

## Allelopathic Influence of *Artemisia lancea* Essential Oil on Selected Broad and Narrow Weeds

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The present study analyzed the chemical composition and phytotoxic activity of *Artemisia lancea* essential oil (EO). Gas chromatography-mass spectrometry (GC-MS) results showed the presence of 55 compounds, representing 82.36% of the entire EO, with eucalyptol (18.7%) being the most abundant constituent. Phytotoxic bioassay results indicated that the EO (concentration ranging from 0.25-5 mg mL<sup>-1</sup>) inhibited the growth of *Amaranthus retroflexus* and *Setaria viridis*. Root length of *A. retroflexus* and *S. viridis* were reduced with the increasing concentration of the EO, especially when the EO was applied at 1-5 mg mL<sup>-1</sup>. When treated with 1-5 mg mL<sup>-1</sup> EO, shoot length of *A. retroflexus* and *S. viridis* were also significantly suppressed. The half maximal inhibitory concentration (IC<sub>50</sub>) values for inhibiting root and shoot growth of *A. retroflexus* by the EO were 0.284 and 0.246 mg mL<sup>-1</sup>; for *S. viridis*, the EO showed IC<sub>50</sub> values of 0.44 and 0.262 mg mL<sup>-1</sup> for root and shoot inhibition, respectively. The most promising findings were observed when *A. lancea* EO concentration reached 1 mg mL<sup>-1</sup> and higher, which completely inhibited seed germination of *A. retroflexus* and *S. viridis*. These findings suggest *A. lancea* EO has potential as an eco-friendly bioherbicide for agricultural weed control.

**Keywords:** phytotoxicity, GC-MS, eucalyptol, allelochemicals, eco-friendly bioherbicide

### Introduction

*Artemisia* is a genus consisting of approximately 500 species in the family Compositae, specifically belonging to the subtribe *Artemisiinae* of the tribe *Anthemideae*, which is one of the largest genera in the tribe.<sup>1</sup> It comprises annual, biennial, or perennial herbs, with some being semi-shrubs or small shrubs, and is known for its strong essential oil (EO) aroma.<sup>2</sup> *Artemisia* is found in almost every continent except Antarctica, with most species concentrated in the Northern hemisphere, particularly in the Eurasian continent. The diversity center of *Artemisia* is mainly located in Central Asia, including Uzbekistan,

Tajikistan, Turkmenistan, Kazakhstan, Kyrgyzstan, parts of Russia, China, and Mongolia. Other centers of diversity include Iran and the Mediterranean region as well as the western part of North America.<sup>2-4</sup> *Artemisia lancea* is a small, fragrant shrub with a height of 80-150 cm and has leaves covered in fine hairs. It is traditionally used in China as a folk remedy for its anthelmintic, antipyretic, and antifebrile properties and is mainly found in South and East Asia.<sup>5</sup> Researchers from around the world have reported on the chemical constituents of EOs in *Artemisia* species and the components of EO in *Artemisia* species exhibit variations based on several factors, including the species,<sup>6</sup> the plant part used for extraction,<sup>7</sup> the season of plant growth,<sup>8</sup> the location of collection,<sup>9</sup> technology for extracting EO,<sup>10</sup> and so on.<sup>11</sup> Research on chemical constituents of *Artemisia* species has revealed a range of

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secondary metabolites, including flavonoids, caffeoylquinic acids, terpenoids, coumarins, sterols, and acetylenic compounds. Due to the existence of EOs, *Artemisia* species exhibit strong aroma and bitterness, which deter animals and other plants from consuming them, thus conferring a selective advantage.<sup>12</sup> Certain EOs produced by *Artemisia* species possess high phytotoxicity and have been acknowledged as highly effective allelochemicals that play a significant role in facilitating the establishment of the plant's competitive advantage.<sup>13</sup>

