Identify potential allelochemicals from *Humulus scandens* (Lour.) Merr. root extracts that induce allelopathy on *Alternanthera philoxeroides* (Mart.) Griseb.

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Although it is well-documented that invasion of invasive plants is promoted with allelopathic effects by inhibiting the growth and phenotypic performance of native plants, little is known conversely. In this study, the allelopathy effects of a native plant, *Humulus scandens* (Lour.) Merr., on a typical invasive species *Alternanthera philoxeroides* (Mart.) Griseb., was investigated by exposing *A. philoxeroides* seedlings to three chemical solvent extracts (i.e., petroleum ether extract (PE), ethyl acetate extract (EE), and n-butanol extract (NE) of *H. scandens* root (HR). The three chemical extracts inhibited the growth, stem length, node number, leaf number, leaf area, and root number, and increased malondialdehyde (MDA) content of *A. philoxeroides* seedlings, which indicated that the extracts inhibited the plant growth by damaging the membrane system of leaves. And the synthetical effect of allelopathy (SE) index indicated that EE had the greatest inhibition on the growth of *A. philoxeroides*. Fifty compounds were identified from the three extracts of HR using GC–MS analysis, among which 5 compounds (dibutyl phthalate, stigmasta-3,5-diene, 2,6-Di-tert-butylphenol campesterol, and neophytadiene) were identified from *H. scandens* root extracts for the first time. And n-hexadecanoic acid exists in all three extracts. The findings of the present study provide a novel method to potentially control the invasion of *A. philoxeroides*. However, field monitoring under natural conditions would be necessary to confirm in practice the results obtained with the bioassays.

Bioinvasion has become a serious environmental problem in the world in general and is considered as the second biggest threat to biodiversity. *Alternanthera philoxeroides* (Mart.) Griseb. (an *Amaranthaceae* family member, generally named alligator weed), is a worldwide invasive plant species of which invasion was reported in 32 different countries. It grows well in terrestrial, aquatic, hygrophytic and other habitats. *A. philoxeroides* was initially introduced into China as animal feed in the 1930s because of its fast growth, high photosynthetic rate and high nitrogen utilization rate. However, *A. philoxeroides* is currently considered as a significant threat to plant diversity because it is highly competitive to replace herbage and other plant species, and is aggressive against cotton, corn, rice, soybean and a variety of vegetables. Its asexual reproduction enables it to easily create new infection by stem fragmentation in most invaded areas. Therefore, it has become a serious issue to control this invasive species.

Three principal means are generally utilized to control the alligator weed, being physical, chemical and biological removal. Physical methods are mainly to remove invasive plants by manual and mechanical methods; chemical methods are to spray chemical herbicides such as glyphosate to cause plant death; biological methods are mainly to control plant growth by natural enemies, soil animals and soil microorganisms. These methods are usually suffering from expensive costs, lack of durability, risks of accelerating invasion and producing herbicide-resistant weeds, leading to poor efficiency of invasion control. However, allelopathy found its role in successful replacement control of invasive weeds, consequently being introduced to combat the challenges of environmental pollution and herbicide resistance development. Simultaneously, allelochemicals could be produced and degraded under natural conditions, avoiding the risks of secondary contamination during chemical control. A diverse array of allelochemicals are produced by plants, such as phenolic compounds,

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terpenoids, glycosides and alkaloids. These chemicals are released by volatilization, leaching, root exudation and decomposition. Rhizosphere biochemistry that is shaped by allelopathy may drive geographic co-evolutionary trajectories, affecting the coexistence of species and the development of plant communities, ultimately resulting in an invasion control.

So far, it is generally accepted in research that invasive plants are able to inhibit the growth of native plants mainly by allelopathic effects. For instance, fresh shoot aqueous extract of Tithonia diversifolia, an invasive species, significantly inhibited the radicle and plumule lengths of the maize (Zea mays L.) seedlings. Nevertheless, the potential allelopathic effects of native species on alligator weed are rarely investigated in China. One of the limited study showed that extracts of Phragmites australis (Cav.) Trin inhibited the growth of invasive plant A. philoxeroides, providing a potential means to control this invasive plant. Using allelopathy of native plants to control invasive plants may become a potential novel method of invasion control. Humulus scandens (Lour.) Merr. (belonging to the Moraceae family) is widely distributed in China, mainly growing at the edge of ditch, wasteland, ruins and forests. H. scandens is more competitive than A. philoxeroides in the field and laboratory.

The present study focused on two questions: (1) Does H. scandens root extract have allelopathic inhibition on the growth of A. philoxeroides? (2) What are the main secondary metabolites in H. scandens that potentially have allelopathic effects on A. philoxeroides? By answering these important questions, this study aimed to develop a potential method to control the invasion of A. philoxeroides by making use of the allelopathy effects of native plants if they exist.

