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Full Length Research Paper

Antimicrobial Potential and Chemical Profiling of *Chrysocoma ciliata L.* Leaf Essential Oil

A. J. Afolayan* and A. O. T. Ashafa

Department of Botany, University of Fort Hare, Alice 5700, South Africa.

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***Chrysocoma ciliata* L. is one of the South African medicinal plants used for the management of pains, stomach and menstrual disorders by the people of the Eastern Cape. The essential oil extracted from the leaves of the plant through hydrodistillation yielded 1.20% (volume/fresh weight). A total of 37 compounds were identified, constituting 85.28% of the total oil composition. The major components of the oil were the monoterpenes (α-pinene, 7.87%; β-pinene, 42.94%; myrcene, 6.13%, cis-ocimene, 7.23%; allo-ocimene, 1.18%) and sesquiterpenes (trans-beta farnesene, 1.49%; germacrene D, 1.82%; bicyclogermacrene, 2.52%; viridiflorol, 7.70%). The oil inhibited both bacteria and fungi species at relatively low concentrations. The 10 bacteria (five gram-positive and five gram-negative) used were inhibited at minimum inhibitory concentration (MIC) range of 0.08 - 10.00 mg/ml. Also, the oil suppressed the growth of four fungi species with inhibition zone ranging from 14.50 - 51.24 mm. The presence of monoterpenes as the major constituents of the essential oil from this herb could be responsible for the notable antimicrobial activity observed in this study. Therefore, the essential oil from *C. ciliata* could be promising natural product for the development of antimicrobial agents.**

Key words: *Chrysocoma ciliata*, hydrodistillation, essential oil, antimicrobial activity.

INTRODUCTION

The importance of aromatic plants is considerable due to their applications in folk medicine and their potential for commercial exploitations. These plants are used as aroma and flavour enhancers, cosmetics and in pharma- ceuticals (Boussaada et al., 2008). Aromatic and medi- cinal plants produce a wide range of volatile terpene hydrocarbons and their corresponding oxygenated deri- vatives, which together are called essential oils. Essential oils have been widely used in folkloric medicine and as such, they have been screened for their potential uses as alternative remedies for the treatment of infectious diseases (Tepe et al., 2004; Kodali et al., 2005), food borne diseases (Aureli et al., 1992; Fabio et al., 2003) and cancer cells (Sylvestre et al., 2006).

Essential oils have also been reported to be useful in aromatherapy (Buttner et al., 1996), food preservation (Faid et al., 1995), and fragrance industries (Van de Braak and Leijten, 1999). The production of essential oils by plants is believed to be primarily as a defence mechanism against pathogens and pests (Oxenham, 2003). Essential oils and their components are gaining increasing interest because of their relatively safe status, wide acceptance by consumers and their exploitation for potential multi-purpose functional uses (Sawamura, 2000).

Chrysocoma ciliata L. otherwise known as bitterbos or bitter cowcurd is a dense, rounded shrub growing up to 50 cm in height. The yellowish green leaves are small and needle shaped, sticky to touch and with bitter taste. The plant is indigenous to southern Africa, becoming invasive in overgrazed parts of the karoo and poorly managed velds (Van Wyk et al., 2002). Our preliminary investigations on its local uses revealed that the species is used in relieving menstrual pains and to reduce heavy blood flow during menstruations. In addition, it is used to boost fertility in women and in the management of stomach disorders. Based on preliminary findings, the aerial parts of the plant is crushed and boiled in water and the infusion taken orally three to four times daily. To the best of our knowledge, the chemical composition and the antimicrobial activity of the volatile constituents from this species have not been reported in literature. This study was therefore undertaken to investigate the chemical

*Corresponding author. E-mail: Aafolayan@ufh.ac.za. Fax: +27866282295.

composition and antimicrobial potential of the essential oil of *C. ciliata* against 10 bacteria and four fungal species.

According to Mathekga and Meyer, (1998), *in vitro* antimicrobial screening could provide the preliminary observations necessary to select among plant materials, those with potentially useful properties for further chemical and pharmacological investigations.

MATERIALS AND METHODS

Plant material and authentication

Plant materials were collected in April 2008 from a single population of *C. ciliata* growing around Ntselamanzi Township in Nkokobe Municipality, Eastern Cape Province (33°11.10'S and 7°10.60'E; altitude 695 m). The mean annual rainfall of the area is about 700 mm and temperature range of 13 to 25°C. The species was authenticated by Mr Tony Dold, Selmar Schonland Herbarium, Rhodes University, South Africa. A voucher specimen (AshMed, 2008/1) was prepared and deposited in the Giffen Herbarium of the Univer- sity of Fort Hare.

