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Allelopathic Potential and Chemical Composition of Essential Oil from the Invasive Plant *Acmella radicans*

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Abstract: *Acmella radicans* (Jacquin) R.K. Jansen is a new invasive species recorded in Yunnan Province, China, and little is known about its allelopathic potential and allelochemicals. In this study, the allelopathic effects of the essential oil (EO) of *A. radicans* on seed germination and seedling growth of four common plants, *Brassica napus*, *Brassica rapa* ssp. *chinensis*, *Digitaria sanguinalis*, and *Lolium multiflorum*, were explored. The results showed that the seed germination index, germination rate, root length, stem length, and biomass of *B. napus* and *B. rapa* ssp. *chinensis* were significantly inhibited at all EO concentrations of *A. radicans*, but there was a 'low-promotion and high-inhibition effect' on the root length of *D. sanguinalis* and *L. multiflorum* at low concentrations of 0.5 μL·mL^{-1} and 0.5–1.0 μL·mL^{-1}, respectively. With increasing concentrations of EO, the inhibition rates of seed germination and seedling growth of four common plants gradually increased, and *D. sanguinalis* and *L. multiflorum* were the most inhibited, followed by *B. rapa* ssp. *chinensis*, and the least inhibited was *B. napus*. Thirty-two components were identified using GC–MS, representing 99.07% of the EO in *A. radicans*. The major components were 2-tridecanone (30.46%), caryophyllene oxide (19.18%), 4,8,11,11-tetramethylbicyclo[7.2.0]undec-3-en-5-ol (7.84%), β-caryophyllene (7.67%), and widdrol (4.7%). Among the compounds we identified, (E,E)-2,4-decadienal, 2-tridecanone, γ-cadinene, δ-cadinene, (E)-α-cadinol, spathulenol, caryophyllene oxide, and widdrol have been previously reported as having possible allelopathic effects. Our study was the first to show that *A. radicans* could potentially release allelochemicals to influence neighboring plants during its invasion and expansion.

Keywords: alien plants; bioassays; phytotoxic; competitive ability; GC-MS; extraction

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1. Introduction

Invasive alien plant species have become one of the greatest threats, leading to global biodiversity loss and other environmental and economic impacts [1,2]. These invasive species can quickly establish a dominant population due to their rapid growth, physiological and ecological adaptability, and in many cases, allelopathic effects [3,4]. If invasive alien plants do possess allelopathic capabilities, they may displace native species through the dual impact of competition for resources and allelopathic impacts [4,5]. Therefore, investigating allelopathy in invasive alien plants, particularly new invasive species, is quite

important in determining the invasion mechanisms and potential impacts on native species or crops.

Acmella radicans (Jacquin) R.K. Jansen is an annual herbaceous plant that originated in Central America and Mexico [6]. It is easily distinguished from other *Acmella* genus species due to white corollas and achenes with well-developed corky margins [7]. It can grow up to 155 cm in height. A single plant can produce up to 14,300 seeds. Having expanded from its native range, *A. radicans* has invaded many countries in various world regions, such as Colombia, Bangladesh, Cuba, Curaçao, India, Tanzania, and Thailand [8–11]. *Acmella radicans* was first recorded as naturalized in China in 2014, specifically in Anhui Province [7]. This plant prefers moist habitats such as riparian areas, roadside ditches, and relatively wet agricultural fields such as rice paddies. Medicinal uses for *A. radicans* include relief of toothaches, and throat and gum infections [9,12,13].

Acmella radicans was first discovered in Yunnan Province in southwestern China during a survey of invasive alien plant species and was already widely distributed in Baoshan City and Lincang City as a serious invasive species, where it has quickly become dominant in many habitats and negatively affected species richness, species diversity, and evenness of local communities as well as soil nutrients [14]. Our previous research showed that *A. radicans* had potential allelopathic effects on four major associated weeds, *Bidens pilosa*, *Ageratum conyzoides*, *Digitaria sanguinalis*, and *Chloris virgata*. These four weeds were markedly more inhibited by aqueous extracts of the above-ground parts of *A. radicans* (leaves and stems) than aqueous extracts of the below-ground parts of the plant [14]. However, little is known about the allelopathic effects and chemical composition of essential oil (EO) from the aboveground part of *A. radicans*.

The objectives of the present study were (1) to examine the allelopathic potential of EO from the above-ground part of *A. radicans* against four commonly associated plants, *Brassica napus*, *Brassica rapa* ssp. *chinensis*, *D. sanguinalis*, and *Lolium multiflorum*, and (2) to characterize the chemical composition of the EO. Elucidating the allelopathy mechanisms of the invasive plant *A. radicans* is needed to provide a scientific basis for early detection and rapid response actions to proactively manage *A. radicans*.

2. Materials and Methods

2.1. Study Species

Acemella radicans plants in the vegetative stage prior to flowering were collected in Mengtong Township, Changning County, Baoshan City of Yunnan Province on 15 September 2022. After harvesting, the above-ground plant parts (leaves and stems) of *A. radicans* were selected and divided into 1–2 cm pieces for EO extraction.

For the bioassay, weed seeds of *D. sanguinalis* and *L. multiflorum* were collected in Mengtong Township in August 2021, and the vegetable crop seeds of *B. napus* and *B. rapa* ssp. *chinensis* were purchased from a local market in Changning County, Yunnan Province. Everything was stored at 4 °C prior to use in the experiment.