Results

Influence of Humulus scandens root (HR) extracts on morphology index of A. philoxeroides seedlings. Different chemical extracts of HR posed a significant influence on the growth of A. philoxeroides (Fig. 1). Stem length, node number and leaf number decreased initially and then increased along with an increase in the extractant polarity. There were significant differences in the morphology index of A. philoxeroides between treatments and control, namely stem length (F = 13.16, P < 0.001), node number (F = 23.34, P < 0.001), leaf number (F = 43.396, P < 0.001), leaf area (F = 144.7, P < 0.001) and root number (F = 20.128, P < 0.001). The solvent extractions of petroleum ether extract (PE) and ethyl acetate extract (EE) had significant inhibitory effects on the total biomass (P < 0.001) and aboveground biomass (P = 0.001) of A. philoxeroides as well, reducing the biomass by 16–68% compared with control (Table 1). The extracts EE and NE significantly reduced the belowground biomass (P < 0.001, P = 0.029) by 72% and 29%, respectively, while PE extract significantly increased it (P = 0.001) by 37%. The PE and EE extracts had significant inhibitory effect on the leaf area ratio (LAR), reducing it by 53% and 50% respectively. The PE extract significantly enhanced the root/shoot ratio of A. philoxeroides (P = 0.004).
being revealed by the fact that three chemical extracts of H. scandens inhibited the seedling growth of A. philoxeroides. The present study identified an inhibitory allelopathy effect of a native plant species on invasive plants to native plants, while the opposite study may become a new way to control invasive weeds. The treatments with the same letter are not significantly different at 0.05 level.

**Table 1.** Effects of three chemical extracts on the biomass, LAR and Root/shoot ratio of A. philoxeroides (Mean ± SD). The treatments with the same letter are not significantly different at the 0.05 level.

| Extract          | Aboveground biomass | Belowground biomass | Total biomass | Leaf area ratio (LAR) | Root/shoot ratio |
|------------------|---------------------|---------------------|--------------|-----------------------|------------------|
| Control          | 21.25 ± 5.73a       | 3.65 ± 0.66b        | 24.9 ± 5.51a | 4.67 ± 0.96a          | 0.18 ± 0.06b     |
| Petroleum ether  | 15.8 ± 2.34b        | 5 ± 0.42a           | 20.8 ± 2.01ab| 2.26 ± 0.19b          | 0.33 ± 0.07a     |
| Ethyl acetate    | 7.04 ± 0.93c        | 1.02 ± 0.29d        | 8.06 ± 1.01d | 2.37 ± 1.03b          | 0.15 ± 0.05b     |
| N-Butanol        | 14.83 ± 2.03b       | 2.58 ± 0.69c        | 17.46 ± 2.63c| 4.34 ± 0.86a          | 0.17 ± 0.03b     |

**Figure 2.** The effect of the three chemical extracts on the leaf enzymes, (A) POD activity and (B) CAT activity and (C) MDA content, of A. philoxeroides (from left to right, the bars are control, PE, EE and NE, respectively). The treatments with the same letter are not significantly difference at 0.05 level.

**Impacts of HR extracts on peroxidases activity of A. philoxeroides leaves.** The three chemical extracts had significant effects on the leaf POD activity ($F = 5.81$, $P = 0.007$) and MDA content ($F = 12.75$, $P < 0.001$) of A. philoxeroides (Fig. 2A,C), while they did not induce any significant differences in CAT activity ($F = 2.35$, $P = 0.111$) (Fig. 2B). The PE and NE extracts significantly stimulated the POD activity ($P = 0.025$ and $P = 0.012$) by 28% and 32%, respectively. The MDA content of A. philoxeroides leaves with PE ($P = 0.001$), EE ($P < 0.001$) and NE ($P = 0.006$) extracts significantly increased by 44–85% compared with control.

**Identification of potential allelochemicals.** A total of 50 compounds were identified from the three extracts (Table 2), mainly being phenols, terpenes, alkaloids. Among them, 30 compounds were identified from petroleum ether extracts, which were 16 phenols and their derivatives, 7 terpenes, 7 alkaloids. The highest relative content was dibutyl phthalate (14.77%), followed by n-Hexadecanoic acid (13.99%), 9,12,15-Octadecatrienoic acid, (Z,Z,Z)- (12.33%), 9,12-Octadecadienoic acid (Z,Z)- (11.79%). Twenty-six compounds were identified from ethyl acetate extract, including 13 phenols and their derivatives, 5 terpenes, 3 alkaloids. Among these 26 chemicals, the highest relative content was presented by methyl palmitate (11.8%), followed by stigmasta-3,5-diene (8.75%), (Z,Z)-Octadeca-9, 12-dienoic acid (7.62%), n-Hexadecanoic acid (7.38%), 9,12-Octadecadienoic acid (Z,Z)-methyl ester (6.13%). Twelve compounds were identified from n-butanol extract, being 5 phenols and their derivatives, 4 terpenes, and 2 alkaloids. Among these 12 chemicals stigmasterane-3,6-dione,(5à) (6.73%), n-Hexadecanoic acid (6.29%), and á-sitosterol (5.63%) were with the highest relative content.

