Nematicidal Effects of Juniperus chinensis Leaf Extracts on the Pine Wood Nematode (Bursaphelenchus xylophilus)

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Abstract

Pine wood nematode (PWN), *Bursaphelenchus xylophilus*, as a plant parasitic nematode, can induce pine wilt disease (PWD) which is a highly destructive forest disease causing huge losses to the global ecological environment and economy. Therefore, it has been a increasingly hot topic to develop eco-friendly and efficient nematicides against PWNs. In this study, three nematicidal compounds were isolated from the *n*-hexane extract of *Juniperus chinensis* leaves through bioactivity-guided fractionation, and identified as abietic acid, 8*α*,11-elemadiol and parabenzlactone by mass spectrometry and nuclear magnetic resonance (NMR) spectroscopy. By the assays on PWN vitality, all the three compounds were confirmed to have inhibitory effect on the motility, oviposition, fecundity, feeding, and development of PWNs, especially, parabenzlactone demonstrated the most pronounced efficacy. The bioassays on PWN pathogenicity showed that all the three compounds alleviated wilting symptoms of Japanese black pine seedlings caused by PWNs. These findings indicated that the three compounds from *J. chinensis* leaves have potential utility in controlling PWNs, providing valuable insights for the development of novel nematicidal agents.

1. Introduction

The pine wood nematode (PWN, *Bursaphelenchus xylophilus*) is a plant-parasitic nematode which infects various species of pine trees, such as black pine (*Pinus thunbergii*), red pine (*Pinus densiflora*) and korean pine (*Pinus koraiensis*) et al, causing pine wilt disease (PWD) (Aikawa et al. 2003; Akiba et al. 2012). PWD, as a devastating forest disease, has the characteristics of high mortality, wide vector transmission, complex pathogenic mechanism, and difficulty in control, which has inflicted substantial losses on the global forest resource and ecological environment (Vicente et al. 2012; Ju et al. 2016; Chen and Song 2021; Pires et al. 2022; Dai et al. 2023; Gao et al. 2024). Herein, effective and sustainable measures for PWD management are greatly needed.

Among the various existing methods for the prevention and control of PWD, chemical methods that are to eliminate PWNs or their vector insects by aerial spraying or injecting pesticides to achieve the purpose of curing the infected pine, are the most commonly used (Takai et al. 2003; Hao et al. 2023; Wang et al. 2024). Especially, trunk injection of nematicides against PWN is considered one of the most effective and sustainable strategies to control PWD owing to its less adverse effect on environment than spraying pesticides (Takai et al. 2003; Faria et al. 2021; Kim at al. 2024). However, frequent utilization of chemically synthesized nematicides exhibits drug resistance, toxicity to non-target organisms even loss of biodiversity, persistent residues, and potential risks to human health (Zhang et al. 2023; Shehata et al. 2024). Accordingly, developing eco-friendly nematicides for PWN control is urgent. Natural plants contain a large number of bioactive compounds (Shin et al. 2019; Gan et al. 2021; Seo et al. 2021), and dozens of plant secondary metabolites have been confirmed to exhibit significant activity against PWNs (Guo et al. 2016; Rajasekharan et al. 2020; Lee et al. 2022; Feng et al. 2023a; Feng et al. 2023b). These active substances possess the potential to be developed into ideal nematicides or serve as lead compounds for the synthesis of high-efficiency nematicidal agents (Guo et al. 2016; Chen and Song 2021; Lee et al. 2022).

Juniperus chinensis as an evergreen tree native to China is widely distributed in East Asia (Park et al. 2018). The branches and leaves of *J. chinensis* were reported to have a variety of unique medicinal effects, such as antibacterial, anti-inflammatory, anti-tumor, and anti-diabetes activities (Keskes et al. 2014; Kim et al. 2016; Khamis and Chai 2021). In this research, we found the *n*-hexane extract of *J. chinensis* leaves was nematicidal against PWNs for the first time. The main nematicidal constituents were obtained and their effects on the motility, oviposition, hatching, fecundity, feeding, development, and pathogenicity of PWNs were investigated, which can provide valuable clues for developing environment-friendly plant-derived nematicidal agents.

2. Materials and methods

2.1 Materials and Instruments

J. chinensis leaves were collected from the campus of Qingdao University, Qingdao City, Shandong Province, China (36°7'47" N, 120°42'92" E). The silica gel (200–300 mesh) utilized for column chromatography was purchased from Qingdao Ocean Chemical Co., Ltd. (Qingdao, China). The PWNs isolated from black pine infected with PWD in Yantai City, Shandong Province, China (37°11'54" N, 121°41'36" E), by using the Bellman funnel method (Beane and Hobbs 1983) were inoculated on *Botrytis cinerea* in potato dextrose agar (PDA) medium and cultured in the dark at 25 ^{o}C. PWNs were observed under a stereomicroscope (Motic BA200, Xiamen, China).

2.2 Isolation and identification of Nematicidal Compounds

Fresh *J. chinensis* leaves (200 g) were finely minced and extracted with absolute ethanol (1 L×2) for 24 h by the aid of intermittent ultrasonic oscillation, and then was successively partitioned using equal volumes of n-hexane and ethyl acetate to yield 1.68 g of n-hexane extract, 0.36 g of ethyl acetate extract, and 7.90 g of aqueous extract from *J. chinensis* leaves.