2.2. Extraction and Bioassay of Essential Oils

The EO from fresh materials of *A. radicans* was extracted by means of steam distil lation (a modified Clevenger apparatus, 1.5-L) for 3–4 h using 1200 g of above-ground parts, and then the extracted EO layer was immediately separated and dried by anhydrous Na_2SO_4. These procedures were performed many times until enough EO samples were collected for further bioassay evaluation and GC-MS analysis. Based on pre-experimental trials of different concentrations, the allelopathic effects of the EO of *A. radicans* on *B. napus*, *B. rapa* ssp. *chinensis*, *D. sanguinalis*, and *L. multiflorum* were tested at the following five concentrations (0.5, 1.0, 2.0, 4.0, and 8.0 $\mu L \cdot mL^{-1}$) along with a control (distilled water) (CK). The bioassay procedures of different concentrations on germination and seedlings of the four species described were the same as followed by Shen et al. [15]. The shoot height, root length, and fresh biomass of the germinated plant seedlings were measured after a 7-day period.

2.3. Gas Chromatography-Mass Spectroscopy (GC-MS) of the Essential Oil

The EO samples from *A. radicans* were analyzed on an Agilent 7890 gas chromatograph equipped with a quadrupole mass spectrometer (Agilent 5975 N, Santa Clara, CA, USA). The chemical compounds were analyzed and identified in accordance with the same procedure described before by Zhao et al. [16]. Gas chromatography working conditions: HP-5MS fused silica capillary column (30 m × 0.25 mm, film thickness 0.25 μm); 280 °C injector and 260 °C detector; 40 °C for 2 min and 5 °C/min to 250 °C oven temperature, with a final hold time of 6 min at 250 °C; helium carrier gas with a flow rate of (1.0 mL/min), a split ratio of (1:5), and a 1.0 μL sample injection volume. Mass spectrometry operating conditions: full scan with range 50–550 m/z; 150 °C quadrupole and 280 °C interface; 230 °C ion source; 70 eV EI source.

The chemical composition of the EO of *A. radicans* was identified by searching the NIST mass spectrum library, aided with CAS data and the related literature. Compounds with values that were more than a 90% match were selected and the relative percentage of each substance was calculated using the GC peak area.

2.4. Statistical Analysis

We followed the same procedure as found in Shen et al. [15] to calculate the germination rate, germination index [17], and allelopathic response index (RI: when T ≥ C, RI = 1-C/T; when T < C, RI = T/C-1; C is the control value and T is the treatment value) [18] of aqueous extracts from *B. napus*, *B. rapa* ssp. *chinensis*, *D. sanguinalis*, and *L. multiflorum*. The synthetical allelopathic index was calculated using the mean value of RI values of germination rate, germination index, root length, shoot length, and biomass. Data were analyzed by analysis of variance (one-way ANOVA) for seed germination and growth parameters. If significant differences were detected by ANOVA, Duncan's multiple range tests were used to detect differences among treatments at a 5% level of significance.

3. Results

3.1. Seed Germination and Seedling Growth

The germination index and germination rate for the four plants *B. napus*, *B. rapa* ssp. *chinensis*, *D. sanguinalis*, and *L. multiflorum* were significantly affected by the EO of *A. radicans* (Table 1). The EO had strong inhibitory effects on the germination rate and germination index of the four bioassay species (Table 1). With increasing concentrations, inhibition by EO was gradually increased, except the germination rate at 0.5 $\mu L \cdot mL^{-1}$ for *L. multiflorum* (Table 1). The suppression rates of the EO on the germination rate and germination index of *B. napus* and *B. rapa* ssp. *chinensis* were generally lower than those of *D. sanguinalis* and *L. multiflorum*.

The EO of *A. radicans* resulted in varying effects on the root and shoot length of the four plants *B. napus*, *B. rapa* ssp. *chinensis*, *D. sanguinalis*, and *L. multiflorum* (Table 1). Nearly all EO concentrations had strong inhibitory effects on the four bioassay species, with the following exceptions: root length at a concentration 0.5 $\mu L \cdot mL^{-1}$ for *D. sanguinalis* and root length at concentrations of 0.5–1.0 $\mu L \cdot mL^{-1}$ for *L. multiflorum* showed stimulatory effects (Table 1). The suppression rates of the EO on the root length of *B. napus* and *D. sanguinalis* were generally greater than those of *B. rapa* ssp. *chinensis* and *L. multiflorum*, but the inhibition rates of the EO on the shoot length of *D. sanguinalis* and *L. multiflorum* were generally greater than those of *B. napus* and *B. rapa* ssp. *chinensis*.

The biomass of the four bioassay species was significantly inhibited by the EO of *A. radicans* (Table 1). The inhibitory rates of the EO on the biomass of the four plants *B. napus*, *B. rapa* ssp. *chinensis*, *D. sanguinalis*, and *L. multiflorum* were significantly increased with increasing concentrations, and the biomass of *D. sanguinalis* and *L. multiflorum* was inhibited more than that of *B. napus* and *B. rapa* ssp. *chinensis*.

Table 1. Effects of essential oil at different concentrations (control, CK = 0 μL·mL^{-1}) of *Acmella radicans* on the seed germination, root, stem, and biomass of different plants.

