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Osmitopsis asteriscoides (Asteraceae)-the antimicrobial activity and essential oil composition of a Cape-Dutch remedy

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Abstract

The essential oil composition and antimicrobial activity of *Osmitopsis asteriscoides*, a medicinal plant used in traditional herbal preparations in South Africa has been investigated. Three different antimicrobial methods (disc diffusion, minimum inhibitory concentration by micro-titer plate and time-kill studies) were comparatively evaluated against *Candida albicans*, *Staphylococcus aureus* and *Pseudomonas aeruginosa*. A preliminary screening was done using the disc diffusion method on nine bacterial and four fungal isolates. Minimum inhibitory concentrations showed some correlation with the disc diffusion method. However, time-kill studies appear to be a more superior method for determining antimicrobial activity of volatile compounds such as essential oils. Two moderately susceptible and one resistant organism were selected to further demonstrate the variability between the three methods. The antimicrobial activity of the essential oil, tested by means of time-kill methodology at concentrations ranging from 0.5 to 2% (v/v) indicate a strong fungicidal activity against *Candida albicans* and the oil was also found to be bacteriostatic against *Staphylococcus aureus* in a concentration-dependent manner. The essential oil rapidly reduced viable counts of *Pseudomonas aeruginosa*, but regrowth was noted after 240 min. The results have been generated in duplicate in separate microbiology laboratories using different time-kill methods and the results are congruent. The two major essential oil components camphor and 1,8-cineole were investigated indicating the positive antimicrobial efficacy of 1,8-cineole independently and in combination with camphor. In addition to (−)-camphor and 1,8-cineole, 40 compounds were identified by GC-MS in the hydro-distilled essential oil. The high concentration of cineole and camphor and their synergistic effect is presented as a possible explanation for the traditional use of *Osmitopsis asteriscoides* for treating microbe-related illnesses.

Keywords: *Osmitopsis asteriscoides*; Essential oil; Antimicrobial activity; Camphor; 1,8-Cineole

1. Introduction

Osmitopsis asteriscoides, known locally as 'bels' or 'belskruie', is restricted to the southwestern Cape region in South Africa (Bremer, 1972). This traditional Cape-Dutch remedy has been used to treat various ailments and may be taken orally in the form of a brandy tincture for chest complaints. The dried plant may also be used externally for inflammation, cuts and swelling (Van Wyk et al., 1997). The leaves of the plant have numerous surface glands containing essential oil and when brushed the plant emits a strong eucalyptus-camphor smell. Previous phytochemical work has been undertaken on *Osmitopsis* (Bohlman and Zdero, 1974; Bohlman et al., 1985) which reported the presence of sesquiterpene lactones. This is the first report on the biological activity and composition of the essential oil, which is the most characteristic feature of this highly aromatic plant. Although the antimicrobial activity of essential oils and their constituents are well known (Hinou et al., 1989; Yousef and Tawil, 1980; Pattnaik et al., 1997; Hammer et al., 1999) the methodology used to assess in vitro antimicrobial action remains a topic of debate. This study has also documented the variability, reproducibility and accuracy in the results obtained using different methods.

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2. Materials and methods

2.1. Plant material

The aerial parts from one individual plant were collected from a natural population near Betty's Bay in the South Western Cape. Due to the large volume of oil required for time-kill studies plant material was collected from a large number of plants in the same population. Population samples were hydro-distilled for 3 h.