Allelopathy derives from the combination of the Greek words “*allelon*” and “*pathos*”, which signify mutual influence and potential harm or suffering, respectively. In 1937, the Austrian professor Hans Molisch coined the term for the first time.<sup>14</sup> According to the definition adopted by the International Allelopathy Society, allelopathy refers to the emission of secondary metabolites, known as allelochemicals, into the environment by various organisms such as plants, fungi, microorganisms and viruses, to impact the surrounding plants' growth and the development of agricultural systems.<sup>15</sup> Allelochemicals are substances involved in allelopathy, also referred to as plant EOs or secondary metabolites of plants. EOs, natural products, primary and secondary metabolites, endogenous substances and surplus metabolites are among the components that make up allelochemicals.<sup>16</sup> EO include monoterpenes, sesquiterpenes, and aromatic compounds, possessing applications such as antibacterial, insecticidal, antiparasitic, and medicinal properties.<sup>17</sup> EO is widespread allelochemicals found in the plant kingdom, as they are secondary metabolites emitted by plants into the atmosphere.<sup>18</sup> EOs are complex mixtures of monoterpenes, sesquiterpenes, fatty acids, benzenoids and other compounds, which are usually obtained from plants using techniques such as hydro-distillation.<sup>19</sup> Plants have the capacity to produce and release diverse types of EOs, such as terpenoids, amino acid derivatives, benzenoids / phenylpropanoids and fatty acid derivatives.<sup>20</sup> Typically, these EOs released by plants perform a variety of ecological roles, including kin recognition, chemical communication, and attracting or repelling insects.<sup>21-25</sup> For centuries, EOs from plants have been utilized in the form of fragrances, medicines, and pleasant aromas.<sup>26</sup> The development of distillation techniques in the Middle Ages made it possible to extract EO, which has been employed in various ancient applications including food, pharmaceuticals, and cosmetics.<sup>27</sup> EO represents a crucial class of secondary metabolites in *Artemisia* species, with *Artemisia* EO demonstrating robust insecticidal efficacy against pests and high antibacterial activity against plant diseases.

According to Pandey and Singh,<sup>28</sup> the main constituents

of EOs found in the majority of *Artemisia* species are camphor, 1,8-cineole,  $\beta$ -pinene, thujone, artemisia ketone, caryophyllene, camphene and germacrene D. Reports<sup>29,30</sup> on EO from *Artemisia californica* communities indicate that these EOs have interspecific allelopathic effects on both woody and herbaceous plant species in annual grassland habitats, exerting negative impacts on the receiving plant species, and simultaneously altering the structure of soil microorganisms.<sup>31,32</sup> EO may significantly contribute to crop protection and have been proposed as environmentally friendly alternatives to synthetic pesticides.<sup>17,33</sup>

Current research on *Artemisia* EO primarily focuses on their chemical composition and insecticidal properties<sup>34</sup> as well as their antibacterial activity.<sup>35</sup> *Artemisia* species have been the subject of numerous studies demonstrating their allelopathic activity.<sup>36-40</sup> Like other *Artemisia* species, *A. lancea* emits a unique fragrance, indicating the production of EO. Studies<sup>5</sup> have reported the components of EO from *A. lancea* and indicated their insecticidal effects on insects. However, no research has been reported on the allelopathic effects of *A. lancea*. Based on this, our study aims to investigate the chemical composition, toxicological effects, and phytotoxic activity of *A. lanceae* EO and its major constituents.

## Experimental

### Experimental material

During flowering stage, in July 2023, aerial parts from *A. lancea* were collected in Baicheng City, Jilin Province, China, at the geographical coordinates of latitude 45.8614N, longitude 123.2636E. Dr Caixia Han at Xinjiang Institute of Ecology and Geography, Chinese Academy of Sciences, identified the plant and preserved a voucher specimen with the accession number XJBI02317080 in their Herbarium. Before EO extraction, the plant material was cut into small fragments and dried at room temperature for two weeks.