Items	Concentration/ μL·mL^{-1}	*Brassica napus*	*Brassica rapa* ssp. *chinensis*	*Digitaria sanguinalis*	*Lolium multiflorum*
Germination rate/%	CK	98.750 ± 1.250 ^{a}	97.500 ± 1.443 ^{a}	93.750 ± 1.250 ^{a}	91.250 ± 3.146 ^{a}
	0.5	90.000 ± 2.887 ^{b}	92.500 ± 2.500 ^{ab}	90.000 ± 2.041 ^{ab}	92.500 ± 2.500 ^{a}
	1	90.000 ± 2.041 ^{b}	93.750 ± 1.250 ^{ab}	82.500 ± 3.228 ^{bc}	87.500 ± 4.787 ^{a}
	2	90.000 ± 2.041 ^{b}	92.500 ± 1.443 ^{ab}	75.000 ± 6.124 ^{cd}	75.000 ± 9.574 ^{ab}
	4	86.250 ± 1.250 ^{bc}	90.000 ± 2.041 ^{b}	68.750 ± 2.394 ^{d}	60.000 ± 5.774 ^{b}
	8	82.500 ± 1.443 ^{c}	87.500 ± 3.228 ^{b}	26.250 ± 3.750 ^{e}	60.000 ± 7.071 ^{b}
Germination index	CK	16.792 ± 0.300 ^{a}	18.292 ± 0.438 ^{a}	6.513 ± 0.114 ^{a}	4.175 ± 0.292 ^{a}
	0.5	13.542 ± 0.463 ^{b}	16.875 ± 0.533 ^{b}	6.075 ± 0.206 ^{ab}	3.321 ± 0.091 ^{b}
	1	11.958 ± 0.427 ^{c}	16.875 ± 0.375 ^{b}	5.579 ± 0.268 ^{bc}	3.146 ± 0.120 ^{b}
	2	11.792 ± 0.502 ^{c}	16.542 ± 0.315 ^{b}	4.900 ± 0.360 ^{c}	2.508 ± 0.292 ^{c}
	4	10.417 ± 0.221 ^{d}	16.167 ± 0.561 ^{b}	4.021 ± 0.275 ^{d}	2.050 ± 0.125 ^{cd}
	8	8.583 ± 0.308 ^{e}	13.042 ± 0.453 ^{c}	1.808 ± 0.304 ^{e}	1.863 ± 0.163 ^{d}
Root length/cm	CK	1.229 ± 0.058 ^{a}	1.142 ± 0.060 ^{a}	1.685 ± 0.030 ^{a}	2.647 ± 0.147 ^{a}
	0.5	0.820 ± 0.025 ^{b}	0.938 ± 0.144 ^{ab}	1.835 ± 0.052 ^{a}	2.723 ± 0.111 ^{a}
	1	0.752 ± 0.058 ^{bc}	0.942 ± 0.115 ^{ab}	1.653 ± 0.100 ^{a}	2.659 ± 0.186 ^{a}
	2	0.705 ± 0.024 ^{bc}	0.834 ± 0.028 ^{b}	1.221 ± 0.039 ^{b}	1.904 ± 0.180 ^{b}
	4	0.669 ± 0.033 ^{c}	0.781 ± 0.039 ^{b}	0.733 ± 0.054 ^{c}	1.550 ± 0.158 ^{bc}
	8	0.640 ± 0.041 ^{c}	0.781 ± 0.042 ^{b}	0.658 ± 0.058 ^{c}	1.407 ± 0.050 ^{c}
Shoot length/cm	CK	0.857 ± 0.030 ^{a}	0.986 ± 0.020 ^{a}	1.183 ± 0.077 ^{a}	3.319 ± 0.122 ^{a}
	0.5	0.824 ± 0.006 ^{ab}	0.893 ± 0.048 ^{b}	0.850 ± 0.105 ^{b}	1.685 ± 0.157 ^{b}
	1	0.816 ± 0.027 ^{abc}	0.833 ± 0.013 ^{bc}	0.780 ± 0.009 ^{b}	1.389 ± 0.058 ^{c}
	2	0.722 ± 0.029 ^{bc}	0.805 ± 0.035 ^{cd}	0.519 ± 0.050 ^{c}	0.648 ± 0.047 ^{d}
	4	0.711 ± 0.054 ^{bc}	0.751 ± 0.018 ^{cd}	0.440 ± 0.042 ^{c}	0.617 ± 0.055 ^{d}
	8	0.704 ± 0.045 ^{c}	0.744 ± 0.003 ^{d}	0.370 ± 0.045 ^{c}	0.544 ± 0.082 ^{d}
Biomass/g	CK	0.145 ± 0.007 ^{a}	0.155 ± 0.008 ^{a}	0.041 ± 0.001 ^{a}	0.100 ± 0.005 ^{a}
	0.5	0.143 ± 0.003 ^{a}	0.150 ± 0.007 ^{a}	0.039 ± 0.002 ^{a}	0.081 ± 0.006 ^{b}
	1	0.143 ± 0.007 ^{a}	0.149 ± 0.014 ^{a}	0.037 ± 0.002 ^{a}	0.066 ± 0.004 ^{c}
	2	0.130 ± 0.003 ^{ab}	0.147 ± 0.011 ^{a}	0.029 ± 0.001 ^{b}	0.030 ± 0.002 ^{d}
	4	0.121 ± 0.007 ^{bc}	0.115 ± 0.007 ^{b}	0.028 ± 0.001 ^{b}	0.025 ± 0.002 ^{d}
	8	0.109 ± 0.005 ^{c}	0.078 ± 0.005 ^{c}	0.009 ± 0.001 ^{c}	0.022 ± 0.004 ^{d}

Data are expressed as mean ± standard deviation. Different letters within the same column signify significant differences at $p < 0.05$.