In this study, 5 compounds were identified from H. scandens root extracts for the first time, which were dibutyl phthalate, stigmasta-3,5-diene, 2,6-Di-tert-butylphenol campesterol, and neophytadiene. And n-Hexadecanoic acid exists in all three extracts.

**Evaluation of allelopathic effects.** The three chemical extracts inhibited the stem length, node number, leaf number, leaf area, above- and total biomass of A. philoxeroides ($RI < 0$, Table 3). The allelopathic inhibition of EE extract on stem, leaf and biomass of A. philoxeroides was significantly greater than that of PE and NE extracts, while EE extract promoted root length ($RI > 0$). The synthetical effect of allelopathy (SE) index indicated that EE had the greatest inhibition on the growth of A. philoxeroides ($RI = -0.539$), followed by PE ($RI = -0.209$) and NE ($RI = -0.197$).

**Discussion** Allelopathy is ubiquitously existing among plant species, generally being tested with the effects on the plant seedling growth 34, 35. Common native species, for instance pueraria lobata and paederia scandens, depressed growth of Ipomoea cairica 36, 37. Allelochemicals inhibited protein synthesis 38 and cell division and elongation 39, consequently affecting plant growth and development. However, the study of allelopathy mainly focuses on the allelopathy of invasive plants to native plants, while the opposite study may become a new way to control invasive weeds. The present study identified an inhibitory allelopathy effect of a native plant species H. scandens on A. philoxeroides, being revealed by the fact that three chemical extracts of HR inhibited the seedling growth of A. philoxeroides ($SE < 0$, Table 3). Inhibition of shoot growth of A. philoxeroides was previously identified as well with extracts, residues and allelochemicals from different plants and fungi 40. Biomass is one of the main important factors controlling the spread of A. philoxeroides 41, 42. The regrowth capacity of alligator weed was weakened by removing and
| Compound Name | Petroleum ether extract (PE) Content % | Ethyl acetate extract (EE) Content % | N-Butanol extract (NE) Content % |
|---------------|--------------------------------------|-------------------------------------|---------------------------------|
| 1 n-Hexadecanoic acid | 13.99 | 7.38 | 6.29 |
| 2 Tetradecanoic acid | 0.81 | 0.55 | – |
| 3 Neophytiadiene | 0.52 | 0.89 | – |
| 4 Hexadecanoic acid, methyl ester | 2.95 | 11.8 | – |
| 5 9,12-Octadecadienoic acid (Z,Z)-, methyl ester | 1.66 | 6.13 | – |
| 6 9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)- | 1.21 | 4.99 | – |
| 7 9,12-Octadecadienoic acid (Z,Z)- | 11.79 | 7.62 | – |
| 8 9,12,15-Octadecatrienoic acid, (Z,Z,Z)- | 12.33 | 4.37 | – |
| 9 (E)-13-Docosenoic acid | 0.41 | 0.62 | – |
| 10 Eicosanoic acid | 1.38 | 1.48 | – |
| 11 Stigmasterol | 0.66 | 2.27 | – |
| 12 Stigmastera-5,22-dien-3-ol, acetate, (3α)- | 2.7 | 1.78 | – |
| 13 Stigmastera-3,5-diene | 2.29 | 8.75 | – |
| 14 1-Heptatriacotanol | 0.4 | – | 2.06 |
| 15 Stigmastane-3,6-dione, (5α)- | 0.73 | – | 6.73 |
| 16 α-Sitosterol | 0.46 | – | 5.63 |
| 17 Betulinaldehyde | – | 0.68 | 1.7 |
| 18 1,3-Dioxolane, 4,5-dimethyl-2-pentadecyl- | – | – | 0.73 |
| 19 Ethanol, 2-[4-(1,1-dimethylthyl) phenoxyl] | – | 0.48 | – |
| 20 Benzoic acid, 2-hydroxy-, butyl ester | – | – | 0.55 |
| 21 1,2,3,4-Tetrahydroquinolin-6-ol-3-carboxylic acid | 0.55 | – | – |
| 22 2,6-Di-t-cresyl phenol | – | 0.69 | – |
| 23 2-Pentadecanone, 6,10,14-trimethyl- | 0.56 | – | – |
| 24 trans-13-Octadecenoic acid | 0.55 | – | – |
| 25 17-Octadecynoic acid | – | 0.72 | – |
| 26 Pentadecanoic acid | 0.96 | – | – |
| 27 1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester | 1.56 | – | – |
| 28 Ethanol, 2-(9-octadecenyl)- (Z)- | – | 0.87 | – |
| 29 9-Hexadecenoic acid, methyl ester, (Z)- | – | 0.43 | – |
| 30 Pentadecanoic acid, 13-methyl-, methyl ester | – | 1.22 | – |
| 31 1,2-Benzenedicarboxylic acid, butyl octyl ester | – | 0.52 | – |
| 32 Methyl stearate | – | 1.26 | – |
| 33 Octadecanoic acid | – | 2.02 | – |
| 34 Octadecanoic acid | 2.67 | – | – |
| 35 Octadecanoic acid, ethyl ester | 0.69 | – | – |
| 36 Bis(2-ethylhexyl) phthalate | 2.24 | – | – |
| 37 Glycolid stearate | 0.7 | – | – |
| 38 Hexadecanoic acid, ethyl ester | 0.83 | – | – |
| 39 Hexadecanoic acid, butyl octyl ester | 1.58 | – | – |
| 40 Cholest-5-en-3-one | 0.92 | 2.59 | – |
| 41 Lupid-20(29)-en-3-one | 2.59 | – | – |
| 42 4,22-Stigmastadiene-3-one | 0.64 | – | – |
| 43 Eicosanoic acid, methyl ester | – | 0.87 | – |
| 44 Docosanoic acid, methyl ester | 1.18 | – | – |
| 45 Trilinolein | 0.8 | – | – |
| 46 Campesterol | 1.43 | – | – |
| 47 Betulin | – | 0.52 | – |
| 48 Urosdesoxycholic acid | – | 1.34 | – |
| 49 Hexadecanoic acid, butyl ester | – | 1.5 | – |
| 50 Oleic acid, eicosyl ester | – | 2.21 | – |