### Experimental method

#### EO extraction and gas chromatography-mass spectrometry (GC-MS) analysis

EO was extracted from 200 g of the dried aboveground parts of *A. lancea* plant by hydro-distillation method for 4 h and its yield was calculated using the formula provided by Han *et al.*<sup>41</sup> The EO obtained were preserved at 4 °C for subsequent chemical analysis and allelopathy tests. The components of *A. lancea* EO was analyzed by a 7890A/5975C gas chromatography-mass spectrometry (GC-MS) system (Agilent Technologies, Palo Alto, CA,

USA) equipped with a non-polar column HP-5MS 5% phenyl methyl siloxane (30 m × 0.25 mm; film thickness 0.25 mm) according to the method of Zhou *et al.*<sup>42</sup> The process was performed as follows: the carrier gas was helium, with a flow rate of 1 mL min<sup>-1</sup>. The oven temperature was held at 50 °C for 10 min then programmed from 50 to 120 °C at 1.5 °C min<sup>-1</sup>; from 120 to 240 °C at 20 °C min<sup>-1</sup> and then held at this temperature for 5 min. Mass spectra were taken at 70 eV. Mass range was *m/z* 35-600 Da. The temperature of both injector and detector were kept at 280 °C; the sample injection volume was 0.1 mL; the split ratio was 50:1. Relative amounts of individual components were calculated based on GC peak areas response factor correction. Identification of the constituents of the EO was made by comparison of their mass spectra and retention indices (RI, calculated by linear interpolation relative to retention times of a standard mixture of C<sub>7</sub>-C<sub>40</sub> *n*-alkanes) with the data stored in the NIST database (National Institute of Standards and Technology). The retention index was calculated using the following formula:

$$RI = 100[n + (N - n) \times (\log t_R(\text{unknown}) - \log t_R(n)) / (\log t_R(N) - \log t_R(n))] \quad (1)$$

where *n*: No. of carbon atoms in the smaller alkane, *N*: No. of carbon atoms of the larger alkane, *t<sub>R</sub>*: retention time of the individual compound.<sup>43</sup>

#### Phytotoxic effect of the EO

The toxicity of the EO was tested against the dicot species *Amaranthus retroflexus* and the monocot species *Setaria viridis* using the Petri dish assay method. In detail, *A. lancea* EO was dissolved in Tween 20 (0.1%, v/v, Tween 20 surfactant diluted in distilled H<sub>2</sub>O) to get the suspension at 0.25, 0.5, 1, 2 and 5 mg mL<sup>-1</sup>, using ultrasonic treatment. The water suspension of 0.1% Tween-20 was used as a parallel control. On top of a filter paper, 10 seeds of the test plants were uniformly distributed onto a 9 cm diameter Petri dish. This process was repeated 5 times for each treatment. The Petri dishes were sealed using paraffin film and placed in a dark plant incubator maintained at a temperature of 25 ± 2 °C for storage. After 5 days of cultivation, the root length and shoot height of *A. retroflexus* were recorded, while the measurements for *S. viridis* were taken after 7 days of cultivation.<sup>34</sup>

#### Phytotoxic activity of major constituent

The phytotoxic activity of the main component eucalyptol (18.7%) was tested against *A. retroflexus* and

*S. viridis*. Eucalyptol (98% purity) was purchased from Aladdin Reagent Co., Ltd. (Shanghai, China). Eucalyptol was dissolved in Tween 20 (0.1%, v/v, Tween 20 surfactant diluted in distilled H<sub>2</sub>O) to get the suspension at 0.25, 0.5, 1, 2 and 5 mg mL<sup>-1</sup>, using ultrasonic treatment. The water suspension of 0.1% Tween-20 was used as a parallel control. On top of a filter paper, 10 seeds of the test plants were uniformly distributed onto a 9 cm diameter Petri dish. This process was repeated 5 times for each treatment. The Petri dishes were sealed using paraffin film and placed in a dark plant incubator maintained at a temperature of 25 ± 2 °C for storage. After 5 days of cultivation, the root length and shoot height of *A. retroflexus* were recorded, while the measurements for *S. viridis* were taken after 7 days of cultivation.<sup>34</sup>