3.2. Allelopathic Index

The measured allelopathic response index and synthetical allelopathic index of the EO of *A. radicans* on the germination and seedling growth of four bioassay species varied, depending on concentrations and species (Table 2). For *B. napus* and *B. rapa* ssp. *chinensis*, all measured allelopathic indices were significantly lower than 0 and were significantly reduced with increasing concentration. For *D. sanguinalis*, all measured allelopathic indices were significantly lower than 0, with the exception of an above-zero index for root length at a concentration 0.5 μL·mL^{-1}. The allelopathic indices were significantly reduced with increasing concentrations. For *L. multiflorum*, some allelopathic indices for the germination rate at low concentrations of 0.5 μL·mL^{-1}, and for root length at low concentrations of 0.5–1.0 μL·mL^{-1}, were higher than 0, whereas other allelopathic indices were well below 0. The allelopathic indices of the EO of *A. radicans* for *L. multiflorum* were significantly reduced with increasing concentrations (Table 2). Comparing the allelopathic response index and synthetical allelopathic index of the EO of *A. radicans* among the four common plants, *D. sanguinalis* and *L. multiflorum* showed the strongest inhibition, followed by *B. napus*, and the least inhibited was *B. rapa* ssp. *chinensis* (Table 2).

Table 2. Allelopathic response index of the essential oil of *Acmella radicans* on different plants.

Items	Concentration/ μL·mL^{-1}	*Brassica napus*	*Brassica rapa* ssp. *chinensis*	*Digitaria sanguinalis*	*Lolium multiflorum*
Germination rate	0.5	−0.089 ± 0.024 ^{a}	−0.051 ± 0.022 ^{ab}	−0.040 ± 0.013 ^{a}	0.015 ± 0.015 ^{a}
	1	−0.089 ± 0.013 ^{a}	−0.038 ± 0.013 ^{a}	−0.121 ± 0.026 ^{ab}	−0.043 ± 0.027 ^{ab}
	2	−0.089 ± 0.013 ^{a}	−0.051 ± 0.001 ^{abc}	−0.202 ± 0.058 ^{bc}	−0.185 ± 0.078 ^{b}
	4	−0.126 ± 0.014 ^{ab}	−0.077 ± 0.015 ^{abcd}	−0.267 ± 0.022 ^{c}	−0.345 ± 0.051 ^{c}
	8	−0.165 ± 0.012 ^{b}	−0.103 ± 0.022 ^{bd}	−0.721 ± 0.038 ^{d}	−0.347 ± 0.054 ^{c}
Germination index	0.5	−0.194 ± 0.016 ^{a}	−0.078 ± 0.012 ^{a}	−0.068 ± 0.016 ^{a}	−0.197 ± 0.036 ^{a}
	1	−0.289 ± 0.013 ^{b}	−0.077 ± 0.007 ^{a}	−0.144 ± 0.033 ^{ab}	−0.241 ± 0.030 ^{a}
	2	−0.298 ± 0.023 ^{b}	−0.095 ± 0.006 ^{ab}	−0.250 ± 0.045 ^{b}	−0.405 ± 0.028 ^{b}
	4	−0.380 ± 0.002 ^{c}	−0.117 ± 0.012 ^{b}	−0.384 ± 0.033 ^{c}	−0.508 ± 0.012 ^{c}
	8	−0.489 ± 0.010 ^{d}	−0.287 ± 0.012 ^{c}	−0.724 ± 0.042 ^{d}	−0.555 ± 0.013 ^{c}
Root length	0.5	−0.331 ± 0.012 ^{a}	−0.187 ± 0.092 ^{a}	0.089 ± 0.029 ^{a}	0.031 ± 0.024 ^{a}
	1	−0.390 ± 0.028 ^{ab}	−0.175 ± 0.090 ^{a}	−0.021 ± 0.045 ^{b}	0.005 ± 0.053 ^{a}
	2	−0.421 ± 0.042 ^{bc}	−0.265 ± 0.034 ^{a}	−0.275 ± 0.017 ^{c}	−0.281 ± 0.058 ^{b}
	4	−0.456 ± 0.009 ^{bc}	−0.309 ± 0.055 ^{a}	−0.566 ± 0.026 ^{d}	−0.417 ± 0.038 ^{c}
	8	−0.479 ± 0.019 ^{c}	−0.309 ± 0.038 ^{a}	−0.611 ± 0.028 ^{d}	−0.463 ± 0.039 ^{c}
Shoot length	0.5	−0.036 ± 0.027 ^{a}	−0.096 ± 0.032 ^{a}	−0.289 ± 0.042 ^{a}	−0.495 ± 0.033 ^{a}
	1	−0.048 ± 0.012 ^{a}	−0.154 ± 0.015 ^{ab}	−0.334 ± 0.034 ^{a}	−0.582 ± 0.008 ^{b}
	2	−0.158 ± 0.008 ^{b}	−0.183 ± 0.034 ^{bc}	−0.563 ± 0.022 ^{b}	−0.806 ± 0.008 ^{c}
	4	−0.173 ± 0.035 ^{b}	−0.238 ± 0.013 ^{c}	−0.630 ± 0.012 ^{bc}	−0.815 ± 0.012 ^{c}
	8	−0.180 ± 0.030 ^{b}	−0.244 ± 0.018 ^{c}	−0.690 ± 0.021 ^{c}	−0.838 ± 0.020 ^{c}
Biomass	0.5	−0.005 ± 0.041 ^{a}	−0.029 ± 0.020 ^{a}	−0.045 ± 0.029 ^{a}	−0.199 ± 0.020 ^{a}
	1	−0.010 ± 0.020 ^{a}	−0.039 ± 0.070 ^{a}	−0.092 ± 0.016 ^{a}	−0.348 ± 0.021 ^{b}
	2	−0.099 ± 0.037 ^{b}	−0.053 ± 0.020 ^{a}	−0.287 ± 0.014 ^{b}	−0.699 ± 0.012 ^{c}
	4	−0.161 ± 0.021 ^{bc}	−0.253 ± 0.028 ^{b}	−0.323 ± 0.004 ^{b}	−0.752 ± 0.009 ^{cd}
	8	−0.244 ± 0.009 ^{c}	−0.497 ± 0.016 ^{c}	−0.775 ± 0.014 ^{c}	−0.784 ± 0.028 ^{d}
Synthetical allelopathic index	0.5	−0.131 ± 0.059 ^{a}	−0.088 ± 0.027 ^{a}	−0.071 ± 0.061 ^{a}	−0.169 ± 0.095 ^{a}
	1	−0.165 ± 0.074 ^{a}	−0.097 ± 0.029 ^{a}	−0.142 ± 0.052 ^{a}	−0.242 ± 0.107 ^{a}
	2	−0.213 ± 0.064 ^{a}	−0.129 ± 0.042 ^{a}	−0.315 ± 0.064 ^{b}	−0.475 ± 0.120 ^{ab}
	4	−0.259 ± 0.066 ^{a}	−0.199 ± 0.044 ^{ab}	−0.434 ± 0.070 ^{b}	−0.567 ± 0.093 ^{b}
	8	−0.311 ± 0.072 ^{a}	−0.288 ± 0.063 ^{b}	−0.704 ± 0.027 ^{c}	−0.597 ± 0.094 ^{b}

