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Isolation of Phytotoxic Compounds from Tree-of-Heaven (*Ailanthus altissima* Swingle)

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The aqueous root extract of *Ailanthus altissima* showed allelopathic activity against radish (*Raphanus sativus* L. cv. "Saxa"), garden cress (*Lepidium sativum* L.), and purslane (*Portulaca oleracea* L.) seeds. A bioassay-oriented purification of active extracts, chromatographic fractions, and compounds demonstrated dose-dependent activity on germination and radicle growth of test seeds; radish seed was the most sensitive to allelochemicals. Active compounds have been isolated: ailanthone, ailanthinone, chaparrine, and ailanthinol B (quassinoid derivatives); the alkaloid 1-methoxycanthin-6-one is not active. The compound with greatest inhibitory activity is ailanthone. The data obtained suggest a possible use of tree-of-heaven root extracts or of its active constituents as natural herbicides.

KEYWORDS: ***Ailanthus altissima*; tree-of-heaven; allelopathic activity; quassinoids; ailanthone; natural herbicides**

INTRODUCTION

The ways in which plants interact with other organisms in an environment are complex. The production and/or the accumulation of secondary metabolites could have different functions: self-defense, sexual attraction, symbiosis, and development (*1*). The study of compounds produced by plants which inhibit or stimulate the germination and the development of other plants is important for understanding the mechanism(s) of the ecological interaction.

Our research group is carrying out a series of studies on the possible allelopathic properties of medicinal plants (*2*) that, being rich in active principles, are considered a primary source of potential allelochemicals. This paper deals with the identification of possible allelochemicals from *Ailanthus altissima* Swingle (synonym *Ailanthus glandulosa* Desf.) (Simaroubaceae) through a bioassay-oriented study.

A. altissima, the tree-of-heaven, is native to China and was introduced in Europe around the end of 18th century. Although they are scentless on the tree, the leaves and flowers have an unpleasant odor when crushed. Tree-of-heaven is today widely naturalized and is adapted to a wide variety of soil conditions. The plant is very difficult to control and to remove once it has established a taproot. A number of characteristics contribute to the success of tree-of-heaven: the versatility of its reproductive methods, its extremely rapid growth, the tolerance to unfavorable conditions, and the probable presence of allelochemicals (*3*).

A. altissima is used in Chinese traditional medicine as a bitter aromatic drug and in the treatment of colds and gastric diseases. Previous phytochemical studies have demonstrated the presence in the plant of quassinoids (*4*−*8*) as well as indole alkaloids (*9*−*13*). Lipids and fatty acids (*14*−*17*), phenolic derivatives (*18*−*20*), and volatile compounds from leaves (*21*) have also been characterized. Extracts of the tree-of-heaven and some isolated compounds have demonstrated medicinal properties (*22*−*30*).

The first investigations on the phytotoxic effects of *A. altissima* extracts go back to Mergen (*31*) and Voigt and Mergen (*32*), who reported that water extracts of foliage and stems of the tree-of-heaven were injurious to tree seedlings of neighboring species. Successive studies demonstrated that the plant extracts possess allelochemicals, inhibiting germination and radicle growth of different species (*33*−*36*). In 1996, Heisey proposed ailanthone as the phytotoxic compound in *A. altissima* (*37*), and in 1995, Lin et al. (*38*) isolated ailanthone as the only allelopathic compound in the plant. Recently, Heisey (*39*, *40*) showed a strong herbicidal activity of ailanthone in greenhouse experiments.

In this paper, we present the results of a bioassay-oriented study, carried out with the aim to isolate phyototoxic compounds from *A. altissima*, by evaluating the activity of different extracts, chromatographic fractions, and pure isolated compounds on germination and radicle growth of three test seeds.

MATERIALS AND METHODS

Plant Material. Plant material was collected in the summer of 2000 at the University Campus in Fisciano. After the picking, the material was separated into its different parts (roots, leaves, leaflets, leafstalks, stems, and fruits) and dried in the air. An aliquot of fresh leaves was submitted to hydrodistillation.

Extraction and Isolation. Different parts of *A. altissima* (roots, leaves, leaflets, leafstalks, stems, and fruits) were separated and then air-dried. Each part was extracted at room temperature at a concentration of 10% w/v and successively with solvents of increasing polarity

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[petroleum ether, chloroform, chloroform−methanol (9:1), methanol, and water].