#### Statistical analysis

A completely randomized design was implemented in the bioassay experiments. Results were presented as mean ± standard error of the mean (SE), and statistical analysis was completed using one-way analysis of variance (ANOVA) with a significance level of *p* < 0.05. The SPSS statistical package version 26.0 (IBM Corp., Armonk, NY, USA) was used for the analysis.<sup>44</sup> To further analyze the data and compare the differences between the treatments, Fisher's Least Significant Difference (LSD) test was employed at a significance level of *p* < 0.05. This statistical method allowed for a more detailed examination of the results. The 50% required inhibitory concentration was determined using probability analysis.

## Results

#### EO composition

The yield of EO from *A. lancea* was 1.4% (volume *per* dry weight), with 55 detected components, accounting for 82.36% of the total EO (Table 1). The primary constituent, eucalyptol, made up 18.7% of the total EO. Overall, the EO contained 48.79% monoterpenes (comprising 15.30% monoterpene hydrocarbons and 33.49% oxygenated monoterpenes) and 27.22% sesquiterpenes (comprising 17.39% sesquiterpene hydrocarbons and 9.83% oxygenated sesquiterpenes), with 6.35% attributed to other chemicals (Table 1).

#### Allelopathic activity of EO

*A. lancea* EO was tested for the allelopathic potential against *A. retroflexus*, a dicot plant, and *S. viridis*, a

