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COMPARATIVE ANALYSIS OF VOLATILE OIL COMPOSITION OF NOXIOUS EXOTIC PLANT *MIKANIA MICRANTHA* AND ITS INDIGENOUS AKIN *MIKANIA CORDATA*

ZHEN WANG[1], RUONAN WANG[1], YANG PENG[1], XIAOXIAN RUAN[1], TING WANG[2,4], WENBO LIAO[1*] AND YINGJUAN SU[3,4*]

[1]School of Life Sciences, Sun Yat-sen University, 510275, Guangzhou, China
[2]College of Life Sciences, South China Agricultural University, 510642, Guangzhou, China
[3]School of Agriculture and Biotechnology, Shenzhen Campus of Sun Yat-Sen University, Sun Yat-Sen University, 518107, China
[4]Research Institute of Sun Yat-sen University in Shenzhen, 518057, Shenzhen, China

*Corresponding author's email: lsslwb@mail.sysu.edu.cn (W.L.); suyj@mail.sysu.edu.cn (Y.S.)

Abstract

Mikania micrantha and *M. cordata* (Asteraceae) are plants of great concern in China. The former is known globally as a "plant killer", while the latter is a harmless indigenous species. They form an ideal congeneric pair for comparative study to elucidate the invasive mechanisms of alien weeds. In this study, we analyzed the volatile oils from various tissues of the two plants using gas chromatography-mass spectrometry and compared their chemical compositions. Both *Mikania micrantha* and *M. cordata* were rich in sesquiterpenes. β-Farnesene, β-caryophyllene, and α-zingiberene were their shared ingredients. Compared to *M. cordata*, *M. micrantha* had more detected compounds in its roots (24), stems (32), and flowers (43). Maximum differences were found in the volatile oil composition and content between the two plants. This study is valuable for exploring the allelopathy, prevention, and management of *M. micrantha*.

Key words: *Mikania micrantha*; *Mikania cordata*; Volatile oil; Composition

Introduction

The genus *Mikania* (Asteraceae) includes only two species in China, *M. micrantha* and *M. cordata* (Shi *et al*., 2011). The former is an alien invasive weed from Central and South America, notorious as the "plant killer", posing a serious threat to local agriculture, ecosystems, and environment (Wang *et al*., 2003). In contrast, the latter is harmless to native species and the environment, regarded as an indigenous plant restricted in Taiwan, Hainan, and Yunnan (Kong *et al*., 2000). As perennial vines, their morphological features are too similar to distinguish easily, including slender multi-branch stems, achene with pappus, and both sexual and vegetative reproduction (Holm *et al*., 1978; Kong *et al*., 2000). Subtle identified differences involve the hairy membranous outgrowth of young vegetative shoots, flower heads, corollas, and the length of involucral bracts (Su *et al*., 2018). Hence, *M. micrantha* and *M. cordata* represent an ideal congeneric plant pair for comparative studies to unravel the invasive mechanisms of alien weeds (Vila and Weiner, 2004). Currently, their comparative investigations focus on mitochondrial genomics (Wang *et al*., 2024), chloroplast genomics (Su *et al*., 2018), genomics (Wang *et al*., 2025), epigenomics (Sang *et al*., 2024), transcriptomics (Guo *et al*., 2018), and photosynthetic traits (Deng *et al*., 2004). However, comparative analysis of volatile oil composition remains unreported.

