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Chemical composition and antimicrobial activity of essential oil of *Tarchonanthus camphoratus*

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Abstract

The essential oil of *Tarchonanthus camphoratus* (Asteraceae), obtained by hydro-distillation, was analysed by gas chromatography–mass spectrometry (GC–MS) and also evaluated for antimicrobial activity. Out of 45 peaks representing 99.8% of the oil, 38 components which constitute 95.8% of the total oil were identified. The oil was dominated by monoterpenes, which accounted for 80.9% of the oil. This study indicates the presence of a high percentage of oxygenated monoterpenes (62.3%), of which the main constituents were fenchol (15.9%), 1,8-cineole (14.3%) and α-terpineol (13.2%). Other monoterpenes present in fairly good amounts were α-pinene (6.87%), *trans*-pinene hydrate (6.51%), terpinen-4-ol (4.74%) and camphene (3.76%). The oil was screened for antimicrobial activity against both Gram positive (*Staphylococcus aureus, Bacillus* ssp.) and Gram negative (*Escherichia coli, Pseudomonas aeruginosa, Salmonella typhi, Klebsiella pneumoniae, Proteus mirabilis*) bacteria and a pathogenic fungus *Candida albicans*. Except for *P. aeruginosa,* which showed resistance, the oil had pronounced antibacterial and antifungal activities.

Keywords: *Tarchonanthus camphoratus*; Asteraceae; Fenchol; 1,8-Cineole; α-Terpineol; Antimicrobial activity; Antifungal activity; MIC

1. Introduction

Tarchonanthus camphoratus is in the well known medicinal plant family of Asteraceae and grows widely in Kenya. The leaves of the plant, either as decoctions or infusions, have been used to relieve bronchitis, asthma, headache, inflammations, chilblains or abdominal pains (Amabeoku, Green, Eagles, & Benjeddou, 2000). Aromatic and medicinal plants produce a wide variety of volatile terpene hydrocarbons and their corresponding oxygenated derivatives known as essential oils. The antimicrobial activities of essential oils have been well recognized for many years (Hammer, Carson, & Riley, 1999). This activity could act as a chemical defence against plant pathogenic diseases. Pathogens can readily penetrate at wound sites, caused, for example, by herbivores. Wounding of leaves, which are covered by volatile glands, results in rupture of the glands, causing the oil to flow over the wound. The existence, therefore, of antimicrobial activity in the oil, would be of considerable benefit to the plant.

The development of bacterial resistance to presently available antibiotics has necessitated the search for new antimicrobial agents. The Gram positive bacteria, such as *Staphylococcus aureus* is mainly responsible for post-operative wound infections, toxic shock syndrome, endocarditis, osteomyelitis and food poisoning (Mylotte, McDermott, & Spooner, 1987). The Gram negative bacterium, such as *Escherichia coli,* is present in human intestines and causes urinary tract infection, coleocystitis or septicemia (Singh, Chandra, Bose, & Luthra, 2000). *Bacillus subtilis* is a rod-shaped aerobic bacterium and there are

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reported to have some pathogenic role (Gorden, Haynes, & Paug, 1973). The aim of the present study was to determine the chemical composition of the essential oil of *Tarchonanthus camphoratus* and examine the antimicrobial and antifungal activities.

2. Materials and methods

2.1. Plant material

The leaves of *T. camphoratus* were collected from the botanical-garden of Egerton University in Kenya which is at an altitude of 2127 m. Voucher specimens were deposited at the Department of Botany, Egerton University.

2.2. Isolation of volatile components

Fresh leaves of *T. camphoratus* were subjected to hydrodistillation 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.

2.3. GC, GC–MS analysis

Samples of essential oils were diluted in methyl-*tert*.-butylether (MTBE) (1:100) and analysed on an Agilent GC–MSD apparatus equipped with an Rtx-5SIL MS ('Restek') (30 m × 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 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 the literature (Adams, 1995) and supplemented by the Wiley and QuadLib 1607 GC–MS libraries. The relative proportions of the essential oil constituents were expressed as percentages obtained by peak area normalization, all relative response factors being taken as one.

2.4. Pharmacological screening

The antimicrobial activity of the essential oil was tested according to the National Committee of Clinical Laboratory Standards 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, Candida albicans.* Freshly grown microbial suspensions in Mueller Hinton Broth were standardized to a cell density of 1.5×10^8 (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 20%, 25%, 40%, 50% and 75%. Neat oil was also used, giving a corresponding concentration of 90×10^2 μg per sensitivity disc. The positive antibacterial and antifungal activities were established by the presence of measurable zones of inhibition after 24 h of incubation at 37 °C. Minimum inhibition concentration (MIC) was defined as the lowest concentration that inhibited growth of the microorganism detected visually. Chloramphenicol was used as the standard antibiotic.