**Table 1.** The chemical composition of the essential oil of *Artemisia lancea* aerial parts

Peak	$t_r$ / min	Compound	CAS	RI <sup>a</sup>	RI <sup>b</sup>	Area / %
1	4.317	1-pentanol, 3-methyl-	000589-35-5	835	843	0.27
2	6.863	(-)- $\alpha$ -pinene	007785-26-4	926	934	2.05
3	7.405	camphene	000079-92-5	940	951	0.49
4	8.411	sabinene	003387-41-5	966	973	3.79
5	9.125	$\beta$ -myrcene	000123-35-3	984	992	5.83
6	9.641	$\alpha$ -phellandrene	000099-83-2	997	1007	0.42
7	10.183	$\alpha$ -terpinene	000099-86-5	1009	1017	0.35
8	10.837	eucalyptol	000470-82-6	1024	1038	18.70
9	11.138	( <i>E</i> )- $\beta$ -ocimene	003779-61-1	1030	1039	0.14
10	11.646	$\beta$ -( <i>Z</i> )-ocimene	003338-55-4	1041	1039	0.22
11	12.076	$\gamma$ -terpinene	000099-85-4	1051	1056	0.79
12	12.437	( <i>Z</i> )-sabinene hydroxide	017699-16-0	1058	1075	1.31
13	13.418	terpinolene	000586-62-9	1080	1097	0.21
14	14.02	linalool	000078-70-6	1093	1104	2.00
15	14.923	( <i>Z</i> )- <i>para</i> -menth-2-en-1-ol	029803-82-5	1112	1123	0.20
16	15.37	allo-ocimene	007216-56-0	1121	1131	0.59
17	15.981	(+)-2-bornanone	000464-49-3	1134	1144	2.43
18	17.022	borneol	000507-70-0	1156	1166	3.60
19	17.555	(-)-4-terpineol	020126-76-5	1168	1175	1.40
20	18.235	$\alpha$ -terpineol	000098-55-5	1182	1190	3.45
21	19.955	( <i>Z</i> )-geraniol	000106-25-2	1219	1229	0.22
22	20.17	( <i>Z</i> )-3-hexenyl- $\alpha$ -methylbutyrate	053398-85-9	1224	1233	0.14
23	20.368	( <i>Z</i> )-3-hexenyl isovalerate	035154-45-1	1228	1238	0.14
24	24.747	$\gamma$ -pyronene	000514-95-4	1325	1338	0.41
25	25.684	eugenol	000097-53-0	1347	1359	0.18
26	26.398	$\alpha$ -copaene	003856-25-5	1363	1376	0.17
27	26.768	(-)- $\beta$ -bourbonene	005208-59-3	1372	1384	0.23
28	27.439	( <i>Z</i> )-jasmone	000488-10-8	1387	1396	1.28
29	28.23	$\beta$ -ylangene	20479-06-5	1406	1439	1.99
30	28.635	$\beta$ -copaene	018252-44-3	1416	1433	0.73
31	29.263	isogermacrene D	317819-80-0	1431	1439	0.23
32	29.624	$\gamma$ -muurolene	030021-74-0	1440	1449	0.42
33	29.951	( <i>Z</i> )- $\beta$ -farnesene	028973-97-9	1448	1442	5.73
34	30.579	2-isopropyl-4 $\alpha$ ,8-dimethyl-1,2,3,4,4 $\alpha$ ,5,6,7-octahydronaphthalene	103827-22-1	1463	1491	0.42
35	30.785	germacrene D	023986-74-5	1468	1480	0.83
36	30.983	$\beta$ -selinene	017066-67-0	1473	1489	0.48
37	31.422	2-isopropyl-5-methyl-9-methylene-bicyclo-1-decene (4.4.0)	150320-52-8	1483	1503	0.92
38	31.989	$\alpha$ -farnesene	000502-61-4	1497	1507	1.62
39	32.144	3,6-dihydrochamazulene	018454-88-1	1501	1518.7	1.61
40	32.54	$\beta$ -sesquiphellandrene	020307-83-9	1511	1525	0.58
41	33.142	1,1,5,6-tetramethyl-1,2-dihydronaphthalene	220766-68-7	1527	1511	0.32
42	34.14	nerolidol	007212-44-4	1552	1565	0.65
43	34.682	neryl 2-methylbutanoate	051117-19-2	1566	1581	5.34
44	35.671	geranyl 2-methylbutyrate	068705-63-5	1592	1596	0.45
45	35.852	neryl $\beta$ -methyl butyrate	003915-83-1	1596	1576	0.18
46	36.127	$\alpha$ -calacorene	021391-99-1	1604	1584	3.05
47	37.882	$\alpha$ -costal	004586-01-0	1651	1695	0.31
48	38.363	naphthalene, 1-methyl-7-(1-methylethyl)	000490-65-3	1664	1715	0.62
49	38.613	$\alpha$ -bisabolol	000515-69-5	1671	1680	2.54
50	40.153	chamazulene	000529-05-5	1713	1735	1.00

**Table 1.** The chemical composition of the essential oil of *Artemisia lancea* aerial parts (cont.)

Peak	t <sub>R</sub> / min	Compound	CAS	RI <sup>a</sup>	RI <sup>b</sup>	Area / %
51	40.798	γ-costol	065018-14-6	1732	1752	0.37
52	41.71	dehydrochamazulene	321732-25-6	1758	1785	0.33
53	42.123	methyl isocostate	132342-55-3	1769	1792	0.36
54	47.998	dibutyl phthalate	000084-74-2	1946	1964	0.11
55	52.669	phytol	000150-86-7	2096	2122	0.04
		monoterpene hydrocarbons				15.30
		oxygenated monoterpenes				33.49
		sesquiterpene hydrocarbons				17.39
		oxygenated sesquiterpenes				9.83
		others				6.35
		total identified				82.36
		oil yield / (% , volume <i>per</i> dry weight)				1.4

