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# Chemical Composition and Bioactivity of the Essential Oil of *Chromolaena odorata* from Nigeria

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**Abstract:** The essential oil from the dried leaves of *Chromolaena odorata* (L.) R.M. King & H. Rob. was obtained by hydrodistillation and analyzed by gas chromatography-mass spectrometry (GC-MS). The major components were α-pinene (42.2%), β-pinene (10.6%), germacrene D (9.7%), β-copaen-4α-ol (9.4%), (*E*)-caryophyllene (5.4%), and geijerene/pregeijerene (7.5%). The oil was screened for antimicrobial activity and showed antibacterial activity against *Bacillus cereus* (MIC = 39 μg/mL) and antifungal activity against *Aspergillus niger* (MIC = 78 μg/mL). DFT (B3LYP/6-31G*) and post-HF (MP2/6-311+G**) indicate that pregeijerene is less stable (0.45 and 3.99 kcal/mol, respectively) than its Cope rearrangement product geijerene.

**Keywords:** *Chromolaena odorata*; *Eupatorium odoratum*; Asteraceae; essential oil; pinene; germacrene D; β-copaen-4α-ol; geijerene; pregeijerene; Cope rearrangement.

## 1. Introduction

There are approximately 165 species of *Chromolaena* distributed in the tropical and warm temperate regions of the Americas [1]. *Chromolaena odorata* (L.) R.M. King & H. Rob. (syn. *Eupatorium odoratum* L.) originally ranged from southern Mexico south to Argentina and the Caribbean [2], but has been introduced into the Old World tropics where it has become an invasive pest [3]. The plant has exhibited allelopathic effects and has been reported to cause livestock death [3]. Medicinally, the plant decoction is taken as a remedy for coughs and colds or in baths to treat skin

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diseases [2]. The plant, locally called 'ewe awolowo', is used in West African traditional medicine as a wound healing and a local antiseptic agent [4,5]. *C. odorata* essential oil has exhibited insecticidal [6], insect repellent [7], and antibacterial [5,8] activities. In this report, we present the essential oil composition of the aerial parts of *C. odorata* from Lagos, Nigeria. *C. odorata* essential oils from Ife, Nigeria [5], Ivory Coast [8], and Thailand [9] have been previously reported.

## 2. Materials and Methods

### *2.1. Plant Material*

Dried leaves of *C. odorata* were collected in March, 2009, from Epe, Lagos, Lagos state, Nigeria, and the plant species was authenticated in the Forestry Research Institute of Nigeria, Ibadan. A 500-g sample of sun-dried leaves was hydrodistilled for 4 h in a modified Clevenger-type apparatus to yield 1.35 g light green essential oil. The essential oil so obtained was stored in a sealed glass bottle with screw lid cover under refrigeration at 4°C.

### *2.2 Gas Chromatography-Mass Spectrometry*

The *C. odorata* essential oil was subjected to GC-MS analysis on an Agilent system consisting of a model 6890 gas chromatograph, a model 5973 mass selective detector (EIMS, electron energy = 70 eV, scan range = 45-400 amu, and scan rate = 3.99 scans/sec), and an Agilent ChemStation data system. The GC column was an HP-5ms fused silica capillary with a (5% phenyl)-methylpolysiloxane stationary phase, film thickness of 0.25 µm, a length of 30 m, and an internal diameter of 0.25 mm. The carrier gas was helium with a column head pressure of 7.07 psi and flow rate of 1.0 mL/min. Inlet temperature was 200°C and MSD detector temperature was 280°C. The GC oven temperature program was used as follows: 40°C initial temperature, hold for 10 min; increased at 3°/min to 200°C; increased 2°/min to 220°C. The sample was dissolved in dichloromethane 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 (RI, determined with reference to a $C_9 - C_{21}$ homologous series of normal alkanes), and by comparison of their mass spectral fragmentation patterns with those reported in the literature [10] and stored on the MS library [NIST database (G1036A, revision D.01.00)/ChemStation data system (G1701CA, version C.00.01.08)].

### *2.3 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 [11]. 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 essential oil 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 ×

$10^7$ CFU/mL. Antifungal activity against *Aspergillus niger* (ATCC No. 16888) was determined as above using potato dextrose broth inoculated with *A. niger* hyphal culture diluted to a McFarland turbidity of 1.0. Amphotericin B was the positive control.

