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Evaluation of nematicidal activity of ethanol extracts of Euphorbiaceae plants and constituents from *Euphorbia fischeriana* to *Meloidogyne incognita* (Kofoid and White) Chitwood

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Abstract
Ethanol extracts of 20 plant species (Euphorbiaceae) were assessed for nematicidal activity against the root-knot nematode, *Meloidogyne incognita* (Kofoid and White) Chitwood. Out of these, four extracts: *Croton tiglium*, *Euphorbia fischeriana*, *Leptopus chinensis* and *Ricinus communis* caused 100% mortalities of the root-knot nematode at 1000 µg/ml for 72 h. The ethanol extract of *E. fischeriana* exhibited nematicidal activity against *M. incognita* with a 72-h LC_{50} value of 69.0 µg/ml. Six nematicidal constituents were isolated from *E. fischeriana* as 1-(2, 4-dihydroxy-6-methoxy-3-methylphenyl)-ethanone (**1**), jolkinolide A (**2**), jolkinolide B (**3**), 12-deoxyphorbol 13-(9*Z*)-octadecenoate 20-acetate (**4**), 17-hydroxyjolkinolide A (**5**), and 17-hydroxyjolkinolide B (**6**). Compound **4** and **1** exhibited strong toxicity against *M. incognita* with 72-h LC_{50} values of 48.6 µg/ml and 87.8 µg/ml, respectively. The other four compounds, compound **5** (LC_{50} = 354.6 µg/ml), **6** (LC_{50} = 539.4 µg/ml), **2** (LC_{50} = 627.7 µg/ml) and **3** (LC_{50} = 825.2 µg/ml), also possessed toxicity against *M. incognita*. The above findings suggested that the four ethanol extracts and constituents of *E. fischeriana* especially compound **4** and **1** may be explored as natural potential nematicides.

Keywords: *Euphorbia fischeriana*; *Meloidogyne incognita*; Nematicidal activity

1. Introduction
Plant-parasitic nematodes cause severe crop losses every year. The root-knot nematode, Meloidogyne incognita (Kofoid and White) Chitwood is the most economically important and widely distributed nematode throughout China and causes considerable crop loss [1]. Control of plant-parasitic nematodes is still relied mainly on repeated applications of synthetic pesticides or soil fumigants rather than on other approaches e.g. using natural enemies, enhancing cultural practices, and cultivating resistant cultivars [1, 2]. Although effective, their repeated use fosters serious environmental and human health concerns. These problems have highlighted the need for development of selective nematode-control alternatives. 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 the soil health [2]. In view of these facts, investigations have been undertaken by various groups of scientists, which have shown effective control of root-knot nematodes [3-7]. Many plant extracts and essential oils have been screened for their nematicidal activity against the plant-parasitic nematodes [8-14].
Euphorbia *fischeriana* Steud is a perennial herbaceous plant with latex, distributed mainly in north China [15]. The dried plant roots of *E. fischeriana* have long been used for the treatment of a wide range of ailments, including edema, ascites, ingestion, as well as liver and lung cancer [16]. Aqueous extract of *E. fischeriana* roots has been used to control aphids (Aphis gossypii), cabbage beetle (*Colaphellus bowringi*) and *Pieris rapae* on vegetables and rice leaf roller (*Cnaphalocrocis medinalis*) as well as spider mites [17]. Petroleum ether extract of *E. fischeriana* roots exhibited strong contact toxicity against the adults and eggs of carmine spider mite, *Tetranychus* cinnabarinus [18]. Moreover, one formulation based on ethanol extract of *E. fischeriana* roots had been evaluated in control of cabbage moth (Barathra brassicae) and green peach aphids (Myzus persicae) [19]. Four feeding deterrents were isolated from ethanol extract of *E. fischeriana* roots against two grain storage insects [20]. Previous phytochemical

investigations led to the isolation of diterpenoids, dimeric diterpenoid, triterpenoids, phenolics, and steroids from *E. fischeriana* [21-37]. However, the bioactive compounds of *E. fischeriana* against plant-parasitic nematodes have not been isolated and identified from this Chinese medicinal herb. In this paper, the ethanol extracts of 20 species of Euphorbiaceae plants were evaluated for nematicidal activity against *M. incognita*. In addition, we reported the isolation of six compounds contained in *E. fischeriana* roots against the root-knot nematodes by bioassay-guided fractionation.