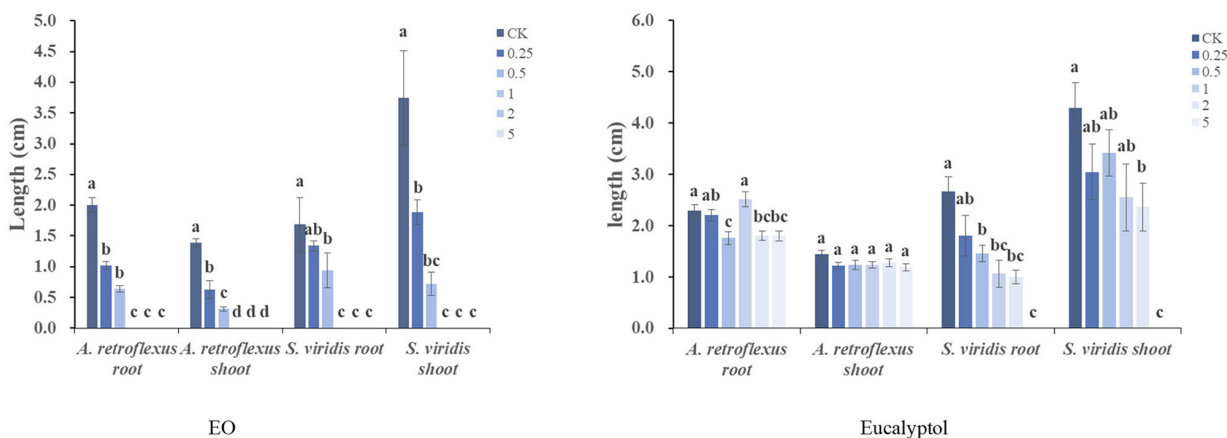
The measured parameters for determining the composition of EO included retention time (t<sub>R</sub>), retention indices calculated by linear interpolation relative to a standard mixture of *n*-alkanes (C8-C40) using a HP-5MS column (RI<sup>a</sup>), retention index obtained from literature (RI<sup>b</sup>), and constituent percentage (area).

monocot plant. The EO released by *A. lancea* considerably slowed down seedling growth at the lowest treatment concentration of 0.25 mg mL<sup>-1</sup>, causing a 48.88% reduction in root elongation for *A. retroflexus* and a 20.34% reduction for *S. viridis*. Increasing the EO concentration to 0.5 mg mL<sup>-1</sup> resulted in a suppression of radical elongation by 68.20% for *A. retroflexus* and 44.24% for *S. viridis*. Moreover, treatments containing 1, 2, and 5 mg mL<sup>-1</sup> completely inhibited the elongation of roots in *S. viridis* and *A. retroflexus*. The impact of EO on seedling height was particularly noticeable, with the lowest concentration of 0.25 mg mL<sup>-1</sup> showing the EO suppressed seedling height by 55.04% for *A. retroflexus* and 49.62% for *S. viridis*. Similarly, at 0.5 mg mL<sup>-1</sup>, radical elongation was suppressed by 77.52% for *A. retroflexus* and 80.76% for *S. viridis*. Higher concentrations of 1, 2, and 5 mg mL<sup>-1</sup> resulted in a complete inhibition of height elongation for both species. Notably, *A. retroflexus* exhibited greater sensitivity to the EO compared to *S. viridis*, as indicated

the half maximal inhibitory concentration (IC<sub>50</sub>) values of 0.284 and 0.246 mg mL<sup>-1</sup> for *A. retroflexus* root and shoot, respectively, as well as 0.44 and 0.262 mg mL<sup>-1</sup> for *S. viridis* root and shoot, respectively (Figure 1). It indicated that *A. lancea* had the capability to generate EO that could be emitted into its surroundings, influencing the growth of neighboring plants.

#### Phytotoxic activity of *A. lancea* EO and its major constituent

Based on the results shown in Table 1, eucalyptol (18.7%) was identified as the major component of *A. lancea* EO. The phytotoxic activity of eucalyptol was assessed across a concentration range of 0 to 5 mg mL<sup>-1</sup>, to determine the effects of the major component. Significant inhibition of root growth in both plant species was observed when the concentration of eucalyptol reached 0.25 mg mL<sup>-1</sup>, reducing root elongation by 3.75% for *A. retroflexus* and 32.33% for *S. viridis*. As the