### *2.4 Ab Initio Calculations*

All calculations were carried out using SPARTAN '08 for Windows [12]. The hybrid B3LYP functional [13,14] and the 6-31G* basis set [15] were used for the optimization of all stationary points in the gas phase. Single-point Hartree-Fock *ab initio* energies were calculated using the DFT geometries (above) at the 6-311+G** [15] level, followed by a correlation energy calculation using the second-order Møller-Plesset model (MP2) [15]. Frequency calculations were employed to characterize stationary points as minima or first-order saddle points. All reaction and activation enthalpies reported are zero-point (ZPE) corrected and thermally corrected. Entropies were calculated using the linear harmonic oscillator approximation.

## 3. Results and Discussion

The essential oil was obtained as light green oil (0.16% of the dried plant material). GC-MS analysis of *C. odorata* essential oil led to identification of 56 components, representing 99.3% of the oil (Table 1). The oil was rich in α- and β-pinenes (42.2% and 10.6%, respectively), germacrene D (9.7%), β-copaene-4α-ol (9.4%), and (*E*)-caryophyllene (5.4%). Both geijerene and pregeijerene were also found in *C. odorata* oil (4.7% and 2.8%, respectively). This essential oil, then, is qualitatively similar to oils reported from Ivory Coast [8] and Thailand [9], but different from an oil reported previously from Nigeria, which was rich in camphor, limonene, and cadinol, but apparently devoid of geijerene and/or pregeijerene [5].

Both geijerene and pregeijerene were abundant components of the *C. odorata* essential oils from Ivory Coast (4.7% and 14.3%, respectively) [8] and from Thailand (3.1% and 17.6%, respectively) [9]. It is interesting that the concentrations of geijerene in these previous studies are less than the concentrations of pregeijerene. Pregeijerene has been found to readily undergo a Cope rearrangement to give geijerene [16,17]. A compilation of recent essential oils obtained by hydrodistillation and analyzed by GC with injection temperatures of around 250°C shows that some have greater pregeijerene concentrations [18-20] while others have greater geijerene [21-25]. Interestingly, subcritical fluid extraction of *Ruta graveolens* using $CO_2$ (40-45°C) also showed greater geijerene than pregeijerene [26]. However, on average, geijerene is slightly more abundant than pregeijerene (55.6:44.4). The variability in geijerene/pregeijerene ratios in the reported essential oils suggests that equilibrium was not achieved during the 3-4 hours of hydrodistillation. Jones and Sutherland had reported that pregeijerene rapidly rearranged to geijerene at 170°C [16], and the MP2 calculated $\Delta G°_r$ of -4.16 kcal/mol is consistent with nearly complete conversion of pregeijerene to geijerene at equilibrium (Table 2, Figure 1).

**Table 1.** Chemical composition of *Chromolaena odorata* leaf essential oil.

| RI | Compound | % | RI | Compound | % |
|---|---|---|---|---|---|
| 938 | α-Pinene | 42.2 | 1502 | γ-Amorphene | 1.2 |
| 980 | β-Pinene | 10.6 | 1504 | α-Muurolene | tr |
| 995 | Myrcene | 0.9 | 1508 | Premnaspirodiene | 0.2 |
| 1018 | α-Terpinene | tr | 1514 | γ-Cadinene | 0.3 |
| 1025 | *p*-Cymene | tr | 1526 | δ-Cadinene | 1.9 |
| 1029 | Limonene | 0.7 | 1531 | *trans*-Cadina-1,4-diene | tr |
| 1033 | 1,8-Cineole | tr | 1535 | α-Cadinene | tr |
| 1039 | (*Z*)-β-Ocimene | 0.2 | 1541 | α-Calacorene | 0.2 |
| 1049 | (*E*)-β-Ocimene | 0.6 | 1554 | Germacrene B | 0.1 |
| 1060 | γ-Terpinene | tr | 1562 | (*E*)-Nerolidol | 0.3 |
| 1146 | Geijerene | 4.7 | 1565 | β-Calacorene | tr |
| 1162 | Pinocarvone | 0.3 | 1581 | Caryophyllene oxide | tr |
| 1174 | *cis*-Pinocamphone | tr | 1593 | β-Copaen-4α-ol | 9.4 |
| 1194 | Myrtenol | 0.4 | 1600 | Guaiol | tr |
| 1207 | Verbenone | tr | 1609 | Humulene epoxide II | 1.1 |
| 1216 | *trans*-Carveol | tr | 1621 | α-Corocalene | tr |
| 1292 | Pregeijerene | 2.8 | 1628 | 1-*epi*-Cubenol | 0.4 |
| 1336 | δ-Elemene | tr | 1631 | γ-Eudesmol | tr |
| 1347 | α-Cubebene | tr | 1640 | τ-Cadinol | 0.2 |
| 1372 | α-Copaene | 1.5 | 1649 | β-Eudesmol | tr |
| 1383 | β-Bourbonene | 0.2 | 1652 | α-Eudesmol | tr |
| 1392 | β-Elemene | 0.7 | 1654 | α-Cadinol | 0.6 |
| 1419 | (*E*)-Caryophyllene | 5.4 | 1659 | *cis*-Calamenen-10-ol | tr |
| 1431 | β-Copaene | 0.3 | 1666 | *trans*-Calamenen-10-ol | tr |
| 1450 | α-Humulene | 1.2 | 1672 | Cadalene | 0.2 |
| 1466 | Precocene I | tr | 1676 | Andro encecalinol | tr |
| 1486 | Germacrene D | 9.7 | 1676 | Mustakone | 0.2 |
| 1498 | *trans*-Muurola-4(14),5-diene | 0.5 | 1683 | Khusinol | tr |