Extraction of essential oil and GC-MS analysis

About 250 g of the fresh leaves of *C. ciliata* was subjected to hydrodistillation for 4 h in Clavenger-type apparatus and the oil was collected in *n*-hexane. The oil was analyzed using a Hewlett Packard 6890 Gas Chromatograph linked with Hewlett Packard 5973 mass spectrometer system and equipped with a HP5-MS capillary column (30 m x 0.25 mm, film thickness 0.25 µm, Agilent Technologies Wilmington, DE, USA). The oven temperature was programmed from 70 - 240°C at a rate of 5°C/min. The ion source was set at 240°C with ionization voltage of 70 eV. Helium was used as the carrier gas. Spectra were analyzed using the Hewlett- Packard Enhanced Chem Station G1701 programme for windows.

Identification of compounds

The components of the oil were identified by matching their spectra and retention indices with those of the Wiley 275 library (Wiley, New York) in the computer library and literature (Shibamoto, 1987). Percentage composition was calculated using the summation of the peak areas of the total oil composition.

Test organisms

Five Gram-positive bacteria (*Staphylococcus aereus, Staphylococcus epidermidus, Bacillus cereus, Micrococcus kristinae, Streptococcus faecalis*), five Gram-negative bacteria (*Escherichia coli, Pseudomonas aeruginosa, Shigelia flexneri, Klebsella pneumoniae, Serratia marcescens*) and four fungi species (*Aspergillus niger, Aspergillus flavus, Penicilium notatum, Candida albicans*) were used in this study. All the test organisms were laboratory isolates obtained from the Department of Biochemistry and Micro- biology, University of Fort Hare, South Africa. The bacteria were maintained on nutrient agar while the fungi were cultured and maintained on potato dextrose agar. The organisms were revived for bioassay by subculturing in their respective medium before use.

Antibacterial activity assay

The minimum inhibitory concentration (MIC) values of the oil on each organism were determined using microplate dilution method (Ellof, 1998), with slight modifications. Briefly, bacterial strains were cultured overnight (24 h) in autoclaved nutrient broth (Biolab, Johannesburg, South Africa), and was adjusted to a final density of 10^6 cfu/ml. This was used to inoculate 96-well microtitre plates containing serial two fold dilutions of essential oil (10 - 0.08 mg/ml) under aseptic condition. In order to increase miscibility, the oil was dissolved in 10% aqueous dimethylsulfoxide (DMSO) in the ratio 1:10. The plates were incubated under aerobic conditions at 37°C and examined after 24 h. As an indicator of bacterial growth, 40 µL of 0.2 mg/mL p-iodonitrotetrazolium (97% purity, Fluka Chemie) 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 the biological activity of the organisms. Each treatment was performed in triplicate and complete suppression of growth at a specific concentration of oil was required for it to be declared active (Ellof, 1998). Streptomycin and clhoramphenicol were used as positive controls in the experiment with pure solvent and sample free solutions as blank controls.

Antifungal activity assay

The quantitative antifungal activity of the essential oil was performed using the agar well diffusion method. Briefly: Potato dextrose agar (PDA) (Biolab, Johannesburg, South Africa) was autoclaved and 5 ml was poured into each 65 mm petri dishes, swirled and allowed to solidify. A portion of actively growing *A. niger, A. flavus, P. notatum* and *C. albicans* was cut out into another plate and diluted with distilled water to approximately 10^5 colony forming unit per milliliter (CFU/ml). Five hundred microliters of the inoculums was spread over the plates already containing solidified PDA and approximately 5 mm well was bored at the center of the plates. The oil at concentrations of 25 µL, 50 µL and 100 µL were added to the wells as appropriate. Plates were sealed with parafilm and left for 30 min at room temperature to allow the diffusion of the oil. The plates were then incubated for 48 h at 25°C, after which the inhibition zone obtained around the well was mea- sured. Controls were plates containing microorganisms without the oils. The experiments were carried out in triplicate under aseptic condition.

RESULTS AND DISCUSSION

Chemical composition of essential oil

The essential oil obtained from the fresh leaves of *C. ciliata* by hydrodistillation had a light yellow colour with a pungent smell at room temperature. The oil yield was 1.20% (volume/fresh weight). A total of 37 compounds were identified, constituting 85.28% of the total oil com- position (Table 1). The oil was dominated by monoter- penes α-pinene (7.87%), β-pinene (42.94%), myrcene (6.13%), cis-ocimene (7.23%) and allo-ocimene (1.18%). The major sesquiterpenes in the oil were trans-beta farnesene (1.49%), germacrene D (1.82%), bicyclogermacrene (2.52%) and viridiflorol (7.70%).

