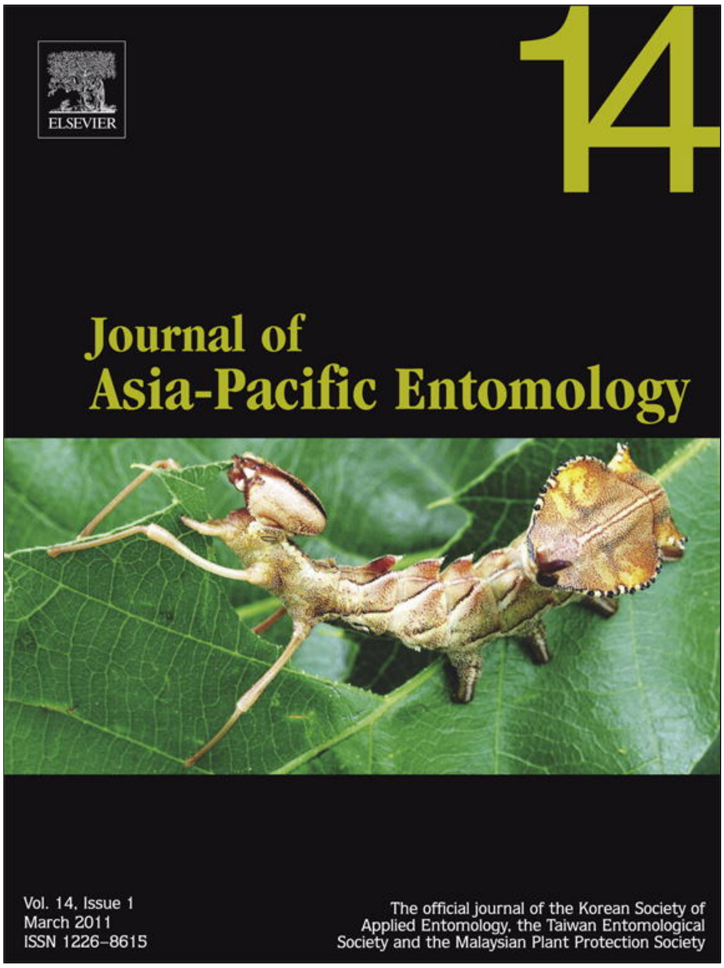
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Short Communication

Nematicidal coumarin from *Ficus carica* L.

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ABSTRACT

The methanol extracts from 40 plant species were screened for their nematicidal activity against the nematodes *Bursaphelenchus xylophilus*, *Panagrellus redivivus* and *Caenorhabditis elegans*. The leaf extract of *Ficus carica* L. exhibited the strongest nematicidal activity, causing 74.3%, 96.2% and 98.4% mortality, respectively, within 72 h. By bioassay-guided fractionation, a coumarin was obtained. The compound was determined to be psoralen based on spectroscopic data. It showed nematicidal activity against the tested nematodes. This is the first report of the nematicidal activity of *F. carica* and psoralen.

Introduction

Plant-parasitic nematodes are economically important pests of many plants worldwide. The pine wood nematode (*Bursaphelenchus xylophilus*) causes a major share of plant loss due to nematodes (Hajime et al., 2001). Many synthetic compounds are used for plant protection, but most of them have resulted in significant environmental pollution and the development of resistance in nematodes (Kerry, 2000). In recent years, the natural compounds have increasingly become the focus of those interested in the discovery of nematicides because they are more environmentally friendly (Akhtar and Farzana, 1996; Shaukat et al., 2002). Plants are a large, potential source of nematicidal chemicals (Chitwood, 2002; Park et al., 2005; Pérez et al., 2003; Saleh et al., 1987; Udalova et al., 2004). In the present work, the methanol extract from 40 plant species were screened for their nematicidal activity against the nematodes *B. xylophilus*, *Panagrellus redivivus* and *Caenorhabditis elegans*. Based on initial results, a coumarin with nematicidal activity was isolated from *Ficus carica* L.

Materials and methods

Plant samples were collected between May and June of 2008 from Kunming Institute of Botany, Yunnan, P. R. China. The preparation of plant extracts, culture of nematodes, bioassay methods and LC_{50} calculation were based on Hong et al. (2007).