2.2. GC-MS analysis

Analysis of samples with GC-MS was performed using HP 1800A GCD system operating under the following conditions; column: HP-Innowax (60 m × 0.25 mm i.d., 0.25 μm film thickness), temperatures: injection port 250 °C, column 60 °C for 10 min, 4 °C/min to 220 °C, 220 °C for 10 min, 1 °C/min to 240 °C (total = 80 min). Chiral separations were performed on a multidimensional gas chromatography/mass spectrometry (MD-GC/MS) system. Two Hewlett-Packard GC 6890 systems with MSD and Gerstel multi column switching (MCS) system were used. The cooled injection system (CIS) was kept at 40 °C for injection. Helium was used as carrier gas (1 ml/min). Precolumn, HP-Innowax fused silica capillary column (60 m × 0.25 mm i.d., with 0.25 μm film thickness). GC oven temperature was kept at 60 °C for 10 min and programmed to 220 °C at a rate of 4 °C/min and kept constant at 220 °C for 10 min, then programmed to 240 °C at a rate of 1 °C/min and kept constant at 240 °C for 40 min. The FID detector temperature was at 250 °C. Main column, Lipodex E [Octakis(3-*O*-butyryl-2,6-di-*O*-pentyl)-γ-cyclodextrin] (70% in OV 1701) (25 m × 0.25 mm i.d.). Temperature program for camphor was 40 °C for 34 min and programmed to 120 °C at a rate of 1 °C/min then kept constant at 120 °C for 6 min. MS were taken at 70 eV. Mass range was from *m*/*z* 35 to 425. Library search was carried out using TBAM Library of Essential Oil Constituents.

2.3. Antimicrobial testing

Three different antimicrobial assays (disc diffusion, minimum inhibitory concentrations and time-kill studies) were performed on the hydro-distilled oil. A comparative study of the different assays was undertaken and validated the need to re-evaluate false negative results often found in disc diffusion methodology (Eloff, 1998).

2.4. Disc diffusion studies

Antimicrobial disc diffusion assays were performed on eight reference bacterial strains: *Pseudomonas aeruginosa* (ATCC 9027), *Escherichia coli* (ATCC 22922), *Staphylococcus aureus* (ATCC 25923), *Enterococcus faecalis* (ATCC 29212), *Serratia odorifera* (ATCC 33132), *Bacillus subtilis* (ATCC 15244), *Salmonella typhimurium* (ATCC 14028), *Staphylococcus epidermidis* (ATCC 2223) and a clinical strain of *Proteus vulgaris* was tested. Four fungal strains: *Candida albicans* (ATCC 10231), *Cryptococcus neoformans* (ATCC 90112), *Aspergillus niger* (clinical strain) and *Alternaria alternata* (clinical strain) were tested. Base layers of Mueller Hinton (Oxoid) agar were prepared for bacterial studies and Sabourauds Dextrose (Oxoid) agar were prepared for fungal cultures. Spore suspensions yielding an inoculum size of approximately 1×10^6 were thoroughly mixed into the overlaying agar surface. With aseptic manipulation, 6 mm discs were saturated with 8–12 mg neat oil and placed onto the set agar. Neomycin discs (30 μg, Oxoid) were used for positive bacterial controls. Nystatin discs (100 iμ, Oxoid) were used for fungal controls. All plates were incubated at 37 °C for 48 h with the exception of the molds, which were incubated at 25 °C for 7 days. Tests were done in triplicate.

2.5. Microplate bioassay

Minimum inhibitory concentrations were determined using the INT microplate method (Eloff, 1998). The hydro-distilled oil at starting concentrations of 64 mg/ml were transferred into the first well of a microtiter plate. Serial dilutions are performed so that essential oil concentrations of 32, 16, 8, 4, 2, 1, 0.5 and 0.25 mg/ml were obtained. A fixed bacterial culture yielding an inoculum size of 1×10^6 was added to all wells and incubated for 37 °C for 24 h. A 0.2 mg/ml *p*-iodotetrazolium violet (INT) solution was transferred to all inoculated wells and examined to determine a colour change in relation to concentration of microbial growth after 30, 60, 120 min and 24 h.

