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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%), germacrene-4(15),5,10(14)-trien-1 α -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 CHCl₃ (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 \times 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 CHCl₃ to give a 1% w/v solution; 1- μ L 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 μ L of 1%, w/w, solutions of essential oil in DMSO plus 50 μ L 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 μ L. After 48 h, supernatant fluid was removed by suction and replaced with 100 μ L growth medium containing 1.0 μ L of DMSO solution of the essential oil (1% w/w in DMSO). This gave a final concentration of 100 μ g/mL in each well. Solutions were added to wells in four replicates. Medium controls and DMSO controls (10 μ L 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 μ L 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-1 α -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 μ g/mL) and *S. aureus* (MIC = 1250 μ g/mL), but did show marginal activity against the *B. cereus* (MIC = 313 μ g/mL). The oil also showed marginal *in-vitro* cytotoxic activity against MDA-MB-231 cells (76.0 \pm 1.5% kill at 100 μ g/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-1 α -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	<i>p</i> -Cymene	1.6
1031	β -Phellandrene	7.3
1375	α -Copaene	1.8
1390	β -Cubebene	0.8
1420	(<i>E</i>)-Caryophyllene	9.2
1427	β -Copaene	tr
1451	α -Humulene	0.5
1459	(<i>E</i>)- β -farnesene	tr
1484	Germacrene D	21.1
1486	β -Selinene	tr
1494	<i>trans</i> -Muurolo-4(14),5-diene	0.4
1496	α -Zingiberene	0.8
1500	α -Muurolole	tr
1512	(<i>E,E</i>)- α -Farnesene	4.5
1524	δ -Cadinene	4.1
1532	<i>cis</i> -Calamenene	tr
1543	α -Calacorene	tr
1563	β -Calacorene	tr
1582	Caryophyllene oxide	0.8
1593	Salvial-4(14)-en-1-one	tr
1630	Muurolo-4,10(14)-dien-1 β -ol	3.9
1634	Caryophylla-4(12),8(13)-dien-5 α -ol	0.5
1638	Caryophylla-4(12),8(13)-dien-5 β -ol	1.7
1661	<i>cis</i> -Calamenen-10-ol	tr
1671	<i>trans</i> -Calamenen-10-ol	0.6
1682	Khusinol	1.0
1688	Germacra-4(15),5,10(14)-trien-1 α -ol	11.9

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