Analysis of Allelochemical Components in Aqueous Extracts of South American Pennywort (Hydrocotyle verticillata) and Its Rhizosphere Soil Postprint

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Abstract

Using seed germination tests and substance identification via gas chromatography-mass spectrometry (GC-MS) and liquid chromatography-mass spectrometry (LC-MS), this study analyzed the effects of Hydrocotyle verticillata extracts prepared with different solvents on seed germination, as well as the composition of extracts from H. verticillata plants and rhizosphere soil, to investigate the influence of H. verticillata on the seed germination of other plants and to screen for the primary chemical substances affecting them. The results showed that: (1) Hydrocotyle verticillata extracts prepared with different solvents all exhibited varying degrees of inhibitory effects on seed germination; (2) Under GC-MS analysis, a total of 35 substances were identified in the aqueous extract of H. verticillata plants, with dibutyl phthalate (15.2%), 10,15-octadecanedioic acid (8.58%), and 2,4-di-tert-butylphenol (6.81%) being the major components; in the aqueous extract of rhizosphere soil, 17 components were identified, with oleamide (26.47%), n-heptacosane (9.63%), and ethyl palmitate (4.83%) as the main substances; (3) Under LC-MS analysis, a total of 109 compounds were identified in the aqueous extract of H. verticillata plants; in ESI+ mode, L-phenylalanine (3,483.99 $ng \cdot mg^{1}$) and luteolin (2,306.64 $ng \cdot mg^{1}$) showed the highest contents, while in ESI- mode, D-quinic acid (21,827.71 $ng \cdot mg^{1}$) and chlorogenic acid (12,589.25 $ng \cdot mg^{1}$) exhibited the highest contents; in the aqueous extract of rhizosphere soil, 93 components were identified; in ESI+ mode, butyric acid (7,660.53 $ng \cdot mg^{1}$) and palmitamide (3,200.36 $ng \cdot mg^{1}$) showed the highest contents, while in ESI- mode, n-octacosanoic acid (18,605.35 $ng \cdot mg^{1}$) and sucrose (12,183.23 $ng \cdot mg^{1}$) exhibited the highest contents; (4) The potential allelochemicals of H. verticillata were primarily fatty acids, amides, esters, and aromatic acids, while the substances that may

directly exert allelopathic effects could be butyric acid, n-octacosanoic acid, glycolic acid, oleamide, palmitamide, ethyl palmitate, and benzoic acid; among these, the input of fatty acid substances may originate from H. verticillata, soil microorganisms, and soil fauna, whereas amides, esters, and aromatic substances are more likely derived from H. verticillata plants.

Full Text

Preamble

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Title: Analysis of Allelochemicals in Water Extracts of Plant and Rhizospheric Soil of *Hydrocotyle vulgaris*

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Abstract

This study employed seed germination experiments, gas chromatography-mass spectrometry (GC-MS), and liquid chromatography-mass spectrometry (LC-MS) to investigate the effects of different solvent extracts of *Hydrocotyle vulgaris* on seed germination and to identify the main chemical constituents in plant and rhizosphere soil extracts. The results showed: (1) All solvent extracts of *H. vulgaris* exhibited inhibitory effects on seed germination to varying degrees; (2) GC-MS analysis identified 35 compounds in the plant water extract, with dibutyl phthalate (15.2%), 10,15-octadecanedioic acid (8.58%), and 2,4-di-tert-butylphenol (6.81%) as the main components. In the rhizosphere soil water extract, 17 compounds were identified, with oleic acid amide (26.47%), n-heptacosane (9.63%), and ethyl palmitate (4.83%) as the main components; (3) LC-MS analysis identified 109 compounds in the plant water extract. In ESI+