2. Materials and Methods

2.1. Plant extracts

The plant samples were collected from Lishui City (27.54° N and 119.20° E, Zhejiang Province), Xiaolongmen National Forest Park (39.48° N and 115.25° E, Mentougou District, Beijing) and Xi Shuan Ba Na National Nature Reserve (22.02° N and 100.80° E, Yunnan Province) (Table 1). The plant samples were identified, and the voucher specimens were deposited at the Museum of Department of Entomology, China Agricultural University. The samples were air-dried and grinded to powdered form using a grinding mill (Retsch Muhle, Haan, Germany). Approximately 100 g of dried power from each of 20 plant species were extracted in 700 ml of 75% ethanol over three weeks at room temperature. The extracts were concentrated using a vacuum rotary evaporator to afford syrupy gums.

Table 1: Nematicidal activity of the ethanol extracts of Euphorbiaceae plants against *Meloidogyne incognita*

Species	Parts used	Harvest Site	Mortality % (Mean ± SD)
Acalypha australis L.	Aerial parts	Beijing	41.8 ± 15.2
Aleurites moluccana (L.) Willd.	Twig and leaf	Yunnan	43.5 ± 12.9
Croton tiglium L.	Seeds	Yunnan	100.0 ± 0
Euphorbia esula L.	Roots	Yunnan	39.9 ± 10.1
Euphorbia fischeriana Steud.	Roots	Yunnan	100.0 ± 0
Euphorbia helioscopia L.	Aerial parts	Zhejiang	62.5 ± 18.5
Euphorbia hirta L.	Aerial parts	Zhejiang	59.7 ± 10.9
Euphorbia humifusa Willd.	Aerial parts	Beijing	37.8 ± 11.2
Euphorbia jolkinii Boiss.	Roots	Yunnan	34.2 ± 9.8
Euphorbia maculata L.	Aerial parts	Beijing	53.5 ± 12.6
Euphorbia pulcherrima Willd. ex Klotzsch	Aerial parts	Beijing	58.9 ± 16.7
Flueggea suffruticosa (Pall.) Baill.	Twig and leaf	Beijing	48.3 ± 14.3
Glochidion eriocarpum Champ.ex Benth.	Twig and leaf	Yunnan	62.7 ± 10.6
Jatropha curcas L.	Stem barks	Yunnan	32.6 ± 7.4
Leptopus chinensis (Bge.) Pojark.	Twig and leaf	Yunnan	100.0 ± 0
Mallotus apelta (Lour.) Müll.Arg.	Roots	Yunnan	43.5 ± 15.1
Mallotus repandus (Willd.) Mull.Arg.	Twig and leaf	Zhejiang	29.5 ± 6.9
Phyllanthus urinaria L.	Aerial parts	Yunnan	69.9 ± 14.4
Ricinus communis L.	Aerial parts	Beijing	100.0 ± 0
Triadica sebifera (L.) Small	Stem barks	Zhejiang	86.8 ± 6.5
Control			0 ± 0

2.2. Nematicidal toxicity

Experiments were performed under laboratory conditions at 26-28 °C. Second stage juveniles (J2) of *M. incognita* were obtained from a pure culture that was previously initiated by egg masses and propagated on tomato (*Solanum lycopersicum*) in the glasshouse. Egg masses were hand-picked using sterilized forceps from heavily infected roots. These egg masses were washed in distilled water, placed in 15 mesh sieves (8 cm in diameter) containing crossed layers of tissue paper at 25-26 °C to obtain freshly hatched second stage juveniles (J2). Only juveniles collected within 48 h were used. For bioassays, the ethanol extracts of each plant were diluted with 10 µl ethanol and the final concentration is 1 mg/ml. Aliquots of H_2O (20 µl) containing ca. 100 juveniles (J2) were transferred to vials to which 980 µl of the solution containing ethanol extract was added. The vials were kept on a hood at 25 °C. The inactive nematodes were counted every 24 h for 72 h. After the last count, the inactive juveniles were maintained in distilled H_2O for 24 h to observe their revival.