The *n*-hexane extract (1.2 g) of *J. chinensis* leaves with nematicidal activity based on the bioassays was fractionated by silica gel column chromatography. Seventeen fractions, Fr.1-Fr.17, were obtained by stepwise gradient elution of *n*-hexane-ethyl acetate (9:1, 4:1, 3:1, 3:2 and 2:3, *v/v*). Fr.7, Fr.11, and Fr.12 confirmed to have significant nematicidal activity were further purified via preparative thin layer chromatography by using *n*-hexane-ethyl acetate solution (1:1, *v/v*) as developing agent, respectively, to yield compound *1* (34.1 mg), compound *2* (10.6 mg) and compound *3* (4.3 mg).

The chemical structures of compounds *1–3* were identified using a nuclear magnetic resonance (NMR) spectrometer (Avance-Neo-500, Brucker, Karlsruhe, Germany) operating at 500 MHz for ^{1}H NMR and 125 MHz for ^{13}C NMR with tetramethylsilane (TMS) as the internal standard and chloroform-d ($CDCl_3$) as the solvent. Additionally, electron impact mass spectrometry (EI-MS) was measured using gas chromatography-mass spectrometry (GC-MS, Agilent 7890A-5975C, Santa Clara, CA, USA).

2.3 Nematicidal assays against PWNs

The nematicidal activity against PWN of the extracts, chromatographed fractions and compounds *1–3* from *J. chinensis* leaves were separately tested using the drug impregnation method reporeted by Feng et al (2023b). PWNs were considered dead if they were rigid and did not respond to stimulation with a fine needle (Seo et al. 2010; Zhang et al. 2011). The corrected mortality of PWNs were calculated according to the following formula (Sun et al. 2022).

$$\text{Corrected mortality\%} = (\text{Mortality\% in treatment} - \text{Mortality\% in control}) / (1 - \text{Mortality\% in control})$$

2.4 Nematicidal mechanism of the Compounds against PWNs

2.4.1 Effect of Nematicidal Compounds on PWN Motility

PWNs were separately immersed in 0.5 mM abietic acid, 8*α*,11-elemadiol, and parabenzlactone solutions (5% DMSO aqueous solution containing 0.5% Triton X-100) in a 96-well plate with approximately 100 nematodes per well. Nematodes immersed in the background solvent served as the control. Each treatment was repeated three times. Following a 24-h incubation at 25 ^{o}C in the dark, 15 nematodes were randomly selected from each well, and the number of head thrashes per minute was recorded under a stereomicroscope.

2.4.2 Effect of Nematicidal Compounds on PWN Oviposition

The impact of nematicidal compounds on the oviposition of PWNs was evaluated following the method of Cheng et al (2017). 20 pairs of adult nematodes were carefully selected and transferred to 48-well plates. Subsequently, 0.5 mM of each aforementioned nematicidal compound solution was separately introduced, along with an appropriate quantity of *B. cinerea* mycelia as nutrient source. Nematodes were immersed in the background solvent served as the control. All treatments were replicated three times. Following a 24-h incubation at 25 ^{o}C in darkness, the number of eggs was recorded using a stereomicroscope.

2.4.3 Effect of Nematicidal Compounds on PWN Egg Hatching

The PWN eggs were obtained following the methodology described by Liu et al (2019). Approximately 100 eggs were individually transferred into the designated wells of a 96-well plate containing the aforementioned nematicidal compound solutions. Eggs immersed in the background solvent were used as the control. Each treatment was replicated three times. The plates were incubated at 25 ^{o}C for 24 h in darkness. Subsequently, the number of hatched nematodes was counted under a stereomicroscope. Hatching rates were calculated according to the formula below.

Hatching rate%=larva/(larva + egg) × 100%

2.4.4 Effect of Nematicidal Compounds on PWN Fecundity

Approximately 200 PWNs were individually transferred into the designated wells of a 96-well plate containing the different nematicidal compound solution. Each treatment was replicated three times. The nematodes of each group were transferred to PDA Petri dishes fully covered with *B. cinerea* and cultured for 8 d, then were isolated and washed three times with sterile water. The number of nematodes was counted under a stereomicroscope.

2.4.5 Effect of Nematicidal Compounds on PWN Feeding

Approximately 200 PWNs were treated as described in section 2.4.4. Nematodes immersed in the background solvent served as the control. Each treatment was replicated three times. After a 24-h incubation at 25 ^{o}C in the dark, the nematodes from each group were washed three times with sterile water, then transferred to PDA Petri dishes fully covered with *B. cinerea* and cultured under the same conditions. The feeding of the PWNs was continuously monitored.

2.4.6 Effect of Nematicidal Compounds on the Development of PWNs

The development of the PWN involves six distinct stages: egg, J1, J2, J3, J4, and adult (Tanaka et al. 2019). To evaluate the impact of the nematicidal compounds on nematode development, approximately 100 J2 larvae were separately exposed to the different nematicidal compound solution (0.5 mM) in the designated wells of a 96-well plate with the appropriate mycelia of *B. cinerea* as nutrient source. J2 larvae immersed in the background solvent were used as the control. Each treatment was replicated three times. The 96-well plate was incubated at 25 ^{o}C in the dark for 48 h. Subsequently, the nematodes at each developmental stage (J2, J3, J4) was quantified using a stereomicroscope.

2.5 Effect of Nematicidal Compounds on PWN Pathogenicity

Sterile 60-d-old black pine seedlings were obtained according to the method described by Sun et al (2022). 10 μL of nematode suspension (30 PWNs/μL) was introduced into pine seedlings using a syringe. On the third day post-inoculation, 10 μL of each 5 mM nematicidal compound solution was separately injected into pine seedlings by syringe. For the control group, 10 μL of the background solvent was injected. Each treatment was repeated four times.

2.6 Statistical Analysis

Corrected mortality data were calculated in Microsoft Excel. Median lethal concentration (LC_{50}) values were determined through probit analysis using SPSS 26.0 (IBM SPSS Statistics, Chicago, IL, USA). Significant differences among treatment means were assessed using Student's t-tests conducted in GraphPad Prism 10.3.0 (GraphPad Software, San Diego, CA, USA).

