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Identification of essential oil components from *Conradina canescens*

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Abstract
Conradina canescens Gray (false rosemary) is a common evergreen shrub, endemic to a small area of west Florida and adjacent Alabama and southern parts of Mississippi, USA. In this work, essential oils of *C. canescens* were obtained by hydrodistillation and analyzed by GC-MS. The analysis revealed a variety of terpenes, terpenic alcohols, ketones, and aldehydes. The major components of *C. canescens* oil were 1,8-cineole, camphor, α-pinene, *p*-cymene, *cis*-pinocamphone, myrtenal, myrtenol, verbenone, and myrtenyl acetate, in addition to other minor constituents. Antimicrobial activities were determined using the microbroth dilution technique, and *in-vitro* cytotoxic activity against MCF-7 cells was tested using the MTT assay. *C. canescens* oils have shown no antimicrobial or anticancer activity although some of their identified constituents are antimicrobial agents.

Keywords: *Conradina canescens*, GC-MS, essential oil composition, monoterpenes, 1,8-cineole, camphor.

1. Introduction
Conradina (Family Lamiaceae) is a small genus of just seven native US species. *Conradina canescens* Gray is a common small (up to 1 m in height), evergreen, compact shrub. Its narrow needle-like whorled leaves are recurved silvery gray, and grow on numerous slender upright stems [1]. The flowers and leaves have an herbal scent similar to those of *Rosmarinus officinalis*, which gives *Conradina* its common name, false rosemary. The leaves release a strong mint-like or terpenoid odor when crushed. Typically, flowers are small pale lavender in color and appear near the tops of the plant in early spring. *C. canescens* originated from the Gulf Coast. Although this species is common in its native range, it is endemic to only a small area of west Florida and adjacent Alabama and southern parts of Mississippi (USDA Symbol: COCA19) [2]. It occurs on coastal dunes, in scrubs and in pineland ecosystems. It shows tolerance for the heat and humidity of the Southeast and is considered a drought-tolerant landscape plant that grows best in lean, sandy soils [3]. There are very limited studies on the chemical composition and bioactivity of this plant species. These studies focused on identifying the compounds responsible for the strong allelopathic activity demonstrated by this species. To our knowledge, this study is the first report of volatile compounds collected by hydrodistillation at two phenological stages of *C. canescens*.

2. Materials and Methods
2.1 Plant Material
The aerial parts of *Conradina canescens* were collected from private properties in Santa Rosa County, near the city of Navarre, Florida, USA in March and again in May of 2010 and 2014. The plant was collected and identified by William J Guthrie. Each sample consisted of apical cuttings over 10 cm long. The plant materials were air-dried for several days. Approximately 25 g of each sample was crushed and hydrodistilled using a Likens-Nickerson apparatus with continuous extraction with chloroform for 4 h to give a clear yellow essential oil, which was stored at 4 ^{0}C until analysis.

2.2 Gas Chromatographic – Mass Spectral Analysis
The essential oils of *C. canescens* were analyzed by GC-MS using an Agilent 6890 GC with Agilent 5973 mass selective detector, an HP-5 ms fused silica capillary column and an

Agilent ChemStation data system as previously described [4]. Identification of the oil components was based on their retention indices (RI) and by comparison of their mass spectral fragmentation patterns with those reported in the literature [5].

2.3 Antimicrobial Screening

The essential oils were screened for antimicrobial activity against *Escherichia coli* (ATCC No. 10798), *Pseudomonas aeruginosa* (ATCC No. 27853), *Bacillus cereus* (ATCC No. 14579), *Staphylococcus aureus* (ATCC No.29213), and *Candida albicans* (ATCC No. 10231) using the microbroth dilution technique as previously reported [6].

2.4 Cytotoxicity Screening

MCF-7 human breast adenocarcinoma cells (ATCC No. HTB-22) were grown in RPMI 1640 supplemented with 10% FBS (Fetal bovine serum), 30mM HEPES, $NaHCO_3$, and Penicillin-Streptomycin. *In-vitro* cytotoxic activity of *C. canescens* oils on MCF-7 cells was performed using the 96-well MTT assay as previously reported [7].

