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NOTE

Chemical composition and cytotoxic activity of the leaf essential oil of *Eugenia zuchowskiae* from Monteverde, Costa Rica

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Abstract The leaf essential oil of *Eugenia zuchowskiae* from Monteverde, Costa Rica, has been obtained by hydrodistillation and analyzed by GC–MS. The principal constituents of *E. zuchowskiae* leaf oil were α-pinene (28.3%), *β*-caryophyllene (13.2%), α-humulene (13.1%), and α-copaene (8.1%). The leaf essential oil of *E. zuchowskiae* showed pronounced in-vitro cytotoxic activity against MCF-7, MDA-MB-468, and UACC-257 human tumor cell lines. The major components showed cytotoxic activities comparable to doxorubicin (LC_{50} 14–70 μg/ml).

Keywords *Eugenia zuchowskiae* · Essential oil · Chemical composition · Cytotoxicity

Introduction

The family Myrtaceae is made up of 129 genera and approximately 4,620 species, mostly from tropical and warm subtropical regions [1]. There are about 550 species of *Eugenia*, 17 of which are found in the Monteverde region of Costa Rica. A number of *Eugenia* species are used in traditional herbal medicine, including *E. axillaris* to treat diarrhea [2, 3], *E. caryophyllata* used as a local anesthetic and analgesic [4], *E. dysenterica* to treat dysentery [5], and *E. uniflora* for a variety of ailments [6].

Eugenia zuchowskiae Barrie [7] is a tree, endemic to Costa Rica, and locally common on the upper Pacific slope at 1,450–1,550 m, found in mature forests and at the forest edge. We have an ongoing investigation of the medicinal potential of higher plants from Monteverde, Costa Rica [8], including essential oils [9]. In this work, we describe the chemical composition and cytotoxic activity of the leaf essential oil of *E. zuchowskiae* from Monteverde, Costa Rica. To our knowledge, neither the cytotoxic activity nor the chemical composition of *E. zuchowskiae* has been investigated previously.

Materials and methods

Plant material

Leaves of *Eugenia zuchowskiae* were collected from a mature tree near Monteverde, Costa Rica (10.3445°N, 84.8317°W, 1,420 m above sea level). The plant was identified by W. A. Haber, and a voucher specimen (Haber & Zuchowski 10036) has been deposited in the herbarium of the Missouri Botanical Garden. The fresh leaves (88.1 g) were chopped and hydrodistilled using a Likens–Nickerson apparatus with continuous extraction with chloroform. The extract was dried over calcium chloride and the chloroform evaporated to give the essential oil (140.5 mg).

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Gas chromatographic–mass spectral analysis

The leaf oil of *E. zuchowskiae* was subjected to gas chromatographic–mass spectral analysis using an Agilent 6890 GC with Agilent 5973 mass selective detector, fused silica capillary column (HP-5 ms, 30 m × 0.25 mm), helium carrier gas, 1.0 ml/min flow rate; injector temperature 200°C, oven temperature program: 40°C initial temperature, held for 10 min, increased at 3°/min to 200°C, increased 2°/min to 220°C, and interface temperature 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 [10] and stored in the MS library [NIST database (G1036A, revision D.01.00)/ChemStation data system (G1701CA, version C.00.01.08)]. The chemical composition of *E. zuchowskiae* leaf oil is summarized in Table 1.

Cytotoxicity assay

The essential oil and components (commercially available from Sigma-Aldrich) were screened for cytotoxic activity using the MTT assay [11] against MCF-7, MDA-MB-468, and UACC-257 human tumor cell lines as described previously [9, 12]. LC_{50} values for the pure compounds were determined using serial dilutions of the compounds in DMSO, and calculated using the Reed–Muench method [13]. Cytotoxic activities of *E. zuchowskiae* leaf essential oil and major components are summarized in Table 2.

Results and discussion

A yield of 0.16% of leaf essential oil was obtained by hydrodistillation of the fresh leaves of *E. zuchowskiae*. The chemical composition of the leaf oil is summarized in Table 1. A total of 40 compounds were identified, accounting for 100% of the total composition. Sesquiterpene hydrocarbons (53.1%) and monoterpene hydrocarbons (33.0%) dominated the leaf oil composition. The most abundant compounds in *E. zuchowskiae* leaf oil were the monoterpene α-pinene (28.3%), and the sesquiterpenes *β*-caryophyllene (13.2%), α-humulene (13.1%), α-copaene (8.1%), and *δ*-cadinene (6.2%). These compounds are common to many species of *Eugenia* [14–19].