The aqueous root extract, which is more active in bioassays, was fractionated in H_2O−BuOH. The BuOH extract, which showed activity in bioassays, was dissolved in methanol, and 2 g of this extract was fractionated by gel permeation chromatography on a Sephadex LH-20 column, eluting with MeOH. Sixty-one fractions of about 10 mL each were obtained and pooled in eight main fractions (I−VIII) on the basis of their TLC similarity in BuOH−AcOH−H_2O (12:3:5) and $CHCl_3$−MeOH−H_2O (70:30:3). Fractions III (157 mg), IV (289 mg), and VII (192 mg), which were active in the bioassays, were purified with RP-HPLC on a C18 μ-Bondapack column (30 cm × 7.8 mm).

From fraction III, eluted by a mixture of H_2O−MeOH (45:55), were purified ailanthone (32 mg), ailanthinone (12 mg), and chaparrine (15 mg). From fraction IV, eluted by a mixture of H_2O−MeOH (1:1), was purified ailanthinol B (9 mg). From fraction VII, containing alkaloids, was isolated the indolic alkaloid 1-methoxycanthin-6-one (27 mg).

Structural determination of the compounds was performed by accurate analyses of ^{1}H NMR, ^{13}C NMR, and ^{13}C NMR DEPT data and their comparison with literature data (*8*, *11*, *41*−*43*). NMR spectra were obtained on a Bruker DRX 600 spectrometer.

Obtainment of the Essential Oil. The fresh leaves of *A. altissima* were subjected to hydrodistillation for 3 h, following the procedure reported in *European Pharmacopoeia* (*44*). A pale yellow essential oil was obtained in 0.91% yield on dry weight basis.

Bioassay. A bioassay based on seed germination and subsequent radicle growth was used to study allelopathic effects of the extracts, chromatographic fractions, pure compounds, and essential oil of *A. altissima*.

Seeds of radish (*Raphanus sativus* L. cv. "Saxa") and garden cress (*Lepidium sativum* L. cv. "Inglese"), collected in 2000, were purchased from Imperatore Co. in Naples; seeds of purslane (*Portulaca oleracea* L.) were collected during the summer of 2000 from wild plants.

The seeds were surface-sterilized in 95% ethanol for 15 s and sown in Petri dishes (90 mm diameter) containing five layers of Whatman filter paper, impregnated with 7 mL of distilled water (control) or 7 mL of the tested extract, chromatographic fraction, pure compound, or the solution of the extracted oil. Material of low solubility in water was dissolved in a water−acetone mixture (97.5:2.5). Controls of an aqueous solution of acetone showed no significant differences in comparison with controls of water.

The germination conditions were as follow: for radish and cress seeds, 22 ± 1 °C and 12-h light photoperiod; for purslane seeds, 28 °C and 14-h light photoperiod. The light intensity was 25 μM photons m^{-2} s^{-1}. The seed germination process was observed directly in Petri dishes, at 4-h intervals, with a stereomicroscope. A seed was considered germinated when the protrusion of the radicle was evident (*45*). After 120 h (on the fifth day), the effects of radicle elongation were measured in light and in dark (the lengths were measured in millimeters). All tests were repeated six times, using Petri dishes containing 10 seeds each. Data are expressed as the mean ± SEM of both germination and radicle elongation.

RESULTS AND DISCUSSION

Different parts of *A. altissima* (roots, leaves, leaflets, leafstalks, stems and fruits) were separated and extracted with solvents and mixtures of solvents of different polarity [petroleum ether, chloroform, chloroform−methanol (9:1), methanol, and water]. The extracts were assayed on the germination and radicle growth of seeds of three different species: radish (*Raphanus sativus* L. cv. "Saxa") and garden cress (*Lepidium sativum* L. cv. "Inglese"), usually used as targets for antigerminative studies, and purslane (*Portulaca oleracea* L.), a common weed. At the same time, as the plant gives off a strong smell due to the presence at the leaf base of glands which produced an oil-resin scent, we obtained, through hydrodistillation, an essential oil from fresh leaflets to check their possible activity on the same seeds.

Tables 1, **2**, and **3** report the effects of the different extracts, at a dose of 1 mg/mL, on the germination and radicle growth of radish, cress, and purslane, respectively. The data clearly show that extracts of roots, and particularly the more polar extracts, inhibit germination of all three tested species. The leaf extracts also exert an inhibition, whereas extracts of leaflets, leafstalks, and stems have no inhibitory activity (data not

Table 1. Effects of Extracts (1 mg/mL) of *A. altissima* on Germination of Radish, 120 h after Sowing[a]

	germination (%)	inhibition (%)	radicle length (mm)	inhibition (%)
control	100 ± 3		58.5 ± 28.4	
leaves				
petroleum ether extract	80 ± 8	20	52.9 ± 21.7	9.5
chloroform extract	75 ± 4	25	43.1 ± 26.2	26.3
chloroform–methanol (9:1) extract	85 ± 3	15	53.7 ± 12.1	8.2
methanol extract	100 ± 4	0	63.3 ± 28.8	+8.2
water extract	35 ± 2	65	6.8 ± 3.3	88.4
roots				
petroleum ether extract	90 ± 3	10	69.3 ± 35.2	+18.5
chloroform extract	35 ± 2	65	1.0 ± 0.1	98.3
chloroform–methanol (9:1) extract	55 ± 5	55	12.3 ± 4.1	79.0
methanol extract	40 ± 1	60	5.3 ± 1.3	91.0
water extract	0	100	0	

[a] Data are expressed as percentage of germinated seeds ± SEM and as mean of radicle length (mm) ± SEM. Each Petri dish contained 10 seeds; each determination was repeated six times.