2.6. Time-kill studies

Death kinetic studies on three different isolates, one fungal strain (*Candida albicans*, ATCC 10231) and two bacterial strains, one a Gram-positive (*Staphylococcus aureus*, ATCC 29523) and a Gram-negative isolate (*Pseudomonas aeruginosa*, ATCC 9027 for UW and ATCC 27853 for UI) were determined on the essential oil in two separate laboratories using different time-kill methodologies. Comparative time-kill studies were done with the two main chemical components identified in the essential oil of *Osmitopsis asteriscoides*, i.e. 1,8-cineole and camphor. *Candida albicans*, the most susceptible organism, was used to demonstrate the efficacy of cineole and camphor independently and in combination in concentrations relative to their ratio in the plant.

The inactivation broth death kinetic method (Christoph et al., 2000) was adopted by the University of the Witwatersrand (hereafter referred to as the UW method). Cultures were grown in Tryptone Soya (Oxoid) broth and centrifuged for 10 min at 5000 rpm. The supernatant was discarded and the pellets resuspended in 10 ml of a 0.9% NaCl solution. Oil concentrations of 0.5, 1, 1.5 and 2% were incorporated into 50 ml Tryptone Soya (Oxoid) broth with 0.5% Tween

and a final inoculum of approximately 1×10^6 CFU/ml. The different concentrations were incubated at 37 °C in a shaking water bath. At time intervals ranging from 0 min to 24 h, aliquots of 1 ml were transferred to 9 ml inactivation broth consisting of 0.1% peptone (Oxoid), 5% lecithin (Merck) and 5% yeast extract (Oxoid). Five serial dilutions were performed in 0.9% NaCl solution, from the inactivation broth 100 μl was plated onto Tryptone Soya (Oxoid) agar for each oil concentration. The plates were incubated at 37 °C for 48 h and colony forming units (CFUs) counted and death kinetics expressed in $\log_{10}$ reduction time-kill plots. Controls were included in the study having the same broth formulation but without the oil. The assay was performed in duplicate.

At the University of Iowa (hereafter referred to as the UI method) time-kill procedures were conducted as described previously (Klepser et al., 1997). Fungi and bacteria were obtained from stored samples and subcultured twice on Potato Dextrose agar or Mueller Hinton agar (Remel) prior to testing. Suspensions containing test organisms were prepared in sterile water for fungi and normal saline for bacteria by touching three to five colonies from a 24-h-old culture plate and adjusting the resulting suspension to approximately 1×10^6 to 5×10^6 CFU/ml using spectrophotometric methods. One milliliter of the suspension containing the test organism, along with 0.05 ml Tween 80 was added to 9 ml of media, RPMI 1640 buffered with MOPS to pH 7.0 for fungi and Mueller Hinton broth for bacteria with or without drug, providing the starting inoculum of approximately 5×10^5 CFU/ml. The range of concentrations tested was 0.5, 1, 1.5 and 2% (v/v) oil. The culture vials were incubated with agitation at 35 °C. At predetermined time points (0, 2, 4, 6, 8 and 24-h following the addition of oil), a 0.1 ml sample was removed from each culture vial, serially diluted 1:10 in sterile water or saline and a 30 μl aliquot was plated on Potato Dextrose or Mueller Hinton agar (Remel). Colony counts were determined after incubation of the plates at 35 °C for 24–48 h. When colony counts were suspected to be less than 1000 CFU/ml, 30 μl samples were removed and plated without dilution. The limit of quantification by these methods is 100 CFU/ml (Klepser et al., 1998). The carryover effect of the oil was evaluated over the range of concentrations used in this study as previously described (Klepser et al., 1998). All time-kill curve experiments were conducted in duplicate.

