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Volatile components of the aerial parts of *Conoclinium coelestinum* from North Alabama

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Abstract
The essential oil of *Conoclinium coelestinum*, growing wild in north Alabama, has been obtained by hydrodistillation from the aerial parts, and the oil analyzed by gas chromatography – mass spectrometry. *C. coelestinum* essential oil was rich in germacrene D (28.6%), (2*E*)-hexenal (9.5%), β-caryophyllene (7.4%), and (*Z*, *Z*)-α-farnesene (7.2%). The essential oil was screened for antifungal activity against *Aspergillus niger*, *Candida albicans*, and *Cryptococcus neoformans*, but showed only marginal activity (MIC = 625, 1250, and 313 μg/mL, respectively).

Keywords: Asteraceae, blue mistflower, essential oil, chemical composition, germacrene D

1. Introduction
Conoclinium coelestinum (L.) DC., the blue mistflower (Asteraceae) is native to the southeastern United States, from eastern Texas east to the Atlantic coast and from southern Missouri, southern Illinois, southern Ohio, south to the Gulf coast [1]. It is a perennial herb with long slender rhizomes and bright blue flower heads (ca. 1-1.5 cm, Figure 1) [2, 3]. Non-volatile compounds isolated from this plant include the pyrrolizidine alkaloid intermedine [4], flavonoids gardenin A and nobiletin [5], luteolin and luteolin derivatives [6], the amorphane sesquiterpenoid 5, 8-epoxy-4, 6-dihydroxy-3-cadinanone, and the benzofuran glucoside 7-hydroxytoxol 7-*O*-glucoside [7]. There are apparently no reports on the volatile components from this plant, however, and as part of our continuing investigations on the essential oils of north Alabama Asteraceae [8–10], we have collected and analyzed the essential oil from the aerial parts of *C. coelestinum*.

Fig 1: *Conoclinium coelestinum* (L.) DC. Photograph by S.K. Lawson.

2. Materials and Methods
2.1 Plant Material
The aerial parts of *C. coelestinum* were collected on 12 August 2018 from the Flint River Greenway in north Alabama (34°38′40″N, 86°27′22″W, elev. 180 m). The plant was identified by S.K. Lawson; a voucher specimen (20180812-111413) has been deposited in the University of Alabama in Huntsville herbarium. The fresh plant material (64.53 g) was hydrodistilled using a Likens-Nickerson apparatus, with continuous extraction with CH_2Cl_2, for 2.5 h to give a pale-yellow essential oil (45.4 mg).

2.2 Gas Chromatographic – Mass Spectral Analysis
The essential oil of *C. coelestinum* was analyzed by GC-MS, as described previously [11], using a Shimadzu GC-MS-QP2010 Ultra fitted with a Phenomenex ZB-5ms column. Identification of the essential oil components was determined by comparison of their retention indices and their mass spectral fragmentation patters with those from the Adams [12], NIST17 [13], and FFNSC 3 [14] databases and our in-house library.

2.3 Antifungal Screening
The *C. coelestinum* essential oil was screened for antifungal activity against *Aspergillus niger* (ATCC 16888), *Candida albicans* (ATCC 18804), and *Cryptococcus neoformans* (ATCC 24607) using the microbroth dilution method as previously described [15]. Amphotericin B was used as the positive control and RPMI medium was used as the negative control.

3. Results and Discussion
Hydrodistillation of the aerial parts of *C. coelestinum* yielded a pale-yellow essential oil in 0.070% yield. A total of 66 compounds were identified in the essential oil of *C. coelestinum* accounting for 95.2% (see Table 1). Sesquiterpene hydrocarbons (73.0%), green leaf volatiles (11.1%), and oxygenated sesquiterpenoids (10.8%) were the predominant chemical classes. The major components in *C. coelestinum* essential oil were germacrene D (28.8%), (2*E*)-hexenal (9.6%), β-caryophyllene (7.4%), and (*Z*, *Z*)-α-farnesene (7.2%).

Table 1: Aerial parts essential oil composition of *Conoclinium coelestinum* (L.) DC.