Column chromatography (CC) was performed on silica gel G (200–300 mesh, Qingdao Marine Chemical Factory, China) on Sephadex LH-20 (Amersham Pharmacia, Sweden). TLC was performed on silica gel GF_{254} (10–40 μm, Qingdao). All solvents were distilled before use. NMR spectra were obtained with Bruker DRX-500 spectrometers with TMS as internal standard. ESI-MS was recorded on Finnigan LCQ-Advantage mass spectrometer, in *m/z*.

The crude methanol leaves extract (2.1 g) of *Ficus carica* L. was subjected to Silica gel G CC (200–300 mesh; petroleum ether//EtOAc 20:1–2:1) and 17 fractions ($Fr.A_1$–$Fr.A_{17}$) were produced. Fraction A_7 was purified further by Sephadex LH-20 CC eluted with acetone to yield compound **1** (12 mg).

Results and discussion

The name and plant part of 40 plant species from 27 families and their nematicidal activity are shown in Table 1. The extracts which had little (<30%, 72 h) or no nematicidal activity were not shown on the table. Ten plant species in eight genera possessed nematicidal activity (>30%, 72 h) against the tested nematodes. The leaf extract of *Ficus carica* exhibited the strongest nematicidal activity against *B. xylophilus*, *P. redivivus* and *C. elegans*, and caused 74.3%, 96.2% and 98.4% mortality, respectively, within 72 h at the concentration of 5 mg/mL. The 5% methanol control caused 9.2%, 4.6% and 3.2% mortality, respectively. Other leaf or branch extracts of nine plant species showed medium or weak activity against *P. redivivus* and *C. elegans*, and no activity against *B. xylophilus*.

Compound **1** was obtained by column chromatography isolation from *F. carica*. The compound was obtained as a colorless needle. ^{1}H-NMR (500 MHz, $CDC1_3$): δ 6.38 (1 H, d, *J* = 9.8 Hz, H-3), 6.83 (1 H, dd, *J* = 2.0, 1.0 Hz, H-3'), 7.46 (1 H, *brs*, H-8), 7.68 (1 H, s, H-5), 7.70 (1 H, d, *J* = 2.0 Hz, H-2'), 7.80 (1 H, d, *J* = 9.8 Hz, H-4). ^{13}C-NMR

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Table 1
The name and tested plant part of the assessed plants and effects of plant extracts on the tested nematodes (mortality (%±SD)).