3. Results

3.1 Nematicidal activity of *J. chinensis* leaf extracts

The corrected mortality of the *n*-hexane extract, ethyl acetate extract, and aqueous extract of *J. chinensis* leaves were 65.85%, 24.67%, and 6.89%, respectively, at 1 mg/mL in 72 h (Table 1). The *n*-hexane extract demonstrated significant activity against PWNs, whereas the ethyl acetate and aqueous extracts exhibited much lower activity.

Table 1
Nematicidal activities of the *J. chinensis* leaf extracts

Samples (1mg/mL)	**Corrected mortality (%, mean ± SD)***		
	24 h	**48 h**	**72 h**
N-hexane extract	41.58 ± 0.80^{a}	54.36 ± 0.52^{a}	65.85 ± 1.61^{a}
Ethyl acetate extract	1.67 ± 0.30^{b}	18.42 ± 1.28^{b}	24.67 ± 0.92^{b}
Aqueous fraction	1.40 ± 0.73^{b}	2.29 ± 1.10^{c}	6.89 ± 1.20^{c}

*Means in the column followed by the same letter did not differ significantly (($p \leq 0.05$) according to the LSD test.

Among the 17 fractions obtained by column chromatography from the *n*-hexane extract of *J. chinensis* leaves, Fr.7, Fr.11, and Fr.12 had prominent nematicidal activity with the corrected mortality against PWNs of 66.13%, 69.59%, and 70.43%, respectively, at 500 μg/mL in 72 h (Table 2).

Table 2
Nematicidal activities of chromatographed fractions from *n*-hexane extracts of *J. chinensis* leaves

Samples (500μg/mL)	Corrected mortality (%, mean ± SD)*		
	24 h	**48 h**	**72 h**
Fr.1	3.40 ± 0.43^{def}	6.39 ± 1.13^{g}	13.45 ± 0.53^{gh}
Fr.2	11.82 ± 1.75^{c}	13.43 ± 0.22^{ef}	23.16 ± 1.93^{de}
Fr.3	2.15 ± 0.27^{ef}	2.71 ± 0.14^{g}	5.57 ± 0.11^{i}
Fr.4	1.99 ± 0.15^{ef}	3.23 ± 0.19^{g}	4.98 ± 1.41^{i}
Fr.5	7.27 ± 0.51^{cde}	8.83 ± 0.75f^{g}	12.57 ± 0.96^{h}
Fr.6	2.65 ± 0.38^{def}	15.52 ± 0.95^{de}	31.46 ± 0.25^{bc}
Fr.7	45.44 ± 0.77^{a}	62.49 ± 1.50^{a}	66.13 ± 1.82^{a}
Fr.8	5.17 ± 0.87^{def}	13.61 ± 1.23^{ef}	23.03 ± 0.15^{de}
Fr.9	4.35 ± 1.37^{def}	14.57 ± 2.09^{def}	18.50 ± 1.18^{ef}
Fr.10	8.71 ± 1.17^{cd}	21.01 ± 0.94^{cd}	27.59 ± 1.84^{cd}
Fr.11	32.24 ± 3.46^{b}	58.38 ± 3.47^{a}	69.59 ± 1.24^{a}
Fr.12	34.92 ± 3.83^{b}	57.19 ± 4.71^{a}	70.43 ± 1.72^{a}
Fr.13	3.76 ± 0.56^{def}	16.03 ± 0.49^{de}	18.07 ± 1.13^{fg}
Fr.14	4.56 ± 0.11^{def}	32.38 ± 2.51^{b}	33.93 ± 2.06^{b}
Fr.15	6.12 ± 0.75^{de}	15.65 ± 1.24^{de}	19.55 ± 1.11^{ef}
Fr.16	4.45 ± 0.38^{def}	23.30 ± 0.50^{c}	25.08 ± 0.12^{d}
Fr.17	0.52 ± 0.02^{f}	3.13 ± 0.38^{g}	4.66 ± 0.79^{i}

*Means in the column followed by the same letter did not differ significantly (($p \leq 0.05$) according to the LSD test.

3.2 Identification of Nematicidal Compounds

Based on the MS and NMR spectral data (Figure S1-S3), the three isolated compounds *1–3* were identified as abietic acid, 8*α*,11-elemadiol and parabenzlactone (Fig. 1).

Compound *1*, yellow crystal (*n*-hexane). EI-MS *m/z*: 302.45. ^{1}H NMR (500 MHz, $CDCl_3$, δ): 0.83 (3H, s, 20-CH_3), 1.02 (6H, d, J = 5.36, 16, 17-CH_3), 1.13–1.15 (2H, m, 1-CH_2), 1.26 (3H, s, 19-CH_3), 1.57–1.59 (2H, m, 2-CH_2), 1.77–1.80 (4H, m, 3, 11-CH_2), 1.87–1.90 (2H, m, 6-CH_2), 1.93 (1H, 9-CH), 2.07–2.10 (3H, m, 5-CH, 12-CH_2), 2.17–2.22 (1H, m, 15-CH), 5.35–5.40 (1H, m, 7-CH), 5.77 (1H, s, 14-CH); ^{13}C NMR (125 MHz, $CDCl_3$, δ): 14.04 (C-20), 16.78 (19-C), 18.06 (2-C), 20.88 (16-C), 21.43 (17-C), 22.48 (11-C), 25.63 (6-C), 27.46 (12-C), 34.49 (10-C), 34.89 (15-C), 37.20 (3-C), 38.28 (1-C), 44.93 (5-C), 46.29 (4-C), 50.94 (9-C), 120.48 (7-C), 122.38 (14-C), 135.61 (8-C), 145.37 (13-C), 183.64 (COOH). The MS and NMR spectral data of Compound 1 were consistent with those of abietic acid reported in the literature (He et al. 2012), thereby it was confirmed as abietic acid (Fig. 1a).