Volatile oils contain complex components such as terpenoids (including monoterpenes and sesquiterpenes), phenylpropanoids, and fatty acid derivatives (Ma *et al*., 2021), which can be extracted using steam distillation from different plant tissues (Caballero-Gallardo *et al*., 2014). Volatile oils from roots, stems, leaves, flowers, and fruits play important roles in allelopathic effects (Ma *et al*., 2021). As signaling molecules, volatiles can not only influence the growth of neighboring plants and inhibit their seed germination but also manipulate ecosystem functions (Nishida *et al*., 2005; He *et al*., 2014; Ma *et al*., 2021). Allelopathy is prominent in invasive plants, serving as a powerful novel chemical weapon against native plants (Kato-Noguchi, 2023; Kato-Noguchi, 2022a; Kato-Noguchi, 2022b; Kato-Noguchi and Kurniadie, 2022; Ma *et al*., 2020). For instance, volatiles from *M. micrantha* leaves and flowers can inhibit the growth of *Lactuca sativa*, *Chrysanthemum coronarium*, *Bidens pilosa*, and *Abutulon theophrasti* (Ma *et al*., 2021). The main identified ingredients include α-terpineol, β-ocimene, β-myrcene, α-pinene and β-caryophyllene (Ma *et al*., 2021). Notably, β-caryophyllene from *M. micrantha* leaves is confirmed to hinder the growth of plants such as *L. sativa, Raphanus sativus*, and *Brassica campestris* (Wang *et al*., 2010).

The composition and content of volatile oils vary among different plant tissues (Dudareva, 2004). Previous studies have only characterized the volatile oils of leaves, flowers, and other tissues of *M. micrantha* separately. Notably, the absence of volatile oil characterization in roots may result in an incomplete assessment of allelopathic potential. Hence, to further explore the invasive mechanism of *M. micrantha*, it is necessary to analyze the volatile oils from different tissues and compare them with the corresponding tissues of its indigenous congener.

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Terpenoids are a large group of natural secondary metabolites with isoprene structural units (Liu *et al*., 2019). Currently, over sixty thousand terpenoids and their derivatives have been identified, which are classified into monoterpenes, sesquiterpenes, diterpenes, and triterpenes (Liu *et al*., 2019). Among them, sesquiterpenes such as caryophyllene have allelopathic properties in the volatile compounds of many plants (Woranoot *et al*., 2019). Sesquiterpenes are biosynthesized through the mevalonate (MVA) pathway with the precursor isopentenyl diphosphate (IPP), while precursors of monoterpenes are formed by the 2-C-methyl-D-erythritol-4-phosphate (MEP) pathway (Liu *et al*., 2022). These precursors are subsequently converted into various terpenoids by terpene synthases (Liu *et al*., 2022).

In this study, the volatile oils from different tissues (roots, stems, leaves, and flowers) of *M. micrantha* and *M. cordata* were analyzed by gas chromatography-mass spectrometry (GC-MS). This study provides valuable information on the allelopathy of *M. micrantha* and lays a foundation for its prevention and control.

Results and Discussion

To better understand the composition and content of volatile oils in various tissues of *M. micrantha* and *M. cordata*, we performed GC-MS analyses on roots, stems, and leaves. We also conducted a comparison analysis of the volatile oil composition in the different tissues between the two plants. Unlike *M. cordata*, we also obtained flowers of *M. micrantha* in addition to roots, stems, and leaves. The base peak chromatograms of the volatile oils of the two plants are shown in Figs. 1 and 2. The compounds were identified by comparing the measured mass spectra with known compounds in the National Institute of Standards and Technology (NIST, USA) database.

For *M. micrantha*, the chemical profiles of the essential oils from roots, stems, leaves, and flowers are shown in Table 1. Specifically, we identified 24, 32, 43, and 39 known essential oil constituents in these tissues, respectively. Compared to leaves and flowers, the other two tissues contained higher amounts of acidic substances such as stearic acid, pentadecanoic acid, myristic acid, and dodecanoic acid. In the leaf essential oil, the major compounds were α-zingiberene (34.55%), γ-curcumene (19.40%), α-bergamotene (11.45%), and β-caryophyllene (9.27%); the remaining compounds ranged from 0.01 to 4.95%. For the stems, the predominant components included hexadecylic acid (40.13%), α-zingiberene (13.41%), and α-curcumene (10.99%). γ-curcumene (6.10%), α-bergamotene (3.99%), linoleic acid (3.76%), and β-caryophyllene (1.49%) were also identified as main constituents. In the roots, the major compound was palmitic acid (54.71%). Other main compounds included α-curcumene (6.64%), dodecanoic acid (5.55%), (-)-isoledene (5.31%), and pentadecanoic acid (4.50%). For the flowers, the main constituents were α-zingiberene (34.862%), γ-curcumene (18.61%), α-bergamotene (11.001%), and α-curcumene (9.525%). Other main components included β-caryophyllene (7.102%), δ-cadinene (3.734%), and palmitic acid (2.077%).