3. Results

3.1. Disc diffusion assay

Of the 13 test organisms studied in the disc diffusion assay, four (*Pseudomonas aeruginosa*, *Bacillus subtilis*, *Aspergillus niger* and *Alternaria alternata*) showed no inhibitory effect. Table 1 lists the zones (mm) and minimum inhibitory values (MIC) for the fungal and bacterial isolates studied. The correlation between the two different screening methods was examined and generally larger zones of inhibition correlated with lower MIC values. Some variation for *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Bacillus subtilis* and *Cryptococcus neoformans*, did occur, where the MIC value is lower than what would be expected when observing inhibition zones. This variation between methods (Pattnail et al., 1996; Chalchat and Garry, 1997; Janssen et al., 1987; Pauli and Kubeczka, 1997; Remmal and Tantaoui-Elaraki, 1993) can be attributed mainly to the variation of neat essential oil on disc, disc size, agar composition as well as the volatility of oil in an open air system. The incubation period for the molds is 7 days, and the volatility of the essential oils may be responsible for the negative inhibition results obtained in the disc diffusion assay. Recently, it was illustrated (Inouye et al., 2001) that the MIC values of essential oils were lowered two- to eightfold when evaporation was prevented.

Table 1
Results of the disc diffusion assay, MICs and MIPs of *Osmitopsis asteriscoides* essential oil

Test organism	Diameter (mm)	DD controls		MIC (mg/ml)	MIP
		Neomycin	Nystatin		
Bacillus subtilis	R*	16		32	ND
Enterococcus faecalis	8	14		>32	ND
Escherichia coli	9	16		>32	ND
Proteus vulgaris	8	18		16	ND
Pseudomonas aeruginosa	R*	8		16	2% (17.8 mg/ml)
Salmonella typhimurium	8	14		32	ND
Serratia odorifera	8	18		>32	ND
Staphylococcus aureus	12	20		32	1.5% (13.35 mg/ml)
Staphylococcus epidermidis	8	8		>32	ND
Alternaria alternata	R*		10	ND	ND
Aspergillus niger	R*		26	ND	ND
Candida albicans	10		20	16	0.5% (4.45 mg/ml)
Cryptococcus neoformans	12		22	8	ND

The diameter of the zone of inhibition is expressed in millimeter including the disc (6 mm). Neomycin and Nystatin served as controls for bacteria and fungi, respectively. R*: resistant, ND: not determined, DD: disc diffusion, MIC: minimum inhibitory concentration, MIP: minimum inhibitory percentage.