mode, L-phenylalanine (3,483.99 ng · mg ^{1}) and luteolin (2,306.64 ng · mg ^{1}) had the highest contents, while in ESI- mode, D-quinic acid (21,827.71 ng · mg ^{1}) and chlorogenic acid (12,589.25 ng · mg ^{1}) had the highest contents. In the rhizosphere soil water extract, 93 compounds were identified. In ESI+ mode, butyric acid (7,660.53 ng · mg ^{1}) and palmitamide (3,200.36 ng · mg ^{1}) had the highest contents, while in ESI- mode, n-octacosanoic acid (18,605.35 ng · mg ^{1}) and sucrose (12,183.23 ng · mg ^{1}) had the highest contents; (4) The potential allelochemicals of *H. vulgaris* were mainly fatty acids, amides, esters, and aromatic acids. The compounds likely exerting direct allelopathic effects may be butyric acid, n-octacosanoic acid, glycolic acid, oleic acid amide, palmitamide, ethyl palmitate, and benzoic acid. Fatty acids may originate from *H. vulgaris*, soil microorganisms, and soil animals, while amides, esters, and aromatic compounds are more likely derived from the *H. vulgaris* plant itself.

Keywords: gas chromatography-mass spectrometry, liquid chromatography-mass spectrometry, allelochemicals, *Hydrocotyle vulgaris*

Introduction

Allelopathy refers to the beneficial or detrimental effects of metabolic secretions from plants or microorganisms on other plants or microorganisms in the environment (Rice, 1984). Plants release chemical substances into soil or air through leaching, volatilization, decomposition of litter and residues, and root exudation. These chemicals accumulate to critical concentrations in soil through physical adsorption and retention, transport, and transformation by soil organisms, ultimately promoting or inhibiting the normal growth of accompanying species (Kobayashi, 2004; Wahren et al., 1998; Li et al., 2009; Gong and Zhang, 2015). Plant allelochemicals are almost exclusively secondary metabolites, generally characterized by small molecular weight and simple structure, and can be broadly classified into 14 categories: water-soluble organic acids, straight-chain alcohols, aliphatic aldehydes and ketones; simple unsaturated lactones; long-chain fatty acids and polyacetylenes; quinones; benzoic acid and its derivatives; cinnamic acid and its derivatives; coumarins; flavonoids; tannins; terpenoids; amino acids and peptides; alkaloids and cyanohydrins; sulfides and glucosinolates; and purines and nucleosides (Callaway & Aschehoug, 2000).

Plant allelopathy is an important mechanism explaining alien species invasion. Identifying allelochemicals that affect the growth of other plants plays a crucial role in understanding invasion mechanisms and developing control strategies for invasive species. The main allelochemicals of *Ageratina adenophora* include chlorogenic acid, flavanones, and eupatoriochromene derivatives (Yang, 2006; Liao et al., 2015; Guo, 2016; Li et al., 2017). These substances can induce physiological and biochemical changes in upland rice (*Oryza sativa*) roots, including alterations in malondialdehyde and catalase activity, and disruption of hormone levels such as abscisic acid and indoleacetic acid, as well as anatomical changes in root apical meristems and cortical parenchyma cells (Yang et al., 2006; Yang et al., 2008). The main allelochemicals of *Dysphania ambrosioides* include -

terpinene and p-cymene (Zhou et al., 2016), which induce caspase-dependent apoptosis in guard cells of *Vicia faba* by regulating ROS and NO-mediated changes in Ca^2 levels (Zhou et al., 2016, 2017). Analytical methods for allelochemicals include GC-MS and LC-MS. GC-MS has a long history in allelopathy studies of invasive species and is commonly used for analyzing plant volatiles and extract constituents (Ji et al., 2012; Gao et al., 2017). However, GC-MS is more suitable for identifying volatile compounds, while LC-MS is better for polar and non-volatile substances (Li et al., 2010; Guo et al., 2011). Using either method alone often fails to completely identify plant allelochemicals, suggesting that combined GC-MS and LC-MS analysis may provide more comprehensive identification and screening.

Hydrocotyle vulgaris, a member of the Apiaceae family, is native to Europe, North America, and Central America. After its introduction to China, it has been commonly used in wetland landscaping (Miu et al., 2011). Due to its biological characteristics, it has been assessed as having high invasion risk in tropical and subtropical humid regions of China (Miu et al., 2011; Liu et al., 2014; Dong et al., 2015; Yang et al., 2013). This study combined GC-MS and LC-MS to analyze the chemical constituents of *H. vulgaris* and its rhizosphere soil, aiming to screen for potentially allelopathic substances secreted into the soil and provide fundamental data for further studies on its chemical ecology.

