with MICs ranging from 107 to 750 mg/ml. It has been already shown that the antimicrobial activity of volatile compounds results from the combined effect of direct vapour absorption on micro- organisms and indirect effect through the medium that absorbed the vapour (Moleyar and Narasimtram, 1986; Bassole et al., 2005). The vapour absorption on microorganisms is determined by their membrane permeability. Gram negative micro-organisms are less susceptible to essential oils than Gram positive ones because they posses outer membrane surrounding the cell membrane (Ratledge and Wilkinson, 1988) which restricts diffusion of hydrophobic compounds through its lopopolysaccharide covering (Vaara, 1992).

Eugenol which was analysed to be the major compound present in the essential oil of this plant has been reported to present antimicrobial (Iwalokun et al., 2003; Janine de Aquino Lemos et al., 2005), insecticidal (Chavan and Nikam, 1982), antihelminthic (Pessoa et al., 2002) and nematicidal (Chatterje et al., 1982) properties. Eugenol is a monoterpene, and so are methyl eugenol, cis-ocimene, - pinene, camphor and trans-ocimene. Methyl eugenol has also antifungal and antibacterial activities (Wright, 2002), in addition to its central nervous system depressant with anesthetic, hypothermic, myore-

Table 2. Antimicrobial activity of the essential oil of *O. gratissimum* L. from Meru.

Microorganism Source		Inhibition zone (mm)										
		Essential oil (µg×10^2)										
		(75.00)	(37.50)	(25.00)	(18.75)	(15.00)	(12.50)	(10.70)	STD [b]	Control	MIC mg/ml	
Gram negative EOx10^2 STD [b]												
E. coli	ATCC25922	21.7±2.1	15.0±0	11.0±1.4	10.0±1.4	9.5±2.1	9.0±1.4	7.5±0.7	28.0±0	0	107	25
S. typhimurium	[a]KEMRI	20.5±0.7	9.5±0.7	9.0±1.4	8.5±0.7	7.5±0.7	0	0	0	0	107	0
K. pneumoniae	[a]KEMRI	18.0±2.8	11.5±0.7	9.5±0.7	9.0±0	8.5±0.7	7.5±0.7	0	24.5±0.7	0	107	22.5
P. mirabilis	[a]KEMRI	16.0±1.7	10.0±0	8.5±0.7	8.0±0	7.5±0.7	0	0	0	0	107	0
P. aeruginosae	ATCC27853	9.0±2.6	0	0	0	0	0	0	0	0	750	0
Gram positive												
S. aureus	ATCC25923	26.6±5.7	13.0±1.4	12.5±0.7	11.5±0.7	11.5±2.1	8.5±2.1	8.0±1.4	23.5±2.1	0	93.7	31.3
Bacillus spp.	[a]KEMRI	22.3±1.5	11.0±1.4	8.0±0	7.5±0.7	7.0±0	0	0	24.5±0.7	0	150	26.3
Fungus		75.00 (µg×10^2)	5.35 (µg×10^2)	5.00 (µg×10^2)					STD [c]		MIC (mg/ml)	
									Control		EOx10^2	STD [c]
C. albicans	[a]KEMRI	S	8.5±0.7	7.0±0					10	0	0.5	N/A

Key: Resistance = 0; S = susceptible. [a]KEMRI = clinical isolate from Kenya Medical Research Institute; [b] = Chloramphenical (30 µg); [c] = Nystatin (100 µg); EO =Essential oil

relaxant and anticonvulsant properties (Emea, 2004). Cis-ocimene, which was found in appreciable amounts in this study, has been found to have antibacterial activity and beta-pinene which has antifungal activities (Wright, 2002). Research into the antimicrobial actions of mono-terpenes suggests that they diffuse into and damage the cell membrane structures (Sikkema et al., 1995). The sesquiterpene found in appreciable amounts like germa-crene D has been reported to activate a major type of antennal receptor neuron of the tobacco budworm moth *Heliothis virescens* (Rostelien et al., 2000). It is known that plants release hundreds of volatiles that are impor-tant in interactions with insects or other organisms, for instance, pollination.

Lima et al. (1993) tested 13 essential oils obtained from plants against dermatophytes. *O. gratissimum* was found to be the most active in inhibiting 80% of the dermato-phyte strains tested and producing zones greater than 10 mm diameter. Similarly, Nwosu and Okafor (1995) repor-ted the antifungal activities of extracts of ten medicinal plants collected from Southeastern Nigeria against seven pathogenic fungi. According to the report, *O. gratissimum* inhibited the growth of *Trichophyton rubrum* and *T. mentagrophytes*. Antidiarrhoeal activities of the leaf extra-cts of *O. gratissimum* have also been reported (Ilori et al., 1986). In addition, Mbata et al. (2005) showed that *O. gratissimum* oils have properties that can inhibit the growth of *psychrophils* and heat resistant organisms and suggested there were need for the use of this plant and its derivatives for the primary purpose of flavouring foods and antimicrobial activities.

This study has shown the composition of the essential oil of *O. gratisimum* L. growing in Meru, Eastern Kenya and its antimicrobial activity. The results show that the essential oil may be used in treatment of diseases cau-sed by the microbes tested.

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