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Full Length Research Paper

Variation in chemical composition and antibacterial activity of the essential oil of fresh and dry leaves and dry stem of *Tarchonanthus camphorates*

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The essential oils of the fresh leaves, dry leaves and dry stem of *Tarchonanthus camphoratus* from Kwa-Zulu Natal, South Africa, were obtained by hydro-distillation and analyzed by gas chromatography mass spectrometry (GC-MS). The essential oils were evaluated for their antibacterial activity against 4 Gram positive and 5 Gram negative bacteria using the disc diffusion method and the minimum inhibitory concentration (MIC) method. A total of 33, 27 and 25 compounds were identified representing 85.9, 73.01 and 72.66% of the total oil composition from the fresh leaves, dry leaves and dry stem, respectively. Sesquiterpene hydrocarbons dominated the essential oil of the fresh leaves (36.46%) and of the dry leaves (59.18) whereas an aldehyde, butanal (35.77%) dominated the essential oil of the dry stem. The oils showed a broad spectrum of antibacterial activity. The zones of inhibition of the fresh leaves, dry leaves, dry stem and chloramphenicol ranged from 7.3 to 14.0, 7.3 to 16.7, 13.5 to 20.4 and 0.00 to 17.5 mm, respectively. The lowest MIC value for the oils was 125 µg/ml.

Key words: *Tarchonanthus camphoratus*, essential oil composition, antibacterial activity.

INTRODUCTION

Tarchonanthus camphoratus L. (family Asteraceae) is a shrub of rarely more than 6 m in height with a greyish appearance and occurs in a wide range of habitats (van Wyk et al., 1997). The strongly scented tree of *T. camphoratus* has many medicinal applications in traditional healing, mainly by smoking from burning leaves or by drinking infusions or decoctions. Traditionally, infusions and tinctures of the leaves are used for stomach trouble, headache, toothache, asthma, bronchitis, inflammation, rheumatism, venereal diseases, indigestion, heartburn, coughs, paralysis and cerebral hemorrhage (Hutchings and Van Staden, 1994; Anthony, 1999). The plant also shows powerful insect repellent action (Omolo et al., 2004) and wild animals that live in the areas where *T. camphoratus* grows, particularly Cape buffaloes and black rhinoceroses, rub themselves against the leaves to get rid of mosquitoes and flies. The plant also seems to drive away tsetse fly, a pathogenic agent of trypanosomiasis (Bekalo et al., 1996).

T. camphoratus has been reported to contain tannins, saponins and reducing sugars (van Wyk et al., 1997; Scott and Springfield, 2005). Leaves of the plant contain various flavanones such as pinocembrin, luteolin, apigenin, nepetin, and hispidulin; a sesquiterpine lactone, parthenolide; a quaternary alkaloid, tarchonanthine (Scott and Springfield, 2005). The essential oil of the leaves of *T. camphoratus* has been reported to be very complex and variable at different locations (Mwangi et al., 1994; Matasyoh et al., 2007; van Vuuren and Sandra, 2008). Antibacterial activity of the essential oil of the leaves of *T. camphoratus* from other ecosystems; Kenya and Eastern Cape, South Africa, has been reported (Matasyoh et al.,

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2007; van Vuuren and Viljoen, 2009). To the best of our knowledge, no studies have been done on the essential oil of the stem of *T. camphoratus.* In addition, there are no reported studies on the chemical composition and antibacterial activity of the essential oils of the fresh leaves, dry leaves and stems of *T. camphoratus* from Kwa-Zulu Natal, South Africa. This study was therefore aimed at investigating the chemical composition and antibacterial activity of the essential oil of the fresh leaves, dry leaves and the stem of *T. camphoratus* from Kwa-Zulu Natal, South Africa.

MATERIALS AND METHODS

Plant

Fresh materials of *T. camphoratus* were collected from Sangoyana in the Northern part of Kwa-Zulu Natal province, South Africa during the month March 2010. The plant was identified by the local people during the time of collection and further identified by Mrs. N.R Ntuli in the Department of Botany, University of Zululand. A Voucher specimen, (NSKN 1) was deposited at the University of Zululand herbarium. The fresh plant material was separated into leaves and the other part with leaves still attached to the stem was dried at room temperature.