Range-finding studies were run to determine the appropriate testing concentrations of the ethanol extract of *E. fischeriana* and isolated compounds. A serial dilution of the ethanol extract (five concentrations, dissolved first in 10 µl ethanol) and pure compounds (five concentrations) was prepared in H_2O with 2% DMSO. Aliquots of H_2O (20 µl) containing ca. 100 juveniles (J2) were transferred to vials to which 980 µl of the solution containing ethanol extract or pure compounds was added. The vials were kept on a hood at 25 °C. The inactive nematodes were counted every 24 h for 72 h. After the last count, the inactive juveniles were maintained in distilled H_2O for 24 h to observe their revival. Six repetitions for each treatment were performed using H_2O and a 2% DMSO in H_2O solution as well as a 2% DMSO in H_2O solution containing 10 µl ethanol as a negative control. The experiments were repeated three times.

2.3. Bioassay-directed fractionation

The powdered roots of *E. fischeriana* were extracted with 95% ethanol (50 l) at room temperature over a period of three weeks, and the extract was evaporated under reduced pressure using a vacuum rotary evaporator to afford a syrupy gum (256 g). This syrup was partitioned between methanol-water and petroleum ether (3×5 l). The petroleum ether extracts were evaporated off to given a residue (38 g). The aqueous layer was re-partitioned with chloroform (3×5 l) to provide a residue (173 g) after evaporation of chloroform. Further partitioning

with ethyl acetate (3× 5 l) gave a residue (76 g) after evaporation of the solvent.

The $CHCl_3$ residue (25 g) (based on bioassay results) was applied to a silica gel column (160-200 mesh, Qingdao Marine Chemical Plant, Shandong, China), eluting with petroleum ether containing increasing accounts of ethyl acetate to give 14 combined fractions according to TLC detection. 1-(2, 4-dihydroxy-6-methoxy-3-methylphenyl)-Ethanone (1, 18.5 mg) and jolkinolide A (2, 59.9 mg) were isolated from Fraction 3 (421 mg) after repeated purification on silica and preparative thin-layer chromatography (PTLC, pre-coated GF254 plates, Qingdao Marine Chemical Plant, Shandong, China). Jolkonolide B (**3**, 31.2 mg) was isolated from Fraction 6 (330 mg) after repeated purification on silica, Sephadex LH-20 and PTLC. Fraction 8 (141 mg) was further chromatographed on silica gel column, Sephadex LH-20 as well as repeated PTLC to provide 12-deoxyphorbol-13-(9*Z*)-octadecanoate-20-acetate (**4**, 16.2 mg). 17-Hydroxyjolkinolide A (**5**, 86.8 mg) was obtained from further chromatographed on silica gel TLC and Sephadex LH-20 and recrystallized from Fraction 10 (409 mg). Fraction 11 (555 mg) was further chromatographed on silica gel column, Sephadex LH-20 and PTLC to obtain 17-hydroxyjolkinolide B (**6**, 73.4 mg). The structures of the compounds were elucidated based on high-resolution electron impact mass spectrometry and nuclear magnetic resonance. ^1H and ^{13}C NMR spectra were recorded on Bruker Avance DRX 500 instruments using $CDCl_3$ as solvent with TMS as internal standard. EIMS were determined on a ThermoQuest Trace 2000 mass spectrometer at 70 eV (probe).

2.4. Statistical analysis

Results from all replicates for the pure compounds and ethanol extract were subjected to probit analysis using the PriProbit Program V1.6.3 to determine LC_{50} (median lethal concentration) values and their 95% fiducial limits (FL 95%) [38].

3. Results and Discussion

3.1. Bioassays

Eleven of the 20 extracts killed at least half of second stage *M. incognita* juveniles (J2s) in an *in vitro* assay at a concentration of 1 mg/ml after 72 h exposure including *Croton tiglium*, *Euphorbia fischeriana*, *E. helioscopia*, *E. hirta*, *E. maculate*, *E. pulcherrima*, *Glochidion eriocarpum*, *Leptopus chinensis*, *Phyllanthus urinaria*, *Ricinus communis*, and *Triadica sebifera* (Table1). Four ethanol extracts, e.g. *C. tiglium*, *E. fischeriana*, *L. chinensis* and *R. communis*, gave 100% mortality of *M. incognita* at a concentration of 1 mg/ml after 72 h exposure.