Table 2. Compounds in petroleum ether extracts that were identified by GC/MS.
Table 3. The allelopathic effects of the extracts of *H. scandens* on *A. philoxeroides*.

| Sample          | Stem length (cm) | Node number | Root length (cm) | Root number | Leaf number | Leaf area (cm²) | Aboveground biomass (g) | Belowground biomass (g) | Total biomass (g) | SE (g) |
|-----------------|------------------|-------------|------------------|-------------|-------------|-----------------|--------------------------|------------------------|-------------------|--------|
| Petroleum ether | 0.17 ± 0.20      | 0.26        | 0.70 ± 0.34      | 0.58 ± 0.26 | 0.27 ± 0.16  | 0.29 ± 0.30      | 0.197 ± 0.209            | 0.67 ± 0.34            | 0.72 ± 0.68       | 0.539  |
| Ethyl acetate   | 0.41 ± 0.40      | 0.00        | 0.60 ± 0.55      | 0.83 ± 0.67 | 0.72 ± 0.68  | 0.30 ± 0.29      | 0.30 ± 0.30              | 0.39 ± 0.30            | 0.39 ± 0.39       | 0.39   |
| N-Butanol       | 0.09 ± 0.13      | 0.13        | 0.31 ± 0.14      | 0.34 ± 0.30 | 0.29 ± 0.30  | 0.30 ± 0.30      | 0.197 ± 0.209            | 0.67 ± 0.34            | 0.72 ± 0.68       | 0.539  |

destroying its above- and below-ground biomass. An inhibited aboveground biomass, belowground biomass, total biomass and the leaf area of *A. philoxeroides* were found in our study with exposure to ethyl acetate extract of HR (RI = − 0.67, − 0.72, − 0.68, − 0.83, respectively). Allocation indicates the investment of plants in resource utilization. Therefore, the reduced biomass of *A. philoxeroides* would likely reduce the ability of this invasive plant to absorb nutrients and capture light energy.

Under optimal conditions, the balance between reactive oxygen species (ROS) formation and consumption is tightly controlled by antioxidant enzymes and redox metabolites. However, allelochemicals were able to induce cell membrane permeability (for example, of saccharomyces, sugar beet, maize and so on) and oxidative stress. In the present study, an increased POD activity in the leaves of *A. philoxeroides* with the treatment of PE and NE extracts (Fig. 2A) indicated an accelerated H₂O₂ stress that was potentially induced by the extracts of HR. Phenolic compounds caused oxidative damage in peanut seedlings and increased the contents of catalase (CAT) and peroxidase (POD) in leaves compared with the control, which is mutually confirmed by this study. MDA is one of the lipid peroxidation products of biofilm system. The higher the content of MDA in the plant, the more obvious the degree of injury. An increased MDA content could damage the membrane system of leaves and consequently inhibit the growth of seedlings. In this study, compared with Ck, MDA content in leaves of *A. philoxeroides* treated with three extracts (PE, EE and NE) increased significantly (Fig. 2C), and EE treatment reached the highest. In conclusion, *H. scandens* may release allelochemicals, which may have negative effects on the ROS of *A. philoxeroides* leaves, thus inhibiting its normal growth.