Table 1 Chemical composition of *Eugenia zuchowskiae* leaf essential oil

Compound	RI[a]	Composition (%)	QI[b] (%)
trans-2-Hexenal	860	0.7	97
α-Pinene	944	28.3	96
Camphene	957	0.1	97
β-Pinene	981	1.9	95
Myrcene	994	Trace[c]	95
Limonene	1,031	2.7	97
Cyclosativene	1,366	Trace[c]	99
α-Ylangene	1,372	0.1	97
α-Copaene	1,380	8.1	99
β-Bourbonene	1,385	Trace	98
α-Gurjunene	1,411	0.3	99
β-Caryophyllene	1,425	13.2	99
Calarene	1,432	0.6	97
Aromadendrene	1,442	4.7	99
α-Guaiene	1,444	0.3	89
α-Humulene	1,459	13.1	99
Alloaromadendrene	1,463	1.1	99
γ-Gurjunene	1,472	0.1	99
γ-Muurolene	1,477	0.3	99
β-Selinene	1,486	0.2	99
Ledene	1,495	1.8	99
Bicyclogermacrene	1,498	1.3	98
α-Muurolene	1,501	0.3	99
γ-Cadinene	1,508	0.8	91
Cubebol	1,515	1.0	90
δ-Cadinene	1,528	6.2	99
Cadina-1,4-diene	1,533	0.1	98
α-Cadinene	1,537	Trace	96
α-Calacorene	1,542	0.3	98
Germacrene B	1,554	Trace[c]	95
Nerolidol	1,568	1.4	90
Spathulenol	1,577	0.8	86
Globulol	1,582	3.9	99
Humulene epoxide II	1,607	0.2	—
1,10-di-*epi*-Cubenol	1,614	Trace[c]	89
10-*epi*-*γ*-Eudesmol	1,628	0.8	91
1-*epi*-Cubenol	1,628	0.8	91
τ-Cadinol	1,642	1.4	94
Torreyol	1,646	0.2	97
α-Cadinol	1,654	0.5	95

[a] *RI* Retention index based on a homologous series of normal alkanes on a HP-5 ms column

[b] *QI* Quality index and reflects the fit comparison of experimental mass spectrum and NIST library mass spectrum

[c] Trace = <0.1%

Table 2 Cytotoxicities of *Eugenia zuchowskiae* leaf essential oil and major components

Material	Cell lines		
	MCF-7	MDA-MB-468	UACC-257
E. zuchowskiae leaf oil	100% kill[a]	100% kill	100% kill
α-Pinene	20.6 (0.5) μg/ml[b]	27.7 (1.6) μg/ml	13.5 (1.1) μg/ml
β-Caryophyllene	19.7 (1.2) μg/ml	39.2 (1.2) μg/ml	16.3 (5.7) μg/ml
α-Humulene	22.1 (1.7) μg/ml	26.1 (3.3) μg/ml	16.5 (2.0) μg/ml
α-Copaene	69.6 (2.5) μg/ml	NT	NT
Doxorubicin[c]	28.6 (1.9) μg/ml	NT	NT
Tingenone[c]	32.5 (2.8) μg/ml	17.9 (2.7) μg/ml	28.2 (2.6) μg/ml

NT Not tested against this cell line

[a] Cytotoxicity of leaf oil expressed as % kill at 100 μg/ml concentration

[b] Cytotoxicity of compounds expressed as LC_{50}, standard deviations *in parentheses*

[c] Positive control

E. zuchowskiae leaf oil was screened for in-vitro cytotoxic activity against MCF-7 (mammary adenocarcinoma), MDA-MB-468 (mammary adenocarcinoma), and UACC-257 (malignant melanoma) human tumor cell lines (Table 2), and proved to be broadly cytotoxic with 100% kill at a concentration of 100 μg/ml on the cell lines tested. The major components, α-pinene, α-copaene, *β*-caryophyllene, and α-humulene, exhibited cytotoxicities comparable to the anticancer agent doxorubicin on MCF-7 cells. The abundance of these components likely accounts for the cytotoxic activity of *E. zuchowskiae* leaf essential oil. α-Pinene has shown in-vitro cytotoxicity to Hep G2 human hepatocellular carcinoma cells [20], and α-humulene has exhibited cytotoxic activity to a number of human tumor cell lines, including MCF-7 [21]. Interestingly, *β*-caryophyllene has been shown to be nontoxic to 153BR human fibroblast cells [22].

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