Family	Plant species	Tested part	Mortality (%, *B. xylophilus*/*P. redivivus*/*C. elegans*)		
			24 h	48 h	72 h
Apocynaceae	*Carissa spinarum*	Leaf, branch	–/–/–	–/–/–	–/–/–
Acanthaceae	*Hypoestes sanguinolenta*	Leaf, branch	–/–/–	–/–/–	–/–/–
Amaranthaceae	*Iresine herbstii*	Leaf, branch	–/–/–	–/–/–	–/–/–
Anacardiaceae	*Pistacia chinensis*	Leaf, branch	–/–/–	–/–/–	–/–/–
	Pistacia weinmannifolia	branch	–/17.8±0.04/12.7±0.05	–/48.7±0.03/40.1±0.04	–/51.4±0.05/53.4±0.05
Begoniaceae	*Begonia 'Little Brother Montecomery'*	Leaf, branch	–/–/–	–/–/–	–/–/–
Buddlejaceae	*Buddleja crispa*	Leaf, branch	–/–/–	–/–/–	–/–/–
Caesalpiniaceae	*Bauhinia brachycarpa*	Leaf, branch	–/–/–	–/–/–	–/–/–
Curcurbitaceae	*Momordica cochinchinensis* (Lour.)Spreng	leaf	–	–	–
		branch	–/–/–/20.7±0.03/36.4±0.01	–/29.6±0.03/41.7±0.01	–/31.8±0.02/43.9±0.02
Dennstaedtiaceae	*Microlepia platyphylla* (D. Don) J. Sm.	Leaf, branch	–/–/–	–/–/–	–/–/–
Ebenaceae	*Diospyros kaki*	Leaf, branch	–/–/–	–/–/–	–/–/–
	Diospyros lotus	Leaf, branch	–/–/–	–/–/–	–/–/–
Elaeagnceae	*Elaeagnus macrantha*	Leaf, branch	–/–/–	–/–/–	–/–/–
Elaeocarpaceoe	*Elaeocarpus decipiens*	leaf	–/1.4±0.02/4.9±0.01	–/32.7±0.02/21.4±0.03	–/47.2±0.04/31.2±0.02
		branch	–/10.3±0.01/2.4±0.01	–/57.6±0.02/23.8±0.02	–/61.3±0.04/38.9±0.02
Fagaceae	*Castanea mollissima* Blume	Leaf, branch	–/–/–	–/–/–	–/–/–
	Lithocarpus harlandii	Leaf, branch	–/–/–	–/–/–	–/–/–
Hamamelidaceae	*Altingia yunnanensis*	leaf	–/4.7±0.01/3.6±0.01	–/39.2±0.01/36.7±0.01	–/59.4±0.01/65.8±0.02
		branch	–/–/–	–/–/–	–/–/–
	Distylium racemosum Siob. Et Zucc	Leaf, branch	–/–/–	–/–/–	–/–/–
	Sycopsis triplinervia	Leaf, branch	–/–/–	–/–/–	–/–/–
	Symingtonia tokinensis	leaf	–/–/–	–/–/–	–/–/–
Hydrangeaceae	*Cardiocrinum giganteum* (Wall.) Makino	leaf	–/–/–	–/–/–	–/–/–
	Deutzia purpurascens (Fr.) Rehd.	Leaf, branch	–/–/–	–/–/–	–/–/–
Hypericaceae	*Hypericum androsaemum* Linn.	Leaf, branch	–/–/–	–/–/–	–/–/–
Juglandaceae	*Platycarya strobilacea*	Leaf, branch	–/–/–	–/–/–	–/–/–
Liliaceae	*Polygonatum kingianum*	Leaf, branch	–/–/–	–/–/–	–/–/–
	Tofieldia thibetica	Leaf, branch	–/–/–	–/–/–	–/–/–
Magnoliaceae	*Manglietia aromatica* Dandy	leaf	–/4.4±0.01/3.2±0.01	–/38.2±0.01/36.7±0.02	–/51.9±0.01/59.6±0.02
	Magnolia officinalis var.*biloba*	Leaf, branch	–/–/–	–/–/–	–/–/–
	Michelia alba	leaf	–/13.9±0.02/–	–/33.2±0.02/–	–/37±0.02/–
		branch	–/–/–	–/–/–	–/–/–
Magnoliaceae	*Tsoongiodendron odorum*	leaf	–/11.2±0.04/6.1±0.03	–/32.4±0.04/18.2±0.03	–/61.3±0.05/43.7±0.05
		branch	–/–/–	–/–/–	–/–/–
Melinceae	*Toona ciliate var. pubescens*	leaf	–/6.4±0.02/–	–/6.9±0.02/–	–/46.6±0.02/–
		branch	–/–/–	–/–/–	–/–/–
Moraceae	*Ficus carica*	leaf	68.5±0.02/64.7±0.04/44.2±0.04	70.7±0.04/80.7±0.03/76.4±0.02	74.3±0.02/96.2±0.02/98.4±0.04
		branch	7.4±0.01/32.1±0.02/11.6±0.01	25.4±0.01/51.2±0.02/48.7±0.02	36.7±0.01/71.4±0.02/57.2±0.01
Onagraceae	*Fuchsia hybrida*	leaf, branch flower	–/–/–	–/–/–	–/–/–
Oleaceae	*Olea europaea*	Leaf, branch	–/–/–	–/–/–	–/–/–
Papilionaceae	*Ormosia fordiana*	leaf	–/3.7±0.01/2.6±0.01	–/30.7±0.02/29.6±0.01	–/39.4±0.02/43.2±0.01
		branch	–/–/–	–/–/–	–/–/–
	Sophora davidii (Franch.) komarov ex Pavol	Leaf, branch	–/–/–	–/–/–	–/–/–
Polypodiaceae	*Neocheiropteris palmatopedata* (Bak.)Christ	Leaf, branch	–/–/–	–/–/–	–/–/–
Rubiaceae	*Serissa japonica*	Leaf, branch	–/–/–	–/–/–	–/–/–
Stemonaceae	*Stemona tuberosa* Lour	Leaf, branch flower	–/–/–	–/–/–	–/–/–
Thymelaeaceae	*Edgeworthia chrysantha* Lindl	Leaf, branch	–/–/–	–/–/–	–/–/–
	5% methanol		2.7±0.01/1.2±0.02/1.4±0.02	3.1±0.02/2.9±0.02/2.5±0.02	9.2±0.02/4.6±0.02/3.2±0.03

Note: –, the mortality of the tested nematodes under 30% in 72 h.