There are many studies on the chemical constituents of *H. scandens*, more in the field of traditional Chinese medicine. Compounds β-sitosterol, carotene, daucosterol, stigmast-3,6-diene, n-hexadecanoic, linoleic acid and stigmasteral were isolated and identified from the whole plant of *H. scandens*. Compounds were obtained from the ethyl acetate fraction of methanol extract stems of *H. scandens* and identified as cis-N-p-coumaroilyltyramine, N-cis-feruloyleptamine, trans-N-p-coumaroilyltyramine, Vomifolioside. In this study, 5 compounds were isolated from *H. scandens* root extracts for the first time, which were dibutyl phthalate, stig masta-3,5-diene, 2,6-Di-tert-butylenol campesterol, and neophytadiene. And n-hexadecanoic acid exists in all three extracts. The compound “stig masta-3,5-diene” is used in medical research and has biological activity against certain inflammatory diseases. Some scholars utilized GC–MS to separate from Solidago Canadensis the compounds 2,6-Di-tert-butylenol which showed certain allelopathy to the growth of *Microcystis aeruginosa*. Compound dibutyl phthalate has certain allelotoxicity to tobacco seedlings and the growth of Microcystis aeruginosa. The results showed that *H. scandens* root extracts significantly inhibited the growth of alligator weeds, mainly being indicated by physiological, biochemical and morphological indices. At the same time, 50 compounds were identified for the first time from *H. scandens* root extracts. However, there are still some limitation in this study. For instance, in the laboratory, allergies are not disturbed; but in the natural environment, it is affected by climate, temperature, soil animals, soil microorganisms and other factors. Therefore, the potential of allelopathy in the prevention and control of alligator weed should be elaborated in future research based on the actual environment.

Materials and methods

Experimental materials. Plants of *A. philoxeroides* and *H. scandens* were collected from the campus of Anhui Agriculture University in China (N31°52’E, 117°16’). Fresh *H. scandens* roots were dried to constant weight under shade, and ground to fine powder passing through 40 mesh sieve, then put in a desiccator. The same components were mixed and concentrated, resulting in three concentrated extraction, being petroleum ether extract (PE), ethyl acetate extract (EE) and n-butanol extract (NE), respectively. The concentrated extractions were then stored in seal at 4 °C in dark place.

Preparation and isolation of the HR extracts. HR extracts were prepared according to Alara and Abdurahman extraction methods with slight modification. Ten gram (total two kilogram) of HR was tightly wrapped with one layer of filter paper and soxhlet extraction with 95% ethanol for 3 h. The extraction was then concentrated into paste using a rotary evaporator (Rotavapor RE-52A coupled with SHE-III circulating water vacuum pump, Shanghai), then it was dissolved in distilled water and extracted three times with petroleum ether, n-butanol and ethyl acetate. The same components were mixed and concentrated, resulting in three concentrated extraction, being petroleum ether extract (PE), ethyl acetate extract (EE) and n-butanol extract (NE), respectively. The concentrated extractions were then stored in seal at 4 °C in dark place.

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**Exposure of A. philoxeroides to three organic extracts.**  *A. philoxeroides* and the fractions of the concentrated extracts (PE, EE and NE, with a concentration of 2 mg mL−1) dissolved in distilled water were added into petri dishes (9 cm diameter) covered with two layers of filter paper. Dishes without any HR extracts were taken as the control (distilled water). Five plant tissues of *A. philoxeroides* were randomly placed in each dish. Each plant tissue contained one node that was 3 cm long and 0.4 cm in stem thick. After 15 days incubation in an incubator (temperature 28°C, light intensity 400 μmol·cm−2·s−2; 16/8 h light/dark), the plant tissues were collected for experimental analysis.

To verify the effects of HR extracts on the seedling development of *A. philoxeroides*, the morphology indices were determined as follows. Stem length and root length were measured directly with a ruler. The number of nodes, leaves and roots were counted. Plant biomass (aboveground and belowground) was measured following dried in oven at 65°C for 48 h till constant weight24. Root/shoot ratio, leaf area ratio and leaf area were calculated as below24,66:

\[
\text{Root/shoot ratio} = \frac{\text{Underground/Boveground biomass}}{\text{Aboveground biomass}};
\]

\[
\text{Leaf area} =\pi \times (\text{Leaf length}) \times (\text{Leaf width})/4;
\]

\[
\text{Leaf area ratio} (LAR) = \frac{\text{Leaf area}}{\text{Total biomass}};
\]

Fresh leaves were separately collected to measure the related enzymes peroxidase (POD), catalase (CAT) and malondialdehyde (MDA) content with kits (Lai Er Bio-Tech). Use POD kit, CAT kit to measure leaf enzyme activity and MDA kit to measure leaf MDA content. Collect fresh clonal plant leaves, rinse 3 times with pure water, wipe dry, weigh 0.2 g and cut into a 2 mL centrifuge tube, add 1.8 mL phosphate buffer, crush with a high-activity and MDA kit to measure leaf MDA content. Collect fresh clonal plant leaves, rinse 3 times with pure water, wipe dry, weigh 0.2 g and cut into a 2 mL centrifuge tube, add 1.8 mL phosphate buffer, crush with a high-activity and MDA kit to measure leaf MDA content.

**Evaluation of allelopathic effects.**  The allelopathic effect of the extract was determined as following61:

\[
RI = \begin{cases} 
1 - \frac{C}{T} & (T \geq C) \\
\frac{T}{C} - 1 & (T < C)
\end{cases}
\]

where T represents growth response of test species treated with extracts and C represents growth response of the test species treated with distilled water (control). A positive RI value indicates that the extract promotes the seedling growth, whereas a negative RI value indicates that the extract inhibits the seedling growth.

