21. Ružičkini dani
DANAS ZNANOST – SUTRA INDUSTRIJA
9. – 11. rujna 2026. • Vukovar • Hrvatska

SVEUČILIŠTE U SPLITU
Agromediteranski fakultet

CEKOM-3LJ
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KARAKTERIZACIJA PROFILA ISPARLJIVIH SPOJEVA SUPERKRITIČNOG EKSTRAKTA *Eucalyptus cinerea* POMOĆU HS-SPME-GC-MS

VOLATILE PROFILE CHARACTERIZATION BY HS-SPME-GC-MS OF *Eucalyptus cinerea* SUPERCRITICAL FLUID EXTRACT

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INTRODUCTION

Eucalyptus cinerea cultivation generates substantial pruning offcuts that are typically discarded, despite their known essential oil content. Leaf oils produced using various species from the Eucalyptus genus are dominated by compounds widely reported to possess antimicrobial, antioxidant and insecticidal or repellent bioactivity, making the offcuts a viable candidate for valorisation as a botanical biopesticide.[1, 2, 3] Supercritical fluid extraction (SFE) using CO_2 as solvent is increasingly favoured for isolating bioactive compounds since it operates at relatively low temperatures, avoids toxic organic solvents and preserves thermolabile compounds that are otherwise degraded in other, more common extraction methods.[4]

The aim of this study was to characterize the volatile organic compound (VOC) profile of a *E. cinerea* offcuts extract, obtained on a pilot-scale SFE system, using headspace solid-phase microextraction coupled with gas chromatography-mass spectrometry (HS-SPME-GC-MS) and to gauge the extract's possible application as a biopesticide based on its compound composition.

Keywords: *Eucalyptus cinerea, volatile compounds, supercritical fluid extraction, HS-SPME-GC-MS, 1,8-Cineole, biopesticide*

METHODOLOGY

15 080 g of air-dried and milled E. cinerea leaves and twigs were processed on a pilot-scale SFE system (Extratex SFI, France; CO_2 as solvent, 300 bar, 45°C, 150 kg/h CO_2 flow rate, 45min extraction time), resulting in 499 g of extract (yield: 3.3%). Volatile compounds were collected using HS-SPME on a DVB/CAR/PDMS fiber (Merck KGaA, Germany; headspace at 40°C, equilibration time 15min, fiber exposure time 40min). The collected volatiles were separated on an Agilent 8890 GC coupled with a 5977B MS detector. Compound identification relied on spectral matching against Wiley 20 and NIST 20 libraries, in addition to retention indices calculated from C8-C40 n-alkanes.

RESULTS & CONCLUSION

- Extraction yield is slightly higher than reported in a laboratory-scale set up with slightly different extraction parameters for *E. cinerea.*[5]
- The higher pressure applied likely enhanced CO_2 density and solvent power, favouring co-extraction of a broader range of compounds, including less volatile constituents not detected at milder laboratory-scale conditions.
- The 35 identified VOCs (Table 1.) were dominated by the oxygenated monoterpene 1,8-Cineole and the monoterpene hydrocarbon α-Pinene, followed by minor constituents (γ-Terpinene, trans-β-Caryophyllene, Bisabolol, Bulnesol).
- VOC composition is broadly consistent with earlier reports on *E. cinerea*, with 1,8-Cineole consistently the dominant compound regardless of extraction method applied.[5]

The most dominant VOCs in the extract, 1,8-Cineole and α-Pinene, have documented insecticidal and repellent activity against various larval and adult insect stages, suggesting potential biopesticide applications. This combination may also present synergistic repellent effects, a phenomenon reported for other Eucalyptus essential oils rich in monoterpenes.[1] Trials for repellent activity against larval and adult insect stages should provide more insight into the extract's prospective use as a botanical biopesticide.

No.	Compound Name	PA (%)	RI
1	α-Pinene	18.37	938
2	Camphene	0.26	954
3	β-Pinene	0.43	981
4	β-Myrcene	0.78	993
5	α-Phellandrene	4.39	1008
6	α-Terpinene	0.16	1021
7	p-Cymene	4.59	1030
8	1, 8-Cineole	47.17	1037
9	(Z)-β-Ocimene	1.59	1041
10	(E)-β-Ocimene	0.53	1051
11	γ-Terpinene	1.50	1061
12	α-Terpinolene	0.35	1088
13	Linalool	0.37	1097
14	Hexyl butanoate	0.11	1102
15	Trans-4-(isopropyl)-1-methylcyclohex-2-en-1-ol	0.16	1140
16	Pinocarvone	0.09	1165
17	Terpinen-4-ol	0.59	1179
18	α-Terpineol	2.07	1192
19	Linalyl acetate	1.00	1257
20	Lavandulyl acetate	0.09	1288
21	Trans-Geranic acid methyl ester	0.32	1322
22	α-Cubebene	7.79	1350
23	α-Copaene	0.11	1373
24	Methyleugenol	0.22	1401
25	α-Gurjunene	0.47	1409
26	Trans-β-Caryophyllene	1.05	1419
27	Trans-α-Bergamotene	1.08	1438
28	Alloaromadendrene	0.61	1458
29	γ-Selinene	0.20	1472
30	Germacrene D	0.56	1490
31	Humulene epoxide	2.27	1610
32	Bulnesol	0.13	1664
33	Bisabolol	0.16	1673
34	Hexadecanol	0.11	1885
35	Bisphenol A	0.28	2157
	Total Peak Area Identified = 99.94%		

Table 1. Composition and content of volatile compounds in the headspace. Retention indices calculated from n-alkanes (C8-C40). PA = Peak Area; RI = Retention Index.

Literature

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ACKNOWLEDGEMENTS

E. cinerea offcuts were kindly donated by local growers from Kaštela, Split-Dalmatia County.

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