Table 2
Effects of compound **1** on the tested nematodes (mortality (%±SD)).

Compound	Concentrations (mg L^{-1})	Mortality (%, *B. xylophilus*/*P. redivivus*/*C. elegans*)		
		24 h	48 h	72 h
1	400	31.8±0.01/39.9±0.03/49.8±0.02	51.7±0.02/52.6±0.02/69.2±0.02	62.1±0.02/70.0±0.03/82.8±0.03
	200	23.4±0.03/24.1±0.03/32.6±0.04	41.1±0.02/37.1±0.02/51.5±0.01	48.7±0.02/51.9±0.02/68.7±0.03
	100	19.7±0.03/19.3±0.02/29.5±0.03	25.4±0.02/22.4±0.02/37.2±0.03	33.8±0.03/39.0±0.03/43.1±0.02
	50	9.0±0.01/12.6±0.02/18.4±0.02	13.7±0.01/16.4±0.02/26.8±0.01	22.1±0.02/28.8±0.02/34.5±0.03
Control (5% acetone, *v*/*v*)		1.3±0.01/1.0±0.02/1.0±0.01	2.4±0.01/1.5±0.02/1.0±0.02	2.6±0.02/1.9±0.02/1.6±0.03

Table 3
Influence of exposure time (48 and 72 h) on nematode mortality at each psoralen concentration.

	50 mg L^{-1}	100 mg L^{-1}	200 mg L^{-1}	400 mg L^{-1}
B. xylophilus	−9.280***[a]	−2.231*	−8.624**	− 12.182***
C. elegans	−6.324**	−4.320*	− 11.307***	− 15.03***
P. redivivus	−5.302**	− 13.329***	− 10.832***	−2.974*

***$P < 0.001$, **$P < 0.01$, *$P < 0.05$.
[a] Values were *t* values from independent sample *t*-test.

(125 MHz, $CDC1_3$): δ 100.3 (d), 106.8 (d), 115.1 (d), 115.8 (s), 120.2 (d), 125.3 (s), 145.2 (d), 147.3 (d), 152.5 (s), 156.8 (s), 161.4(s). ESIMS: 187 $[M+H]^+$. According to the data reported in the reference (Takahiro et al., 1998), the compound was identified as psoralen.

Results of the nematcidal activity of compound **1** (psoralen) are shown in Table 2. Influences of exposure time (48 and 72 h) on the mortality of nematodes at each concentration of psoralen are shown in Table 3. The median lethal concentrations (LC_{50}) of compound **1** against *B. xylophilus*, *P. redivivus* and *C. elegans* were 258.8, 181.1 and 119.40 mg L^{-1} at 72 h, respectively. The nematicidal activity of compound **1** is much weaker than the leaf extract of *F. carica*. There may be other nematicidal compounds that were not obtained in our experiment. Psoralen has been isolated from *Psoralea corylifolia*, *Glehnia littoralis* and *Ficus carica*, and has diverse biological activities, including antioxidant, antibacterial, and antifungal activities (Guo et al., 2005; Hu et al., 2007; Takahiro et al., 1998; Zhao et al., 2005). However, until now, there has been no report about the nematicidal activity of psoralen.

Plants are large, potential source for nematicidal chemicals. Many nematicidal compounds have been obtained from plants, such as alkaloids, sesquiterpenoids, diterpenoids, triterpenoids, fatty acids, cyanogenic glycosides, polythienyls, polyacetylenes, quassinoids, steroids, phenolics, sothiocyanates and glucosinolates (Chitwood, 2002). As science and technology develop, more compounds with strong nematicidal activities will be discovered from plants.

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