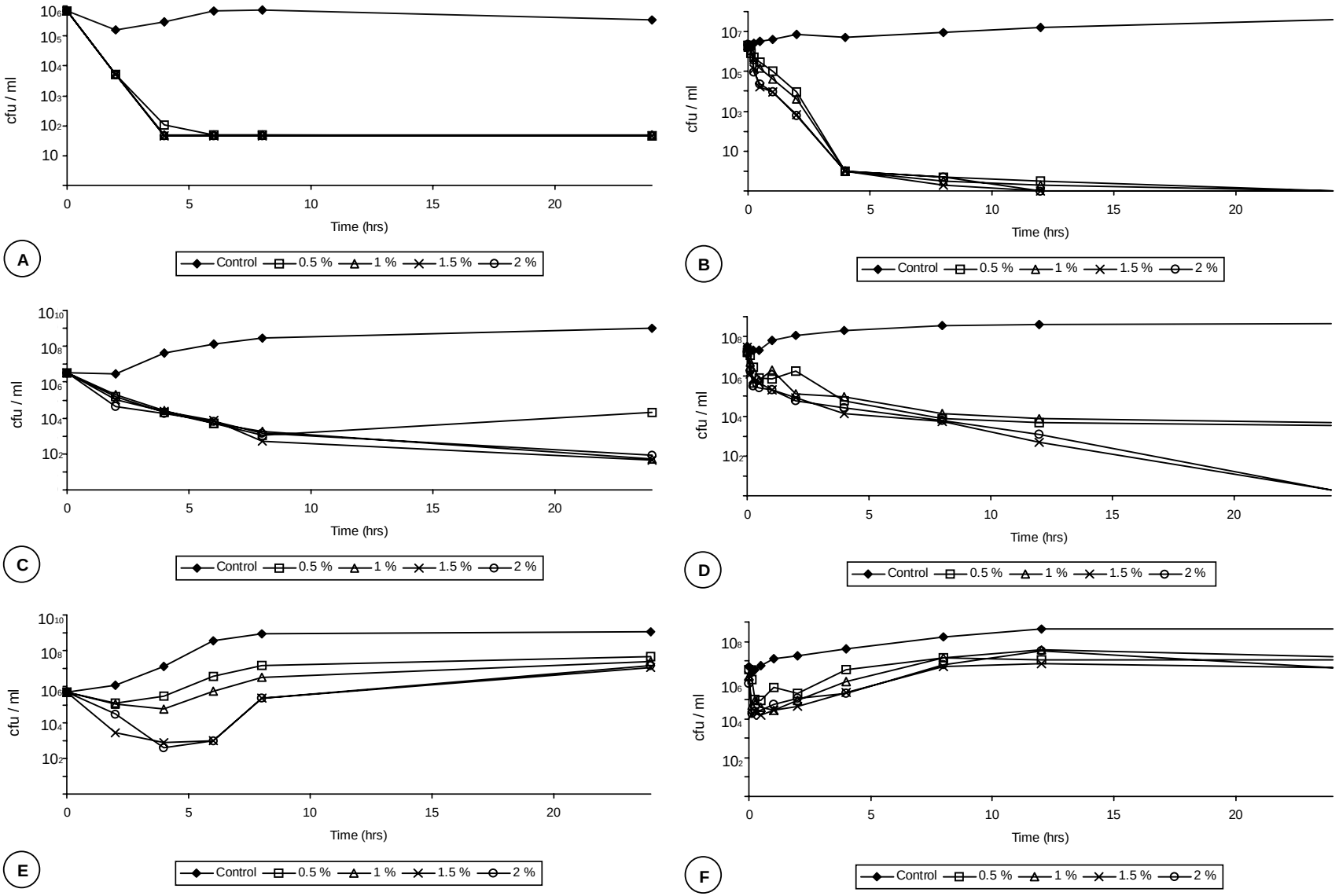

Fig. 1. Plots of mean values for $\log_{10}$ of the numbers of CFU/ml vs. time for *Osmitopsis asteriscoides* essential oil (oil concentrations expressed as a %v/v) tested on *Candida albicans* (ATCC 10231) UI (A), UW (B), *Staphylococcus aureus* (ATCC 29523) UI (C), UW (D), *Pseudomonas aeruginosa* (ATCC 27853) UI (E) and *Pseudomonas aeruginosa* (ATCC 9027) UW (F).

3.2. Time-kill studies

Results of the time-kill study are presented in Fig. 1A–F. Researchers often find the reproducibility of plant-based antimicrobial studies problematic. For this reason we duplicated this part of the study in a collaborative investigation. Fig. 1A (UI) and B (UW) display the cidal effect of the essential oil on *Candida albicans* (ATCC 10231). The cidal activity was exhibited for concentrations ranging between 0.5 and 2.0%. The candidacidal activity is rapidly exhibited and viable counts were considerably lowered at 240 min for all the concentrations and then increasing cidal action with increasing oil concentration. At 24 h complete fungicidal activity was exhibited at 2%. Both, the MIC and disc diffusion method indicate an increased antimicrobial activity in comparison with the studies on *Staphylococcus aureus* and *Pseudomonas aeruginosa*. For *Staphylococcus aureus* (ATCC 29523), the essential oil generally follows a concentration-dependent antibacterial activity (Fig. 1C (UI) and D (UW)). There is an initial drop in viable counts for all concentrations over the first 60 min after which the 1.5 and 2% oil concentrations show increasing bactericidal activity with time. Concentrations 0.5 and 1% become bacteriostatic after 8 h with viable counts neither increasing nor decreasing after 24 h. The role of oil concentration variation is evident again when comparing the disc diffusion method (8 mg oil per disc with a 12 mm zone) and MIC value of 32 mg/ml. Concentrations of 0.5% (v/v) in the time-kill method is 4.45 and 8.90 mg/ml at 1%.