1.1 Experimental Materials

Plant materials were collected from the Waigang River in Nanjing. Whole *H. vulgaris* plants with uniform growth, good health, and no pests or diseases were selected, washed clean, and air-dried in a room. The plants were then ground into powder using a Mixer Mill MM 400 (Retsch, Germany). Soil materials were collected from the rhizosphere of *H. vulgaris* using the shaking method (Liu, 2011). Soil samples were air-dried indoors, ground into powder, and sieved for later use. Test seeds included three common species: cucumber (*Cucumis sativus*), radish (*Raphanus sativus*), and pakchoi (*Brassica chinensis*), purchased from Nanjing Xingguang Seed Company.

1.2 Preparation of Different *H. vulgaris* Extracts

Five grams of *H. vulgaris* powder were weighed and mixed with extraction solvents (distilled water, methanol, ethyl acetate, n-hexane) at a ratio of 1 g plant powder to 40 mL solvent. The mixtures were shaken well and left to stand for 48 h, then filtered twice through qualitative filter paper. The filtrates (methanol, ethyl acetate, and n-hexane extracts) were concentrated using a rotary evaporator (Hei-VAP Value, Heidolph, Germany) under reduced pressure to obtain extract pastes. The pastes were placed in an 80 °C water bath for 2 h to remove residual solvents, then scraped into 200 mL distilled water and stirred thoroughly to obtain *H. vulgaris* extract suspensions.

1.3 Effects of Different *H. vulgaris* Extracts on Seed Germination

Cucumber, radish, and pakchoi seeds were used as test subjects (30 cucumber seeds, 50 radish seeds, and 50 pakchoi seeds per treatment). Seeds were disinfected with 2% potassium permanganate solution for 15 min, then rinsed three times with distilled water. Clean seeds were evenly placed in 90 mm diameter petri dishes lined with two layers of filter paper. Five milliliters of different solvent extract suspensions were added (distilled water for the control group), with three replicates per treatment. Dishes were placed in an illumination incubator (GXZ-338A, Ningbo Jiangnan Instrument Factory) under a 13 h light: 11 h dark photoperiod at 27 °C. Seed germination was defined as radicle emergence through the seed coat. Germination counts were recorded every 24 h, with an additional 5 mL of extract suspension added each time, for a total monitoring period of 10 days.

1.4 Preparation of Water Extracts from *H. vulgaris* and Rhizosphere Soil

Five grams each of *H. vulgaris* powder and rhizosphere soil powder were extracted with distilled water at a ratio of 1 g powder to 40 mL water. The mixtures were shaken on a rotary shaker and filtered twice for later use.

1.5 GC-MS Analysis Method

Water extracts of *H. vulgaris* and rhizosphere soil were passed through 0.22 m organic membranes. Additionally, water extracts were freeze-dried and extracted with ethyl acetate, then passed through 0.22 m organic membranes for analysis. GC-MS analysis was performed using an Agilent 6890N-G5795B gas chromatograph-mass spectrometer (Agilent Technologies, USA) with an HP-5MS capillary column (30 m × 0.25 mm × 0.25 m). The temperature program started at 40 °C (held for 2 min), increased at 8 °C · min ^{1} to 200 °C (held for 1 min), then increased at 15 °C · min ^{1} to 280 °C (held for 20 min). The carrier gas was high-purity He (99.999%) at a flow rate of 1.0 mL · min ^{1} and column head pressure of 7.62 psi. Samples were injected in splitless mode with a 1 min solvent delay. The ion source temperature was 230 °C, quadrupole temperature 180 °C, and injector temperature 250 °C.

1.6 LC-MS Analysis Method

Water extracts of *H. vulgaris* and rhizosphere soil were freeze-dried. Fifty milligrams of freeze-dried sample were mixed with 800 L methanol and 10 L internal standard (2.9 mg · mol ^{1} dichlorophenylalanine), then ground in a tissue grinder at 65 Hz for 45 s, vortexed for 30 s, and centrifuged at 12,000 r · min ^{1} for 15 min at 4 °C. Two hundred microliters of supernatant were collected for LC-MS analysis.