Compared to previous studies, the number of volatile compounds obtained from *M. micrantha* in this study was moderate. For instance, 42 and 19 compounds were identified in the volatile oil of *M. micrantha* flowers in Shenzhen and Hainan, respectively (Shao *et al*. 2001; Ma *et al*., 2021), while 66 compounds were identified from flower volatile oil in East India (Saikia *et al*., 2020). For the *M. micrantha* whole plant, 27 compounds, mainly terpenoids, were distinguished in the essential oil (Nicollier and Thompson, 1981), whereas 49 components were identified in essential oil from Dongguang (Hao *et al*., 2007). Previous investigations into volatile oils have focused on *M. micrantha* leaves. Leaves exhibit varying numbers of essential oil compounds in different growing regions. Specifically, 65, 38, and 49 volatile constituents were identified in *M. micrantha* leaves in Southern Brazil, México, and Vietnam, respectively (Limberger *et al*. 2001; Bravo-Monzón *et al*., 2014). In Hainan Province (China), 19 and 42 compounds were identified in Chengmai County and three other areas (Danzhou, Haikou, and Qionghai), respectively (Ma *et al*., 2020; Ma *et al*., 2021). In Yunnan Province (China), 56 and 50 compounds were detected in different woodlands of Ruili, respectively (Sun *et al*., 2013; Ji *et al*., 2012). Regarding stems, 49 components were detected in Vietnam, whereas 61 constituents were found in Yunnan (Nguyen *et al*., 2023; Sun *et al*., 2013). 51 and 19 compounds were detected in the aerial parts of *M. micrantha* from Shenzhen Fairy Lake Botanical Garden and other areas of Guangdong Province (China), respectively (Feng *et al*., 2004; Huang *et al*., 2020). In addition, 66 compounds were identified in the inflorescence in Yunnan (Sun *et al*., 2013). In the present study, we identified 24, 32, 43, and 39 compounds in the roots, stems, leaves, and flowers, respectively. These results showed that the chemical composition of the volatile oil of *M. micrantha* is related to harvest period, geography, light conditions, temperature, humidity, nutrients, growth state, and extraction time (Nguyen *et al*., 2023).

Similarly, the number of volatile compounds in *M. cordata* is also related to the harvest period and geographical location. In this study, 73, 66, and 52 compounds were identified in the roots, stems, and leaves of *M. cordata* (Table 2). In Abidjan, 59 compounds were found in the leaves of *M. corrlata* from southern Ivory Coast (Bedi *et al*., 2003), while 47 compounds were identified in the dried leaves from Côte d'hoire (Pélissier & Marion, 2001).

We performed further in-depth comparisons of the chemical profiles of *M. micrantha* and *M. cordata* essential oil with other published data. Our obtained volatile oil compositions were distinctly different from previous investigations and other *Mikania* species. Even though there were different specific chemicals and percentages, terpenes were still the major components in *M. micrantha* and *M. cordata*. As the dominant secondary metabolites in Asteraceae (Liu *et al*., 2022), terpenoids had a relatively high proportion in the two species. β- farnesene, β-caryophyllene, and α-zingiberene were the only three components consistently found in the three tissues of the two plants. These compounds exhibited different contents and abundances in the roots, stems, and leaves of *M. micrantha* and *M. cordata*. The stems and leaves of *M. micrantha* had high α-zingiberene (13.413% and 34.549%), whereas the corresponding tissues of *M. cordata* possessed high β-caryophyllene (16.731% and 27.816%). Only the roots of *M. cordata* presented high β-farnesene (18.637%). β-Caryophyllene was present in all essential oil studies of *M. micrantha* and *M. cordata* with different contents.