Among 20 extracts of Euphorbiaceae plants used in bioassays, 18 of them were evaluated for their nematicidal activity for the first time and only *Jatropha curcas*, *L. chinensis* and *R. communis* extracts had been screened for nematicidal activity in previous report [39, 40]. Aqueous extracts of 15 species of plants (fresh leaf) were assessed for hatching inhibition effects on *M. incognita* and extracts derived from *Calotropis procera* and *R. communis* gave the best results against the nematodes [39]. Moreover, Gao *et al.* [41] measured nematicidal activity of *R. communis* extract and alkaloid ricinine, an active compound against *M. incognita* juveniles and *R. communis* extract also exhibited toxicity to *M. arenaria* [42]. In addition, *R. communis* extracts have been shown to possess insecticidal activity against several insects e.g. leave-cutting ants (*Atta sexdens rubropilosa*), the pulse beetle (*Callosobruchus chinensis*) and the fall armyworm (*Spodoptera frugiperda*) [43-46]. The above findings suggested that the four extracts (100% mortality): *C. tiglium*, *E. fischeriana*, *L. chinensis* and *R. communis* may have potential for development as new natural nematicides to control plant-parasitic nematodes. Thus, the ethanol extract of *E. fischeriana* roots was chosen for further isolation in the present study.

3.2. Isolated bioactive compounds

1-(2,4-dihydroxy-6-methoxy-3-methylphenyl)-Ethanone(**1**). Colorless needle (MeOH), m.p. 223-224 °C. EI-MS *m/z* (%): 196 [M^+] (38), 181 (100), 166 (10), 43 (15), 32 (17), 28 (72). ^1H-NMR (500MHz, $CDCl_3$) δ ppm: 6.01 (1H, s, H-5), 3.34 (3H, s, OCH_3), 2.58 (3H, s, $COCH_3$), 1.96 (3H, s, $ArCH_3$). ^{13}C-NMR (125 MHz, $CDCl_3$) δ ppm: 202.8 (C=O), 164.4 (C-6), 162.7 (C-4), 161.4 (C-2), 104.5 (C-1), 103.3 (C-3), 89.6 (C-5), 54.4 (6-OCH_3), 31.7 (1-$COCH_3$), 5.87 (3-CH_3). The ^1H and ^{13}C NMR data were in agreement with the reported data [27, 28].

Jolkinolide A (**2**). Colorless needle. EI-MS m/z: 314 [M^+] (15), 271 (10), 176 (100), 160 (37), 95 (58), 81 (62), 69 (100). ^1H-NMR (500MHz, $CDCl_3$) δ ppm: 5.45 (1H, d, *J* = 4.6 Hz, H-11), 3.72 (1H, s, H-14), 2.63 (1H, d, *J* = 4.6 Hz, H-9), 2.05 (3H, s, H-17), 0.94 (3H, s, H-18), 0.85 (3H, s, H-19), 0.71 (3H, s, H-20). ^{13}C-NMR (125 MHz, $CDCl_3$) δ ppm: 170.0 (C-16), 147.5 (C-12), 145.0 (C-13), 125.1 (C-15), 104.0 (C-11), 61.1 (C-8), 54.4 (C-14), 53.4 (C-5), 51.8 (C-9), 41.5 (C-3), 41.4 (C-10), 39.8 (C-1), 34.1 (C-7), 33.5 (C-4), 33.4 (C-18), 21.9 (C-19), 20.8 (C-6), 18.4 (C-2), 14.9 (C-20), 8.66 (C-17). The ^1H and ^{13}C NMR data were in agreement with the reported data [28].

Jolkinolide B (3). White needle-like crystals. EI-MS m/z (%): 329 [M^+] (4), 306 (14), 193 (30), 177 (36), 164 (38), 149 (40), 148 (98), 141 (72), 109 (94), 96 (100), 69 (43). ^1H-NMR (500 MHz) 4.07 (s, 1H, H-11), 3.71 (s, 1H, H-14), 2.32 (s, 1H, H-9), 2.11 (s, 3H, H-17), 0.97 (s, 3H, H-18), 0.88 (s, 3H, H-19), 0.85 (s, 3H, H-20). ^{13}C-NMR (125 MHz) 169.0 (C-16), 148.6 (C-13), 130.2 (C-15), 85.2 (C-12), 66.0 (C-8), 60.9 (C-11), 55.3 (C-14), 53.4 (C-5), 48.0 (C-9), 41.2 (C-3), 39.2 (C-10), 39.1 (C-1), 35.6 (C-7), 33.5 (C-4), 33.4 (C-18), 21.8 (C-19), 20.8 (C-6), 18.4 (C-2), 15.4 (C-20), 8.75 (C-17). The ^1H- and ^{13}C-NMR data were in agreement with the reported data [20].