LC-MS analysis was performed using a Thermo Ultimate 3000 LC system coupled with an Orbitrap Elite mass spectrometer (Thermo Fisher Scientific, USA)

with a Hypersil GOLD C18 column (100 × 2.1 mm, 1.9 m). Chromatographic separation was conducted at 40 °C with a flow rate of 0.3 mL · min ^{1}. Mobile phases consisted of A: water + 0.1% formic acid and B: acetonitrile + 0.1% formic acid. The injection volume was 4 L and autosampler temperature was 4 °C.

1.7 Data Analysis

The germination rate, relative germination rate, and allelopathic effect index were calculated using the following formulas (Zeng, 1999; Gui et al., 2011):

(1) Rg = (Mg/M) × 100%, where Rg is the germination rate, Mg is the number of germinated seeds, and M is the total number of test seeds.

(2) Rrg = (Rt/Rc) × 100%, where Rrg is the relative germination rate, Rt is the germination rate of the treatment group, and Rc is the germination rate of the control group.

(3) RI = (1 - Rt/Rc) × 100 or RI = (Rt/Rc - 1) × 100, where RI is the allelopathic effect index, Rt is the germination rate of the treatment group, and Rc is the germination rate of the control group.

Data were processed and analyzed using Excel, SPSS 22.0, and Origin 9.0. One-way ANOVA was used to compare significant differences in root and shoot lengths among different extract treatments.

2.1 Effects of Different *H. vulgaris* Extracts on Seed Germination

Compared with the control group, *H. vulgaris* extracts had minimal effects on cucumber seed germination rates, with little difference observed among the four solvent extracts. In contrast, pakchoi and radish seeds showed more pronounced inhibition, with radish being the most strongly affected, followed by pakchoi [Figure 1: see original paper]. The allelopathic effect index is an important indicator of extract impact intensity. All four solvent extracts showed very weak inhibition on cucumber seeds (|RI| 0.02), but more significant inhibition on pakchoi and radish seeds (maximum |RI| of 0.81 for pakchoi and 0.75 for radish) [Figure 2: see original paper].

Note: CS = *Cucumis sativus*; RS = *Raphanus sativus*; BC = *Brassica chinensis*. Different letters indicate significant differences. The same notation applies below.

2.2 GC-MS Analysis of Water Extracts from *H. vulgaris* and Rhizosphere Soil

GC-MS analysis was performed on water extracts from *H. vulgaris* and its rhizosphere soil, with total ion chromatograms shown in [Figure 3: see original paper]

and [Figure 4: see original paper]. As shown in , 35 organic compounds were identified in the plant water extract, belonging to alkanes, alcohols, acids, phenols, ketones, carbohydrates, and glycosides. Esters were the most abundant class (35.34%), followed by acids (13.26%) and alcohols (10.14%). The main compounds were dibutyl phthalate (15.2%), 10,12-octadecadiynoic acid (8.58%), 2,4-di-tert-butylphenol (6.81%), 6-[(1E)-3-hydroxy-1-butenyl]-1,5,5-trimethyl-7-oxabicyclo[4.1.0]heptan-3-ol (6.10%), ethyl palmitate (4.52%), and (6R,7E,9R)-9-hydroxy-4,7-megastigmadien-3-one (4.78%).

In the rhizosphere soil water extract, 17 organic compounds were identified, belonging to alkanes, alkenes, phenols, esters, amides, and other classes. Amides were the most abundant (26.47%), followed by alkanes (15.75%) and esters (11.93%). Oleic acid amide was the most abundant compound (26.47%), followed by n-heptacosane (9.63%) and ethyl palmitate (4.38%). Eight compounds were shared between plant and soil extracts, belonging to alkanes, esters, and carbohydrates, but these shared components were not major constituents in either plant or soil.