Antimicrobial screening (Table 3) revealed the leaf essential oil of *C. odorata* to exhibit marginal antibacterial activity against *Bacillus cereus* (MIC = 39 μg/mL) and antifungal activity against *Aspergillus niger* (MIC = 78 μg/mL). These antibacterial results are in contrast to those previously reported by Inya-Agha et al. [5] and Bamba et al. [8] who did observe activity against *S. aureus* [5], *E. coli* [5,8], and *P. aeruginosa* [8].

**Table 2.** *Ab initio* thermodynamic properties for the pregeijerene – geijerene Cope rearrangement.

| | *H*° (kcal/mol) | | Relative enthalpy (kcal/mol) | | *G*° (kcal/mol) | | Relative free energy (kcal/mol) | |
|---|---|---|---|---|---|---|---|---|
| | B3LYP | MP2 | B3LYP | MP2 | B3LYP | MP2 | B3LYP | MP2 |
| Pregeijerene | -293531.96 | -292671.06 | 0 | 0 | -293563.37 | -292700.15 | 0 | 0 |
| Transition State | -293501.57 | -292648.08 | 30.39 | 22.98 | -293532.50 | -292676.81 | 30.87 | 23.34 |
| Geijerene | -293532.25 | -292674.92 | -0.29 | -3.86 | -293564.02 | -292704.31 | -0.64 | -4.16 |