12-Deoxyphorbol 13-(9Z)-octadecenoate 20-acetate (**4**). White powder ($CHCl_3$), EI-MS m/z (%): 654 [M^+] (3), 337 (24), 311 (22), 313 (35), 239 (23), 108 (26), 97 (67), 69 (63), 55 (100). ^1H-NMR (500 MHz) 7.63 (1H, s, H-1), 5.73 (1H, d, *J* = 4.1Hz, H-7), 5.36 (2H, m, H-9' and H-10'), 4.48 (2H, ABq, *J* = 12.3, 7.3 Hz, H-20), 3.30 (1H, brd, *J* = 2.2 Hz, H-10), 3.02 (1H, t, *J* = 5.1 Hz, H-8), 2.02 (3H, s, OAc-20). ^{13}C-NMR (125 MHz) 209.1 (C-3), 161.4 (C-1), 135.0 (C-6), 133.8 (C-7), 132.8 (C-2), 75.9 (C-9), 73.6 (C-4), 69.4 (C-20), 63.2 (C-13), 55.7 (C-10), 39.4 (C-8), 38.9 (C-5), 36.3 (C-11), 32.4 (C-14), 31.9 (C-12), 23.2 (C-16), 22.6 (C-15), 18.5 (C-18), 15.3 (C-17), 10.0 (C-19), 175.9 (C-1'), 34.2 (C-2'), 24.9 (C-3'), 28.9-29.8 (C-4'-C-8' and C-12'-C-15'), 31.8 (C-16'), 22.6 (C-17'), 14.1 (C-18'), 173.4 and 21.2 (OAc-20). The ^1H- and ^{13}C-NMR data were in agreement with the reported data [20, 31]. *17-Hydroxyjolkinolide A* (**5**). White needle-like crystals. EI-MS m/z (%): 329 [M^+] (5), 175 (58), 173 (100), 162 (24), 94 (38), 78 (45), 68 (53), 54 (42), 40 (55). ^1H-NMR (500 MHz) 5.60 (d, *J* = 4.6 Hz, 1H, H-11), 4.05 (s, 1H, H-14), 2.68 (d, *J* = 4.6 Hz, 1H, H-9), 4.65 (s, 2H, H-17), 0.97 (s, 3H, H-18), 0.88 (s, 3H, H-19), 0.76 (s, 3H, H-20). ^{13}C-NMR (125 MHz) 169.2 (C-16), 147.3 (C-12), 146.6 (C-13), 127.3 (C-15), 106.5 (C-11), 61.3 (C-8), 56.5 (C-17), 54.4 (C-14), 53.5 (C-5), 51.8 (C-9), 41.5 (C-3), 41.3 (C-10), 39.9 (C-1), 34.0 (C-7), 33.6 (C-4),

33.4 (C-18), 21.9 (C-19), 20.8 (C-6), 18.4 (C-2), 14.1 (C-20). The ^{1}H- and ^{13}C-NMR data were in agreement with the reported data [20].

17-Hydroxyjolkinolide B (**6**). White needle-like crystals. EI-MS m/z (%): 346 [M^{+}] (3), 257 (12), 114 (32), 104 (38), 94 (43), 90 (66), 78 (63), 68 (65), 54 (82), 40 (100). ^{1}H-NMR (500 MHz) 4.70 (d, *J* = 12.0 Hz, 2H, H-17), 4.14 (s, 1H, H-14), 4.09 (s, 1H, H-11), 2.32 (s, 1H, H-9), 0.96 (s, 3H, H-18), 0.88 (s, 3H, H-19), 0.88 (s, 3H, H-20). ^{13}C-NMR (125 MHz) 168.1 (C-16), 151.0 (C-13), 132.9 (C-15), 85.4 (C-12), 66.7 (C-8), 61.5 (C-11), 56.5 (C-17), 55.2 (C-14), 53.5 (C-5), 47.8 (C-9), 41.2 (C-3), 39.2 (C-10), 39.1 (C-1), 35.6 (C-7), 33.5 (C-4), 33.4 (C-18), 21.8 (C-19), 20.8 (C-6), 18.4 (C-2), 15.5 (C-20). The ^{1}H- and ^{13}C-NMR data were in agreement with the reported data [20].