Table 2. Effects of Extracts (1 mg/mL) of *A. altissima* on Germination of Cress, 120 h after Sowing[a]

	germination (%)	inhibition (%)	radicle length (mm)	inhibition (%)
control	100 ± 2		105.0 ± 26.0	
leaves				
petroleum ether extract	100 ± 3	0	39.6 ± 22.2	62.2
chloroform extract	100 ± 4	0	40.9 ± 26.2	61.0
chloroform–methanol (9:1) extract	90 ± 7	10	63.4 ± 39.1	39.6
methanol extract	100 ± 8	0	73.4 ± 38.3	30.1
water extract	45 ± 5	55	1.0 ± 0.1	99.0
roots				
petroleum ether extract	95 ± 3	5	73.5 ± 35.3	30.0
chloroform extract	95 ± 1	5	3.2 ± 0.7	96.9
chloroform–methanol (9:1) extract	85 ± 1	15	6.2 ± 1.2	94.1
methanol extract	75 ± 1	25	4.3 ± 1.8	95.9
water extract	20 ± 3	80	2.5 ± 0.6	97.6

[a] Data are expressed as percentage of germinated seeds ± SEM and as mean of radicle length (mm) ± SEM. Each Petri dish contained 10 seeds; each determination was repeated six times.

Table 3. Effects of Extracts (1 mg/mL) of *A. altissima* on Germination of Purslane, 120 h after Sowing[a]

	germination (%)	inhibition (%)	radicle length (mm)	inhibition (%)
control	90 ± 3		31.7 ± 3.4	
leaves				
petroleum ether extract	75 ± 2	17	25.7 ± 1.2	13.2
chloroform extract	90 ± 5	0	30.9 ± 1.7	2.5
chloroform–methanol (9:1) extract	70 ± 3	22	15.3 ± 6.1	51.7
methanol extract	65 ± 2	28	31.1 ± 4.1	1.9
water extract	60 ± 6	33	12.3 ± 4.1	61.2
roots				
petroleum ether extract	70 ± 2	22	26.3 ± 6.1	20.2
chloroform extract	60 ± 2	33	27.2 ± 4.3	14.2
chloroform–methanol (9:1) extract	70 ± 5	22	30.5 ± 6.5	3.8
methanol extract	85 ± 7	6	28.3 ± 3.1	10.7
water extract	25 ± 1	72	10.3 ± 4.1	67.5

[a] Data are expressed as percentage of germinated seeds ± SEM and as mean of radicle length (mm) ± SEM. Each Petri dish contained 10 seeds; each determination was repeated six times.

Table 4. Effects of Sephadex Fractions (1 mg/mL) on Germination and Radicle Elongation of Radish, Cress, and Purslane, 120 h after Sowing[a]

	germination (%)	inhibition (%)	radicle length (mm)	inhibition (%)
		Radish		
control	100 ± 2		60.7 ± 3.1	
fraction I	70 ± 2	30	48.2 ± 2.1	20.6
fraction II	30 ± 3	70	7.2 ± 1.7	88.1
fraction III	0	100		
fraction IV	0	100		
fraction V	65 ± 2	35	18.6 ± 3.1	69.4
fraction VI	80 ± 3	20	23.9 ± 2.7	60.6
fraction VII	20 ± 1	80	3.5 ± 0.7	94.2
fraction VIII	90 ± 4	10	57.5 ± 13.7	5.3
		Cress		
control	100 ± 3		99.7 ± 16.2	
fraction I	100 ± 1	0	71.4 ± 15.6	28.4
fraction II	100 ± 2	0	8.8 ± 1.2	91.1
fraction III	100 ± 3	0	5.7 ± 1.8	94.3
fraction IV	75 ± 1	25	3.6 ± 1.3	96.4
fraction V	90 ± 3	10	11.9 ± 2.6	88.1
fraction VI	95 ± 2	5	49.1 ± 19.4	50.1
fraction VII	80 ± 4	20	7.1 ± 1.4	92.8
fraction VIII	100 ± 2	0	66.2 ± 16.1	33.6
		Purslane		
control	90 ± 3		32.6 ± 13.1	
fraction I	70 ± 4	22	19.8 ± 12.1	39.2
fraction II	70 ± 3	22	2.5 ± 0.9	92.3
fraction III	35 ± 2	61	2.1 ± 0.3	93.5
fraction IV	75 ± 1	17	2.7 ± 0.4	91.7
fraction V	85 ± 3	6	4.4 ± 1.0	86.5
fraction VI	85 ± 4	6	21.7 ± 4.3	33.4
fraction VII	90 ± 5	0	26.4 ± 3.5	19.0
fraction VIII	90 ± 3	0	31.0 ± 6.7	4.9