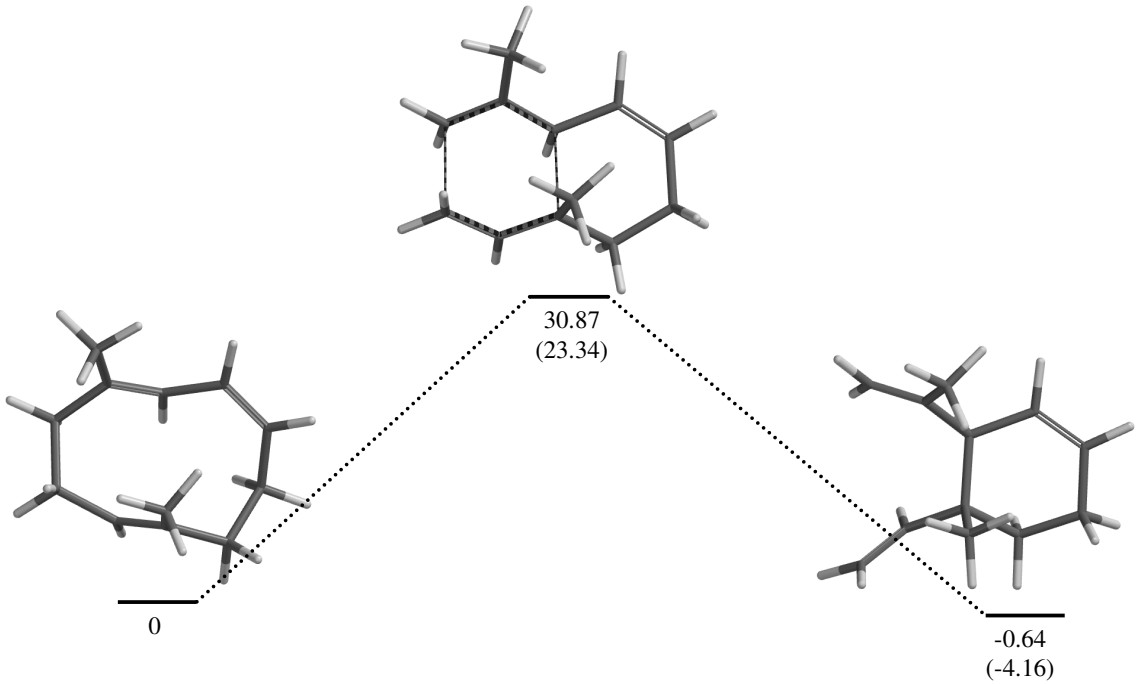


**Figure 1.** Energy profile for Cope rearrangement of pregeijerene to geijerene, relative energies in kcal/mol (MP2 in parentheses).

**Table 3.** Antimicrobial activity (MIC, μg/mL) of *Chromolaena odorata* essential oil and major components.

| Material | *B. cereus* | *S. aureus* | *E. coli* | *P. aeruginosa* | *C. albicans* | *A. niger* |
|---|---|---|---|---|---|---|
| *C. odorata* oil | 39 | 1250 | 1250 | 1250 | 1250 | 78 |
| α-Pinene | 312 | 625 | 312 | 625 | 156 | 625 |
| β-Pinene | 312 | 625 | 625 | 1250 | 625 | 156 |
| Germacrene D | 625 | 156 | 625 | 1250 | 625 | 39 |
| Positive Control | 1.22[a] | 0.61[a] | 2.44[a] | 1.22[a] | 0.61[b] | 0.61[b] |

[a]Geneticin. [b]Amphotericin B

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## References

[1] D.J. Mabberley (1997). The Plant Book, 2nd Ed. Cambridge University Press, UK, p 155.
[2] J.F. Morton (1981). Atlas of Medicinal Plants of Middle America, Vol. II. Charles C. Thomas, Publisher, Springfield, Illinois, USA, pp 932-933.

[3] C. Zachariades, M. Day, R. Muniappan and G.V.P. Reddy (2009). *Chromolaena odorata* (L.) King and Robinson (Asteraceae). In: Biological Control of Tropical Weeds Using Arthropods, *eds:* R. Muniappan, G.V.P. Reddy and A. Raman. Cambridge University Press, UK, pp 130-160.

[4] E. Adjanohoun and L. Ake-Assi (1979). Contribution au Recensement des Plantes Médicinale de Côte d'Ivoire. Centre National de Floristique, Abidjan, Ivory Coast, 359 pp.

[5] S.I. Inya-Agha, B.O. Oguntimein, A. Sofowora and T.V. Benjamin (1987). Phytochemical and antibacterial studies on the essential oil of *Eupatorium odoratum*. *Int. J. Crude Drug Res.* **25**, 49-52.

[6] H. Bouda, L.A. Tapondjou, D.A. Fontem and M.Y.D. Gumedzoe (2001). Effect of essential oils from leaves of *Ageratum conyzoides*, *Lantana camara* and *Chromolaena odorata* on the mortality of *Sitophilus zeamais* (Coleoptera, Curculionidae). *J. Stored Prod. Res.* **37**, 103-109.

[7] S. Cui, S. Tan, G. Ouyang, S. Jiang and J. Pawliszyn (2009). Headspace solid-phase microextraction gas chromatography-mass spectrometry analysis of *Eupatorium odoratum* extract as an oviposition repellent. *J. Chromatogr. B* **877**, 1901-1906.

[8] D. Bamba, J.M. Bessière, C. Marion, Y. Pélissier and I. Fourasté (1993). Essential oil of *Eupatorium odoratum*. *Planta Med.* **59**, 184-185.

[9] N. Pisutthanan, B. Liawruangrath, S. Liawruangrath, A. Baramee, A. Apisariyakul, J. Korth and J.B. Bremner (2006). Constituents of the essential oil from aerial parts of *Chromolaena odorata* from Thailand. *Nat. Prod. Res.* **20**, 636-640.