2.3 LC-MS Analysis of Water Extracts from *H. vulgaris* and Rhizosphere Soil

In positive ion mode (ESI+), the plant water extract was dominated by amino acids and their derivatives (6,495.50 ng · mg ^{1}), phenolic acids (2,651.16 ng · mg ^{1}), and flavonoids (2,306.64 ng · mg ^{1}). The most abundant compounds were L-phenylalanine (3,483.99 ng · mg ^{1}), caffeic acid (2,045.91 ng · mg ^{1}), and luteolin (2,306.64 ng · mg ^{1}). In the rhizosphere soil, fatty acids were the dominant class (10,561.55 ng · mg ^{1}), followed by amines (3,200.36 ng · mg ^{1}) and vitamins (2,989.29 ng · mg ^{1}). The most abundant compounds were butyric acid (7,660.53 ng · mg ^{1}), palmitamide (3,200.36 ng · mg ^{1}), and styrene (2,704.13 ng · mg ^{1}).

In negative ion mode (ESI-), the plant water extract was dominated by dicarboxylic acids (23,455.88 ng · mg ^{1}), phenolic acids (16,959.38 ng · mg ^{1}), and carbohydrates and their derivatives (11,570.66 ng · mg ^{1}). The most abundant compounds were D-quinic acid (21,827.71 ng · mg ^{1}), malic acid (12,369.30 ng · mg ^{1}), and chlorogenic acid (12,589.25 ng · mg ^{1}). In the rhizosphere soil, fatty acids (19,801.71 ng · mg ^{1}) and carbohydrates and their derivatives (12,945.74 ng · mg ^{1}) were the main classes. The most abundant compounds were n-octacosanoic acid (18,605.35 ng · mg ^{1}), sucrose (12,183.23 ng · mg ^{1}), and vitamin A acid (8,187.75 ng · mg ^{1}).

3.1 Inhibitory Effects of Different *H. vulgaris* Extracts on Germination

The results demonstrate that 25 g · L ^{1} extracts of *H. vulgaris* in different solvents inhibited both pakchoi and radish, consistent with the findings of Yang et al. (2013). This indicates that *H. vulgaris* possesses the ability to inhibit seed germination of other plants. The weaker inhibition on cucumber seeds

compared to their results may be attributed to the lower extract concentration used in this study (25 g · L ^{1}) versus their concentration of 50 g · L ^{1}.

3.2 Comparison of Chemical Components in Water Extracts from *H. vulgaris* and Rhizosphere Soil

Allelochemicals are the primary carriers of allelopathic effects and are almost exclusively plant secondary metabolites with small molecular weights and simple structures. These compounds are typically released into soil through root exudation, rainwater leaching, and decomposition of residues, and can affect other plants through physical or chemical actions or be transformed by soil microbial activity into compounds with such effects (Sun et al., 2016; Guo et al., 2018; Bonanomi et al., 2006; Fernandez et al., 2008). Therefore, this study analyzed the chemical constituents of plant and rhizosphere soil water extracts to explore the potential allelopathic substances of *H. vulgaris*.

Since chemical constituents in soil directly affect other organisms, this analysis focused on comparing major components in soil and shared major components between plant and soil. Ester and amino acid contents were significantly reduced in soil, while fatty acids, amides, phenols, nucleic acids and bases, vitamins, aromatic acids, hydroxy acids, and carbohydrates showed marked increases. Fatty acids, amides, phenols, aromatic acids, vitamins, and hydroxy acids were present in relatively high concentrations in soil, and these compound classes have been demonstrated to possess allelopathic activity (Xie et al., 2018; Li et al., 2017; Gao et al., 2011; Dong et al., 2018). Although phenolic acid content decreased in soil, phenolic compounds are typically important allelochemicals in previous studies (Li et al., 2010), necessitating further verification to determine whether they are primary allelochemicals of *H. vulgaris*.