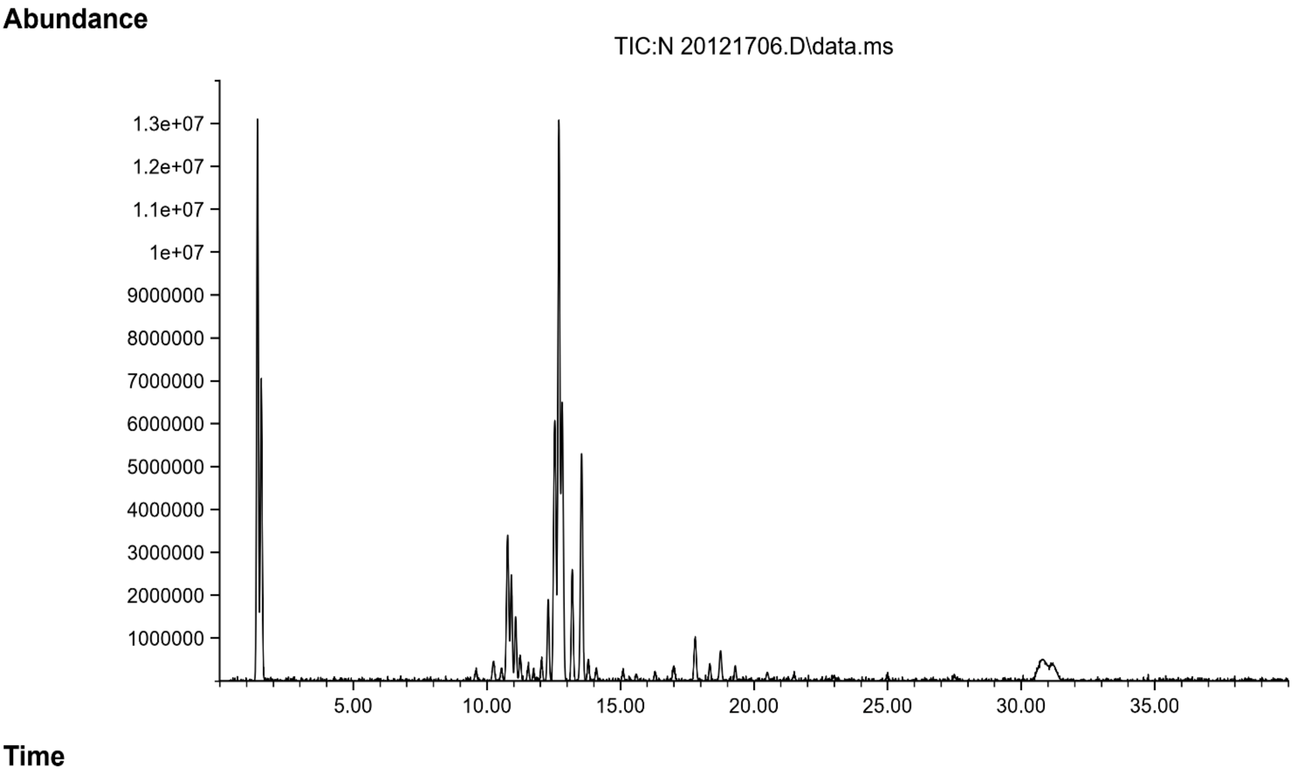

Fig. 1. The base peak chromatograms of the leaf volatile oils of *Mikania micrantha*.