In Fig. 1E (UI) and F (UW), the death kinetics show a decrease in the number of surviving *Pseudomonas aeruginosa* (ATCC 9027 for UW and ATCC 27853 for UI) cells within 60 min, but then an increase in CFUs over time indicates that after initial bacteriostatic effect, regrowth of the organism occurs. The 24 h time-kill plot for *Pseudomonas aeruginosa* show a more detailed progression of bacteriostatic activity, which can obviously not be recorded by the disc diffusion method. The regrowth effect gives some rationale for the differences of results obtained for disc diffusion.

Comparison of the time-kill plots for the three organisms studied showed that the killing rate was the greatest for *Candida albicans*, then *Staphylococcus aureus* and then with initial activity followed by subsequent regrowth for *Pseudomonas aeruginosa*.

For *Candida albicans*, both the UI and UW method showed congruent killing rates before 4 h. For *Staphylococcus aureus*, steady killing rates for both methods were obtained with slight variations at 2% concentration where minor regrowth was noted for the UI method. With *Pseudomonas aeruginosa*, regrowth before 4 h was noted in both methods. The UW method noted an initial growth reduction for the initial 60 min whereas with the UI method, a steady growth reduction for the first 4 h was noted. This may be attributed to the different *Pseudomonas aeruginosa* strains.

3.3. Chemical composition analysis

Forty-two compounds were identified in the essential oil representing 96.1% of the total composition (Table 2). The first column (A) represents the essential oil composition of a single plant in a natural population. Due to the large amount of oil required for this study leaf material had to be collected from a number of plants. The essential oil composition of this pooled sample is presented in column B (Table 2) and shows negligible quantitative and qualitative variation when compared to the essential oil composition of a single plant. 1,8-Cineole and camphor are the main compounds in the oil representing 72% accumulatively. Further studies on the role of the two major chemical components 1,8-cineole (60%) and camphor (12%) on the antimicrobial activity of *Candida albicans* was demonstrated with death kinetic studies. Fig. 2 demonstrates that both

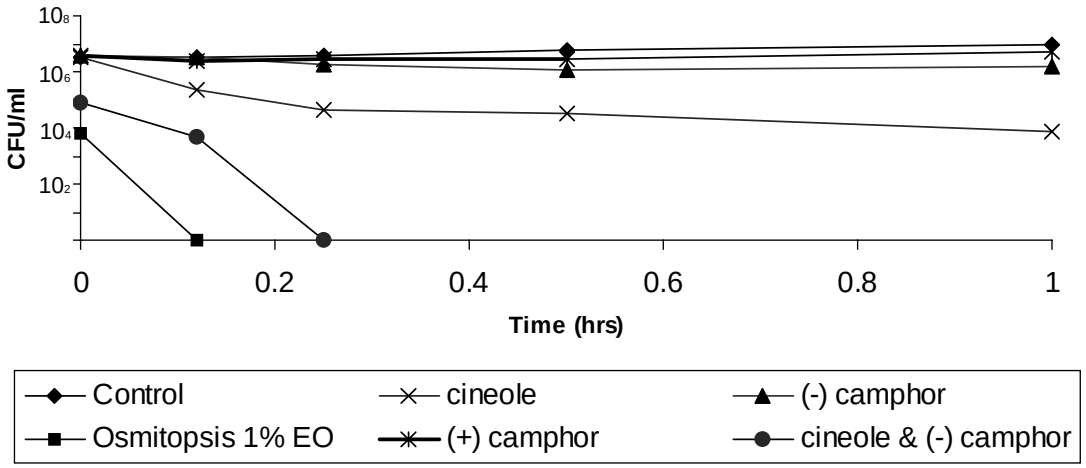

Fig. 2. Plots of mean values for $\log_{10}$ of the numbers of CFU/ml vs. time for *Osmitopsis asteriscoides* essential oil (1%), cineole, (+)-camphor, (−)-camphor, cineole and (−)-camphor tested on *Candida albicans* (ATCC 10231).