Extraction of the essential oil

The fresh leaves (250 g), dry leaves (250 g) and dry stem (300 g) were separately subjected to hydro-distillation using a Clevenger-type apparatus in 350 ml distilled water. The essential oils were collected 4 h after boiling, and were weighed and kept at 4°C in sealed glass vials before analysis and bioassay.

Gas chromatography-mass spectrometry (GC-MS) analysis

GC-MS analysis was carried out using an Agilent 6890 GC with an Agilent 5973 mass selective detector [MSD, operated in the EI mode (electron energy = 70 eV), scan range = 45 to 400 amu, and scan rate = 3.99 scans/s] and an Agilent ChemStation data system. The GC was equipped with a fused silica capillary HP-5 MS column of an internal diameter of 0.25 mm, film thickness of 0.25 µm and a length of 30 m.

The initial temperature of the column was 70°C and was heated to 240°C at a rate of 5°C min^{-1}. Helium was used as the carrier gas at a flow rate of 1 ml/min. The split ratio was 1:25. Scan time was 50 min with a scanning range of 35 to 450 amu. A 1% w/v solution of the samples in hexane was prepared and 1 µl was injected using a splitless injection technique.

Identification of components

The identification of the oil constituents was based on their retention indices determined by reference to a homologous series of *n*-alkanes (C_8-C_{30}), and by comparison of their mass spectral fragmentation patterns with those reported in the literature (Joulain and Koenig, 1998; Adams, 2007) and stored in the MS library [NIST database (G1036A, revision D.01.00)/ChemStation data system (G1701CA version C.00.01.080)]. The percentages of each component were reported as raw percentages based on the total ion current without standardization.

Evaluation of the antibacterial activity

Microorganisms

Four Gram-positive bacteria *Staphylococcus aureus* (ATCC 6538), *Bacillus cereus* (ATCC 10702), *Enterococcus faecalis* (ATCC 29212)*, Staphylococcus epidermidis* (KZN) and five Gram-negative bacteria *Klebsiella pneumonia* (ATCC 10031), *Pseudomonas aeruginosa* (ATCC 19582), *Escherichia coli* (ATCC 25922)*, Shigella flexneri* (KZN) and *Salmonella* species (KZN) were used in this study. The micro-organisms have been reported to be multi-drug resistant and causative agents for nosocomial infections (Anonymous, 2012a, b). All the microorganisms were laboratory isolates obtained from the Department of Biochemistry and Microbiology, University of Fort Hare, Alice, South Africa. The bacteria cultures were grown and maintained on Nutrient Broth medium at 37°C for 24 h prior to use.

Agar disc diffusion method

The disc diffusion method was employed for the determination of antibacterial activities of the oils (Lesueur et al., 2007). The nutrient agar plates were seeded with a suspension of approximately10^5 CFU/ml of the bacteria strains. The empty sterilized Whatman No.1 filter paper disc (6 mm) was impregnated with 10 µl of the oil dissolved in dimethyl sulfoxide (DMSO) (final concentration of 10% (v/v)), dried and placed aseptically on seeded plates with the help of sterile forceps. The standard antibiotic, chloramphenicol (2 µg/disc) was used as a positive control, while DMSO solvent was used as the negative control. The plates were incubated at 37°C for 24 h after which the diameters of inhibition zones were measured in millimeters. Tests were carried out in triplicate.