Synthetical effect of allelopathy index (SE) was applied to evaluate the allelopathic effect by the average of several RI values and determined as following62:

\[
SE = \left( \frac{RI_{\text{stemlength}} + RI_{\text{leafnumber}} + RI_{\text{leafarea}} + RI_{\text{rootnumber}} + RI_{\text{rootlength}} + RI_{\text{abovegroundbiomass}} + RI_{\text{belowgroundbiomass}} + RI_{\text{totalbiomass}}}{9} \right)
\]

**Identification of potential allelochemicals from the EE, PE and NE extractions.**  The extracted samples were dissolved in n-hexane (chromatographically pure) and analyzed by GC–MS (TRACE ISQ, Thermo Scientific). The injector temperature was 280°C. The initial column temperature was constant at 60°C for 5 min, increased to 100°C at a rate of 3.5°C/min for 5 min, then ramped to 200°C at 8°C/min for 5 min. The temperature was then brought to 280°C at a rate of 15°C/min and held until the end of the 15-min run. Helium was the carrier gas and the program was not divided.

Agilent data analysis software and NIST11 library were used to determine the retention time of chromatography, and peak area was used to calculate the content of the substance62. According to the 80% principle, compounds with library matching coefficient greater than or equal to 80% are used for analysis63.

**Data analysis.**  The experiment followed a completely randomized design, composed of three extracts (petroleum ether, ethyl acetate, and n-butanol) of *A. philoxeroides*. Data were tested for normality and homogeneity of variance. ANOVA was conducted on morphology and physiological indices with exudate treatments for *A. philoxeroides*. Differences between means were assessed with LSDs and Duncan’s test (*P* < 0.05), using the SPSS v.21.0 for Windows. Graphs are performed by Sigmaplot 12.0.

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**References**

1. Pomella, A. W. V., Barreto, R. W. & Charudattan, R. Nimbya alternantherae a potential biocontrol agent for alligatorweed, *Alternanthera philoxeroides*. *Biocontrol* 52, 271–288 (2007).
2. Barreto, R. W. & Torres, A. N. L. Nimbya alternantherae and Cercospora alternantherae: two new records of fungal pathogens on *Alternanthera philoxeroides* (alligatorweed) in Brazil, Australas. *Plant Pathol* 28, 103–107 (1999).
3. Ridenour, W. M. & Callaway, R. M. The relative importance of allelopathy in interference: the effects of an invasive weed on a native bunchgrass. *Oecologia* 126, 444–450 (2001).
4. Tanveer, A., Ali, H. H. & Manalil, S. Eco-biology and management of alligator weed [Alternanthera philoxeroides (Mart.) Griseb.] a review. *Wetlands* **38**, 1067–1079 (2018).

5. Garbari, F. & Pedullà, M. L. *Alternanthera philoxeroides* (Mart) Griseb (Amaranthaceae), a new species for the exotic flora of Italy. *I. Plant Taxon Geogr* **56**, 139–143 (2018).

6. Chen, X., Wang, R. & Cao, Q. The relationship between the distribution of invasive plant *Alternanthera philoxeroides* and soil properties is scale-dependent. *Pol. J. Environ. Stud.* **24**, 1931–1938 (2015).

7. Wang, T., Su, J. & Miao, L. The invasive stoloniferous clonal dwarf *Phragmites australis* *L* and its resistance to allelochemical stress. *J. Chem. Ecol.* **35**, 605–606 (2009).

8. Wang, B., Li, W. & Wang, J. Genetic diversity of *Alternanthera philoxeroides* in China. *Aquat. Bot* **81**, 277–283 (2005).

9. Shen, J., Shen, M. & Wang, X. Effect of environmental factors on shoot emergence and vegetative growth of alligatorweed (*Alternanthera philoxeroides*). *Weed Sci.* **53**, 471–478 (2005).

10. Basset, L., Paynert, Q. & Hankin, B. Characterising alligator weed (*Alternanthera philoxeroides*, *Amaranthaceae*) invasion at a northern New Zealand lake. *N. Z. J. Ecol.* **36**, 216–222 (2012).

11. Pan, X. Y. *Invasive Alternanthera philoxeroides*: biology, ecology and management. *Acta Phytotaxonomica Sinica* **45**, 884–900 (2007).

12. Phung, T., Xuan, T. & Tu, A. T. Weed suppressing potential and isolation of potent plant growth inhibitors from Castanea crenata Sieb. et Zucc.*. Molecules **23**, 345 (2018).

13. Yu, Z. & Bi, H. Status Quo of research on ecosystem services value in China and suggestions to future research. *Energy Procedia* **5**, 1044–1048 (2011).