**Figure 1.** Phytotoxic effects of *A. lancea* EO and eucalyptol on root and shoot growth of *A. retroflexus* and *S. viridis*. Different letters (a, b, c, d) represent a significant difference at  $p < 0.05$  level according to Fisher's LSD test.



concentration increased to 5 mg mL<sup>-1</sup>, eucalyptol continued to significantly reduce root elongation, with reductions of 20.36% for *A. retroflexus* and 100% for *S. viridis*. It is worth noting that at 0.25 mg mL<sup>-1</sup>, eucalyptol consistently inhibited shoot development of *S. viridis*, while it did not affect shoot development in *A. retroflexus*. Even at the highest concentration tested (5 mg mL<sup>-1</sup>), the growth of *A. retroflexus* was not completely inhibited, whereas both shoot and root growth of *S. viridis* were completely suppressed. The results showed that *A. retroflexus* was less sensitive to the EO than *S. viridis*, as indicated by their respective IC<sub>50</sub> values. The IC<sub>50</sub> values for *A. retroflexus* root and shoot were 10.5 and 3.78 mg mL<sup>-1</sup>, respectively, which were higher than those of *S. viridis* root and shoot (0.64 and 1.148 mg mL<sup>-1</sup>, respectively) (Figure 1).

## Discussion

In studies investigating the allelopathic effects of *Artemisia* EO, to identify chemical constituents, GC-MS was employed by most researchers.<sup>45</sup> Some researchers utilized GC-MS to analyze the chemical composition of EOs from ten *Artemisia* species on the Qinghai-Tibet Plateau. They further employed principal component analysis (PCA) for multivariate statistical analysis of the obtained data. GC-MS analysis demonstrated the presence of 65 compounds, which collectively constituted 83.82% of the total relative content of the EO. Notably, the major components identified were 1,8-cineole (16.53%), camphor (15.20%), and dehydrocostus lactone (13.59%).<sup>46</sup>

Our GC-MS results showed the presence of 55 compounds, representing 82.36% of the entire EO, with eucalyptol (18.7%) being the most abundant constituent. This study showed that the yield of EO from *A. lancea* was 1.4% (volume *per dry weight*) was higher than the yield of EO from *A. lancea* conducted by Zhu *et al.*,<sup>5</sup> which was 0.63%. This fluctuation in the chemical composition of *A. lancea* EO in both studies may be due to both external and internal factors such as geographical location, the method used for EO extraction and the plants' growth conditions, etc.

*Artemisia*, the largest genus within the *Asteraceae* family, possesses diverse medicinal applications.<sup>28</sup> *Artemisia* plants are abundant in bioactive compounds, making them valuable sources for various secondary metabolites with diverse biological activities. These activities are attributed to the presence of different active ingredients and are mediated through various modes of action.<sup>47</sup> There are currently various methods available for extracting EOs from *Artemisia* plants, like hydro-distillation,<sup>34</sup> headspace extraction and solid-phase microextraction (SPME). For instances, some researchers extracted EO from *A. vulgaris*

using both hydro-distillation and headspace extraction methods. They then investigated the impact of the climate of the plant growth region on the composition of the EO from *A. vulgaris*. The findings of the study unveiled a total of 96 EO, which accounted for 91-97% of the overall composition. Specifically, when using hydro-distillation, the EO was predominantly composed of monoterpenes (44.49%) and sesquiterpenes (29.98%). On the other hand, headspace extraction primarily detected monoterpenes (80.33%) in the EO,<sup>48</sup> conducted a seasonal study on the EO of the whole plant of *A. absinthium* from Spanish populations. Hydro-distillation and simultaneous distillation-extraction were employed to obtain extracts from *A. absinthium* plants collected from wild populations in Spain, for aerial parts and roots respectively. These extracts were then analyzed using gas chromatography mass spectrometry (GC-MS) and gas chromatography flame ionization detection (GC-FID) to investigate the seasonal variations of the compounds. The results provide evidence supporting the potential application of *A. absinthium* as a natural herbicide.<sup>49</sup> SPME method is a rapid and straightforward technique commonly employed for the characterization of EO in aromatic and medicinal plants. In comparison to steam distillation, headspace extraction offers several advantages, including faster extraction and reduced plant material requirements. This method has also been utilized in the analysis of *Artemisia* EO, owing to its advantageous features such as the elimination of organic solvents and minimal sample requirement.<sup>50</sup>