Minimal inhibitory concentration (MIC) tests

The essential oils of the fresh leaves, dry leaves and dry stem *T. camphoratus* dissolved in 10% DMSO were first diluted to the highest concentration (500 µg/ml) to be tested, and then serial 2-fold dilutions were made in order to obtain a concentration range from 7.8 to 500 µg/ml. The inoculation of the bacterial strains was prepared from 12 h broth cultures, and suspensions were adjusted to approximately 10^5 CFU/m. This was used to inoculate 96-well microtitre plates containing serial 2-fold dilutions of the essential oils (7.8 to 500 µg/ml) under aseptic condition. The plates were incubated under aerobic conditions at 37°C and examined after 24 h. As an indicator of bacterial growth, 40 of 0.2 mg/ml of p-Iodonitrotetrazolium (INT) solution was added to each well and incubated for 30 min at 37°C. The colourless tetrazolium salt was reduced to a red coloured product by biological activity of the organisms. Each treatment was done in triplicate and complete suppression of growth at a specific concentration of the oil was required for it to be declared active (Oyedeji and Afolayan, 2005). Chloramphenicol was used as a positive control.

RESULTS AND DISCUSSION

Chemical composition

Yellowish green oils with yields of 0.14, 0.09 and 0.03% were obtained from the fresh leaves, dry leaves and dry stem of the plant, respectively. The oils gave a total of 33, 27 and 25 identified compounds representing 85.9, 73.01 and 72.66% of the total oil composition from the fresh leaves, dry leaves and dry stem, respectively (Table 1)

Table 1. Chemical constituents of the essential oils of the fresh leaves, dry leaves and dry stem of *T. camphoratus*.

Name of compound	Percentage composition			
	KI	FL	DL	DS
Monoterpene hydrocarbons	0	**9.57**	**1.61**	**0.00**
α- Pinene	938	2.52	0.45	-
Camphene	952	2.15	0.33	-
β- Pinene	978	0.65	-	-
α-Terpinene	1017	2.61	0.65	-
p-Cymene	1026	1.64	0.18	-
Oxygenated monoterpenes	0	**11.79**	**6.26**	**0.79**
1,8-Cineole	1033	9.19	1.94	0.15
Linalool	1098	1.42	1.77	0.33
Camphor	1145	-	0.62	-
(-)-Borneol	1169	-	-	0.07
Terpinene-4-ol	1180	0.56	0.43	0.08
(-)-α-Terpineol	1190	0.62	0.82	0.16
Carvacrol	1299	-	0.68	-
Sesquiterpene hydrocarbons	-	**36.46**	**59.18**	**18.54**
α-Copaene	1378	1.45	2.33	1.21
Isocomene	1392	-	-	1.35
α-Elemene	1393	-	2.98	-
Calarene	1403	2.02	3.60	-
δ-gurjunene	1410	1.25	-	-
(-)-Isoledene	1419	1.22	2.72	0.63
Beta-caryophyllene	1427	2.73	5.48	1.05
α-gurjunene	1436	-	-	0.13
α-Guaiene	1439	-	2.73	0.86
α-Humulene	1461	0.64	0.97	0.29
γ-Gurjunene	1472	-	0.43	-
Aromandrene	1475	2.05	6.12	0.16
α-Muurolene	1476	3.50		1.35
γ-Muurolene	1480	3.19	5.13	2.11
Ledene	1482	0.93	-	-
Germacrene D	1484	-	-	0.69
Eremophilene	1486	1.25	0.10	1.04
α-Selinene	1488	1.08	-	-
Valencene	1491	2.39	-	-
β-Guaiene	1500	1.20	10.70	-
γ-Cadinene	1513	1.50	9.09	2.13
cis-calamenene	1520	3.17	-	-
δ-Cadinene	1526	6.89	6.80	5.54
Oxygenated sesquiterpenes	-	**19.5**	**3.19**	**14.26**
Elemol	1549	**3.5**	2.76	-
Spathulenol	1578	-	0.43	-
Caryophyllene oxide	1580	4.21	-	2.90
Hinesol	1632	2.39	-	-
t-Cadinol	1640	-	-	1.03
t-Muurolol	1642	-	-	10.33
α-Cadinol	1650	9.40	-	-

Table 1. Contd.

