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Full Length Research Paper

Nematicidal activity of the alkaloids from *Macleaya cordata* against certain nematodes

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***Macleaya cordata* R. Br. is a source of bioactive compounds which are used in phytopreparations with anti-inflammatory and antimicrobial activities. In this study, four isoquinoline alkaloids were isolated from the crude alkaloid extract of the aerial parts of *M. cordata*, and were identified as sanguinarine, chelerythrine, protopine and allocryptopine. These alkaloids were screened for their nematicidal activity against the nematodes *Bursaphelenchus xylophilus*, *Caenorhabditis elegans* and *Meloidogyne incognita*. Three alkaloids, sanguinarine, chelerytherine and allocryptopine, showed nematicidal activity with the median lethal concentration (LC_{50}) values as 28.52, 34.50 and 37.45 µg/ml, respectively, against *B. xylophilus*; 22.78, 40.25 and 38.90 µg/ml, respectively, against *C. elegans*; and 67.52, 61.00 and 76.56 µg/ml, respectively, against *M. incognita* at 24 h. This is the first report on the nematicidal activity of the alkaloids from *M. cordata* against the nematodes, *B. xylophilus*, *C. elegans* and *M. incognita*.**

Key words: Nematicidal activity, alkaloids, *Macleaya cordata*, *Bursaphelenchus xylophilus*, *Caenorhabditis elegans*, *Meloidogyne incognita*.

INTRODUCTION

Plant parasitic nematodes, which constitute one of the most important pest species groups affecting a wide variety of plants, cause significant economic losses to agriculture and forest (Chitwood, 2002). It has been estimated that plant parasitic nematodes caused as much as $ 100 billion in annual losses of crops and other plants worldwide (Bird, 2004). The primary damage of pine trees caused by pinewood nematode (*Bursaphelenchus xylophilus*) could lead to rapid wilting and death of pine trees (Kosaka et al., 2001). Root-knot nematode (*Meloidogyne incognita*) is extremely polyphagous, and can infect a great number of plant species typically causing root galls (Natarajan et al., 2006). The use of synthetic nematicides has resulted in significant environmental pollution, and the resistance among nematodes to synthetic nematicides has developed (Kerry, 2000). So, it is necessary to search for environmentally and toxicologically safe, and more selective and efficacious nematicides (Oka et al., 2000; Bar-Eyal et al., 2006). Plants are a prominent source of new nematicidal chemicals, since many plants have been reported to possess nematicidal activity, and a series of nematicidal substances of plant origin such as terpenoids, alkaloids, steroids, and flavonoids have been identified (Chitwood, 2002). Furthermore, the use of plants and plant products is one of the promising methods for nematode control. They are cheap, easy to apply, produce no pollution hazards, and have the capacity to structurally and nutritionally improve soil health (Zasada et al., 2010).

Macleaya cordata R. Br., also known as plume poppy or *Bocconia cordata*, is a perennial herb of the family Papaveraceae. The aerial parts of *M. cordata* have long been used as a traditional Chinese medicine for its analgesic and anti-inflammatory properties in humans (Xinrong, 2003). This herb is mainly distributed in the southeast area of China (Wu, 1999). Previous chemical studies led to the isolation of several alkaloids which exhibited cytotoxic (Ulrichova et al., 1996), protease inhibitory (Sedo et al., 2002), antimicrobial (Liu et al., 2009; Kosina et al., 2010), and molluscicidal (Ming et al.,

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Figure 1. The aerial parts of *Macleaya cordata*.

2011) activities. To the best of our knowledge, there is no reported study on the nematicidal activity of the alkaloids from *M. cordata* against plant parasitic nematodes though there were a few reports about the alkaloids from this plant for their nematicidal activity on non-parasitic and free-living (Matsuhashi et al., 2002) or animal parasitic nematodes (Satou et al., 2002).