Detailed analysis of specific compounds revealed that soil water extract mainly contained oleic acid amide, n-heptacosane, ethyl palmitate, palmitamide, acetic acid, butyric acid, benzoic acid, glycolic acid, n-octacosanoic acid, and vitamin A acid. Oleic acid amide is an important allelochemical in reed leaves (Ye et al., 2016). Butyric acid is a major allelochemical in *Iris pseudacorus* and Chinese fir litter (Chen et al., 2013; Liu, 2006). Palmitic and linoleic acids are important allelochemicals (Shu et al., 2016), suggesting that the related compound palmitamide may be an important allelochemical in *H. vulgaris* rhizosphere soil. N-octacosanoic acid has been reported to inhibit algae in eutrophic water bodies (Zhang, 2009). Glycolic acid may be an allelochemical in the forest soil of *Rhododendron irroratum* (Li et al., 2018). Ethyl palmitate and 2,4-di-tert-butylphenol have been verified to possess allelopathic activity (Shu et al., 2016), and benzoic acid is a major component in decomposing leaf litter of *Toona ciliata* var. *pubescens* (Guo et al., 2018).

Among the main compounds in soil water extract, butyric acid, n-octacosanoic acid, and glycolic acid belong to fatty acids; oleic acid amide and palmitamide are amides; ethyl palmitate is an ester; benzoic acid is an aromatic acid; and

n-heptacosane is an alkane. Comparison of major compounds between plant and soil water extracts revealed that those abundant in soil were present at low concentrations in plant extracts, suggesting transformation or other input processes in soil. Soil fatty acids originate primarily from plants, microorganisms, and soil animals (Otto & Simpson, 2005). The significantly higher fatty acid content in rhizosphere soil compared to plants indicates that microbial and soil animal inputs may represent important sources beyond plant-derived contributions. Additionally, decomposition of plant-secreted esters may provide another pathway for fatty acid input in soil. Oleic acid amide and palmitamide were major components in soil water extract, while large amounts of oleic and palmitic acids were identified in plant extracts. Although the synthesis kinetics of these amides in soil remain unclear, plant-derived oleic and palmitic acids may serve as precursors for amide formation in soil. Ethyl palmitate was present in substantial amounts in both soil and plant water extracts, suggesting that plant input is an important source of this compound in soil. Alkanes are important soil components that are relatively stable and present in low amounts in plants, indicating that n-heptacosane may not originate from *H. vulgaris*. Aromatic acids were present at low concentrations in plants, but plants contained abundant phenols and phenolic acids, suggesting that soil benzoic acid may be derived from plant input.

Therefore, comparative analysis of chemical constituents in *H. vulgaris* plant and rhizosphere soil water extracts suggests that fatty acids, amides, esters, and aromatic acids are potential allelochemicals of *H. vulgaris*. Butyric acid, n-octacosanoic acid, glycolic acid, oleic acid amide, palmitamide, ethyl palmitate, and benzoic acid may be the chemical compounds in *H. vulgaris* rhizosphere soil that directly affect other plants. Among these, fatty acids (butyric acid, n-octacosanoic acid, and glycolic acid) may originate not only from *H. vulgaris* but also from microorganisms and soil animals, with the latter potentially representing a larger proportion of inputs. Amides, esters, and aromatic compounds (oleic acid amide, palmitamide, ethyl palmitate, and benzoic acid) likely depend primarily on input from *H. vulgaris* plants.

References

BONANOMI G, SIEUREZZA MG, CAPORASO S, et al., 2006. Phytotoxicity dynamics of decaying plant materials[J]. New Phytol, 169(3): 571-578.

CALLAWAY RM, ASCHEHOUG ET, 2000. Invasive plants versus their new and old neighbors: a mechanism for exotic invasion [J]. Science, 290: 521-523.

CHEN GY, LI QS, TANG K, 2013. Allelopathic effect of organic acids from *Iris pseudacorus* L. on *microcystis aeruginosa* [J]. Environ Sci Technol, 9: 32-36+71.

DONG BC, WANG JZ, LIU RH, et al., 2013. Effects of heterogeneous competitor distribution and ramet aggregation on the growth and size structure of a clonal plant [J]. PLoS ONE, 8(7): e68557

DONG BC, WANG JZ, LIU RH, et al., 2015. Soil heterogeneity affects ramet placement of *Hydrocotyle vulgaris* [J]. Plant Ecol, 8(1): 91-100.