Others	-	**8.58**	**2.77**	**39.07**
Butanal	620	6.10	2.77	35.77
Isoaromadendrene epoxide	1579	1.5	-	-
α-Costol	1801	0.98	-	-
Hexadecanoic acid	2117	-	-	3.30

Sesquiterpene hydrocarbons dominated the oils of the fresh and dry leaves. There were no monoterpene hydrocarbons in the oil of the dry stem. The oil of the dry leaves had less chemical constituents than the oil of the fresh leaves. This observation is in agreement with that reported by Fatemeh et al. (2006), that there could be a 50-fold reduction in chemical composition when plant materials are dried. The oil of the fresh leaves was dominated by α-cadinol (9.40%), 1,8 cineole (9.19%), δ-cadinene (6.89%), butanal (6.10%) and caryophyllene oxide (4.21%), while the dry leaf oil was found to be rich in β-guaiene (10.7%), γ-cadinene (9.09%), δ-cadinene (6.80%), aromandrene (6.12%), β-caryophyllene (5.48%) and γ-muurolene (5.13%).

On the other hand, the major components in the dry stem oil were butanal (35.77%), T-muurolol (10.33%) and δ-cadinene (5.54%). δ-cadinene featured in all the oils as one of the major compounds. 1,8-cineole, a major compound in the fresh leaf oil also featured as a minor compound in the dry leaf oil and in the dry stem oil, while other major compounds in the oil of the fresh leaves like α-cadinol and caryophyllene oxide were not found at all in the dry leaf oil. T-muurolol whose concentration has been reported to increase with the age of a plant (Anonymous, 2000), featured as a major compound in the oil of the dry stem, but was absent in the oils of both the fresh and dry leaves. This study reinforced the fact that there are quantitative and qualitative differences in the essential oil components of the fresh and dry plant materials.

Earlier investigations on the chemical composition of the fresh leaves of *T. camphoratus* reported major constituents as: 1,8 cineole (16.5%), α-fenchol alcohol (29.1%) (Mwangi et al., 1994); 1,8-cineole (14.3%), α-terpineol (13.2%) and α-fenchol (15.9%) (Maytosh et al., 2007); β-caryophyllene (13.4%), 1,8-cineole (9.3%) (vanVuuren and Sandra, 2008). δ-cadinene, present as one of the major compounds in all the oils in this study was not present at all in the other oils of the fresh leaves of *T. camphoratus* reported. Geographical variation in the essential oil constituents is not uncommon (Viljoen et al., 2006) and the variance of the compounds in the essential oils of *T. camphoratus* can be attributed to differences in locality. It is worth noting that camphor, a compound suspected to be responsible for the camphor smell of the essential oils of *T. camphoratus* (Mwangi et al., 1994) was not found in all of the essential oils and in some it was in trace amounts. 1,8-cineole, present either as a major compound or a minor compound in all the investigated essential oils of *T. camphoratus* has a fresh camphor-like smell (Anonymous, 2011) and it is likely to be the compound responsible for the strong camphor smell of *T. camphoratus.*

Antibacterial results

The antibacterial activity of the essential oils of the fresh leaves, dry leaves and dry stem of *T. camphoratus* were determined against four Gram positive and five Gram negative bacteria. Mean diameters of the zones of inhibition were calculated and the results are shown in Table 2. The essential oils had a broader spectrum of antibacterial activity than the reference antibiotic chloramphenicol. The MIC results (Table 2) revealed that most of the bacteria showed similar susceptibility to the essential oils of the fresh leaves, dry leaves and dry stem of *T. camphoratus* with MIC value of 250 µg/ml. The least sensitive bacteria to the oils of the fresh leaves, dry leaves and dry stem was *S. aureus* with a MIC value of 500 µg/ml. It is noted that the essential oil of the dry stem of *T. camphoratus* in this study was found to be active against all bacteria strains including those that were resistant to chloramphenicol.