Antibacterial activity of the essential oil

The essential oil from *C. ciliata* inhibited all the organisms tested (Table 2), at MICs ranging from 0.08 to 10.00 mg/ml. The activity of the oil was more pronounced on the Gram-positive bacteria than the Gram-negative

Table 1. Chemical composition of essential oil from *Chrysocoma ciliata* L. leaves.

Compound	KI value	% Composition
N-hexanal	920	0.12
Trans-2-hexanal	970	0.07
α -pinene	1055	7.87
β-pinene	1132	42.94
Myrcene	1145	6.13
Cis-ocimene	1183	7.23
α -terpinolene	1218	0.17
Allo-ocimene	1259	1.18
2-cyclohexen-1-ol	1261	0.05
β -ocimene	1280	0.12
Terpinen-4-ol	1321	0.31
(E)-4,8-Dimethyl-1,3,7-nonatriene	1341	0.06
β -fenchyl alcohol	1345	0.44
Thymyl methyl ether	1392	0.71
Phenol, 2-methyl-5-(1-methylethyl)	1476	0.37
β -bisabolene	1587	0.58
Allo-aromadendrene	1600	0.17
4,5-dimethyl-2-(2-propenyl)phenol	1509	0.63
β -elemene	1514	0.27
α -gurjunene	1532	0.15
Trans-cryophyllene	1544	0.50
1-methoxy-2-tert-butyl-6-methylben	1562	0.52
Trans-beta-fernesene	1586	1.49
Germacrene D	1617	1.82
Bicyclogermacrene	1635	2.52
α -copane	1668	0.24
α-calacorene	1678	0.08
Viridiflorol	1731	7.70
Torregol	1779	0.64
t-muurolol	1790	0.62
(+)- beta-costol	1801	0.32
1,12-tridecadiene	1835	0.09
Mintsulfide	1861	0.15
Tetradecanoic acid	1911	0.26
Valerenol	1929	0.14
Neophytadiene	1974	0.10
Hexadecanoic acid	2117	0.34
Total		85.28%

bacteria. The results from this study are in agreement with many studies on other plant species (Bougatsos et al., 2003, Yayli et al., 2005 and Baussaada et al., 2008), that plant essential oils normally have more inhibitory activity against Gram-positive than Gram-negative bac- teria. Of interest is the ability of the oil to inhibit *P. aeruginosa* at 5 mg/ml a well known antibiotic resistant gram-negative bacterium. According to several authors, this bacterium is generally less sensitive to the actions of plants essential oils (Marino et al., 2001; Pintore et al., 2002; Wilkinson et al., 2003; Baussaada et al., 2008). According to van Wyk et al. (2002), *C. ciliata* is responsible for purging disease (severe diarrhoea) in adult animals when they are forced to ingest large quantity of this species during winter or drought season. In contrast, the oil from this plant inhibited *S. flexneri* and *E. coli* which are notable diarrhoea causing bacteria at low concentrations (Table 1). The antibacterial activity exhibited by the essential oil from *C. ciliata* against all bacteria strains could, in part, be associated with the major oil constituents such as α-pinene, β-pinene, myrcene and cis-ocimene. Alpha-pinene and beta-pinene have been reported to display strong antibacterial effects (Dorman and Deans, 2000; Sassi et al., 2008). Essential oils rich in terpenes have also been shown to possess good antibacterial activity (Taylor et al., 1996). The appreciable presence of these terpenes in the essential oil of this species, could explain its antibacterial activity against the tested bacteria strains.

