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**Der Pharma Chemica, 2009, 1(2): 14-18**
*(http://derpharmachemica.com/archive.html)*

ISSN 0975-413X

# Chemical composition and antimicrobial activity of the bark essential oil of *Cedrela odorata* from Monteverde, Costa Rica

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## Abstract

The pale yellow volatile oil from the bark of *Cedrela odorata* from Monteverde, Costa Rica, was obtained by hydrodistillation and analyzed by gas chromatography – mass spectrometry. The bark oil was dominated by sesquiterpenes: β-elemene (20.3%), germacrene D (15.4%), 1,10-di-epi-cubenol (7.2%), and β-acoradiene (7.0%). *C. odorata* bark essential oil was screened for antimicrobial activity and showed appreciable activity against the Gram-positive bacterium *Bacillus cereus* and the mold *Aspergillus niger*.

**Keywords:** *Cedrela* odorata, Melicaceae, β-elemene, germacrene D, *Bacillus cereus*, *Aspergillus niger*.

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## Introduction

*Cedrela odorata* L. (Meliaceae), "Cedro amargo" is a medium to large tree (30+ m tall) that ranges from Mexico to South America and the West Indies. The tree is best known for its pink-red, rot-resistant wood that is used to make furniture, moth-proof chests, and guitars [1]. Teas and decoctions of the bark are used in folk medicine to treat fever, bronchitis, indigestion and other gastrointestinal ailments. Externally, the bark is used in baths to relieve colds, aches, and wounds [2]. A number of *Cedrela* essential oils have been previously investigated, including *C. odorata* leaf and bark oil from Brazil [3], *C. odorata* leaf oil from Nigeria [4], the leaf [3] and bark [5] oils of *C. fissilis* from Brazil, and the bark essential oil of *C. tonduzii* from Costa Rica [6]. In this work, we present the chemical composition and antimicrobial activity of the bark essential oil of *C. odorata* from the Monteverde region of northwestern Costa Rica.

## Results and Discussion

Hydrodistillation of the fresh bark of *C. odorata* yielded a pale yellow essential oil in 0.0857% yield. GC-MS analysis (Table 1) revealed the bark oil to be composed largely of sesquiterpene hydrocarbons (71%) and oxygenated sesquiterpenoids (28%). The major components were the sesquiterpene hydrocarbons β-elemene (20.3%), germacrene D (15.4%), and β-acoradiene (7.0%), along with the sesquiterpene alcohol 1,10-di-epi-cubenol (7.2%). *C. odorata* bark oil from Brazil was also dominated by sesquiterpenoids, but with a very different composition: (*E*)-caryophyllene (17.2%), (*Z*)-caryophyllene (9.1%), *cis*-4(14),5-muuroladiene (10.5%), and lesser amounts of β-elemene (5.4%) and germacrene D (0.4%) [3]. *C. tonduzii*, a higher elevation *Cedrela* from Monteverde, also contained abundant sesquiterpenes: α-humulene (4-34%), α-selinene (4-32%), germacrene D (13-17%), (*E*)-caryophyllene (2-13%), β-elemene (7-9%), and β-selinene (6-8%) [6]. *C. fissilis* bark oil had notable amounts of sesquiterpenoid alcohols (52.0%) in addition to sesquiterpene hydrocarbons (40.5%) [5].

**Table 1. Chemical composition of *Cedrela odorata* bark essential oil**

| RI[a] | Compound | % Composition[b] |
|---|---|---|
| 936 | α-Pinene | 1.1±0.3 |
| 1338 | δ-Elemene | 1.7±0.4 |
| 1375 | α-Copaene | 0.6±0.2 |
| 1384 | β-Bourbonene | 1.5±0.3 |
| 1395 | β-Elemene | 20.3±1.1 |
| 1412 | β-Funebrene (= 1,7-di-*epi*-β-Cedrene) | 0.4±0.2 |
| 1420 | (*E*)-Caryophyllene | 2.6±0.2 |
| 1434 | γ-Elemene | 0.3±0.1 |
| 1437 | Unidentified sesquiterpenoid | 0.9±0.2 |
| 1443 | 6,9-Guaiadiene | 0.8±0.2 |
| 1449 | *cis*-Muurola-3,5-diene | 0.1±0.1 |
| 1453 | α-Humulene | 0.5±0.1 |
| 1462 | α-Acoradiene | 2.5±0.6 |

| | | |
|---|---|---|
| 1469 | β-Acoradiene | 7.0±0.5 |
| 1476 | *trans*-Cadina-1(6),4-diene | 2.9±0.1 |
| 1484 | Germacrene D | 15.4±0.7 |
| 1489 | β-Selinene | 1.5±0.1 |
| 1492 | *trans*-Muurola-4(14),5-diene | trace |
| 1498 | β-Alaskene | 3.5±0.1 |
| 1502 | α-Muurolene | 0.1±0.1 |
| 1507 | Premnaspirodiene | 3.2±0.4 |
| 1513 | δ-Amorphene | trace |
| 1516 | γ-Cadinene | 1.5±0.1 |
| 1525 | δ-Cadinene | 1.3±0.1 |
| 1551 | Hedycaryol/Elemol | 1.0±0.1 |
| 1557 | Germacrene B | 2.5±0.1 |
| 1577 | Spathulenol | 0.2±0.1 |
| 1587 | Unidentified sesquiterpenoid | 0.8±0.2 |
| 1601 | Juniperol + Cedrol | 7.1±1.1 |
| 1616 | 1,10-di-*epi*-Cubenol | 7.2±0.9 |
| 1621 | Unidentified sesquiterpenoid | 1.3±0.3 |
| 1628 | 1-*epi*-Cubenol | 1.1±0.4 |
| 1641 | τ-Cadinol | 3.0±0.4 |
| 1646 | α-Muurolol | 0.1±0.1 |
| 1655 | α-Cadinol | 3.6±0.5 |