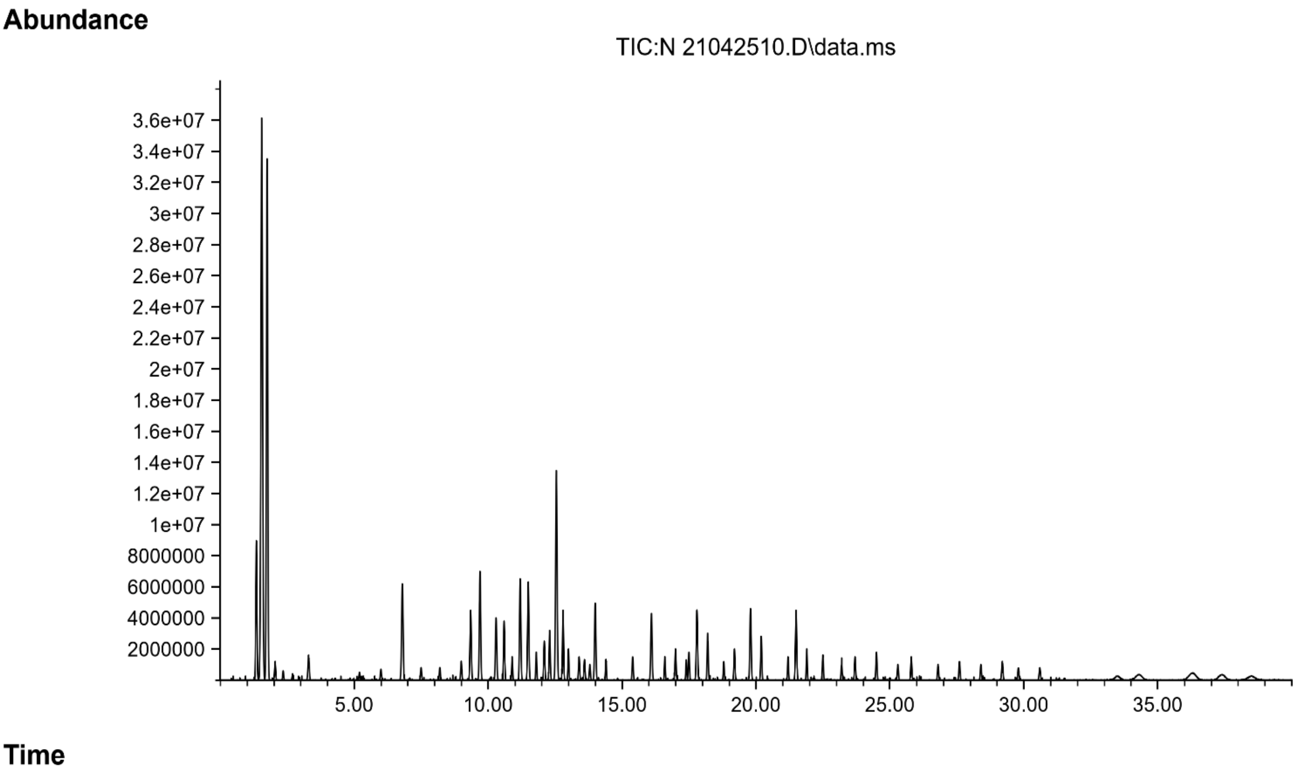

Fig. 2. The base peak chromatograms of the root volatile oils of *Mikania cordata*.

β-Caryophyllene is a sesquiterpene that occurs greatly in the volatile oils of plants such as *Polygonum lapathifolium* (Demirpolat *et al*., 2022). It has antioxidant and anti-inflammatory properties and especially inhibits seedling growth (Muthusamy *et al*., 2020). β-Caryophyllene is synthesized from farnesyl diphosphate by β-caryophyllene synthase (Cai *et al*., 2002). In this study, β-caryophyllene was present in *M. micrantha* roots (1.151%), stems (1.487%), and leaves (9.27%), and flowers (7.102%). The contents of the latter two tissues were similar to published data (9.49% and 9.17%) (Wang *et al*., 2009). Meanwhile *M. cordata* roots, stems, and leaves contained 8.43%, 16.73%, and 27.82%, respectively. β-Caryophyllene has been confirmed as an important allelopathic component in the essential oils of *M. micrantha* (Wang *et al*., 2009). In addition, β-farnesene and α-zingiberene are also important terpenoids. Similar to β-caryophyllene, they can act either individually or synergistically as allelochemicals for *M. micrantha*.

Table 1. Constituents of essential oils from *Mikania micrantha*.