The aim of this study was to evaluate the nematicidal activity of the crude alkaloid extract and its four alkaloids from the aerial parts of *M. cordata* in order to clarify their potential application as nematicidal agents for nematode control. The median lethal concentration (LC_{50}) values of the alkaloids were determined on two plant parasitic nematodes, *B. xylophilus* and *M. incognita* as well as a model nematode, *Caenorhabditis elegans*.

MATERIALS AND METHODS

Plant materials

The aerial parts of *M. cordata* R. Br. (Figure 1) were collected from the heart of Hunan Province of China in September 2009, and were authenticated by Dr. Fanglu Du from Hunan University of Chinese Medicine of China. A voucher specimen was deposited in the Herbarium of the Institute of Chinese Medicinal Materials, China Agricultural University.

Preparation of the crude alkaloid extract and its main alkaloids

The aerial parts of *M. cordata* were air-dried and ground. The powder (3 kg) was then extracted with 1% sulfuric acid-water at room temperature (20 to 25°C) for three days (3×5 L). The pH value of the combined filtrate was adjusted to 9 with 10% sodium hydroxide solution to precipitate the alkaloid part, which was further extracted with 95% ethanol under reflux (3×1 L) for 5 h at 80°C. The ethanol extract was acidified with 50% sulfuric acid to pH 2 to give the crude alkaloid extract (21 g). The crude alkaloid extract was fractionated by repeated column chromatography and preparative thin layer chromatography (TLC) to give four compounds.

Culture of nematodes

The pinewood nematode, *B. xylophilus*, was kindly supplied by Dr. Bingyan Xie at the Institute of Vegetables and Flowers, Chinese Academy of Agricultural Sciences. The fungus *Botrytis cinerea* was cultured on a potato dextrose agar (PDA) plate at 25°C. When the fungus was fully grown, the plate was inoculated with the pinewood nematodes, and then cultured until the fungal mycelia had been completely consumed.

C. elegans, which was kindly supplied by Dr. Yuanmei Zuo at the College of Resources and Environmental Sciences, China Agricultural University, was inoculated on the nematode growth medium (NGM) that was cultured previously with *Escherichia coli* OP_{50} according to methods of Steiernagle (1999). The NGM plate was full of the worms after 4 to 5 days at 16°C in darkness.

The root-knot nematode *M. incognita*, which was kindly supplied by Dr. Heng Jian at the College of Agronomy and Biotechnology, China Agricultural University, was cultured on tomatoes under greenhouse conditions, and the fresh egg masses were extracted according to the methods of Li et al. (2005). Fresh eggs were then kept in water for egg hatching. The second stage juveniles (J_2) that emerged from the eggs after 48 h were incubated at 30°C and were used for nematicidal assay.

Nematicidal assay

In order to test the nematicidal activity of the alkaloids at 200 μg/ml, 50% acetone water stock solution for each sample at 2 mg/ml was prepared. The test nematode dilution (90 μl containing 40 to 50 nematodes) was added into each well of the sterile 96-well microplate and then, 10 μl of sample stock solution was added into each well and mixed thoroughly. 5% of acetone-water solution was used as the negative control. Five replicates were carried out for each treatment, and the experiments were repeated twice. Dead and active nematodes were counted after 24 h. The nematodes were considered to be dead when they did not move on physical stimuli with a fine needle (Hong et al., 2007). The mean percentage of mortality was then calculated. The net percentage of mortality was about 3% by using 5% acetone-water solution as the negative control after 24 h. Nematode recovery was not observed in the dead nematodes.

To further determine the LC_{50} values of the alkaloids, 5% acetone water solutions of each sample at 200, 100, 50, 25, 10, 5 and 1 μg/ml were assayed for nematicidal activity by the afore described method. Avemectin B1 was used as the positive control with the purity of 97.2%. It was a mixture of avermectin B1a and avermectin B1b in the ratio of 9.5 to 0.5 (w/w). Avemectin B1 was kindly provided by Dr. Shankui Yuan at the Institute for the Control of Agrochemicals, Chinese Ministry of Agriculture.