Compound *2*, colorless oil liquid. EI-MS *m/z*: 238.37. ^{1}H NMR (500 MHz, $CDCl_3$, δ): 1.08 (3H, s, 14-H), 1.21 (3H, s, 12-H), 1.25 (3H, s, 13-H), 1.28 (1H, q, J = 12.2, 6b-H), 1.45–1.51 (3H, m, 6a-H, 7a-H, 9a-H), 1.60–1.65 (1H, m, 9b-H), 1.71 (3H, s, 15-H), 2.04 (J = 3.1, 13.7, 5a-H), 4.11–4.15 (1H, m, 8b-H), 4.61 (1H, s, 3a-H), 4.85–4.93 (3H, 3b-H, 2a-H, 2b-H), 5.77 (1H, dd, J = 10.9, 17.4, 1-H); ^{13}C NMR (125 MHz, $CDCl_3$, δ): 17.50 (CH_3, C-14), 24.68 (CH_3, C-12), 26.17 (CH_3, C-15), 28.58 (CH_2, C-6), 29.10 (CH_3, C-13), 41.10 (C, C-10), 44.79 (CH_2, C-9), 52.09 (CH, C-7), 52.15 (CH, C-5), 72.80 (CH, C-8), 76.72 (C, C-11), 110.84 (CH_2, C-3), 112.83 (CH_2, C-2), 146.26 (CH, C-4) and 148.29 (C, C-1). The MS and NMR data were consistent with the reported ones of 8*α*,11-elemadiol (Barrero et al. 2011). Compound 2 was identified as 8*α*,11-elemadiol (Fig. 1b).

Compound *3*, white powder. EI-MS *m/z*: 370.36. ^{1}H NMR (500 MHz, $CDCl_3$, δ): 2.32–2.36 (1H, m, H-8'), 2.60 (2H, d, J = 9.48 Hz, H-7), 2.97-3.00 (1H, m, H-8), 3.73 (2H, d, J = 7.76 Hz, H-9'), 4.24 (1H, d, J = 6.28 Hz, H-7'), 5.93 (2H, s, O-CH_2-O), 6.03 (2H, s, O-CH_2-O), 6.63 (1H, dd, J = 8.4, 1.00, H-6'), 6.73 (1H, d, J = 1.0, H-2'), 6.86–7.08 (4H, m, H-2, H-5, H-5', H-6); ^{13}C NMR (125 MHz, $CDCl_3$, δ): 32.02 (C-7), 37.63 (CH, C-8'), 40.02 (CH, C-8), 69.61 (CH_2, C-9'), 76.71 (CH, C-7'), 101.15 (OCH_2O), 101.84 (OCH_2O), 104.92 (CH, C-2'), 108.61 (CH, C-5), 108.76 (CH, C-5'), 109.27 (CH, C-2), 122.19 (CH, C-6'), 126.23 (CH, C-6), 131.58 (C-1), 137.44 (C-1'), 146.66 (C-4'), 148.05 (C-4), 148.45 (C, C-3'), 149.30 (C-3), 172.70 (C-9). Based on the reported spectral data (Arruda et al. 2018), compound 3 was identified as parabenzlactone (Fig. 1c).

3.3 Nematicidal Activity of the Compounds

The three compounds, abietic acid, 8*α*,11-elemadiol, and parabenzlactone isolated from *J. chinensis* leaves, were testified to have significant nematicidal activity against PWN with the LC_{50} values of 1.77 mM, 1.32 mM, and 1.30 mM, respectively, in 24 h (Table 3). Notably, parabenzlactone showed the strongest nematicidal activity.

Table 3
LC_{50} values of the compounds isolated from *Juniperus chinensis* leaves against PWNs at 24 h

Compound	LC_{50} (mM)	Confidence Interval(mM)	χ^2
Abietic acid	1.77	1.54–2.77	0.30
8α,11-Elemadiol	1.32	0.75–1.51	0.23
Parabenzlactone	1.30	1.13–2.43	0.61

3.4 Nematicidal mechanism of the Compounds against PWNs

3.4.1 Effects of three nematicidal compounds on PWN motility, oviposition, hatching, fecundity

The thrashing frequencies of PWNs exposed to abietic acid, 8α,11-elemadiol, and parabenzlactone with 39.85, 27.77, and 36.69 thrashes per minute, respectively) were much lower than that of control with 62.38 thrashes per minute (Fig. 2a). It was indicated that the three nematicidal compounds significantly reduced the motility of PWNs, and compound 8α11-elemadiol showed the strongest inhibitory effect.

Following the treatment of 20 pairs of PWNs with nematicidal compounds, the number of eggs oviposited within 24 h in the control and experimental groups was counted (Fig. 2b). The average number of eggs oviposited in 24 h was 146 in the control group, while it was 125, 90, and 53 in the abietic acid, 8α,11-elemadiol, and parabenzlactone groups, respectively. The oviposition rate of PWNs in the experimental group was significantly lower than that in the control group, and the parabenzlactone group had the lowest oviposition rate, indicating that the three nematocida compounds had obvious inhibitory effects on the oviposition of PWNs, and parabenzlactone had the strongest inhibitory effect on the oviposition of PWNs.