3. Results and discussion

The leaves of *T. camphoratus* afforded an essential oil on hydro-distillation which was analysed by gas chromatography–mass spectroscopy (GC–MS). Out of 45 peaks (representing 99.8% of the oil), 38 components were identified, representing 95.8% of the total oil. The constituents identified by GC–MS analysis, their retention times and area percentages are summarized in Table 1.

The oil was dominated by monoterpenes, which accounted for 80.9% of the oil. This study indicated the presence of a high percentage of oxygenated monoterpenes (62.3%) of which the main constituents were fenchol (15.9%), 1,8-cineole (14.3%) and α-terpineol (13.2%). An earlier analysis of the leaf oil of *T. camphoratus* indicated that these three oxygenated monoterpenes were the major constituents but with a higher amount of fenchol (29.1%) (Mwangi & Achola, 1994). Other monoterpenes present in fairly good amounts were α-pinene (6.87%), *trans*-pinene hydrate (6.51%), terpinen-4-ol (4.74%) and camphene (3.76%). On the other hand, β-eudesmol (5.79%) was the major oxygenated sesquiterpene present in the oil. Other sesquiterpene hydrocarbons, such as δ-curcumene (2.15%), α-cadinol (1.75%) and *ar*-curcumene (1.69%) were also present.

The essential oil was evaluated for antimicrobial activity against pathogenic strains of Gram positive (*Staphylococcus aureus, Bacillus* ssp.) and Gram negative (*Escherichia coli, Pseudomonas aeruginosa, Salmonella typhim, Klebsiella pneumoniae, Proteus mirabilis*) bacteria. It was found to be active against all the bacterial strains except for *Pseudomonas aeruginosa.* It also showed a marked antifungal activity against *Candida albicans*. However, the activity of the oil varies with its concentration and kind of bacteria. 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). 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 90×10^2 μg) as that of chloramphenicol (30 μg). *P. aeruginosa* was considered resistant to the essential oil and even to the reference antibiotic chloramphenicol, since no inhibition zone was observed. This bacterium has shown resistance to antimicrobial agents and diterpenes present in *Salvia* species

Table 1
Chemical composition of *Tarchonanthus camphoratus* leaves oil

	Compound	KI	Concentration (%)	Method of identification
Monoterpenes				
1	Thujene	935	0.16	RI, GC–MS
2	**α-Pinene**	**938**	**6.87**	RI, GC–MS
3	**Camphene**	**952**	**3.76**	RI, GC–MS
4	Sabinene	976	0.32	RI, GC–MS
5	β-Pinene	978	2.03	RI, GC–MS
6	δ-2-Carene	1006	0.93	RI, GC–MS
7	1-Phellandrene	1011	0.48	RI, GC–MS
8	α-Terpinene	1018	0.53	RI, GC–MS
9	*p*-Cymene	1029	1.29	RI, GC–MS
10	Limonene	1030	1.18	RI, GC–MS
11	**1,8-Cineole**	**1034**	**14.3**	RI, GC–MS
12	γ-Terpinene	1062	1.02	RI, GC–MS
13	*trans*-Sabinene hydrate	1068	0.64	RI, GC–MS
14	α-Terpinolene	1089	1.03	RI, GC–MS
15	Fenchone	1091	0.86	RI, GC–MS
16	β-Terpineol	1120	0.44	RI, GC–MS
17	Nonanal	1122	0.51	RI, GC–MS
18	**Fenchol**	**1125**	**15.9**	RI, GC–MS
19	***trans*-Pinene hydrate**	**1137**	**6.51**	RI, GC–MS
20	*trans*-Pinocarveol	1142	0.36	RI, GC–MS
21	1-Terpineol	1143	0.38	RI, GC–MS
22	*Trans*-Verbenol	1144	0.37	RI, GC–MS
23	Exo-methyl-camphenilol	1150	0.65	RI, GC–MS
24	Borneol	1166	2.26	RI, GC–MS
25	**Terpinen-4-ol**	**1177**	**4.74**	RI, GC–MS
26	**α-Terpineol**	**1191**	**13.2**	RI, GC–MS
27	Fenchyl acetate	1223	0.20	RI, GC–MS
	Total		**80.9**	
Sesquiterpenes				
28	α-Copaene	1376	0.32	RI, GC–MS
29	β-Caryophyllene	1418	0.63	RI, GC–MS
30	Aromadendrene	1461	0.49	RI, GC–MS
31	γ-Curcumene	1471	2.15	RI, GC–MS
32	*ar*-Curcumene	1483	1.69	RI, GC–MS
33	Valencene	1491	0.19	RI, GC–MS
34	γ-Cadinene	1513	0.32	RI, GC–MS
35	δ-Cadinene	1524	0.74	RI, GC–MS
36	Caryophyllene oxide	1582	0.82	RI, GC–MS
37	α-Cadinol	1653	1.75	RI, GC–MS
38	**β-Eudesmol**	**1655**	**5.79**	RI, GC–MS
	Total		**14.9**	

KI – Kovat index, RI – Retention index.