3. Results and Discussion

Chemical analysis of *Conradina* essential oils (Table 1) revealed a variety of terpenes, terpenic aldehydes and ketones, and terpenic alcohols. *Conradina* was reported to produce large amounts of terpenes as an allelopathic weapon to inhibit the germination and growth of nearby grasses which helps to prevent fire damage [8, 9] and as a chemical defense against insects [10]. The major components of *C. canescens* oil were 1, 8-cineole, camphor, α-pinene, *p*-cymene, *cis*-pinocamphone, myrtenal, myrtenol, verbenone, and myrtenyl acetate with other minor constituents (3% or less of each). The previous studies on *Conradina* used the passive extraction method in order to avoid thermal degradation of its volatile components [2, 8, 10]. Our results using the hydrodistillation-extraction procedure have shown that there is little loss of volatile compounds of this plant. Interestingly, we were able to extract and identify a larger number of compounds and larger quantities using this hydrodistillation-extraction method.

The most abundant constituent was 1,8-cineole (eucalyptol), a well-known cyclic ether and a monoterpenoid. 1,8-Cineole was also reported as the major compound in *Eucalyptus camaldulensis* and *E. tereticornis* [11], *E. globulus* [12] and *Melaleuca leucadendron* [13]. 1,8-Cineole and camphor were also found to be the major components of *C. brevifolia, C. glabra and C. verticillata* [2]. *p*-Cymene was found in several plant species such as *Tarchonanthus camphoratus* [14], and *Lantana camara* [15]. There is a clear variation between the samples in the amount of some constituents especially 1,8-cineole, which might be due to seasonal changes during growth and environmental differences during the time of collection. Another possibility could be the normal chemical differences between individuals of the same population as well as the chemical variations between one leaf and another on the same individual. Terpene biosynthesis is high at the first few weeks of leaf development and then declines to very low levels by age [16]. In addition, flowering can be a reason for these differences. A lower level or lack of a certain component at the time of flowering might be the result of being catabolized and used as a carbon source. Gershenzon [16] reported that in *Mentha*, about 50-75% of the monoterpenes stored in mature leaves were degraded at the time of flowering in order to recover some of the metabolic costs of this stage.

The monoterpenoid major components in *C. canescens* compare favorably with those of *Rosmarinus officinalis* [17-20]. Thus, for example, in *R. officinalis*, α-pinene concentration generally ranges 14-26%; 1,8-cineole around 1-3% in Sardinian samples [18] and as much as 61% in samples from Turkey [19]; camphor around 3-24%; borneol 2-18%; verbenone ranges from 0% to 45% in Turkish samples [19]; bornyl acetate about 1% in Serbian samples [20] and 14-17% in Corsican samples [17]; and *p*-cymene 0-4%. *Conradina canescens* may represent, therefore, a potential herb for culinary use, herbal teas, or aromatherapy.

The essential oils of *C. canescens* were screened for potential antimicrobial activity against *Escherichia coli*, *Pseudomonas aeruginosa, Bacillus cereus*, *Staphylococcus aureus*, and *Candida albicans. C. canescens* essential oils showed no antibacterial activity (MIC = 1250 µg /mL with *P. aeruginosa*, *S. aureus* and *C. albicans*; MIC = 625 µg /mL with *E. coli* and *B. cereus*). Interestingly, some of the identified constituents were reported to be effective antimicrobial agents such as 1,8-cineole [21-23], α-terpineol, terpinen-4-ol, α-pinene, β-pinene, β-caryophyllene, α-phellandrene, and *p*-cymene [24]. One possible explanation is that the concentrations of these components in *Conradina* oil were not high enough to show their antimicrobial effects. The essential oils of *C. canescens* were screened for *in-vitro* cytotoxic activity against MCF-7 cells but showed no significant activity (0% kill at 100 µg/mL).

Table 1: Chemical composition of *Conradina canescens* essential oils.