Components	% in samples and retentions time (RT, min)							
	Leaf	RT	Flower	RT	Root	RT	Stem	RT
Hexadecanoic acid	0.22	30.550	2.08	30.562	54.71	30.509	40.13	30.506
Phytol	0.20	23.805	—	—	—	—	1.84	23.692
α-bisabolol	0.48	18.542	0.51	18.533	2.87	19.123	0.94	18.420
Nerolidol	0.64	17.173	0.52	17.176	—	—	0.54	17.091
Bicyclogermacrene	0.47	16.608	—	—	—	—	—	—
Germacrene b	0.52	14.620	0.28	14.620	0.66	14.598	1.13	14.598
Farnesol	0.09	14.221	0.11	14.221	—	—	0.15	14.202
α-curcumene	4.95	13.772	9.53	13.766	6.64	13.719	10.99	13.725
δ-cadinene	2.40	13.612	3.73	13.600	0.69	13.562	1.41	13.552
α-cedrene	2.06	13.339	1.80	13.320	—	—	0.92	13.279
α-serinene	0.26	13.251	—	—	—	—	—	—
α-gurjunene	0.42	13.201	—	—	—	—	—	—
β-bisabolene	0.53	13.179	—	—	2.77	13.091	—	—
α-zingiberene	34.55	13.153	34.86	13.110	1.95	13.003	13.41	13.012
Germacrene-d	2.39	12.959	1.51	12.946	0.85	12.902	0.76	12.902
γ-curcumene	19.40	12.657	18.61	12.626	1.43	12.566	6.10	12.570
α-humulene	0.62	12.353	1.05	12.350	—	—	1.09	12.325
β-farnesene	0.98	12.177	1.02	12.174	0.67	12.149	0.51	12.146
Aromadendrene	0.26	11.998	0.26	11.998	—	—	—	—
β-sesquiphellandrene	0.72	11.885	0.69	11.894	—	—	0.34	11.854
γ-elemene	0.05	11.828	0.07	11.819	1.85	11.797	0.30	11.791
β-caryophyllene	9.27	11.317	7.1	11.292	1.15	11.241	1.49	11.238
α-bergamotene	11.45	11.097	11.00	11.075	1.72	11.012	3.99	11.021
Valenene	0.12	10.908	0.10	10.899	—	—	—	—
α-farnesene	0.14	10.764	0.16	10.770	—	—	—	—
β-cubebene	2.15	10.324	1.31	10.315	—	—	—	—
α-copaene	1.70	9.646	0.64	9.640	—	—	—	—
δ-elemene	0.64	9.206	0.26	9.247	—	—	—	—
α-cubebene	0.63	9.034	0.20	9.031	—	—	0.27	9.608
2-hexen-1-ol	0.06	7.866	0.09	7.866	—	—	0.15	7.866
3-hexen-1-ol	0.13	7.514	0.08	7.517	—	—	0.15	7.511
n-hexyl alcohol	0.04	7.008	—	—	0.31	7.018	1.07	7.005
4,8-dimethyl-1,3,7-nonatriene	0.01	6.402	—	—	—	—	—	—
β-ocimene	0.12	5.501	0.02	5.511	—	—	—	—
2-hexenal	0.04	5.030	0.01	5.043	—	—	—	—
Limonene	0.20	4.760	0.04	4.766	—	—	0.13	4.760
β-myrcene	0.03	4.192	0.02	4.195	—	—	—	—
β-pinene	0.02	3.576	—	—	—	—	—	—
α-pinene	0.11	2.725	0.06	2.719	—	—	0.14	2.732
α-cadinol	—	—	0.13	19.402	—	—	—	—
Fitone	—	—	0.36	18.344	—	—	—	—
Caryophyllene oxide	—	—	0.48	16.617	0.62	16.633	0.72	16.617
γ-muurolene	—	—	0.15	16.008	—	—	—	—
Hexyl formate	—	—	0.09	7.015	—	—	—	—
Caproaldehyde	—	—	0.01	3.240	—	—	—	—
Octadecanoic acid	—	—	—	—	1.75	39.738	1.72	39.703
Pentadecanoic acid	—	—	—	—	4.50	27.419	1.88	27.413
Xanthorrhizol	—	—	—	—	0.87	25.275	0.82	25.265
Myristic acid	—	—	—	—	2.02	25.111	0.76	25.108
Linoleic acid	—	—	—	—	—	—	3.76	22.106
Dodecanoic acid	—	—	—	—	5.55	21.918	1.19	21.918
Octanoic acid	—	—	—	—	0.54	17.365	—	—
Isoledene	—	—	—	—	5.31	12.334	—	—
Ylangene	—	—	—	—	0.21	9.615	1.21	19.101
Italicene	0.73	19.223	0.85	19.211	—	—	—	—
Shogaol	0.10	18.093	0.21	18.090	—	—	—	—
Sesquisabinene	0.11	16.705	—	—	—	—	—	—
8-hydroxy-α-copaene	—	—	—	—	0.38	17.697	—	—
Sabinene	0.02	3.708	—	—	—	—	—	—