DONG YN, FENG B, WANG BX, et al., 2018. Allelopathy of aqueous extract of cattail on *Microcystis aeruginosa*[J]. Chin J Ecol, 37(2): 498-505.

FERNANDEZ C, VOIRIOT S, MEVY JP, et al., 2008. Regeneration failure of *Pinus halepensis*, mill: The role of autotoxicity and some abiotic environmental parameters[F]. For Ecol Environ, 255(7): 2928-2936.

GAO Y, LI GP, SHI H, et al., 2017. Allelopathic effect of endophyte-infected *Achnatherum sibiricum* on *Stipa grandis*[J]. Acta Ecol Sin, 37(4): 1063-1073.

GAO YN, LIU BY, GE FJ, et al., 2011. Isolation and identification of allelopathic fatty acids exuded from three submerged hydrocharitaceae species[J]. Acta Hydrobiol Sin, 35(1): 170-174.

GONG JT, ZHANG ZL, 2015. Research advance on influencing factors of plant allelopathy[J]. J Biol, 32(3): 73-77.

GUI FR, JIANG ZL, JIN JB, et al., 2011. Allelopathic effect of *Ageratina adenophora* on seed germination of nine herbaceous species[J]. J Biosafety, 20(4): 331-336.

GUO J, 2016. Effects of mechanical damage and biocontrol factors on the contents of allelochemicals from the rhizosphere of *Ageratina adenophora* Sprengel [J]. Yangzhou: Yangzhou University.

GUO XW, LI K, SUN YN, et al., 2011. Allelopathic effects and identification of allelochemicals in grape root exudates[J]. J Fruit Sci, 37(6): 861-868.

GUO XY, WEN T, ZHANG L, et al., 2018. Allelopathy and chemical composition of decomposing products from leaf litter of *Toona ciliate* var. *pubescens* [J]. Sci Silv Sin, 54(6):24-32.

JI XQ, HAN XT, YANG BJ, et al., 2012. Analysis on allelochemicals in the cell-free filtrates of *Amphidinium carterae*[J]. Acta Ecol Sin, 32(6): 1745-1754.

KOBAYASHI K, 2004. Factors affecting phytotoxic activity of allelochemicals in soil [J]. Weed Biol Manag, 4(1):1-7.

LI CC, QIANG CY, QUAN WX, et al., 2018. Allelopathic potential evaluation of different soil decomposition layers in wild *Rhododendron irroratum* forest[J]. Acta Ecol Sin, 38(13): 4909-4916.

LI GY, HOU XW, DENG X, et al., 2009. Effects of microorganisms on the allelopathy of three invasive weeds [J]. Ecol Environ, 18(3): 1045-1048.

LI G, BAI WB, CAO CL, et al., 2017. Effects of continuous cropping duration of sorghum on components of root exudates and contents of allelochemicals[J]. Chin J Ecol, 36(12): 3535-3544.

LI K, GUO XW, GUO YS, et al., 2010. Allelopathy of grape root aqueous extracts[J]. Chin J Appl Ecol, 21(7): 1779-1784.

LI PD, WANG XX, LI YL, et al., 2010. The contents of phenolic acids in continuous cropping peanut and their allelopathy[J]. Acta Ecol Sin, 30(8): 2128-2134.

LI YJ, CHEN S, LI CY, et al., 2008. Isolation and identification of allelochemicals from *Mikania micrantha* [J]. J S Chin Agric Univ, 29(4): 26-30.

LI X, ZHANG QD, ZHU XZ, 2017. Progress of the research on invasive plant species *Eupatorium adenophorum* over the last decade[J]. Pratac Sci, 34(2): 283-293.

LIAO F, HU YC, WU l, et al., 2015. Induction and mechanism of HeLa cell apoptosis by 9-oxo-10,11-dehydroageraphorone from *Eupatorium adenophorum*[J]. Oncol Rep, 33(4): 1823-

LIU XZ, 2006. Allelopathy of Chinese fir litter by bioassy and identification in different decomposition stage [D]. Fuzhou: Fujian Agriculture and Forestry university.