RI[a]	Compound	2010			2014	
		%(veg)[b]	%(fl)[c]		%(veg)[b]	%(fl)[c]
885	Santene	1.0	1.6		1.3	0.6
916	Tricyclene	0.1	0.1		---	---
935	α-Thujene	0.7	0.3		---	0.1
941	α-Pinene	5.6	4.5		3.2	5.4
954	Camphene	1.2	1.3		2.0	2.1
958	Thuja-2,4(10)-diene	0.7	0.3		---	---
963	Benzaldehyde	0.2	0.3		---	---
976	Sabinene	0.4	0.4		0.2	1.0
979	β-Pinene	1.1	1.2		0.7	---
981	1-Octen-3-ol	0.2	0.2		---	---

992	Myrcene	0.5	tr[d]		---	---
992	Dehydro-1,8-cineole	tr	0.4		0.3	0.9
1004	α-Phellandrene	1.7	0.3		tr	0.8
1016	α-Terpinene	0.3	0.3		---	0.1
1026	*p*-Cymene	5.9	6.6		3.3	12.9
1028	Limonene	tr	0.8		---	---
1029	β-Phellandrene	4.5	tr		---	---
1035	1,8-Cineole	5.2	20.5		25.2	10.7
1038	Lavender lactone	0.1	tr		---	---
1043	Phenylacetaldehyde	0.2	0.3		---	---
1048	(*E*)-β-Ocimene	0.2	0.2		---	---
1060	γ-Terpinene	0.4	0.2		0.1	0.4
1068	*cis*-Sabinene hydrate	0.2	0.1		---	0.1
1070	*p*-Mentha-3,8-diene	---	0.1		---	---
1073	*cis*-Linalool oxide (furanoid)	0.9	0.1		0.5	0.9
1079	*p*-Cresol	0.1	---		---	---
1082	Camphenilone	0.2	0.2		---	---
1088	*trans*-Linalool oxide (furanoid)	1.7	tr		---	---
1090	*p*-Cymenene	---	1.2		1.4	1.6
1097	*trans*-Sabinene hydrate	0.2	0.1		---	---
1102	Linalool	0.5	tr		---	0.9
1114	1-Octen-3-yl acetate	0.2	0.1		---	---
1116	*trans*-Thujone (= β-Thujone)	0.2	0.1		---	---
1121	Dehydrosabina ketone	1.2	1.1		1.2	1.3
1124	*cis*-*p*-Menth-2-en-1-ol	0.2	tr		1.4	---
1127	α-Campholenal	0.8	0.7		---	1.5
1139	*trans*-Pinocarveol	1.4	1.6		---	---
1139	Nopinone	1.0	0.9		0.7	---
1141	*cis*-Verbenol	tr	0.5		---	---
1146	Camphor	5.7	4.4		8.0	6.8
1159	*trans*-Pinocamphone	0.3	0.2		---	---
1160	Sabina ketone	0.3	0.3		1.0	0.7
1161	Pinocarvone	0.4	0.3		---	---
1165	Borneol	1.7	1.1		---	---
1167	*p*-Mentha-1,5-dien-8-ol	0.7	0.8		---	---
1175	*cis*-Pinocamphone	1.3	2.1		5.5	2.8
1178	Terpinen-4-ol	1.6	1.4		---	2.1
1183	*p*-Methylacetophenone	0.3	0.4		---	---
1187	Cryptone	2.1	2.6		2.0	2.3
1188	*p*-Cymen-8-ol	0.8	tr		---	---
1191	α-Terpineol	1.4	1.0		---	---
1199	Myrtenal	5.2	7.6		8.1	10.2
1205	3,6,6-Trimethylnorpinan-2-one	0.5	0.6		---	---
1205	Myrtenol	9.2	3.8		3.4	4.7
1215	Verbenone	4.0	3.2		4.5	4.2
1218	*trans*-2-Pinanol	---	---		0.2	0.1
1224	*trans*-Carveol	1.2	1.0		1.3	1.1
1227	*exo*-2-Hydroxycineole	0.7	0.9		1.8	2.2
1228	*nor*-Davanone	---	0.7		---	---
1233	*m*-Cumenol	1.3	0.4		1.1	0.5
1240	Cuminaldehyde	1.1	1.0		0.6	1.2
1246	Carvone	0.7	0.8		0.8	0.8
1251	*trans*-2-Hydroxypinocamphone	0.5	0.6		1.5	0.9
1255	Piperitone	0.2	0.1		---	0.3
1256	*cis*-Myrtanol	0.2	0.3		---	---
1264	*trans*-Piperitone epoxide	---	---		0.3	0.3
1274	*cis*-Tetrahydrojasmine	0.8	0.9		---	---
1274	*p*-Mentha-1-en-7-al (= Phellandral)	---	---		0.5	0.6
1278	Unidentified	0.2	0.2		1.4	1.2
1285	Bornyl acetate	0.5	0.2		0.6	0.5
1292	*p*-Cymen-7-ol	1.2	1.1		1.7	1.7
1299	Perilla alcohol	0.1	0.3		0.3	0.3
1299	*trans*-Pinocarvyl acetate	0.3	0.1		---	---
1305	Carvacrol	0.7	0.4		0.8	0.6