Table 2 Constituents of essential oils from *Mikania cordata*.

Components	% in samples and retentions time (RT, min)					
	Root	RT	Stem	RT	Leaf	RT
α-cubebene	0.22	8.848	0.52	8.836	0.94	8.835
α-longleaf pinene	0.22	9.059	1.52	9.044	0.93	9.043
Aristolene alkene	0.17	9.090	—	—	—	—
α-copaene	0.69	9.440	—	—	—	—
Cyperene	1.47	10.003	—	—	—	—
β-cubebene	5.54	10.138	12.62	10.128	13.72	10.122
Bornyl acetate	0.18	10.695	—	—	—	—
α-bulnesene	0.54	10.830	—	—	—	—
β-elemene	0.42	10.920	—	—	—	—
β-caryophyllene	8.43	11.085	16.73	11.079	27.82	11.070
β-farnesene	18.64	12.027	0.89	11.982	0.48	11.979
α-humulene	1.17	12.164	2.15	12.151	2.58	12.149
Alloaromadendren	0.33	12.297	—	—	—	—
β-cedrene	1.81	12.741	9.09	12.734	5.15	12.727
α-zingiberene	0.44	12.842	2.92	12.834	1.15	12.830
α-cedrene	0.87	12.935	—	—	—	—
Lepidozene	1.23	13.099	10.18	13.092	13.65	13.089
δ-cadinene	0.66	13.391	2.56	13.385	—	—
Geranyl isovalerate	0.25	14.724	—	—	—	—
Caryophyllene oxide	1.16	16.526	0.60	16.505	0.84	16.509
n-docosane	1.43	19.183	—	—	—	—
Nerolidol	—	—	—	—	1.56	17.029
Tricosane	2.08	20.342	—	—	—	—
Tetracosane	6.01	21.499	—	—	—	—
Pentacosane	4.89	22.874	—	—	—	—
Hexacosane	4.59	24.571	—	—	—	—
Heptacosane	3.71	26.728	—	—	—	—
Octacosane	2.76	29.529	—	—	—	—
Nonacosane	1.55	33.203	—	—	—	—
γ-curcumene	—	—	1.65	12.397	0.66	12.394
α-curcumene	—	—	0.91	13.559	0.22	13.564
Spathulenol	—	—	0.46	18.173	0.50	18.172
Italicene	—	—	5.39	19.239	2.16	19.240
Phytol	—	—	1.91	24.489	3.42	24.492
Hexadecanoic acid	—	—	1.77	32.807	1.05	32.804
Thymol	0.74	18.865	—	—	—	—

Besides terpenes, acids identified in the essential oils also exhibit allelopathic effects. For instance, benzoic acid and lactic acid have been confirmed to play key roles in the allelopathic effects of *M. micrantha* (Ma *et al*., 2020). In the present study, the roots contained three acids with high contents, including palmitic acid (54.71%), dodecanoic acid (5.546%), and pentadecanoic acid (4.497%). The stems had a relatively high concentration of palmitic acid (40.125%), whereas its content in flowers (2.077%) was low. In contrast, the content of palmitic acid was lower in the stems (1.77%) and leaves (1.05%) of *M. cordata.* These results imply that acidic substances may promote the allelopathic effect of *M. micrantha*.

