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Chemical composition of the leaf essential oil of *Clibadium leiocarpum* from Monteverde, Costa Rica

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Abstract

The leaf essential oil of *Clibadium leiocarpum* from Monteverde, Costa Rica, has been obtained by hydrodistillation and the chemical composition determined by GC-MS. The major components in the leaf oil were germacrene D (21.1%), sabinene (16.0%), germacra-4(15),5,10(14)-trien-1α-ol (11.9%), (*E*)-caryophyllene (9.2%), and β-phellandrene (7.3%). The leaf oil showed slight *in-vitro* cytotoxic activity against MDA-MB-231 human breast tumor cells and slight antibacterial activity against *Bacillus cereus*.

Keywords: *Clibadium leiocarpum*; leaf oil; composition; germacrene D; sabinene.

1. Introduction

There are some 29 species of *Clibadium* (Asteraceae) distributed in the Neotropics[1], eight of which occur in the Monteverde region of northwestern Costa Rica. A number of these are used as fish poisons[2, 3] and as traditional medicines[4-9], especially *C. sylvestre* and *C. surinamense*. In this work, we present the leaf essential oil composition of *Clibadium leiocarpum* Steetz collected from Monteverde, Costa Rica. Outside of a brief report of fatty acids, fatty acid esters, and squalene from an extract of *C. leiocarpum*[10], there have been no phytochemical investigations of this plant, and this is the first report on the essential oil composition.

2. Materials and Methods

2.1 Plant Material

Leaves of *C. leiocarpum* were collected from a mature plant growing in the Monteverde Cloud Forest Preserve, Costa Rica (10° 20.9′ N, 84° 45.8′ W, 1530 m elevation) on May 6, 2008. The plant was identified by W. A. Haber, and a voucher specimen (Haber 10247) has been deposited in the herbarium of the Missouri Botanical Garden. The fresh leaves (24.8 g) were chopped and hydrodistilled for 4 h using a Likens-Nickerson hydrodistillation apparatus with continuous extraction with $CHCl_3$ (50 mL). The chloroform extract was evaporated to give the essential oil (83.5 mg) as a pale yellow oil.

2.2 Gas Chromatographic – Mass Spectral Analysis

The leaf oil of *C. leiocarpum* was subjected to gas chromatographic-mass spectral analysis using an Agilent 6890 GC with Agilent 5973 mass selective detector, fused silica capillary column (HP 5ms, 30 m × 0.25 mm), helium carrier gas, 1 mL/min flow rate; injection temperature 200 °C, oven temperature program: 40°C initial temperature, hold for 10 min; increased at 3 °C/min to 200 °C; increased 2°/min to 220 °C, and interface temp 280 °C; EIMS, electron energy, 70 eV. The sample was dissolved in $CHCl_3$ to give a 1% w/v solution; 1-μL injections using a splitless injection technique were used. Identification of oil components was achieved based on their retention indices (determined with reference to a homologous series of normal alkanes), and by comparison of their mass spectral fragmentation patterns with those reported in the literature[11] and stored on the MS library [NIST database (G1036A, revision D.01.00)/ChemStation data system (G1701CA, version C.00.01.08)].

2.3 Antibacterial Screening

The leaf oil of *C. leiocarpum* was screened for antibacterial activity against *Bacillus cereus* (ATCC No. 14579) *Staphylococcus aureus* (ATCC No. 29213) and *Escherichia coli* (ATCC No. 10798). Minimum inhibitory concentrations (MIC) were determined using the microbroth dilution technique[12]. Dilutions of the essential oil were prepared in cation-adjusted Mueller Hinton broth (CAMHB) beginning with 50 µL of 1%, w/w, solutions of essential oil in DMSO plus 50 µL CAMHB. The extract solutions were serially diluted (1:1) in CAMHB in 96-well plates. Organisms at a concentration of approximately 1.5×10^8 colony forming units (CFU)/mL were added to each well. Plates were incubated at 37 °C for 24 h; the final minimum inhibitory concentration (MIC) was determined as the lowest concentration without turbidity. Geneticin was used as a positive antibiotic control; DMSO was used as a negative control.