Table 2
Essential oil composition of *Osmitopsis asteriscoides*

RRI	Compound name	Column A (%)	Column B (%)
1014	Tricyclene	tr	tr
1032	α-Pinene	0.8	3.0
1035	α-Thujene	tr	0.1
1048	2-Methyl-3-buten-2-ol	tr	–
1076	Camphene	1.4	1.8
1118	β-Pinene	0.2	0.6
1132	Sabinene	1.1	1.4
1174	Myrcene	tr	tr
1188	α-Terpinene	tr	tr
1195	Dehydro-1,8-cineole	0.2	0.3
1203	Limonene	0.1	tr
1213	1,8-Cineole	56.0	59.9
1224	*o*-Mentha-1(7),5,8-triene	tr	–
1255	γ-Terpinene	tr	0.3
1280	*p*-Cymene	0.9	0.9
1290	Terpinolene	tr	tr
1348	6-Methyl-5-hepten-2-one	tr	–
1360	Hexanol	tr	–
1384	α-Pinene oxide	0.2	–
1391	(*Z*)-3-Hexen-1-ol	0.2	tr
1450	*trans*-Linalool oxide (furanoid)	0.1	tr
1451	β-Thujone	tr	–
1458	*cis*-1,2-Limonene epoxide	tr	–
1474	*trans*-Sabinene hydrate	1.3	0.1
1482	Longipinene	2.4	2.9
1493	α-Ylangene	0.1	tr
1499	α-Campholenal	0.1	tr
1522	Chrysanthenone	0.2	tr
1532	(−)-Camphor	14.8	12.4
1553	Linalool	tr	0.3
1556	*cis*-Sabinene hydrate	1.1	0.1
1571	*trans*-*p*-Menth-2-en-1-ol	0.1	0.1
1586	Pinocarvone	0.1	0.1
1611	Terpinen-4-ol	0.4	2.3
1638	*cis*-*p*-Menth-2-en-1-ol	tr	tr
1642	Thuj-3-en-10-al	tr	–
1648	Myrtenal	tr	tr
1651	Sabinaketone	0.1	tr
1657	Umbellulone	tr	–
1664	*trans*-Pinocarveol	0.1	0.1
1682	δ-Terpineol	0.5	0.4
1683	*trans*-Verbenol	0.2	–
1706	α-Terpineol	3.9	7.8
1719	Borneol	0.6	4.8
1725	Verbenone	0.2	–
1729	*cis*-1,2-Epoxy-terpin-4-ol	0.3	–
1748	Piperitone	tr	–
1751	Carvone	tr	–
1758	*cis*-Piperitol	tr	tr
1786	*ar*-Curcumene	0.1	–
1798	Methyl salicylate	0.2	0.1
1802	Cumin aldehyde	tr	–
1804	Myrtenol	0.1	tr
1831	2-Hydroxypiperitone	0.3	–
1845	*trans*-Carveol	0.1	–
1864	*p*-Cymen-8-ol	0.3	tr
1875	*trans*-2-Hydroxy-1,8-cineole	0.7	–
1889	Ascaridole	tr	–
1946	4-Hydroxy piperitone	tr	–
2008	Caryophyllene oxide	1.1	0.4
2008	*p*-Menta-1,8-dien-10-ol	–	0.1
2074	Caryophylla-2(12),6(13)-diene-5-one	0.3	–
1957	Cubenol	0.2	–
2113	Cumin alcohol	0.2	tr
2144	Spathulenol	0.3	–
2265	Longiverbenone (=vulgarone B)	0.5	0.2
Total		92.1	96.1

RRI: relative retention indices calculated against *n*-alkanes. Percentage calculated from TIC data; tr: trace (<0.1%).