Based on the egg hatching assays, the hatching rates for eggs treated with abietic acid, 8α,11-elemadiol, and parabenzlactone were 89.31%, 25.01%, and 39.73%, respectively, and the hatching rate of the control group was 83.97% within 24 h. (Fig. 2c). The results indicated that compounds 8α,11-elemadiol and parabenzlactone significantly reduced hatching rate of PWN eggs, while abietic acid had no significant effect on hatching of PWN eggs. Especially, 8α,11-elemadiol had the greatest effect on the hatching of PWN eggs.

On the eighth day of incubation, nematodes from both the control and treatment groups were isolated and counted (Fig. 2d). 4950, 7875, and 2590 nematodes were isolated from the abietic acid, 8α,11-elemadiol, and parabenzlactone groups, respectively, in contrast, 10403 nematodes were obtained from the control group. The results demonstrated that all three compounds significantly decreased the reproductive capacity of PWNs, and parabenzlactone exhibited the highest effect.

3.4.2 Effect of Nematicidal Compounds on PWN Feeding

The feeding situation of PWNs in each group was continuously observed (Fig. 3). On the sixth day post-inoculation, the *B. cinerea* mycelia in the control group had been significantly consumed by PWNs, while the mycelia in the experimental group had little change. On the eighth day, the *B. Cinerea* mycelia in the control group had been completely consumed by the nematodes, whereas the mycelia still remained in the treatment groups, with the parabenzlactone group exhibiting the most residual. The results suggested that the feeding activity of PWNs was markedly inhibited by the three compounds, and parabenzlactone showed strongest inhibitory effect.

3.4.3 Effect of Nematicidal Compounds on PWN Development

After 48 h of incubation, the number of PWNs at J2, J3, and J4 stages in each group was counted (Fig. 4). The majority of nematodes in the control group had developed to the J4 stage with the percentage of 51.72% in 48 h. In contrast, most nematodes in both the abietic acid and 8*α*,11-elemadiol groups remained at the J3 stage with the percentage of 55.34% and 52.48%, respectively, while those in the parabenzlactone group predominantly remained at the J2 stage with the percentage of 56.82%. These findings suggested that abietic acid and 8*α*,11-elemadiol mainly inhibited nematode development from the J3 to J4 stages, whereas parabenzlactone mostly inhibited development from the J2 to J3 and J4 stages. Collectively, all three compounds exhibited inhibitory effects on nematode development, with parabenzlactone demonstrating the highest inhibition.

3.5 Effect of Nematicidal Compounds on PWN Pathogenicity

The growth status of the black pine seedlings in each group was observed continuously (Fig. 5). On the 7th day post-inoculation, the seedlings in the control group began to wilt, and on the 15th day, the seedlings completely wilted. In contrast, the seedlings in the treatment groups all remained healthy during the entire experimental period. It was indicated that the three nematicidal compounds could effectively the PWN pathogenicity in black pine seedlings and had the potential to control PWD through trunk injection.

4. Discussion

PWD is currently the most serious forest disease in China, spreading rapidly and causing significant economic losses and ecological damage (Song et al. 2024). Developing eco-friendly and efficient nematicides against PWN is a promising strategy for PWD control. *J. chinensis*, an evergreen tree of the Cupressaceae family, is widely distributed across China with ready accessibility, providing its high value of exploring bioactive substances. In this study, the nematicidal activity of *n*-hexane extract of *J. chinensis* leaves was first testified. By bioactivity-guided separation, three nematicidal compounds, abietic acid, 8*α*,11-elemadiol, and parabenzlactone were obtained from *n*-hexane extracts of *J. chinensis* leaves. These three compounds showed inhibitory effects on motility, oviposition, fecundity, feeding, and development of PWNs, which could be essentially helpful to control the growth of PWN population. Coincidentally, the bioassays on black pine seedlings infected with PWNs demonstrated that the three compounds significantly alleviated the symptoms of wilting caused by PWN, indicating they effectively controlled PWN development and inhibited PWN pathogenicity in the seedlings. Among the three isolated compounds from *J. chinensis*, parabenzlactone with the strongest nematicidal actvity demonstrated the highest inhibitory effects on PWN oviposition, fecundity, feeding, and development, and 8*α*,11-elemadiol with moderate nematicidal actvity showed the most significant inhibitory effects on the motility and hatching of PWNs. Based on these findings, parabenzlactone and 8*α*,11-elemadiol had the potential as lead compound candidates for nematicidal drugs against PWN, especially parabenzlactone.

The functional group of a nematicidal compound is critical for its activity (Fawzia et al. 2013). Li and Zhang (2023) suggested that the hydroxyl group of terpenoids was key for the higher nematicidal activity according to the nematicidal efficacy evaluation on a series of monoterpenoids. 8*α*,11-elemadiol as a terpenoid contains two hydroxyl groups, which may be the key to its high nematicidal activity. Parabenzlactone with excellent anti-PWN effects has two 1,3-benzodioxole as its core structure, which may be related to its high nematicidal activity. In addition, the lactone moiety of parabenzlactone may also contribute to its nematicidal activity since some lactone compounds have been reported to show strong nematicidal activity against PWN (Wang et al. 2008; Liu et al. 2016). These findings provided valuable clews for the structural modification of nematicidal compounds and the development of high-efficiency nematicides against PWN.

5. Conclusion

The nematicidal activity of *n*-hexane extract of *J. chinensis* leaves was first found and three anti-PWN compounds, abietic acid, 8*α*,11-elemadiol and parabenzlactone were identified. All the three compounds could inhibit the motility, oviposition, fecundity, feeding, and development of PWNs. Specifically, 8*α*,11-elemadiol exhibited the strongest inhibitory effects on the movement and egg hatching of PWNs, and parabenzlactone demonstrated the most significant inhibition of oviposition, fecundity, feeding, and development. Based on PWN-infected pine seedling assays, the three compounds could effectively alleviate the wilting symptoms caused by PWNs. In summary, these compounds demonstrated potential for controlling PWNs and the development of novel, eco-friendly, and high-efficient nematicides.