2.4 Cytotoxicity Screening

Human MDA-MB-231 breast adenocarcinoma cells (ATCC No. HTB-26)[13] were grown in an air environment at 37 °C in Leibovitz's L-15 medium with L-glutamine, supplemented with 10% fetal bovine serum, 100,000 units penicillin and 10.0 mg streptomycin per liter of medium, and buffered with 30 mM Hepes, pH 7.35. Cells were plated into 96-well cell culture plates at 2.5×10^4 cells per well. The volume in each well was 100 µL. After 48 h, supernatant fluid was removed by suction and replaced with 100 µL growth medium containing 1.0 µL of DMSO solution of the essential oil (1% w/w in DMSO). This gave a final concentration of 100 µg/mL in each well. Solutions were added to wells in four replicates. Medium controls and DMSO controls (10 µL DMSO/mL) were used. Tingenone[14] was used as a positive control. After the addition of oil, plates were incubated for 48 h at 37 °C; medium was then removed by suction, and 100 µL of fresh medium was added to each well. In order to establish percent kill rates, the MTT assay for cell viability was carried out[15]. After colorimetric readings were recorded (using a Molecular Devices Spectra MAX Plus microplate reader, 570 nm), average absorbencies, standard deviations, and percent kill ratios ($\%kill_{oil}/\%kill_{DMSO}$) were calculated.

3. Results and Discussion

Hydrodistillation of fresh leaves of *C. leiocarpum* yielded a low yield of pale yellow leaf oil (0.337%). The leaf essential oil composition of *C. leiocarpum* is summarized in Table 1. A total of 30 compounds were identified in the essential oils accounting for 100% composition. The leaf essential oil was dominated by sesquiterpenoids (63.4%), including germacrene D (21.1%), (*E*)-caryophyllene (9.2%), and germacra-4(15),5,10(14)-trien-1α-ol (11.9%), as well as an abundant quantity of the monoterpene sabinene (16.0%). The leaf oil of *C. leiocarpum* as revealed in this study is very different from the leaf oil composition observed for *C. surinamense*, which was dominated by β-pinene, but having no sesquiterpenoids[16]. Czerson and co-workers found only squalene, fatty acids, and fatty acid esters in a petroleum ether/diethyl ether extract of *C. leiocarpum*[10], none of which were found in the leaf essential oil in this study.

Clibadium leiocarpum leaf essential oil was screened for antibacterial activity against *Bacillus cereus*, *Escherichia coli*, and *Staphylococcus aureus*, and for cytotoxic activity against the human breast tumor cell line MDA-MB-231. *C. leiocarpum* leaf oil was inactive against *E. coli* (MIC = 1250 µg/mL) and *S. aureus* (MIC = 1250 µg/mL), but did show marginal activity against the *B. cereus* (MIC = 313 µg/mL). The oil also showed marginal *in-vitro* cytotoxic activity against MDA-MB-231 cells (76.0±1.5% kill at 100 µg/mL). The slight antibacterial activity and cytotoxic activity of *C. leiocarpum* leaf oil can be attributed to the major components. (*E*)-Caryophyllene and germacrene D have both shown antibacterial activity against *B. cereus* as well as cytotoxic activity against MDA-MB-231 cells[17]. Sabinene has shown *in-vitro* cytotoxicity on MCF-7, Hep-G2 and HCT-116 tumor cell lines[18] as well as antibacterial activity against several bacterial species[19].

4. Conclusions

The leaf essential oil of *Clibadium leiocarpum* was dominated by germacrene D (21.1%), sabinene (16.0%), germacra-4(15),5,10(14)-trien-1α-ol (11.9%), (*E*)-caryophyllene (9.2%), and β-phellandrene (7.3%). The slight *in-vitro* cytotoxic activity against MDA-MB-231 cells and slight antibacterial activity against *Bacillus cereus* can be attributed to the major essential oil components.

Table 1: Leaf oil composition of *Clibadium leiocarpum*.

RI	Compound	area %
939	α-Pinene	4.1
974	Sabinene	16.0
980	β-Pinene	7.5
1021	*p*-Cymene	1.6
1031	β-Phellandrene	7.3
1375	α-Copaene	1.8
1390	β-Cubebene	0.8
1420	(*E*)-Caryophyllene	9.2
1427	β-Copaene	tr
1451	α-Humulene	0.5
1459	(*E*)-β-farnesene	tr
1484	Germacrene D	21.1
1486	β-Selinene	tr
1494	*trans*-Muurola-4(14),5-diene	0.4
1496	α-Zingiberene	0.8
1500	α-Muurolene	tr
1512	(*E,E*)-a-Farnesene	4.5
1524	δ-Cadinene	4.1
1532	*cis*-Calamenene	tr
1543	α-Calacorene	tr
1563	β-Calacorene	tr
1582	Caryophyllene oxide	0.8
1593	Salvial-4(14)-en-1-one	tr
1630	Muurola-4,10(14)-dien-1β-ol	3.9
1634	Caryophylla-4(12),8(13)-dien-5α-ol	0.5
1638	Caryophylla-4(12),8(13)-dien-5β-ol	1.7
1661	*cis*-Calamenen-10-ol	tr
1671	*trans*-Calamenen-10-ol	0.6
1682	Khusinol	1.0
1688	Germacra-4(15),5,10(14)-trien-1α-ol	11.9

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