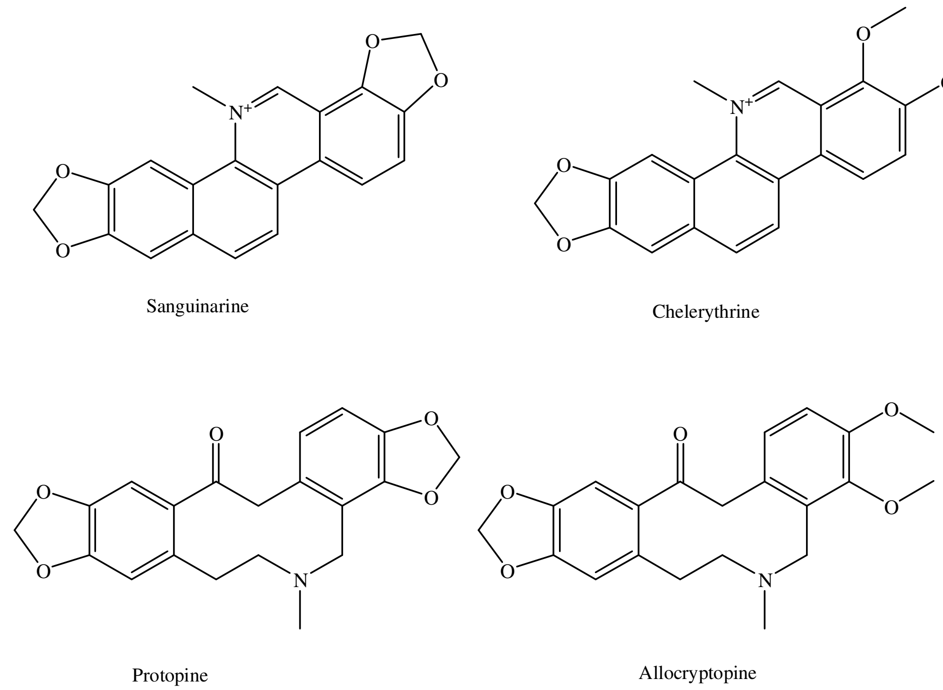

Figure 2. Structures of the alkaloids.

Data analysis

Treatments of mortality experiments were replicated five times, and each experiment was performed twice. The percentages of dead/paralyzed nematodes observed in the microplate assays after 24 h were corrected by eliminating the natural death/paralysis (about 3%) in negative control according to the Schneider-Orelli formula (Ntalli et al., 2011):

Adjusted mortality (%) = (Mortality in treatment - Mortality in negative control) ÷ (1 - Mortality in negative control) × 100

The mortality (%) values were represented by their means and standard deviations (S.D). To describe the nematicidal effects of the samples against the nematodes, the median lethal concentration (LC_{50}) values were calculated using the linear relation between the inhibitory probability and concentration logarithm according to the method of Sakuma (1998).

RESULTS AND DISCUSSION

Preparation of the crude alkaloid extract and alkaloids

The extracted yield of the crude alkaloid extract from the aerial parts of *M. cordata* was 0.7%. The crude alkaloid extract was fractionated by repeated column chromate-graphy and preparative TLC to give four alkaloids, which were identified as sanguinarine, chelerythrine, protopine and α-allocryptopine by physicochemical and spectrometric analysis as mentioned in our previous report (Liu et al., 2009). The structures of the alkaloids are shown in Figure 2.

Screening of the nematicidal activity of the alkaloids

The results of the nematicidal activity of the crude alkaloid extract and alkaloids at concentration of 200 µg/ml are shown in Table 1. Among the alkaloids, protopine exhibited a weak inhibitory activity with the inhibitory rate as 41.39% on *B. xylophilus*, 39.92% on *C. elegans*, and 31.76% on *M. incognita*, respectively. The inhibitory activities of the other alkaloids were in the range of 60 to 90%. All the samples except protopine were selected for further testing of their median lethal concentration (LC_{50}) values.