RI[a]	Compound	%	RI[a]	Compound	%
800	(3*Z*)-Hexenal	0.2	1482	(*Z*,*Z*)-α-Farnesene	7.2
801	Hexanal	0.6	1486	Eremophilene	0.3
848	(2*E*)-Hexenal	9.6	1488	β-Selinene	3.5
860	(2*E*)-Hexenol	0.3	1491	*trans*-Muurola-4(14),5-diene	0.7
854	1-Hexanol	0.4	1493	α-Zingiberene	1.1
932	α-Pinene	0.1	1494	α-Selinene	2.9
948	Camphene	0.3	1497	α-Muurolene	0.4
999	δ-2-Carene	0.1	1504	*trans*-β-Guaiene	0.8
1024	*p*-Cymene	0.1	1506	β-Bisabolene	0.4
1028	Limonene	0.1	1511	γ-Cadinene	0.1
1099	Linalool	0.1	1514	Cubebol	1.1
1191	Methyl salicylate	0.3	1516	δ-Cadinene	1.3
1283	Bornyl acetate	0.5	1518	7-*epi*-α-Selinene	0.2
1334	δ-Elemene	1.4	1522	β-Sesquiphellandrene	0.6
1346	α-Cubebene	0.1	1531	*trans*-Cadina-1,4-diene	0.1
1350	Eugenol	0.3	1539	*trans*-α-Bisabolene	0.2
1368	α-Ylangene	0.1	1542	α-Copaen-11-ol	2.9
1374	α-Copaene	0.3	1547	Unidentified sesquiterpenoid[b]	0.6
1382	β-Bourbonene	0.2	1557	Germacrene B	3.2
1386	β-Cubebene	1.6	1575	Germacra-1(10),5-dien-4β-ol	0.7
1388	β-Elemene	1.9	1580	Caryophyllene oxide	0.2
1411	*cis*-α-Bergamotene	0.1	1588	Viridiflorol	0.6
1418	β-Caryophyllene	7.4	1596	Gleenol	0.4
1428	γ-Elemene	1.8	1612	Zingiberenol	0.2
1431	*trans*-α-Bergamotene	3.3	1616	Neointermedeol	0.5
1433	α-Guaiene	0.1	1626	1-*epi*-Cubenol	0.3
1440	6,9-Guaiadiene	0.2	1641	τ-Cadinol	0.3
1443	Isogermacrene D	0.1	1642	τ-Murrolol	0.2
1448	*cis*-Muurola-3,5-diene	0.2	1645	α-Muurolol (= Torreyol = δ-Cadinol)	0.3
1450	*trans*-Muurola-3,5-diene	0.2	1654	Pogostol	1.8
1451	(*E*)-β-Farnesene	0.2	1668	Unidentified sesquiterpenoid[c]	1.9
1454	α-Humulene	1.3		Green leaf volatiles	11.1
1469	4,5-di-*epi*-Aristolochene	0.5		Monoterpene hydrocarbons	0.6
1472	Selina-4,11-diene	0.1		Oxygenated monoterpenoids	0.6
1473	γ-Muurolene	0.1		Sesquiterpene hydrocarbons	73.0
1476	γ-Curcumene	0.3		Oxygenated sesquiterpenoids	10.8
1480	Germacrene D	28.8		Benzenoids	0.6
				Total Identified	95.2

[a] Retention Index determined with reference to a homologous series of *n*-alkanes on a ZB-5ms column.
[b] MS(EI): 220(23%), 205(19%), 163(20%), 120(35%), 110(100%), 105(19%), 95(36%), 69(40%), 55(21%), 43(16%), 41(23%).
[c] MS(EI): 202(42%), 187(63%), 174(37%), 162(60%), 159(100%), 147(87%), 134(31%), 119(59%), 105(66%), 91(48%), 59(60%), 43(20%), 41(22%).

The aerial parts essential oil of *C. coelestinum* was screened for antifungal activity against the opportunistic fungal pathogens *Aspergillus niger*, *Candida albicans*, and *Cryptococcus neoformans* using the microbroth dilution technique. However, the essential oil exhibited only marginal antifungal activity. The minimum inhibitory concentrations (MIC) were 625, 1250, and 313 μg/mL, respectively.

4. Conclusions
This work presents the first analysis of the volatile

composition of *Conoclinium coelestinum* (or any *Conoclinium* species). The yield of essential oil was low and the antifungal activity was marginal, so the essential oil of this plant is unlikely to be considered for further investigation as anantifungal agent.

5. Acknowledgments

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6. Conflicts of Interest

The authors declare no conflicts of interest.

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