Antifungal activity of essential oil

The antifungal activity of the essential oil from *C. ciliata* is presented in Table 3. The oil inhibited all the four fungal strains in a dose dependent manner, with the zone of inhibition ranging from 14.50 ± 1.76 to 51.24 ± 3.18 mm. The susceptibility of the fungal species to the essential oil of this plant is noteworthy, as these organisms have recently been implicated in cases of immuno-compromised patients that frequently develop opportunistic and superficial mycosis (Portillo et al., 2001; Magwa et al., 2006). The essential oil from *C. ciliata* has a great variety of phytochemicals that could be considered as responsible for the significant part of the antimicrobial activity. Essential oils usually occur as complex mixtures and their activity can generally be accounted for in terms of their major monoterpenoid components. According to Sikkema et al. (1995), the antimicrobial action of monoterpenes suggests that they diffuse into and damage cell membrane structures of microorganisms. The strong antifungal activity exhibited by the essential oil of *C. ciliata* in this study could partly be accounted for by the presence of constituents like α-pinene, a compound that has been reported to possess strong antifungal activity (Magiatis et al., 1999; Matosyoh et al., 2007). In addition, some components that occur in lesser amount may also contribute to the antimicrobial activity of the oil, involving probably some type of synergism with the other active compounds. This screening is an important evaluation of the potential antimicrobial activity of essential oil of this herb.

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Table 2. Antibacterial activity of the essential oil from the leaves of *Chrysocoma ciliata* L.

Bacteria	Gram +/-	Essential oil	Chloramphenicol. (μgmL^{-1})	Streptomycin. (μgmL^{-1})
Staphylococcus aereus	+	0.08	<2	<2
Staphylococcus epidermidus	+	0.08	<2	<2
Bacillus cereus	+	0.08	<2	<2
Micrococcus kristinae	+	1.25	<0.5	<2
Streptococcus faecalis	+	0.08	<2	<4
Escherichia coli	-	10.00	<2	<2
Pseudomonas aeruginosa	-	5.00	<10	<4
Shigelia flexneri	-	1.25	<2	<2
Klebsella pneumoniae	-	5.00	<2	<2
Serratia marcescens	-	2.50	<2	<2

Table 3. Antifungal activity of the essential oil from the leaves of *C. ciliata* L. Data in $\overline{X}$ ± S.D. Percentage inhibitions are in parenthesis.

	Inhibition zone (mm)			
Concentration	***A. niger***	***A. flavus***	***P. notatum***	***C. albicans***
Control	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
25 (µL)	14.50 ±1.76 (24.17)	15.50 ± 2.07 (25.83)	24.50 ± 3.56 (40.83)	13.67 ± 3.27 (21.03)
50 (µL)	19.00 ± 3.03 (29.03)	19.17 ± 2.14 (31.95)	36.67 ± 2.94 (61.12)	47.84 ± 2.89 (79.73)
100 (µL)	23.33 ± 1.63 (38.88)	37.00 ± 2.61 (61.67)	37.00 ± 2.61 (61.67)	51.24 ± 3.18 (85.40)

REFERENCES

Aureli PA, Costantini J, Zolea S (1992). Antimicrobial activity of some plant essential oils against *Listeria monocytogenes*. J. Food Prod. 55: 344-348

Bougatsos C, Meyer JJM, Magiatis P, Vagias C, Chinou IB (2003). Composition and antibacterial activity of the essentialoil of *Helichrysum krausii* Sch. Bip and *Helichrysum rugulosum* Less. from South Africa. Flavour and fragrance J. 18: 48-51.

Boussaada O, Ammar S, Saidana D, Chriaa J, Chraif I, Daami M, Helal AN, Mighri, Z (2008). Chemical composition and antimicrobial activity of volatile components from capitula and aerial parts of *Rhaponticum acaule* DC growing wild in Tunisia. Microbiol. Res. 163: 87-95

Buttner MP, Willeke K, Grinshpun SA (1996). Sampling and analysis of airborne microorganisms. In manual of Environmental Microbiology. Edited by: Hurst CJ, Knudsen GR, Mclnerney MJ, Stezenbach LD,Walter MV. ASM Press: Washington DC. Pp. 629-640.

Dorman HJD, Deans SG (2000). Antimicrobial agents from plants: antibacterial activity of plant volatile oils. J. Appl. Microbial. 88: 308- 316

Ellof JN (1998). A sensitive and quick microplate method to determine the minimum inhibitory concentration of plant extracts for bacteria. Planta Medical 64: 711- 713.

Fabio A, Corona A, Forte E, Quaglio P (2003). Inhibitory activity of spices and essential oils on psychrotrophic bacteria. New Microbiol. 26: 115-120.