LIU CC, 2011. NO -N concentration in rhizosphere soil solution and leaching characteristics in typical crops [D]. Baoding: Hebei Agricultural University.

LIU RH, CHEN QW, DONG BC, et al., 2014. Effects of vegetative propagule pressure on the establishment of an introduced clonal plant, *Hydrocotyle vulgaris* [J]. Sci Rep, 4(1): 5507-

MIU LH, JI MC, WANG YY, et al., 2011. Study on invasion risk of *Hydrocotyle vulgaris* as an alien species in wetlands [J]. J Zhejiang Univ (Agric Life Sci), 30(3): 354-358.

OTTA A, SIMPSON MJ, 2005. Degradation and preservation of vascular plant-derived biomarkers in grassland and forest soils from western Canada[J]. Biogeochemistry, 74(3):

RICE EL, 1984. Allelopathy [M]. New York: Academica Press.

SHU J, CHENG XR, YU MK, et al., 2016. Effect of mowing on *Miscanthus floridulus* allelopathy and secondary metabolites [J]. Acta Agrect Sin, 24(1):76-83.

SUN QH, ZHANG C, LIU GB, et al., 2016. Allelopathy of extracts from the pioneer population, *Artemisia capillaries*, during succession of the herbaceous community in the hilly-gully region of loess plateau[J]. Acta Ecol Sin, 36(8): 2233-2242.

WAHREN J, FELIG P, CERASI E, et al., 1998. The role of soil community in plant population dynamics: Is allelopathy a key component? [J]. Trend Ecol Evolut, 13(10): 407.

XIE DF, ZHANG GC, XIA XX, et al., 2018. Effect of phenolic acids on the growth and photosynthesis of *Populus* × *euramericana* 'Neva' [J]. Acta Ecol Sin, 38(5): 1789-1798.

YANG GQ, 2006. Main allelochemicals isolated and identified from the leachates of *Ageratina adenophora* Sprengel (Asteraceae) and their action mechanisms on Upland Rice (*Oryza sativa*) seedlings [J]. Peking: Chinese Academy of Agricultural Science.

YANG GQ, LIU WX, WAN FH, et al., 2006. Physiological effects of allelochemicals from leachates of *Ageratina adenophora* (Spreng.) on rice seedlings[J]. Allelopathy J, 18(2): 237-

YANG GQ, LIU WX, WAN FH, et al., 2008. Influence of two allelochemicals from *Ageratina adenophora* Sprengel on ABA, IAA and ZR contents in roots of upland rice seedlings[J]. Allelopathy J, 21(2): 253-262.

YANG QQ, MIU LH, HONG CT, et al., 2013. Allelopathic effects of *Hydrocotyle vulgaris* extract on seed germination and seedling growth[J]. J Zhejiang A & F Univ, 30(3): 354-358.

YE XQ, WU M, SHAO XX, et al., 2016. Analysis on allelopathic activity and screening on potential allelopathic compound in water extracts from leaf of *Phragmites australis* [J]. J Plant Resour Environ, 25(2): 41-47.

ZENG RS, 1999. Review on bioassay methods for allelopathy research[J]. Chin J Appl Ecol, 10(1): 123-126.

ZHANG SH, 2009. Studies on two species of submerged the allelopathy between Potamogetonaceae and planktonic algae [D]. Wuhan: Institute of Hydrobiology, Chinese Academy of Sciences.

ZHAO XM, 2009. The effect of continuous cropping on the growth of American ginseng and allelochemical [D]. Peking: Peking Union Medical College.

ZHOU J, MA DW, CHEN YT, et al., 2016. Effects of volatile allelochemicals from *Chenopodium ambrosioides* on the stoma guard cells in leaf epidermis of *Vicia faba*[J]. Guihaia, 36(8):963-968.

ZHOU J, WANG YN, MA DW, et al., 2017. Volatile allechemicas from *Chenopodium ambrosiodes* L. induced guard cells apoptosis and its signal regulation in *Vicia faba* L.[J]. Acta Ecol Sin, 37(17): 5713-5721.

Note: Figure translations are in progress. See original paper for figures.