Declarations

The authors declare no any conflict of interest.

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Author contributions

Conceptualization: Yandong Liu, Guicai Du, and Qunqun Guo; Methodology: Yandong Liu, Guicai Du, and Qunqun Guo; Formal analysis and investigation: Yandong Liu, Yujiang Sun, Lijiao Wang, Fan Wang, Xu Chen, Bing Zhang, and Yiting Han; Writing-original draft preparation: Yandong Liu; Writing-review and editing: Qunqun Guo and Guicai Du; Funding acquisition: Guicai Du and Qunqun Guo; Resources: Guicai Du and Qunqun Guo; Supervision: Guicai Du and Qunqun Guo.

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Figures

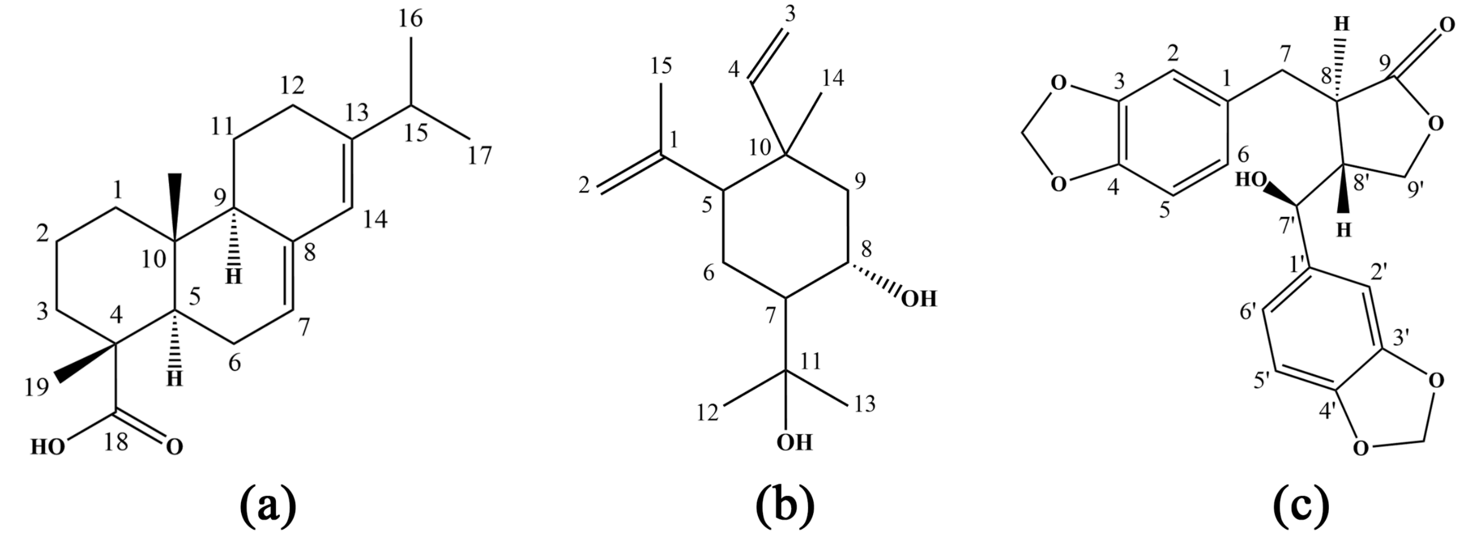

Figure 1

Chemical structures of abietic acid (a), 8*α*,11-elemadiol (b), and parabenzlactone (c)