These bacteria, *S. aureus*, *B. cereus*, *E. faecalis*, and *P. aeruginosa* which were not susceptible to chloramphenicol but susceptible to the essential oil of the dry stem, have been reported to cause nosocomial infections (Hoffman and Roggenkamp, 2003). Of special interest is *P. aeruginosa,* a well known antibiotic resistant Gram-negative bacterium which is generally less sensitive to the actions of plants essential oils (Boussaada et al., 2008), but was sensitive to the oil of the dry stem. The fresh and dry leaves also showed antibacterial activity against most of the tested bacteria strains except *E. faecalis*, *P. aeruginosa* and *S. flexneri*. Earlier antibacterial studies on the essential oil of the fresh leaves of *T. camphoratus* from different ecosystems revealed that the oils had good antibacterial activity but the species from Eastern Cape, South Africa, lacked activity on *E. faecalis* and *K. pneumonia* (Vuuren and Viljoen, 2009) and the species from Kenya lacked activity on *P. aeruginosa* (Matasyoh et al., 2007).

The antimicrobial activity of an essential oil is linked to its chemical composition (Cimanga et al., 2002). The antibacterial action of the essential oils may be due to their major chemical constituents. The major compounds,

Table 2. Antimicrobial activity of the essential oil of the fresh leaf, dry leaf and dry stem of *T. camphoratus.*

Micro organism	FL		DL		DS		C	
	DD	MIC	DD	MIC	DD	MIC	DD	MIC
Gram positive								
S. aureus	11.7	500	15.0	500	19.8	500	0.000	125
S. epidermidis	7.30	250	7.30	250	16.6	250	16.3	250
B. cereus	10.3	250	10.0	250	20.0	250	0.000	250
E. faecalis	0.00	ND	0.000	ND	13.5	250	0.000	125
Gram negative								
E. coli	12.3	250	16.7	250	20.4	250	11.7	125
P. aeruginosa	0.000	ND	0.000	ND	20.0	250	0.000	ND
K. pneumonia	14.0	250	13.7	250	19.3	250	13.7	31.25
S. flexneri	0.000	ND	0.000	ND	17.9	250	11.7	62.5
S. spp.	13.0	250	10.0	125	19.2	250	17.5	125

DD = Diameters of zones of inhibition (disc diameter (6 mm) included), values represent an average of three determinations. FL = Flesh leaves; DL = Dry leaves; DS = Dry stem; C = Chloramphenicol; ND = Not Determined

T-muurolol (10.33%) in the dry stem oil, 1,8-cineole (9.19%), α-cadinol (9.40%), caryophyllene oxide (4.21%), in the fresh leaves oil, globulol (10.70%) and β-caryophyllene (5.48%) in the dry leaves oil have been reported to show antibacterial activity (Chang et al., 2000; Tzakou et al., 2001; Magiatis et al., 2002; Blagojevic et al., 2006).

Possible synergistic effect of major and minor compounds in the essential oils has been reported (Bagamboula et al., 2004). Caryophyllene oxide (2.90%), hexadecanoic acid (3.30%), β-caryophyllene (1.05%) found in the dry stem oil, linalool (1.42%), β-caryophyllene (2.73%), α-pinene (2.52%), germacrene D (2.02%), p-cymene (1.64%), terpinen-4-ol (0.56%) and α-terpineol (0.62%) in the fresh leaves oil and 1,8 cineole (1.94%), linalool (1.77%), terpinene-4-ol (0.43%) found in the dry leaves oil as minor constituents have been reported to show anti-bacterial activity (Carson and Riley, 1995; Raman et al., 1995; Bourkhiss et al., 2000; Chang et al., 2000; Magiatis et al., 2002; Kalemba and Kunicka, 2003; Pauli and Schilcher, 2004; Jovanovic et al., 2005; Togashi et al., 2007). The antibacterial activity of the oils may be due to compounds acting individually or synergetically.

Conclusion

The essential oils of the fresh leaves, dry leaves and dry stem of *T. camphoratus* from Kwa-Zulu Natal, South Africa are mainly dominated by sesquiterpene hydrocarbons and possess significant antibacterial activity. These results suggest the rationale for the use of the plant in folk medicine.

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