[a] Data are expressed as percentage of germinated seeds ± SEM and as mean of radicle length (mm) ± SEM. Each Petri dish contained 10 seeds; each determination was repeated six times.

shown). Water extract of roots completely inhibits germination in radish seeds and inhibits germination by 80% in cress and by 72% in purslane. A considerable inhibitory effect on radish and cress is also exerted by aqueous leaf extract. Radicle elongation of radish and cress is severely inhibited by root extracts, whereas, also in this case, the inhibition of radicle elongation of purslane is lower.

The essential oil from leaflets, tested at concentrations ranging from 62.5 to 500 μg/mL, is completely inactive (data not shown).

Aqueous extract of roots was fractionated in H_2O−BuOH, and the BuOH extract, active in bioassays, was dissolved in methanol and then purified on Sephadex LH-20 column, and its eight main fractions were tested by the same bioassay method. Fractions III and IV, each at a dose of 1 mg/mL, completely inhibit germination of radish, and fraction VII inhibits germination by 80%; radish radicle elongation was inhibited by 94.2% by the same fraction. The effects on cress germination are dramatically minor, whereas radicle growth was severely affected by six of eight chromatographic fractions. Germination of purslane was inhibited by 61% by fraction III, and fractions II−V exerted a strong inhibitory effect on radicle growth of purslane (**Table 4**).

Pure compounds isolated from active fractions were assayed on radish at a concentration of 10^{-4} M. Ailanthone showed the highest inhibitory activity on both germination (88%) and radicle elongation of radish (87.2%); other compounds were less active, with an order of potency ailanthone > ailanthinone > chaparrine > ailanthinol B (**Table 5**). The alkaloid 1-methoxycanthin-6-one was completely inactive.

Table 5. Effects of Pure Compounds (10^{-4} M) on Germination and Radicle Elongation of Radish, 120 h after Sowing[a]

	germination (%)	inhibition (%)	radicle length (mm)	inhibition (%)
control	100 ± 4		81.1 ± 5.7	
ailanthone	12 ± 2	88	10.3 ± 3.2	87.2
ailanthinone	30 ± 3	70	14.6 ± 3.4	82.0
chaparrine	38 ± 3	62	22.2 ± 4.5	72.6
ailanthinol B	70 ± 2	30	49.1 ± 3.1	39.5
1-methoxycanthin-6-one	99 ± 3	1	80.1 ± 3.1	1.2

[a] Data are expressed as mean of germinated seeds ± SEM and radicle length (mm) ± SEM. Each Petri dish contained 10 seeds; each determination was repeated six times.

Our data showed some notable observations. (i) The isolated active principles are quassinoids, thus confirming previous reports on allelopathic properties of ailanthone (*38*−*40*); on the other hand, we identified other active quassinoids that had a good inhibitory activity on germination of radish seed, e.g., ailanthinone (inhibitory activity 70%) and chaparrine (inhibitory activity 62%). (ii) Pure compounds are less active with respect to whole aqueous extract of roots, suggesting a possible synergistic action of various compounds of different biological potency. (iii) Radish seed is more sensitive to allelochemicals of *A. altissima* in comparison with the other seeds, in particular purslane that needs of a longer time of germination. (iv) Quassinoids seems generally to be more soluble in low-polarity solvents: in our experiment, we hypothesize a greater concentrations of such compounds in roots and/or a more effective extraction from roots, for example by diffusion from radicle cells.

The in vitro complete inhibition on radish germination and the strong effects on cress and purslane germination by the aqueous extract of *A. altissima* appear to be important, considering the possible use of the extract as an herbicide in development of sustainable agriculture practices, enabling farmers to use natural pesticides against weeds (*1*, *46*). Our data contribute to the knowledge of the ecological impact of tree-of-heaven on neighboring plants, and we have identified five phyotoxic compounds that contribute to the total activity of *A. altissima* against test seeds. The data obtained agree in part with previous reports (*33*−*40*) that ailanthone is the only active substance in the plant. Moreover, one can hypothesize a variability of quality and quantity of allelochemicals in different organs of *A. altissima.*

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