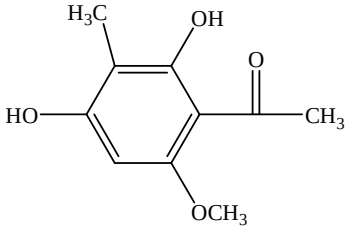
3.3. Nematicidal activity

Compound 4 and **1** exhibited strong nematicidal activity against the root-knot nematode with LC_{50} values of 48.6 µg/ml and 87.8 µg/ml, respectively while the ethanol extract of *E. fischeriana* roots had a LC_{50} value of 59.3 µg/ml (Table 2). When compared with the positive control, carbofuran (LC_{50} = 72.3 µg/ml) [12], compound 4 and the crude ethanol extract showed a little more potent activity and compound 1 had the same level of toxicity against the root-knot nematode. It is suggested that the activity of the crude ethanol extract of *E. fischeriana* roots against the root-knot nematode was mainly attributed to compound **4** and **1**. The other three compounds, compound **2** (LC_{50} = 627.7 µg/ml), compound **5** (LC_{50} = 354.6 µg/ml) and compound **6** (LC_{50} = 539.4 µg/ml) also possessed toxicity against the root-knot nematode. Among the six isolated constituents, compound **3** exhibited the weakest toxicity against the root-knot nematode with a LC_{50} value of 825.2 µg/ml (Table 2). In previous studies [47, 48], several ingenane diterpenes derived from *Euphorbia kansui* were found to exhibit nematicidal activity against the pine wood nematode (*Bursaphelenchus xylophilus*). There were no reports on nematicidal activity of the isolated compounds against nematodes so far. The above finding suggest that nematicidal activities of the crude extract of *E. fischeriana* roots and the isolated compounds, especially compound **4** and **1** are quite promising and they show potential for development as novel natural nematicide for the control of nematodes by considering the currently used nematicides are synthetic and usually have strong toxicity against mammals.

Among the five isolated diterpenoids (Figure 1), compound 4 belongs to tigliane types and the four others belong to abietane types. It seems that tigliane types of diterpenoids exhibit stronger nematicidal activity against the root-knot nematode because the weakest nematicidal diterpenoid, compound **3** was almost 17 times less active than compound **4**. Moreover, among abietane types of diterpenoids, group 17-hydroxy (Figure 1) seems to increase nematicidal activity because compound **5** exhibited stronger activity than compound **2** and compound **6** also possessed stronger nematicidal activity than compound **3**. In traditional Chinese medicine, the roots of *E. fischeriana* have been used for the treatment of a wide range of ailments, including edema, ascites, ingestion, as well as liver and lung cancer [16]. However, no experimental data about the safety of extracts of this medicinal herb and the six isolated constituents is available so far. Therefore, any attempt to develop an *E. fischeriana*-derived agrochemical must be carefully evaluated for harmful effects. Further studies will be extended to evaluate the mode of action of the nematicidal principles as well as the practical challenge of their integration in phytonematode management practices. Moreover, further studies on the safety of the isolated compounds to humans and on development of formulations are necessary to improve the efficacy and stability and to reduce cost.

Table 2: Nematicidal activity of the compounds isolated from *Euphorbia fischeriana* roots against *Meloidogyne incognita*

Treatments	Concentrations (µg/ml)	LC_{50} (µg/ml)	95% fiducial limits	Chi square (χ^2)
Compound 1	10.0-200.0	87.76	71.31- 85.37	11.87
Compound 2	250.0-4000.0	627.73	566.44 - 691.93	16.16
Compound 3	160.0-2000.0	825.20	739.17 - 908.72	12.04
Compound 4	4.0-100.0	48.64	43.96 - 53.86	7.95
Compound 5	100.0-1500.0	354.60	321.45 - 389.22	17.65
Compound 6	100.0-1500.0	539.41	578.42 - 584.30	12.39
Carbofuran*	-	72.29	-	-
E. fischeriana	4.0-100.0	59.03	54.61 - 66.56	9.56