1327	Myrtenyl acetate	5.0	6.4		5.4	3.2
1328	*p*-Mentha-1,4-dien-7-ol	0.1	---		0.1	0.1
1332	3-Oxo-*p*-menth-1-en-7-al	0.2	0.2		0.2	0.3
1341	Piperitenone	0.1	---		0.1	0.1
1350	α-Terpinyl acetate	0.6	0.3		0.6	0.5
1361	Eugenol	---	---		---	tr
1372	4-Hydroxymyrtenal	2.6	3.1		---	---
1385	β-Bourbonene	0.1	---		1.7	2.3
1419	(*E*)-Caryophyllene	0.2	tr		---	0.3
1422	Chrysanthenone	---	---		---	0.1
1454	α-Humulene	0.1	---		0.1	0.1
1461	Alloaromadendrene	0.1	---		---	tr
1483	Germacrene D	1.4	0.1		0.2	0.3
1511	(*E*,*E*)-α-Farnesene	0.5	0.1		0.2	0.2
1561	1-Norbourbanone	0.1	---		---	---
1570	8-Acetoxycarvotanacetone	0.1	---		0.2	0.1
1574	Spathulenol	0.1	0.1		---	---
1585	Caryophyllene oxide	0.6	1.2		1.3	1.6
1594	Viridiflorol	0.3	---		tr	0.4
1605	Ledol	0.1	---		---	0.2
1610	Humulene epoxide II	0.2	0.4		0.2	0.2
1619	Unidentified	0.3	0.4		0.5	---
1637	Caryophylla-4(12),8(13)-dien-5-ol	0.1	---		0.1	0.2
1654	α-Cadinol	0.6	0.3		0.3	0.6
	Total Identified (%)	97.4	97.8		96.1	96.9
	Compounds Identified	85	72		48	57

[a] RI determined with respect to a homologous series of *n*-alkanes on an HP-5ms column.
[b] Sample collected in March, prior to flowering.
[c] Sample collected in May, during flowering.
[d] tr = "trace" (< 0.05%).

4. Conclusions

The essential oil of *Conradina canescens* was analyzed by GC-MS and found to be rich in monoterpenoids, particularly 1,8-cineole, myrtenal, *p*-cymene, camphor, myrtenol, myrtenyl acetate, and α-pinene. *C. canescens* essential oil was screened for antimicrobial activity and cytotoxic activity but was found to be inactive. Because its chemical composition is comparable to rosemary, *C. canescens* may be a useful and beneficial herb.

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6. References

1. Bell CR, Taylor BJ. Florida Wild Flowers and Roadside Plants. Laurel Hill Press. Chapel Hill, North Carolina, USA, 1982, 79.
2. Peterson CL. Analysis of the Essential Oils, Leaf Ultrastructure, and the *In Vitro* Growth Response of the Mint Genus *Conradina*. M.S. Thesis, Florida Institute of Technology, Melbourne, Florida, USA, 1998.
3. Harrison M. Groundcovers for the South. Pineapple Press, Sarasota, Florida, USA, 2006, 69.
4. Monzote L, Nance MR, Garcia M, Scull R, Setzer WN. Comparative chemical, cytotoxicity and antileishmanial properties of essential oils from *Chenopodium ambrosioides*. Natural Product Communications, 2011; 6:281-286.
5. Adams RP. Identification of Essential Oil Components by Gas Chromatography / Mass Spectrometry, 4th Ed. Allured Publishing, Carol Stream, Illinois, 2007.
6. Setzer MC, Setzer WN, Jackes BR, Gentry GA, Moriarity DM. The medicinal value of tropical rainforest plants from Paluma, North Queensland, Australia. Pharmaceutical Biology, 2001; 39:67-78.
7. Palazzo MC, Wright HL, Agius BR, Wright BS, Moriarity DM, Haber WA, Setzer WN. Chemical compositions and biological activities of leaf essential oils of six species of Annonaceae from Monteverde, Costa Rica. Records of Natural Products 2009; 3:153-160.
8. Williamson GB, Fischer NH, Richardson DR, de la Peña A. Chemical inhibition of fire-prone grasses by fire-sensitive shrub, *Conradina canescens*. Journal of Chemical Ecology, 1989; 15(5):1567-1577.
9. Fischer NH, Williamson GB, Weidenhamer JD, Richardson DR. In search of allelopathy in the Florida scrub: The role of terpenoids. Journal of Chemical Ecology, 1994; 20(6):1355-1380.
10. Quinn BP, Bernier UR, Booth MM. Identification of compounds from Etonia rosemary (*Conradina etonia*). Journal of Chromatography A, 2007; 1160: 306-310.
11. Doran JC, Brophy JJ. Tropical red gums – a source of 1, 8-cineole-rich *Eucalyptus* oil. New Forests 1990; 4(3):157- 178.
12. Yang YC, Choi HY, Choi WS, Clark JM, Ahn YJ. Ovicidal and adulticidal activity of *Eucalyptus globulus* leaf oil terpenoids against *Pediculus humanus* capitis