14. Yang, S., Wang, Q. & Hu, T. Physiological responses to allelopathy of decomposing *Cinnamomum septentrionale* leaf litter of three crops (corn, cucumber, and cowpea). *Chin. J. App. Environ. Biol.* **29**, 292–298 (2018).

15. Dong, B. C., Fu, T. & Luo, F. L. Herbivory-induced maternal effects on growth and defense traits in the clonal species *Alternanthera philoxeroides*. *Sci. Total Environ.* **605–606**, 114–123 (2017).

16. Dugdale, T. M., Clements, D. & Hunt, T. D. Alligatorweed produces viable stem fragments in response to herbicide treatment. *J. Aquat. Plant Manag.* **48**, 84–91 (2010).

17. Clements, D., Dugdale, T. M. & Butler, K. L. Management of aquatic alligator weed in an early stage of invasion. *Manag. Biol. Invas. 5*, 327–339 (2014).

18. Clements, D., Dugdale, T. M. & Butler, K. L. Herbicide efficacy for aquatic *Alternanthera philoxeroides* management in an early stage of invasion: integrating above-ground biomass, below-ground biomass and viable stem fragmentation. *Weed Res.* **57**, 257–266 (2017).

19. Bond, W. & Grundy, A. Non-chemical weed management in organic farming systems. *Weed Res.* **41**, 383–405 (2001).

20. SchooLer, S., Cook, T. & Bourne, A. Selective herbicides reduce alligator weed (*Alternanthera philoxeroides*) biomass by enhancing competition. *Weed Sci.* **56**, 259–264 (2008).

21. Sainty, G., Mccorkelle, G. & Julien, M. Control and spread of alligator weed (*Alternanthera philoxeroides*) (Mart.) Griseb., in Australia: Lessons for other regions. *Wetlands Ecol. Manage.* **5**, 195–201 (1997).

22. Annett, R., Habibi, H. R. & Hontela, A. Impact of glyphosate and glyphosate-based herbicides on the freshwater environment. *J. Appl. Toxicol.* **34**, 458–479 (2014).

23. Bai, H., Vepachedu, R. & Gilroy, S. Allelopathy and exotic plant invasion: from molecules and genes to species interactions. *Science* **301**, 1377–1380 (2003).

24. Zhang, Z., Deng, L. L. & Wang, L. C. Allelopathic potential of *Phragmites australis* extracts on the growth of invasive plant *Alternanthera philoxeroides*. *Allelopathy* **45**, 54–63 (2018).

25. Jabran, K., Mahajan, G. & Sardana, V. Allelopathy for weed control in agricultural systems. *Crop Prot.* **72**, 57–65 (2015).

26. Kumbhar, B. A. & Patel, D. D. Allelopathic effects of different weed species on crop. *J. Pharm. Sci. Biosci. Res.* **5**, 292–298 (2018).

27. Weston, L. A. & Duke, S. O. Weed and crop allelopathy. *Crit. Rev. Plant Sci.* **23**, 770–779 (2019).

28. Rice, E. L., Allelopathy, 2nd Ed, A. Press, Editor. 1-50 (1984).

29. Da Silva, I. F. & Vieira, E. A. Phytotoxic potential of *Baccharis pilularis* L. and its resistance to allelochemical stress. *J. Chem. Ecol.* **31**, 659–666 (2005).

30. Kumbhar, B. A. & Patel, D. D. Allelopathic effects of different weed species on crop. *Crit. Rev. Plant Sci.* **23**, 345 (2018).

31. Otusanya, O. O., Ilori, O. I. & Adelusi, A. A. Allelopathic effects of tithonia diversifolia (Hemsl) L. and its resistance to allelopathic stress. *J. Chem. Ecol.* **31**, 534–547 (2019).

32. Huang, Y. M., Zhang, Y. & Liu, Q. Research on allelopathy of aqueous extract from *Tagetes patula* to four garden plants. *J. Chem. Ecol.* **25**, 124–130 (1999).

33. Noctor, G., Reichheld, J.-P. & Foyer, C. H. ROS-related redox regulation and signaling in plants. *Semin. Cell Dev. Biol.* **25**, 2289–2303 (2014).

34. Kaur, N., Chugh, V. & Gupta, A. K. Essential fatty acids as functional components of foods-a review. *J. Food Sci. Technol.* **51**, 2289–2303 (2014).

35. Sun, C. H., Li, Y. & He, H. Y. Physiological and biochemical responses of *Chenopodium album* to drought stresses. *Acta Ecologica Sinica* **25**, 2556–2561 (2005).

36. Li, P., Wang, X. & Li, W. The contents of phenolic acids in continuous cropping peanut and their allelopathy. *Acta Ecol. Sin.* **30**, 2128–2134 (2010).

37. Wang, Y. X., Sun, G. R. & Wang, J. B. Relationships among MDA content, plasma membrane permeability and the chlorophyll fluorescence parameters of *Puccinellia tenuiflora* seedlings under NaCl stress. *Acta Ecol. Sin.* **26**, 122–129 (2006).