Numerous *Artemisia* species have demonstrated a significant overlap in the chemical composition of their EOs,<sup>51</sup> additionally, compounds like camphor and 1,8-cineole have been identified for their diverse roles, including the inhibitory effects on deoxyribonucleic acid (DNA) synthesis, cell proliferation, and elongation.<sup>52</sup> The EO of plants contain common chemical constituents like oxygenated monoterpenes, which confer phytotoxic effects. The phytotoxicity is also caused by multiple mechanisms: moreover, these compounds have been found to inhibit cell division, and impair mitochondrial respiration. Photosynthetic pigments and photosynthesis can be associated with various detrimental effects, including the overproduction of reactive oxygen species leading to oxidative damage, disturbance of the waxy cuticle layer, inhibition of enzyme activity, and impaired water absorption.<sup>53,54</sup> In pot experiments, the inhibitory effect of *A. fragrans* EO on the germination and growth of *Convolvulus arvensis* seeds was observed at concentrations ranging from 1 to 4%. It caused significant reductions in photosynthetic pigments and antioxidant enzyme levels, while inducing the production of hydrogen peroxide and malondialdehyde.<sup>55</sup> Extensive research has been

conducted on artemisinin, for instance: compounds isolated from *A. annua* were tested for phytotoxicity against two monocotyledonous and five dicotyledonous plants. The results indicated that the degree of inhibition on seed germination and seedling growth followed the order of artemisinin > deoxyartemisinin > arteannuin B. Deoxyartemisinin and arteannuin B, which lack an internal peroxide bridge, exhibited weaker activity, suggesting that this part of the structure is crucial for phytotoxic action.<sup>56</sup> Moreover, the addition of 0.22-0.81% artemisinin to soil inhibited the growth of maize when applied via leaves of *A. annua*.<sup>57</sup> Artemisinin demonstrates phytotoxic properties with good activity and stability in soil, indicating its potential as a commercial herbicide.<sup>58</sup> The primary constituent, eucalyptol, present in the EO of *A. lancea*, demonstrated a lesser inhibitory effect on the growth of *A. retroflexus* and *S. viridis* compared to the overall inhibitory effect of the EO derived from *A. lancea*. The present study indicated that the EO of *A. lancea* as well as its major constituent are promising candidates to be further studied as eco-friendly agrochemicals for the purpose of weed management. On the other hand, future work is needed to improve their efficacy, which includes but not limited to optimizing the extraction methods of the EO to increase its yield, optimizing the EO concentrations applied to maximize its phytotoxic activity, and adding adjunct reagents to enhance its effect. It is also necessary to consider the possible side effects that might be triggered by the EO on non-target plants and other organisms.

## Conclusions

The chemical composition of *A. lancea* EO and its phytotoxic activities were studied in this work. The monoterpenes accounting for 48.79% followed by oxygenated monoterpenes (33.49%) were discovered as the most prominent constituents of *A. lancea* EO. Meanwhile, the EO of *A. lancea* exhibited strong phytotoxic activity against broad leaf weeds, completely inhibited seed germination and seedling growth of *A. retroflexus* and *S. viridis* at a concentration of more than 1 mg mL<sup>-1</sup>, indicating that the EO of *A. lancea* is a promising candidate to be further studied as an eco-friendly herbicide against weeds.

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## Author Contributions

The experiments were conceived and designed, and the manuscript was written by Zixiang Shan. The experiments and data analysis were carried out by Shi Kai, Zhou Shixing, and Yasir Arafat. The manuscript was written by Hua Shao. Shao Hua is responsible for the revision of the manuscript for the paper.

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