Data are expressed as mean ± standard deviation. Different letters within the same column signify significant differences at $p < 0.05$.

3.3. Identification of Chemical Composition

Thirty-two components representing 99.07% of the EO of *A. radicans* were identified using GC–MS (Figure 1 and Table 3). The major components were 2-tridecanone (30.46%), caryophyllene oxide (19.18%), 4,8,11,11-tetramethylbicyclo[7.2.0]undec-3-en-5-ol (7.84%), β-caryophyllene (7.67%), widdrol (4.7%), (Z,Z)-heptadeca-1,8,11-triene (2.29%), spathulenol (2.28%), 1H-cyclobut[e]inden-5-ol,decahydro-2,2,4a,7a-tetramethyl-,[2aR-(2aα,4aβ,5α,7aα,7bα)]-(9CI) (2.15%), (E)-α-cadinol (2.08%), and (-)-globulol (2.03%) (Table 3). The allelopathic potentials of some of these EO components have been previously reported as potential allelochemicals produced by other plant species.

Table 3. Chemical compounds of the essential oil of *Acmella radicans*.

No.	Compound	Retention Time (min)	Percentage (%)	Molecular Ion (*m*/*z*)	Main Fragment Ions (*m*/*z*)
1	(E,E)-2,4-Decadienal	39.587	0.56	152.23	81 (100.0); 67 (19.9); 83 (17.0); 55 (14.4); 152 (10.5)
2	β-Patchoulene	42.250	0.64	204.35	161 (100.0); 105 (58.1); 133 (54.1); 189 (53.5); 91 (53.4); 119 (48.5); 93 (46.0); 148 (25.2); 107 (24.0); 55 (23.8)
3	2-Methylene-4,8,8-trimethyl-4-vinyl bicyclo[5,2,0]nonane	42.739	0.70	204.35	93 (100.0); 91 (44.9); 105 (44.8); 79 (36.6); 94 (36.3); 107 (36.0); 133 (33.1); 119 (32.9); 121 (32.9); 69 (28.0)
4	1-Methoxydodecane	44.650	0.57	200.36	58 (100.0); 55 (36.9); 57 (35.8); 59 (34.9); 71 (32.8); 83 (25.2); 56 (23.6); 69 (21.0); 97 (20.5); 70 (19.5)