(Anoplura: Pediculidae). Journal of Agricultural and Food Chemistry 2004; 52(9):2507-2511.
13. Pujiarti R, Ohtani Y, Ichiura H. Physicochemical properties and chemical compositions of *Melaleuca leucadendron* leaf oils taken from the plantations in Java, Indonesia. Journal of Wood Science 2011; 57(5):446-451.
14. Omolo MO, Okinyo D, Ndiege IO, Lwande W, Hassanali A. Repellency of essential oils of some Kenyan plants against *Anopheles gambiae*. Phytochemistry 2004; 65(20):2797-2802.
15. Sundufu AJ. Shoushan H. Chemical composition of the essential oils of *Lantana camara* L. occurring in south China. Flavour and Fragrance Journal 2004; 19:229-232.
16. Gershenzon J. Metabolic costs of terpenoid accumulation in higher plants. Journal of Chemical Ecology 1994; 20:1281-1328.
17. Pintore G, Usai M, Bradesi P, Juliano C, Boatto G, Tomi F *et al*. Casanova J. Chemical composition and antimicrobial activity of *Rosmarinus officinalis* L. oils from Sardinia and Corsica. Flavour and Fragrance Journal, 2002; 17:15-19.
18. Angioni A, Barra A, Cereti E, Barile D, Coïsson JD, Arlorio M *et al*. Chemical composition, plant genetic differences, antimicrobial and antifungal activity investigation of the essential oil of *Rosmarinus officinalis* L. Journal of Agricultural and Food Chemistry, 2004; 52:3530-3535.
19. Celiktas OY, Kocabas EEH, Bedir E, Sukan FV, Ozek T, Baser KHC. Antimicrobial activities of methanol extracts and essential oils of *Rosmarinus officinalis*, depending on location and seasonal variations. Food Chemistry, 2007; 100:553-559.
20. Bozin B, Mimica-Dukic N, Samojlik I, Jovin E. Antimicrobial and antioxidant properties of rosemary and sage (*Rosmarinus officinalis* L. and *Salvia officinalis* L. Lamiaceae) essential oils. Journal of Agricultural and Food Chemistry, 2007; 55:7879-7885.
21. Sökmen A, Gulluce M, Askin AH, Daferera D, Tepe B, Polissiou M *et al*. The *in vitro* antimicrobial and antioxidant activities of the essential oils and methanol extracts of endemic *Thymus spathulifolius*. Food Control, 2004; 15(8):627-634.
22. Randrianarivelo R, Sarter S, Odoux E, Brat P, Lebrun M, Romestand B *et al*. Composition and antimicrobial activity of essential oils of *Cinnamosma fragrans*. Food Chemistry, 2009; 114:680-684.
23. Gilles M, Zhao J, An M, Agboola S. Chemical composition and antimicrobial properties of essential oils of three Australian *Eucalyptus* species. Food Chemistry, 2010; 119(2):731-737.
24. Dorman HJD, Deans SG. Antimicrobial agents from plants: Antibacterial activity of plant volatile oils. Journal of Applied Microbiology, 2000; 88:308-316.