1-(2,4-dihydroxy-6-methoxy-3-methylphenyl)-ethanone (**1**)

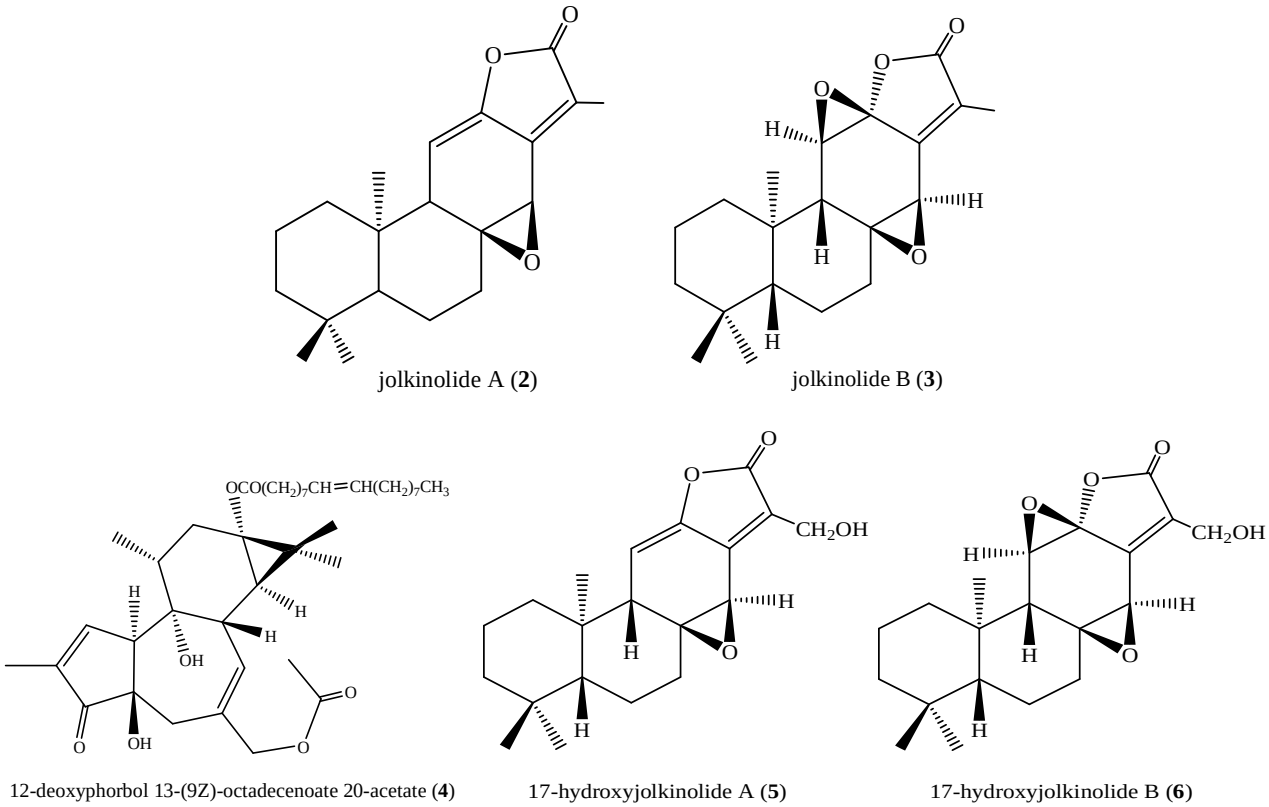


Fig 1: Nematicidal constituents isolated from *Euphorbia fischeriana* roots.

4. Conclusions

Ethanol extracts of 20 species of Euphorbiaceae plants have been evaluated for nematicidal activity against *M. incognita*. The extracts of *C. tiglium*, *E. fischeriana*, *L. chinensis* and *R. communis* exhibited strong nematicidal activity in mass screening bioassay. Based on bioactivity-directed fractionation, six compounds were isolated and identified from *E. fischeriana*. Compared with carbofuran, compound **4** and the crude extract exhibited stronger toxicity and compound **1** had the same level of toxicity against the root-knot nematode. These findings suggest that the extracts and the isolated compounds have potential for development as novel nematicides for the control of the root-knot nematodes. Further studies are needed to evaluate the safety of the extracts and isolated compounds to humans.

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