Table 3. *Cont.*

No.	Compound	Retention Time (min)	Percentage (%)	Molecular Ion (*m*/*z*)	Main Fragment Ions (*m*/*z*)
5	β-Caryophyllene	45.885	7.67	204.35	93 (100.0); 133 (83.4); 91 (79.0); 69 (71.8); 79 (64.7); 105 (53.2); 120 (45.1); 119 (41.1); 107 (41.1); 81 (39.9)
6	α-Caryophyllene	48.061	0.97	204.35	93 (100.0); 80 (33.2); 121 (28.5); 91 (18.3); 92 (18.1); 147 (16.0); 79 (15.8); 107 (14.3); 100 (13.9); 77 (10.7)
7	Precocene I	48.490	0.58	190.24	175 (100.0); 190 (16.6); 132 (14.3); 176 (11.8); 160 (11.6)
8	(Z,Z)-Heptadeca-1,8,11-triene	49.587	2.29	234.42	67 (100.0); 81 (97.4); 55 (66.9); 54 (50.7); 82 (50.4); 95 (40.9); 79 (39.2); 96 (35.0); 110 (29.4); 68 (27.3)
9	2-Tridecanone	50.907	30.46	198.35	58 (100.0); 59 (38.0); 71 (29.8); 57 (24.5); 55 (21.8); 83 (13.1); 85 (12.8); 56 (11.9); 97 (11.9); 69 (10.8)
10	γ-Cadinene	51.645	0.50	204.35	161 (100.0); 105 (56.5); 119 (50.1); 91 (43.8); 93 (34.7); 133 (31.0); 79 (30.3); 81 (28.2); 204 (20.4); 120 (19.0)
11	δ-Cadinene	52.032	0.57	204.35	159 (100.0); 161 (36.1); 119 (35.3); 105 (31.6); 134 (24.6); 204 (20.7); 131 (20.3); 91 (17.5); 160 (14.8); 81 (14.8)
12	4,8,13-Duvatriene-1,3-diol	52.360	1.56	306.48	93 (100.0); 108 (43.9); 95 (42.7); 81 (41.2); 55 (39.4); 71 (38.2); 121 (37.4); 80 (33.3); 107 (31.1); 123 (29.7)
13	Ageratriol	53.830	1.18	252.35	79 (100.0); 96 (71.6); 91 (56.6); 93 (56.4); 69 (48.0); 109 (46.8); 55 (46.6); 95 (44.9); 83 (44.6); 106 (44.0)
14	1,5-Epoxysalvial-4(14)-ene 4,8-epoxyazulene	54.682	0.46	220.35	81 (100.0); 93 (97.0); 96 (96.7); 123 (90.8); 69 (75.2); 95 (67.0); 107 (66.2); 55 (58.0); 67 (56.3); 79 (51.7)
15	4,8,11,11-tetramethylbicyclo[7.2.0]undec-3-en-5-ol	55.107	7.84	222.37	111 (100.0); 123 (33.7); 55 (33.1); 81 (28.8); 95 (23.3); 121 (17.8); 161 (17.8); 69 (16.4); 109 (13.5); 93 (12.1)
16	Spathulenol	55.249	2.28	220.35	119 (100.0); 91 (96.6); 93 (78.2); 205 (74.8); 105 (66.2); 107 (58.4); 133 (57.6); 79 (56.5); 159 (52.9); 131 (47.0)
17	Caryophyllene oxide	55.502	19.18	220.35	79 (100.0); 93 (93.3); 91 (75.6); 95 (66.7); 69 (62.1); 55 (54.1); 109 (49.8); 107 (47.8); 81 (45.5); 67 (44.8)
18	1H-Cyclobut[e]inden-5-ol,decahydro-2,2,4a,7a-tetramethyl-, [2aR-(2aα,4aβ,5α,7aα,7bα)]-(9CI)	55.870	2.15	222.37	111 (100.0); 123 (50.4); 81 (40.6); 55 (36.3); 69 (26.2); 108 (23.9); 95 (23.8); 93 (22.3); 97 (19.7); 151 (17.1)
19	(-)-Isopyrethrin-I	56.069	1.53	328.45	161 (100.0); 123 (66.9); 81 (38.3); 187 (33.2); 119 (27.1); 105 (26.7); 93 (26.1); 205 (24.5); 91 (23.8); 107 (18.12)
20	β-Cubebene	56.350	0.60	204.35	107 (100.0); 163 (63.2); 59 (53.7); 81 (50.0); 93 (39.8); 79 (30.6); 91 (30.3); 67 (28.4); 55 (25.7); 164 (24.6)
21	1,2-Cyclohexanediol, 4-methyl-1-(1-methylethenyl)-, [1S-(1α,2α,4β)]- (9CI)	56.617	0.46	170.25	108 (100.0); 95 (81.2); 93 (76.4); 81 (75.0); 123 (70.7); 84 (58.2); 137 (47.8); 109 (39.6); 97 (37.6); 67 (35.7)
22	(-)-Globulol	56.920	2.03	222.37	93 (100.0); 177 (87.1); 107 (76.8); 81 (74.7); 121 (73.8); 55 (71.7); 91 (69.9); 95 (68.8); 79 (67.1); 133 (62.4)
23	(-)-Humulene epoxide II	57.058	1.26	220.35	109 (100.0); 96 (93.4); 67 (80.8); 138 (76.1); 93 (55.7); 95 (47.7); 55 (47.1); 81 (45.0); 123 (37.4); 82 (36.7)
24	12-Oxatricyclo [6.3.1.02,5]dodecane, 1,4,4,8-tetramethyl-, (1α,2β,5α,8α)- (9CI)	57.281	0.57	222.37	95 (100.0); 108 (89.5); 83 (66.6); 55 (58.8); 123 (57.9); 81(53.1); 93 (49.1); 109 (45.3); 121 (41.4); 69 (35.2)
25	Widdrol	57.674	4.70	234.34	81 (100.0); 93 (92.6); 109 (77.7); 95 (59.1); 133 (58.0); 151 (57.0); 55 (49.9); 108 (49.3); 79 (46.4); 67 (45.5)
26	Caryophylladienol II	58.410	0.54	220.35	136 (100.0); 91 (48.5); 69 (43.2); 67 (40.5); 79 (37.4); 93 (33.8); 105 (29.3); 107 (26.5); 117 (24.4); 82 (24.2)
27	Methenolone	58.710	1.94	302.45	166 (100.0); 136 (91.1); 123 (70.3); 81 (50.0); 95 (48.9); 91 (48.1); 93 (47.9); 79 (46.7); 55 (44.5); 69 (43.5)
28	β-Eudesmol	59.551	0.54	222.37	59 (100.0); 149 (39.4); 108 (26.5); 122 (19.6); 164 (18.0); 81 (15.6); 82 (15.2); 105 (15.1); 79 (14.8); 93 (14.2)