(Darias, Bravo, Rabanal, Sanchez-Mateo, & Martin-Herrera, 1990). The reference antibiotic showed the highest antimicrobial activity against all tested microorganisms except *P. aeruginosa* and *S. typhi,* which showed resistance to it. Interestingly, the oil exhibited activity against *S. typhi* and, therefore, was superior to the reference antibiotic in this particular instance. The oil showed more or else similar activity, across the concentration range, to *K. pneumoniae* and *P. mirabilis*.

The minimum inhibition concentration (MIC) of oil for Gram negative bacteria ranged from 113 to 900 mg/ml and 100 to 129 mg/ml for Gram positive bacteria. The MIC for the fungus *Candida albicans* is 113 mg/ml. The MIC values for chloramphenicol range from 22.5 to 31.3 mg/ml. The higher concentrations of the essential oil than chloramphenicol can be explained by the fact that only a fraction of the oils constitutes the active compounds.

The essential oils evaluated in this work have a great variety of phytochemicals that could be considered as responsible for a larger or smaller part of the antimicrobial activity. Although they usually occur as complex mixtures, their activity can generally be accounted for in terms of their major monoterpenoid components. Research into

Table 2
Antimicrobial activity of the essential oil of *Tarchonanthus camphoratus*

Microorganism	Source	Inhibition zone (mm)							MIC (mg/ml)	
		Essential oil ($\mu g \times 10^2$)					STD[b] (30 μg)	Control	EO[c]	STD[b]
		(90.0)	(67.5)	(45.0)	(22.5)	(18.0)				
Gram negative										
E. Coli	ATCC 25922	26.5 ± 1.5	11.0 ± 1	10.0 ± 0	8.0 ± 0	0	30 ± 0	0	113	25
S. Typhi	KEMRI[a]	11.5 ± 0.5	0	0	0	0	0	0	900	25
K. pneumoniae	KEMRI[a]	13.0 ± 2	11.0 ± 1	0	0	0	25.0 ± 0	0	450	22.5
P. Mirabilis	KEMRI[a]	13 ± 0	11.0 ± 0	10.0 ± 0	9.0 ± 0	0	0	0	225	0
P. aeruginasae	ATCC 27853	0	0	0	0	0	0	0	0	0
Gram Positive										
S. Aureus	ATCC 25923	26.5 ± 1.5	17.5 ± 2.5	15.5 ± 1.5	10.0 ± 1	9.0 ± 1	27.0 ± 2	0	100	31.3
Bacillus ssp.	KEMRI[a]	21.0 ± 1	15.5 ± 0.5	1.0 ± 1	8.5 ± 0.5	0	30 ± 0	0	129	26.3
Fungus										
Candida albicans	KEMRI[a]	20.5 ± 0.5	15.0 ± 0	12.0 ± 0	10.5 ± 0.5	7.5 ± 0.5	0	0	113	0

[a] Clinical isolates from Kenya Medical Research Institute (KEMRI).
[b] Chloramphenicol standard.
[c] Essential oil.

the antimicrobial actions of monoterpenes suggests that they diffuse into and damage cell membrane structures (Sikkema, de Bont, & Poolman, 1995).

α-Pinene, which was found to be in appreciable amounts in the oil of this study, has been reported to be the cause of the antifungal activity of oil from *Pistacia lentiscus* (Anacardiaceae) (Magiatis, Melliou, Skattsounis, Chinou, & Mitaku, 1999). One of the major components of this oil, 1,8-cineole, has been known to exhibit antimicrobial activity against the bacterial strains (*E. coli*, *P. aeruginosa*, *S. typhi*, *S. aureus, rhizobium leguminosarum, bacillus subtilis*) (Sivropoulou et al., 1997). The other major constituent of this oil, α-terpineol, has been reported (Carson & Riley, 1995; Raman, Weir, & Bloomfield, 1995) to inhibit the growth of quite a number of bacteria and fungi that include *S. aureus*, *E. coli*, *S. epidermis* and *C. albicans*. Terpinen-4-ol, which occurs in appreciable amounts in this oil, is also reported (Carson & Riley, 1995) to show activity against these organisms. It is also said to be responsible for the broad spectrum activity of the essential oil of *Melaleuea alternifolia* (tea tree oil) (Sean et al., 2001). This monoterpene, isolated from *Achillea* species, showed antibacterial activity (Magiatis, Skaltsounis, Chinou, & Haroutounian, 2002). Caryophyllene oxide, although a minor constituent in the oil under study, is known to have very efficient antibacterial properties (Magiatis et al., 2002). Another minor monoterpene alcohol, linalool, is reported to have a wide range of antibacterial and antifungal activity (Pattnaik, Bapaji, & Kole, 1997).

4. Conclusion

The composition of the essential oil of *T. camphoratus* growing in Kenya has been analysed and its antimicrobial activity investigated. The results indicate that the oil may be used in the treatment of diseases caused by the microorganisms tested. Further toxicological and clinical studies are required to prove the safety of the oil as a medicine.

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