(+)-camphor, (−)-camphor have negligible antimicrobial activity on *Candida albicans* whereas cineole indicated total microbial reduction after 240 min. As *Osmitopsis asteriscoides* only accumulates levorotatory camphor, it was tested in combination with cineole and a total reduction of CFUs is observed at 15 min. From this we deduce the positive antimicrobial response due to the synergistic property between 1,8-cineole and (−)-camphor. From Fig. 2, it is evident that some minor compounds should also be taken into consideration as 1% essential oil still had a greater killing rate than cineole and (−)-camphor in combination.

4. Discussion and conclusion

Investigation of the two major chemical constituents 1,8-cineole and (−)-camphor, both independently and in combination show that synergistically they have a higher antimicrobial effect on *Candida albicans* than when they are studied independently.

The role of camphor and 1,8-cineole as an antimicrobial has been investigated previously. Camphor is known to be a decongestant and antiseptic (Bruneton, 1995) and it is further known for its topical use as a counter-irritant in fibrositis (Martindale, 1996). Inactivity of cineole against Gram-positive bacteria has been reported previously (Nakatsu et al., 2000). However, with *Staphylococcus aureus*, relatively good activity (Table 1) for *Osmitopsis asteriscoides* was noted. Cineole-rich essential oils have been used historically for respiratory infections (Silvestre et al., 1999). Previously, studies (Pattnaik et al., 1997) have shown that cineole (23.2%) has an antimicrobial effect against 18 microbial strains with the exception of *Pseudomonas aeruginosa*, showing no inhibitory effect. In antimicrobial studies done on *Osmitopsis asteriscoides* having 60% cineole, no lasting antimicrobial effect was noted with *Pseudomonas aeruginosa*. The antifungal activity of cineole, specifically with *Candida albicans* was also noted (Steinmetz et al., 1988), where a decrease of fungal activity was reported after 6 h for rosemary essential oil (composition, 51% cineole and 11% camphor). Similarly with *Osmitopsis asteriscoides*, there was a 90% reduction in fungal activity after 6 h. *Jasonia montana*, another commonly used folk medicine having an essential oil composition of camphor (21.79%) and

4.6% cineole showed antifungal activity against *Candida albicans* and *Cryptococcus neoformans* (Hammerschmidt et al., 1993). This corroborates our findings summarized in Table 1.

In a plant screening (Tzakou et al., 2001) of *Salvia ringens* containing concentrations of cineole (46.42–50.74%), comparable to that obtained for *Osmitopsis asteriscoides* no activity was reported for *Staphylococcus aureus* and *Staphylococcus epidermidis*, but very good activity for Gram negatives. Results of *Osmitopsis asteriscoides* show that the oil is moderately active for *Staphylococcus aureus* and *Staphylococcus epidermidis*. It was thought to be due to the high concentration of camphor (14.78%). Also of note was the fact that camphor was only present in small quantities (1.57–1.77%) for *Salvia ringens*.

Very little information has been reported on the synergistic role of chemical compounds in antimicrobial activity. One study (Nakatsu et al., 2000) reported the significant role of minor compounds in biological activity where a mixture of all compounds found in the essential oil of Thyme showed increased biological activity in comparison to the biological activity of the six major compounds when investigated independently. Most studies focus on the antimicrobial activity of single compounds as seen in a recent study (Magiatis et al., 2002) where camphor and 1,8-cineol were investigated for their independent microbial activities. Both compounds showed increased activity with the Gram negatives. In this study the camphor and 1,8-cineol were investigated independently and in combination at concentrations corresponding to that found in the plant. When looking at antimicrobial properties of chemical constituents within plant studies, researchers should be not only be encouraged to investigate various synergistic properties, but also confine to the concentration of compound to that found within the plant studied.

With comparative method evaluation of the antimicrobial activity of the essential oil the traditional use for antimicrobial therapy is confirmed. The presence of the sesquiterpene lactones having a wide range of biological activity (Bruneton, 1995) and the monoterpenes contained in the essential oil, especially cineole and (−)-camphor provides a chemical rationale for the established traditional use of *Osmitopsis asteriscoides* as a Cape-Dutch remedy which is still widely used today.

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