The allelopathic potential of plant roots generally differ from that of aboveground parts (Wu *et al*., 2024). Roots can release volatile compounds to protect themselves and compete with neighboring plants (Nicol *et al*., 2003). For instance, β-farnesene from the roots of *C. morifolium* cultivars and *Calamintha nepeta* can inhibit root elongation in *Echinochloa crusgalli* seedlings and root growth in *Arabidopsis thaliana*, respectively (Araniti *et al*., 2013; Wu *et al*., 2024). In addition, Arabidopsis roots treated with β-farnesene lose gravitropism, which further leads to microtubule disorganization (Araniti *et al*., 2016). The β-farnesene content varies within the same species from different regions. For instance, the volatile oil of *Leucanthemum vulgare* from Georgia contains 38.3% β-farnesene, whereas samples from North Estonia contain only 7.3% (Sagareishvili *et al*., 2002; Magharri *et al*., 2015).

Similarly, α-zingiberene is an important sesquiterpenes in volatile oils. Compared to the mild, sweet, and warm odour of α-farnesene, α-zingiberene presents a warm, woody, spicy, and persistent odour (Aleem *et al*., 2020), which has potential value in the food, agriculture, and pharmaceutical industries (Zhang *et al*., 2021). In roots, the content of α-zingiberene was higher in *M. micrantha* (1.947%) than in *M. cordata* (0.44%). Hence, β-farnesene and α-zingiberene were considered key components related to the allelochemical effects of the roots, playing a pivotal role in the allelopathic effects of *M. micrantha* in the study.

Experimental

Essential oil extraction and determination: Fresh roots, stems, and leaves were harvested from *M. micrantha* and *M. cordata* at the same growth stage. *Mikania micrantha* plants were collected from Neilingding Island, Shenzhen, Guangdong Province, China (22°24′35.13"N, 113°48′37.09"E). *M. cordata* plants were originally collected from Green Island, Taiwan, China (22°39′40.0"N, 121°28′59.2"E) (2018-12) and subsequently cultivated in the nursery at Sun Yat-sen University, Guangdong, China.

The plant materials were ground and weighed. The essential oil yield was determined according to the method described by Hou *et al*. (2020) as follows: Volatile oil content (%) = W1/W2×100, where W1 represents the mass of obtained volatile oil, and W2 represents the mass of fresh tissue.

Gas chromatography-mass spectrometry analysis: The volatile oils analysis was performed on a gas chromatography system (Agilent 7890B, USA) equipped with an AB-INOWAX capillary column (30 m × 0.25 mm internal diameter, 0.25 µm film thickness). Helium was used as the carrier gas at a flow rate of 0.7 mL/min. The injector temperature was maintained at 250°C. The initial temperature of the column was set at 70°C (held for two min) and increased to 220°C at a rate of 8°C/min, and held for 20 min. The mass spectrometer was operated in full scan mode over a range of m/z 29-550. Electron impact (EI) ionization was performed at 70 eV. The temperatures of the quadrupole, ion source, and transfer line were set to 150°C, 230°C, and 280°C, respectively. Compounds were identified based on their relative retention times and mass spectra data.

Conclusions

We performed a comparative analysis of the volatile oils from different tissues of *M. micrantha* and *M. cordata*. Both plants were rich in terpenoids, especially sesquiterpenes, which were present in high contents. Three sesquiterpenes, including β-farnesene, β-caryophyllene, and α-zingiberene, were detected in all analyzed tissues. More compounds were identified in the tissues of *M. micrantha* than in *M. cordata*. The maximum differences between the two plants were observed in the composition and concentrations of volatile oils, with each tissue exhibiting unique characteristics. Given the abundance of allelopathic components in these oils, our study is key for the prevention and management of *M. micrantha*.

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Author's Contributions: W.L and Y.S designed the experiments; Z.W conducted data analysis and wrote manuscript; T.W checked English grammar; R.W, Y.P, and X.R performed experiment and analysis. All authors read and approved the final version of the manuscript.

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