Table 3. *Cont.*

No.	Compound	Retention Time (min)	Percentage (%)	Molecular Ion (*m*/*z*)	Main Fragment Ions (*m*/*z*)
29	(E)-α-cadinol	59.715	2.08	222.37	95 (100.0); 121 (87.1); 93 (47.8); 81 (46.4); 109 (44.3); 105 (41.1); 79 (38.5); 161 (32.9); 55 (31.4); 204 (31.2)
30	11,11-Dimethyl-8-methylenebicyclo[7.2.0]undec-4-ene-4-methanol	60.527	1.71	220.35	91 (100.0); 93 (96.3); 79 (78.8); 92 (68.5); 107 (67.3); 55 (66.9); 81 (63.8); 95 (63.31); 105 (63.0); 109 (59.0)
31	Bicyclo[5.3.1]undec-1-en-8-ol,7-methyl-4-(1-methylethylidene)-, [7S-(7R*,8R*)]- (9CI)	61.548	0.49	220.35	159 (100.0); 93 (70.3); 220 (62.1); 91 (59.2); 105 (57.8); 119 (49.7); 117 (43.8); 109 (41.4); 131 (40.1); 55 (36.3)
32	Erucamide	94.140	0.46	337.58	59 (100.0); 72 (57.6); 55 (51.2); 69 (22.9); 83 (19.2); 57 (17.2); 126 (14.7); 97 (14.6); 122 (14.3); 56 (14.0)

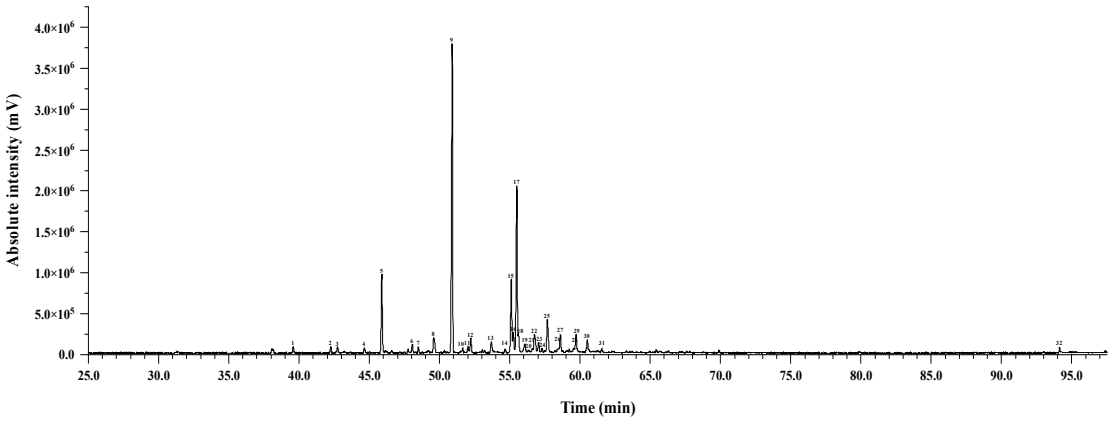

Figure 1. Total ion flow gas chromatogram (by GC-MS) of the essential oil of *Acmella radicans*.

4. Discussion

Allelopathy is considered to be an important mechanism for explaining the invasion and expansion of many invasive alien species [19,20]. Invasive alien plant species may inhibit the growth and development of neighboring plant species or even lead to the decrease and extinction of native plant species through releasing allelochemicals [5,21,22]. As a new invasive species recorded in Yunnan Province, China, *A. radicans* has caused serious damage to local plant diversity and the ecological environment [14], but its allelopathic potential is still poorly studied. This study found that *A. radicans* not only had a certain allelopathic effect on vegetable crops (*B. napus* and *B. rapa* ssp. *chinensis*) but also had a significant inhibitory effect on major weeds (*D. sanguinalis* and *L. multiflorum*).

The allelopathic potential tests of most invasive alien plant species depend on seed germination and seedling growth [21,22]. Seed germination is important to establish populations of invasive plant species as they arrive in a new habitat [21]. The lower root length, prolonged germination time, delayed seedling emergence, reduced root hairs, and senescence or death caused by the allelopathy of invasive alien plants will seriously affect the competitiveness of native plants for both above-ground and underground resources [21,23]. Allelopathic inhibition of germination and root length usually causes a reduction in both water and fertilizer absorption ability, which reduces the effective utilization of resources and affects later growth and development, the status of the species, and eventually leads to a reduction in plant populations [21,23,24]. Our previous study indicated that aqueous extracts (0.00125-0.1 g/mL) of *A. radicans* had a strong inhibitory effect on seed germination and root length of some major weeds, such as *B. pilosa*, *A. conyzoides*, *D. sanguinalis*, and *C. virgate* [14]. Similarly, the inhibition of germination and root length by *A. radicans* in this study demonstrates the allelopathic potential for *A. radicans* to suppress vegetables and neighboring plants in farming systems by attacking them at their most vulnerable stage.