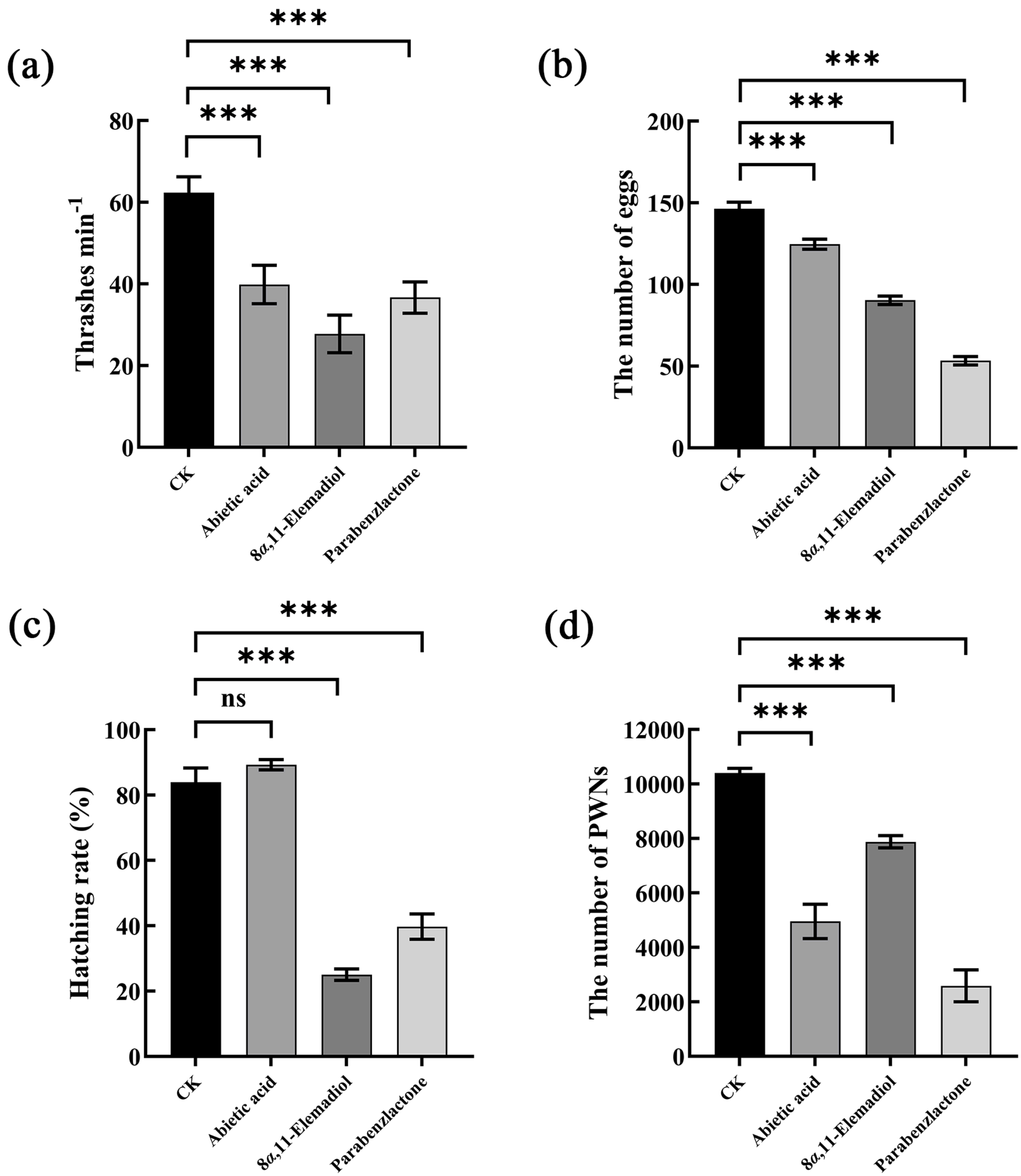

Figure 2

Effects of three nematicidal compounds on motility (a), oviposition (b), hatching (c), fecundity (d). Mean values of three replications were plotted with SD. *** represents significant difference at $p<0.001$, compared to control according to Student's t-test.

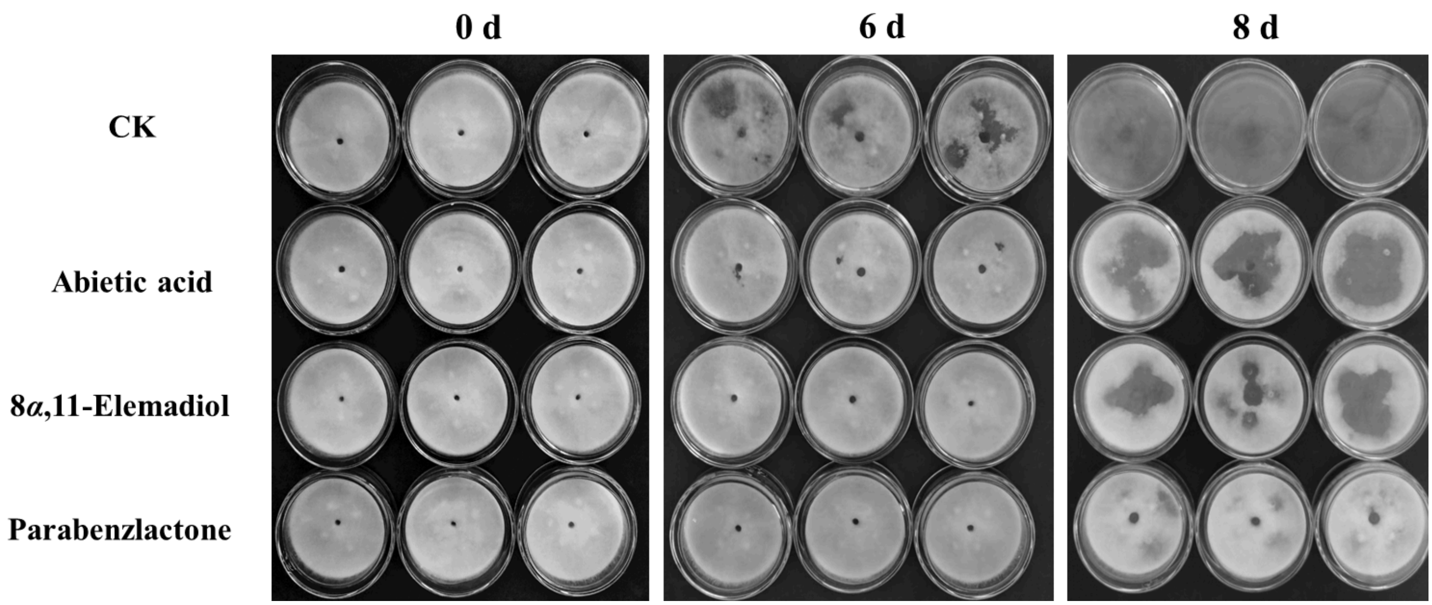

Figure 3

The feeding status of different groups of PWNs treated with 5% DMSO, abietic acid, 8α,11-elemadiol, and parabenzlactone for 24 h.