| | | |
|---|---|---|
| 1685 | Unidentified sesquiterpenoid | 1.4±0.3 |
| | Total Identified | 94.5 |
| | Monoterpene hydrocarbons | 1.1 |
| | Sesquiterpene hydrocarbons | 71.0 |
| | Oxygenated sesquiterpenoids | 27.9 |

[a]RI = "Retention Index", determined in reference to a homologous series of *n*-alkanes on an HP-5ms column.
[b]Average of three chromatograms.

*C. odorata* bark essential oil was screened for antimicrobial activity against *Bacillus cereus* (MIC = 78 μg/mL), *Staphylococcus aureus* (MIC = 625 μg/mL), *Escherichia coli* (MIC = 1250 μg/mL), *Pseudomonas aeruginosa* (MIC = 1250 μg/mL), *Candida albicans* (MIC = 625 μg/mL), and *Aspergillus niger* (MIC = 78 μg/mL). Thus, *C. odorata* bark oil only showed appreciable activity against the Gram-positive bacterium *B. cereus* and the mold *A. niger*. The antibacterial and antifungal activity of *C. odorata* bark essential oil is consistent with the traditional uses of the bark.

## Materials and Methods

*Plant materials*

Bark of *C. odorata* was collected from a mature tree growing in the Monteverde region of northwestern Costa Rica (10° 15.7′ N, 84° 50.4′ W, 903 m above sea level), on May 14, 2009. The plant was identified by William Haber. A voucher specimen (Haber 10645) has been deposited in the herbarium of the Missouri Botanical Garden. The fresh bark (200 g) was chopped and hydrodistilled for 4 hours using a Likens-Nickerson hydrodistillation apparatus [7] with continuous extraction with $CHCl_3$ (50 mL). The chloroform extract was then evaporated to yield a pale yellow essential oil (171.3 mg).

*Gas Chromatographic-Mass Spectral Analysis*

A chromatographic-mass spectral analysis was performed on the bark essential oil of *C. odorata* using an Agilent 6890 GC with Agilent 5973 mass selective detector (EIMS, electron energy = 70 eV, scan range = 45-400 amu, and scan rate = 3.99 scans/sec), using a fused silica capillary column (HP 5ms, 30 m × 0.25 mm) coated with 5% phenyl-polymethylsiloxane (0.25 μm phase thickness). The carrier gas was helium with a flow rate of 1 mL/min and the injection temperature was 200°C. The oven temperature was programmed to initially hold for 10 minutes at 40°C, then ramp to 200°C at 3°C/min and finally to 220°C at 2°/min. The interface temperature was 280°C. A 1% w/v solution of each sample in $CHCl_3$ was prepared and 1 μL was injected using a splitless injection technique. Identification of the oil components was based on their retention indices determined by reference to a homologous series of *n*-alkanes, and by comparison of their mass spectral fragmentation patterns with those reported in the literature [8] and stored on the MS library [NIST database (G1036A, revision D.01.00)/ChemStation data system (G1701CA, version C.00.01.080]. The percentages of each component are reported as

raw percentages based on total ion current without standardization. The chemical composition of *C. odorata* bark essential oil is summarized in Table 1.

*Antimicrobial Screening*

The essential oil was screened for antimicrobial activity against Gram-positive bacteria, *Bacillus cereus* (ATCC No. 14579), *Staphylococcus aureus* (ATCC No. 29213); Gram-negative bacteria, *Pseudomonas aeruginosa* (ATCC No. 27853) and *Escherichia coli* (ATCC No. 10798). Minimum inhibitory concentrations (MIC) were determined using the microbroth dilution technique [9]. Dilutions of the crude extracts were prepared in cation-adjusted Mueller Hinton broth (CAMHB) beginning with 50 μL of 1% w/w solutions of crude extracts 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 $1.5 \times 10^8$ colony forming units (CFU)/mL were added to each well. Plates were incubated at 37°C for 24 hr; 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. Antifungal activity was determined as described above using *Candida albicans* (ATCC No.90028) in yeast-mold (YM) broth with approximately $7.5 \times 10^7$ CFU/mL. Antifungal activity against *Aspergillus niger* (ATCC No. 16888) was determined as above using YM broth inoculated with *A. niger* hyphal culture diluted to a McFarland turbidity of 1.0. Amphotericin B was the positive control.

## Acknowledgments

We are very grateful to the Monteverde Cloud Forest Preserve and the Tropical Science Center for permission to collect plant materials from the Preserve and to an anonymous private donor for the generous gift of the GC-MS instrumentation. We thank Bernhard Vogler for technical assistance with GC-MS measurements.

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