Faid M, Bakhy K, Anshad M, Tantaoui-Elaraki A, Alomondpaste (1995). Physiochemical and microbial characterizations and preservation with sorbic acid and cinnamon. J. food Prod. 58: 547-550

Kordali S, Kotan R, Mavi A, Cakir A, Ala A, Yildirim A (2005). Determination of the chemical composition and antioxidant activity of the essential of *Artemisi, dracunculus* and of the antifungal and antibacterial activities of Turkish *Artemisia absinthium, Artemisia dracunculus, Artemisia santonicum* and *Artemisia spicigera* essential oils. J. Agric. Food. Chem. 53: 9452-9458

Magiatis P, Mellion E, Skahsounis AL, Chinou I, Mitaku S (1999). Composition and antimicrobial activity of the essential oils of *Pistacia lentiscus* var Chia. In book of abstracts: 2000 years of natural products research – past present and future. Amsterdam: Leiden University P. 662.

Magwa ML, Gundiza M, Grewu N, Humphrey G (2006). Chemical composition and biological activities of essential oil from the leaves of *Sesuvium portulacastrum*. J. Ethnopharmacol. 103: 85-89.

Mathekga ADM, Meyer JJM (1998). Antibacterial activity of South African *Helichrysum* species. S. Afr. J. Bot. 64: 239-295.

Marino M, Bersani C, Comi G (2001). Impedence measurements to study the antimicrobial activity of essential oils from Lamiaceae and Compositeae. Int. J. Food Microbiol. 67: 187-195.

Matasyoh J, Kiphino JJ, Karubiu NM, Hailstorks TP (2007). Chemical composition and antimicrobial activity of essential oil of *Tarchonanthus camphoratus*. Food Chem. 100: 1183-1187.

Oxenham SK (2003). Classification of an *Ocimum basilicum* germplasm collection and examination of the antifungal effects of the essential oil of basil. Ph.D. thesis, Glasgow, UK, University of Glasgow.

Pintore G, Usai M, Bradesi P, Juliano C, Boatto G, Tomi F, Mario C, Riccardo C, Joseph C (2002). Chemical composition and antimicrobial activity of Rosmarinus officinalis L. oils from Sardina and Corsica. Flavour and fragrance J. 17: 15-19.

Portillo A, Vila R, Freixa B, Adzet T, Canigueral S (2001). Antifungal activity of Paraguayan plants used in traditional medicine. J. Ethnopharmacol. 76: 93-98.

Sassi AB, Harzallah-Skhiri F, Chraief I, Bourgougnon N, Hamman M, Aouni M (2008). Chemical composition and antimicrobial activities of the essential oil of (Tunisian) Chrysanthemum trifurcatum (Dest.) Batt. And Trab. flowerheads. Chimie 11: 324-330.

Sawamura M (2000). Aroma and functional properties of Japaneseyuzu (*Citrus junos* Tanaka) essential oil. Aromatic Res. 1: 14–19.

Shibamoto T (1987). Retention indices in essential oil analysis. In P. Sandra & C. Bicchi (Eds.), Capillary gas chromatography in essential oils analysis. New York: Huethig, A. Verlag. pp. 259–274.

Sikkema J, de Bront JAM, Poolman B (1995). Mechanisms of membrane toxicity of hydrocarbons. Microbial reviews 59: 201-222.

Sylvestre M, Pichette A, Longtin A, Nagau F, Legault J (2006). Essential oil analysis and anticancer activity of leaf essential oil of *Croton flavens* L. from Gaudeloupe. J. Ethnopharmacol. 103: 99-102.

Taylor RSL, Edel F, Manandhar NP, Towers GHN (1996). Antimicrobial activity of Southern Nepalese medicinal plants. J. Ethnopharmacol. 50: 97-102.

Tepe B, Daferera D, Sokmen M, Polissiou M, Sokmen A (2004). In vitro antimicrobial and antioxidant activity of the essential oils and various extracts of *Thymus eigii* M Zohary and P.H Davis. J. Agric. Food Chem. 52, 1132-1137.

Van de Braak SAAJ, Leijten GCJJ (1999). Essential Oils and Oleoresins: A survey in the Netherlands and other major Markets in the European Union. CBI, Centre for the Promotion of Imports from Developing Countries, Rotterdam. p. 116.

Van Wyk BE, van Heerden F, van Oudtshoorn B (2002). Poisonous plants of South Africa Briza publications, Pretoria. pp. 66-67.

Wilkinson JM, Hipwell M, Ryan T, Cavangh HMA (2003). Bioactivity of Backhousiacitriodora: antibacterial and antifungal activity. J.Agric. and Food Chem. 51: 76-81

Yayli N, Ahmet Y, Cana G, Asu U, Sevgi K, Kamil C, Karaoğlu S (2005). Composition and antimicrobial activity of essential oils from *Centaurea sessilis* and Centaurea *armena.* Phytochem. 66: 1741-1745.