38. Li, Q. & Li, J. T. & Bing, J. The role analysis of APX gene family in the growth and developmental processes and in response to abiotic stresses in *Arabidopsis thaliana*. *Hereditas (Beijing)* **41**, 534–547 (2019).

39. Tang, K., Ming, L. & Shan, D. Allelopathy autotoxicity effects of aquatic extracts from rhizospheric soil on rooting and growth of stem cuttings in *Pogostemon cablin*. *J. Chin. Med. Mater.* **37**, 935–939 (2014).

40. Coelho, E. M. P., Barbosa, M. C. & Mito, M. S. The activity of the antioxidant defense system of the weed species *Senna obtusifolia* L. and its resistance to allelochemical stress. *J. Chem. Ecol.* **43**, 725–738 (2017).

41. Li, J. & Wang, X. Advance of research on *Humulus scandens*. *Qilu Pharm. Affairs* **26**, 353–355 (2007).
48. Xu, B., Jin, Y. & Yihan, W. Chemical constituents from stems and leaves of *Humulus scandens*. Chin. Tradit. Herb. Drugs **45**, 1228–1231 (2014).
49. Zhang, J., Liu, J. & Dai, L.-F. Unlocking the potential antioxidant and anti-inflammatory activities of *Rhododendron molle*. G. Don. *Pak. J. Pharm. Sci.* **32**, 2375–2383 (2019).
50. Chen, Z., Guo, Q. & Huang, K. Analysis of volatile components of solidago canadensis by SPME/GC-MS. *Acta Agriculturae Jiangxi (Chinese)* **26**, 1 (2008).
51. Wang, Y., Yu, L. & Zhang, Y. Effects of two allelochemicals on growth and physiological characteristics of eggplant seedlings. *J. Gansu Agric. Univ. (Chinese)* **3**, 47–50 (2007).
52. Yu, J., Zhang, Y. & Niu, C. Effects of two kinds of allelochemicals on photosynthesis and chlorophyll fluorescence parameters of *Solanum melongena* L. seedlings. *J. Appl. Ecol.* **17**, 1629–1632 (2006).
53. Lande, M. L., Kanedi, M. & Zulkifli, Z. Suppressive effects of lantana camara leaf extracts on the growth of red chilli (*Capsicum annuum*). *World J. Pharm. Life Sci.* **3**, 543–551 (2017).
54. Erdia, G. & Saidi, N. Allelopathic screening of several weed species as potential bioherbicides. *IOP Conf. Ser. Earth Environ. Sci.* **334**, 12–34 (2019).
55. Cimmino, A., Masi, M. & Rubiales, D. Allelopathy for parasitc plant management. *Nat. Prod. Commun.* **13**, 289–294 (2018).
56. Deng, J., Zhang, Y. & Hu, J. Autotoxicity of phthalate esters in tobacco root exudates: Effects on seed germination and seedling growth. *Pedosphere* **27**, 1073–1082 (2017).
57. Gu, S., Zheng, H. & Xu, Q. Comparative toxicity of the plasticizer dibutyl phthalate to two freshwater algae. *Aquat. Toxicol.* **191**, 122–130 (2017).
58. Perveen, S., Yousan, M. & Zahoor, A. F. Extraction, isolation, and identification of various environment friendly components from cocks comb (*Celosia argentea*) leaves for allelopathic potential. *Toxicol. Environ. Chem. Rev.* **96**, 1523–1534 (2014).
59. Alara, O. R., Abdurahman, N. H. & Ukaegbu, C. I. Extraction and characterization of bioactive compounds in *Vernonia amygdalina* leaf ethanolic extract comparing soxhlet and microwave-assisted extraction techniques. *J. Taibah Univ. Sci.* **13**, 414–422 (2019).
60. Wei, W., Hou, Y. & Peng, S. Effects of light intensity on growth and biomass allocation of invasive plants *Mikania micrantha* and *Chromolaena odorata*. *Acta Ecol. Sin.* **37**, 6021–6028 (2017).
61. Williamson, G. B. & Richardson, D. Bioassays for allelopathy: measuring treatment responses with independent controls. *J. Chem. Ecol.* **14**, 181–187 (1988).
62. Gao, Y. B., Li, G. P. & Shi, H. Allelopathic effect of endophyte-infected *Achnatherum sibiricum* on *Stipa grandis*. *Acta Ecol. Sin.* **37**, 1063–1073 (2017).
63. Zhang, L., Wang, X. & Guo, J. Metabolic profiling of Chinese tobacco leaf of different geographical origins by GC-MS. *Agric. Food Chem.* **61**, 2597–2605 (2013).

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**Author contributions**

L.W. and Z.Z. participated in the design of the study, collected experimental data, carried out lab and statistical analyses. Z.Z. conceived, designed and coordinated the study, participated in lab and guided the allelopathy work. Y.L. and X.H. participated in the laboratory work and the collection of experimental data and X.Z. contributed to the writing of the paper. All authors reviewed and approved the final version of this manuscript.

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**Competing interests**

The authors declare no competing interests.

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