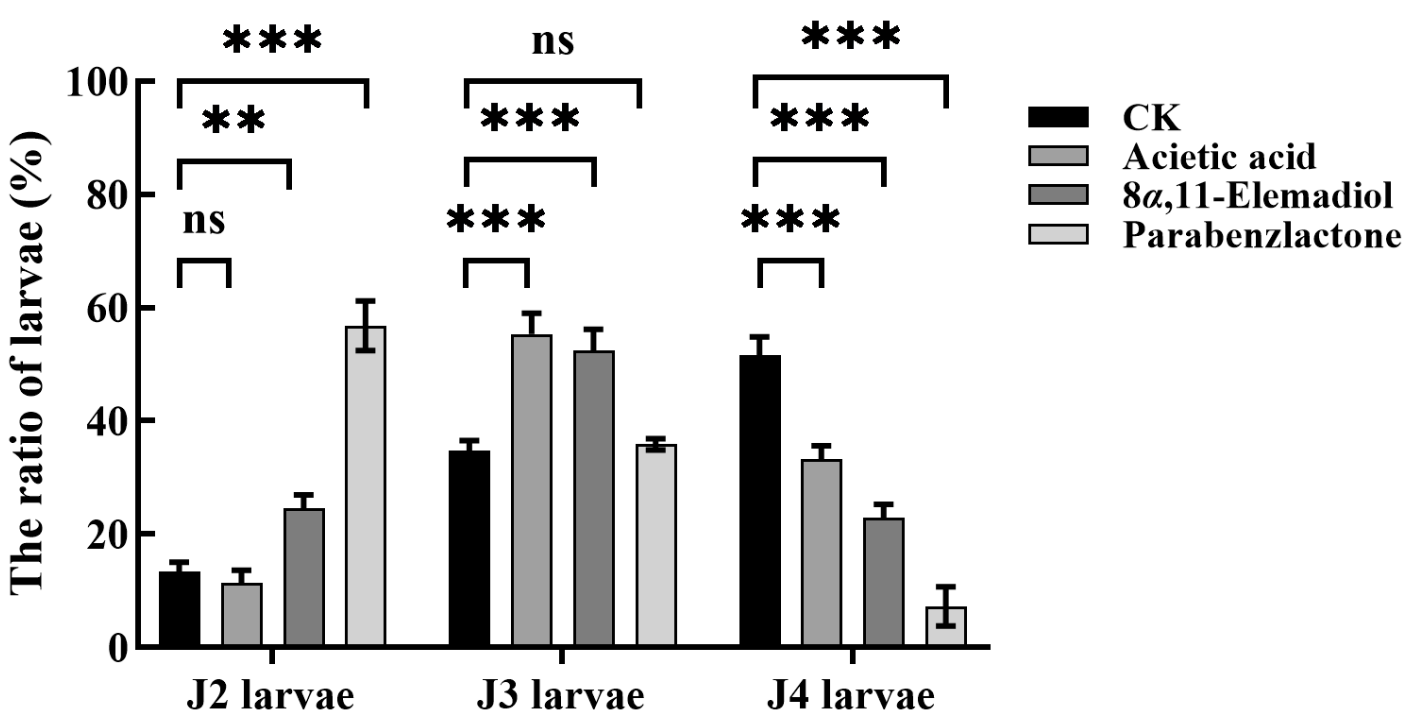

Figure 4

The percentage of PWNs at J2, J3, and J4 stage in different groups after 48 h. Mean values of three replications were plotted with SD. ** represents significant difference at $p<0.01$, *** represents significant difference at $p<0.001$, compared to control according to Student's t-test.

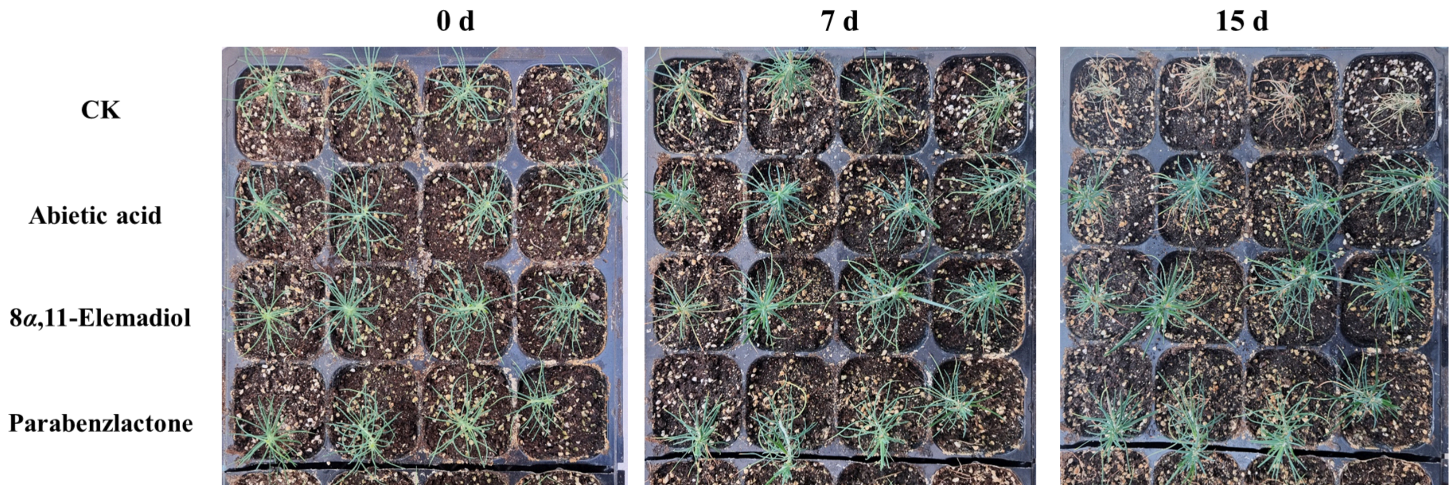

Figure 5

Effect of abietic acid, 8α,11-elemadiol, and parabenzlactone on black pine seedlings infected by PWNs.

Supplementary Files

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