Under natural conditions, a plant may produce allelochemicals at any time during its life cycle, and the allelopathic potential of different plant parts varies greatly [24–26]. These include release as volatile materials, shoot or root leachates, root exudates, and chemicals produced by plants as they decompose in the soil [27]. Many studies have shown that the plant leaf usually has greater allelopathic effects than other parts [21,22]. Our previous study showed that the inhibition rates of aqueous extracts of the above-ground part of *A. radicans* on the seed germination and seedling growth of four major associated weeds, *B. pilosa*, *A. conyzoides*, *D. sanguinalis*, and *C. virgata*, were distinctly higher than those of the underground part [14]. The current results indicated that the EO from the aboveground parts of *A. radicans* also had strong allelopathic inhibition. Among the thirty-two components identified from the EO, the major components were 2-tridecanone (30.46%), caryophyllene oxide (19.18%), 4,8,11,11-tetramethylbicyclo[7.2.0]undec-3-en-5-ol (7.84%), β-caryophyllene (7.67%), and widdrol (4.7%). Among these compounds, (E,E)-2,4-decadienal [28], 2-tridecanone [29], γ-cadinene [29], δ-cadinene [29,30], (E)-α-cadinol [30], spathulenol [31,32], caryophyllene oxide [30,33,34], and widdrol [35] have been previously reported to have possible allelopathic effects. However, the phytotoxic potential of most of the compounds we identified from the EO of *A. radicans* is not clear and needs to be further tested.

The strength of the allelopathic potential of invasive alien plant species is usually determined by the target species, extract concentration, and the plant tissues from which the chemicals are released [24–26]. Seed germination or seedling growth of the target species is generally inhibited by high extract concentrations, whereas a low extract concentration may actually promote seed germination and seedling growth, suggesting that a stimulatory or inhibitory effect is a function of concentration [23–26]. Our previous study showed that the seed germination and seedling growth of *B. pilosa* and *A. conyzoides* were significantly inhibited at all concentrations of two aqueous extracts of *A. radicans*, but also demonstrated a 'low-promotion and high-inhibition effect' on root length, stem length, and biomass of *D. sanguinalis* and *C. virgate* at low concentrations [14]. Likewise, the current study demonstrated a 'low-promotion and high-inhibition effect' on the root length of *D. sanguinalis* and *L. multiflorum* at low concentrations for the EO of *A. radicans*. The allelopathic response index and synthetical allelopathic index are generally the most important indicators to measure the intensity of allelopathy [15,18]. Comparing the allelopathic response index and synthetical allelopathic index of the EO of *A. radicans* on four common plants, the strongest inhibition was seen in *D. sanguinalis* and *L. multiflorum*, followed by *B. rapa* ssp. *chinensis*, and the least inhibited was *B. napus*, showing that the weeds we tested were more sensitive to the allelopathy of *A. radicans*, but the crops were relatively more resistant.

Nowadays, *A. radicans* has become a serious invasive alien plant in northwest Yunnan Province, China. It is widely distributed in Baoshan City and Lincang City as a serious invasive species, primarily invading farmland, tea gardens, orchard land, roadsides, and ditches [14]. In invaded habitats, the population density and importance values of the dominant plant species *A. conyzoides*, *B. pilosa*, *Borreria latifolia*, *C. virgata*, *Cynodon dactylon*, *D. sanguinalis*, and *Setaria plicata* were significantly reduced by *A. radicans*, demonstrating that *A. radicans* has strong competitive ability. This competitive ability is partly due to its rapid growth and relatively large leaf area. Moreover, *A. radicans* also may change the soil environment by absorbing more soil nutrients than other plants, which may facilitate its growth and invasion [14]. The present study showed that the EO of *A. radicans* has strong allelopathic potential against four common plants in Yunnan Province and could inhibit these dominant plant species through releasing allelochemicals. Further work is needed to evaluate how the experimental EO concentrations relate to field conditions.

5. Conclusions

Our results indicated that the EO from the above-ground parts of *A. radicans* strongly inhibited the growth of four common plants, *B. napus*, *B. rapa* ssp. *chinensis*, *D. sanguinalis*, and *L. multiflorum*. Generally, the germination rate, germination vigor, root length, shoot

length, and biomass of four bioassay species were shown to be significantly inhibited with increasing concentrations of the EO of *A. radicans*. The inhibitory rates of the EO of *A. radicans* on seed germination and seedling growth of *D. sanguinalis* and *L. multiflorum* were generally higher than the rates for *B. rapa* ssp. *chinensis*, and the least inhibited was *B. napus*. Most allelopathic indices for the EO from *A. radicans* exhibited negative values and significantly declined with increasing concentrations, which provided clear evidence for potential allelopathic inhibition by *A. radicans*. Thirty-two components were identified from the EO of *A. radicans* and eight of these compounds were reported to have possible allelopathic effects and considered as potential allelochemicals in previous research. However, the phytotoxic potential of most compounds from the EO of *A. radicans* is not clear and needs to be further tested. Thus the allelochemicals and biochemical mechanisms of allelopathy of *A. radicans* under different conditions should be researched further.

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Data Availability Statement: All data needed to evaluate the conclusions in this paper are present in the text.

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