The LC_{50} values of the alkaloids are shown in Table 2. By using *B. xylophilus* and *C. elegans* as the tested nematodes, sanguinarine showed the most effective nematicidal activity with LC_{50} values of 28.52 µg/ml on *B. xylophilus*, and 22.78 µg/ml on *C. elegans* among the alkaloids. *M. incognita* was less sensitive to the alkaloids as compared with *B. xylophilus* and *C. elegans*.

In summary, four nematicidal alkaloids were success-fully separated and structurally identified from the crude alkaloid extract of *M. cordata*. These alkaloids as well as the crude alkaloid extract were first screened for their nematicidal activity against the nematodes, *B. xylophilus*, *C. elegans* and *M. incognita*.

Three alkaloids, sanguinarine, chelerytherine and allocryptopine were screened to show their strong nematicidal activity. They could be the main nematicidal components in *M. cordata*. The results of the present study provide a significant basis for use of the alkaloids from the aerial parts of *M. cordata* for the control of

Table 1. Nematicidal activity of the alkaloids at 200 µg/ml.

Treatment	Mortality (%) of the sample at 200 µg/ml		
	B. xylophilus	***C. elegans***	***M. incognita***
Crude alkaloid extract	68.91 ± 3.09	82.03 ± 2.23	80.98 ± 1.00
Sanguinarine	92.76 ± 2.37	92.99 ± 1.57	94.78 ± 0.62
Chelerythrine	90.57 ± 1.62	91.04 ± 1.57	94.24 ± 2.33
Protopine	41.39 ± 1.01	39.92 ± 3.16	31.76 ± 0.92
Allocryptopine	90.68 ± 1.81	91.27 ± 1.14	90.51 ± 3.31
Avermectin B1	100.00 ± 0.00	100.00 ± 0.00	100.00 ± 0.00

Each value is the mean ± S.D. of five repetitions.

Table 2. Nematicidal activity (LC_{50}) of the alkaloids.

Treatment	Nematode species	Regress equation and correlation coefficient	LC_{50} (µg/ml)
Crude alkaloid extract	*B. xylophilus*	$Y = 1.353X + 2.332$ ($R = 0.968$)	96.96
	C. elegans	$Y = 1.638X + 1.927$ ($R = 0.972$)	75.17
	M. incognita	$Y = 1.210X + 2.618$ ($R = 0.989$)	93.02
Sanguinarine	*B. xylophilus*	$Y = 1.314X + 3.088$ ($R = 0.984$)	28.52
	C. elegans	$Y = 1.275X + 3.269$ ($R = 0.975$)	22.78
	M. incognita	$Y = 1.032X + 3.112$ ($R = 0.994$)	67.52
Chelerythrine	*B. xylophilus*	$Y = 1.746X + 2.315$ ($R = 0.998$)	34.50
	C. elegans	$Y = 1.523X + 2.556$ ($R = 0.973$)	40.25
	M. incognita	$Y = 1.025X + 3.170$ ($R = 0.995$)	61.00
Allocryptopine	*B. xylophilus*	$Y = 2.017X + 1.826$ ($R = 0.992$)	37.45
	C. elegans	$Y = 1.919X + 1.949$ ($R = 0.995$)	38.90
	M. incognita	$Y = 1.000X + 3.116$ ($R = 0.966$)	76.56
Avermectin B1	*B. xylophilus*	$Y = 1.239X + 4.600$ ($R = 0.973$)	2.10
	C. elegans	$Y = 1.034X + 4.804$ ($R = 0.989$)	1.55
	M. incognita	$Y = 1.488X + 4.638$ ($R = 0.964$)	1.75

certain nematodes. The crude alkaloid extract as well as the isolated alkaloids found to be active in this study could be useful for the development of new nematicidal agents. The mechanism of action about these alkaloids might be related to their cytotoxicity which needs to be clarified (Matsuhashi et al., 2002). Furthermore, nematicidal efficacy also needs to be evaluated under field conditions.

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