[10] R.P. Adams (2007). Identification of Essential Oil Components by Gas Chromatography/Mass Spectrometry, 4th Ed. Allured Publishing Corporation. Carol Stream, Illinois.

[11] D.H. Sahm, D.H. and J.A. Washington (1991). Antibacterial susceptibility tests: Dilution methods. In: Manual of Clinical Microbiology, *eds:* A. Balows, W.J. Hausler, K.L. Herrmann, H.D. Isenberg and H.J. Shamody. American Society for Microbiology, Washington DC, USA.

[12] SPARTAN '08 for Windows (2008). Wavefunction, Inc., Irvine, California.

[13] A.D. Becke (1993). Density-functional thermochemistry. III. The role of exact exchange. *J. Chem. Phys.* **98**, 5648-5652.

[14] C. Lee, W. Yang and R.G. Parr (1988). Development of the Colle-Salvetti correlation-energy formula into a functional of the electron density. *Phys. Rev. B* **37**, 785-789.

[15] W.J. Hehre, L. Radom and P.v.R. Schleyer (1986). Ab initio Molecular Orbital Theory. Wiley, New York.

[16] R.V.J. Jones and M.D. Sutherland (1968). 1,5-Dimethylcyclodeca-1,5,7-triene, the precursor of geijerene in *Geijera parviflora* (Lindley). *Aust. J. Chem.* **21**, 2255-2264.

[17] S.R. Kiran, A.S. Reddy, P.S. Devi and K.J. Reddy (2006). Insecticidal, antifeedant and oviposition deterrent effects of the essential oil and individual compounds from leaves of *Chloroxylon swietenia* DC. *Pest Manag. Sci.* **62**, 1116-1121.

[18] P.M. Santos, A.C. Figueiredo, M.M. Oliveira, J.G. Barroso, L.G. Pedro, S.G. Deans, A.K.M. Younus and J.C. Scheffer (1998). Essential oils from hairy root cultures and from fruits and roots of *Pimpinella anisum*. *Phytochemistry* **48**, 455-460.

[19] R.P. Adams, T.A. Zanoni, T.A. van Beck, M.A. Posthumus and C. van de Haar (1998). Essential leaf oil of *Amyris diatrypa* Sprengel from the Dominican Republic. *J. Essent. Oil Res.* **10**, 175-178.

[20] J. Lei, J. Yu, H. Yu and Z. Liao (2008). Composition, cytotoxicity and antimicrobial activity of essential oil from *Dictamnus dasycarpus*. *Food Chem.* **107**, 1205-1209.

[21] K.H.C. Baser, N. Kirimer and B. Demirackmak (1996). Composition of the essential oil of *Wiedemannia orientalis* Fisch. et Mey. from Turkey. *J. Essent. Oil Res.* **8**, 543-544.

[22] A. Velasco-Negueruela, M.J. Pérez-Alonso, P.L. Pérez de Paz, C. Garcia Vallejo, J. Palá-Paúl and A. Iñigo (2002). Chemical composition of the essential oils from the roots, fruits, leaves and stems of *Pimpinella cumbrae* Link growing in the Canary Islands (Spain). *Flavour Fragr. J.* **17**, 468-471.

[23] S.R. Kiran, A.S. Reddy, P.S. Devi and K.J. Reddy (2006). Insecticidal, antifeedant and oviposition deterrent effects of the essential oil and individual compounds from leaves of *Chloroxylon swietenia* DC. *Pest Manag. Sci.* **62**, 1116-1121.

[24] N. Tabanca, B. Demirci, K.H.C. Baser, E. Mincsovics, S.I. Khan, M.R. Jacob and D.E. Wedge (2007). Characterization of volatile constituents of *Scaligeria tripartita* and studies on the antifungal activity against phytopathogenic fungi. *J. Chromatogr. B* **850**, 221-229.

[25] V. Mohammadreza (2008). Chemical composition and antimicrobial activity of *Pimpinella affinis* Ledeb. essential oil growing in Iran. *Int. J. Green Pharm.* **2**, 138-140.

[26] E.E. Stashenko, R. Acosta and J.R. Martinez (2000). High-resolution gas-chromatographic analysis of the secondary metabolites by subcritical-fluid extraction from Colombian rue (*Ruta graveolens* L.). *J. Biochem. Biophys. Methods* **43**, 379-390.