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Study on the anti-nematode activity of extracts from 18 medicinal plants

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Abstract
The 96 well plate method was used to screen the anti nematode activity of 18 kinds of medicinal plant extracts, and the medicinal plant extracts with fatality rate of more than 90% were screened, and then the effects on different development stages of nematodes were explored. The results showed that the extract of *Melia azedarach* had the best anti-nematode activity and had a strong inhibitory effect on nematodes at different developmental stages. The inhibition rate of 10 mg/mL treatment on the hatching of nematode eggs and the fatality rate rate of larval nematode and adult nematode were 100%. However, the extracts of *Stellera chamaejasme*, *Thladiantha dubia* and *Houpoea officinalis* seed only have higher fatality rate to adult nematodes. Moreover, the extract of *Melia azedarach* has the strongest inhibitory effect on adult nematodes at different concentrations, and the weakest inhibitory effect on larval nematodes.

Keywords: Medicinal plants, extract, anti-nematode activity

Introduction
Nematodes, also known as worms, are mostly saprophytic in water and soil, with a few parasitic on humans, animals, and plants. Nematodes, as a major infectious pathogen that endangers plant growth, mainly parasitize vegetables, food crops, trees, fruit trees, and ornamental plants. Nematodes not only plunder the nutrients of the host plant, but also cause a series of pathological changes in the host plant and spread other pathogens (such as fungi, bacteria, etc.), causing secondary harm. Medicinal plants contain abundant anti nematode active ingredients. On the one hand, these nematicidal ingredients can be directly used as nematicides, on the other hand, they can be used as Lead compound to create new nematicides [1]. Compared to chemical nematicides, plant-based nematicides have less environmental impact, strong targeting, and are less likely to cause nematode resistance. Studying the anti nematode activity of medicinal plant extracts provides a foundation for the development of plant-based anti nematode agents, which is currently an important direction of research on nematode control [2].

Materials and Methods
1 Materials
1.1 Medicinal Plants
Coptis chinensis Franch Franch, *Cortex fraxini*, *Gentiana cruciata*, *Pulsatilla chinensis*, *Atractylodes macrocephala*, *Glycyrrhiza uralensis*, *Thradiantha dubia*, *Houpoea officinalis* seed, *Stellera chamaejasme*, *Chaenomes speciosa*, *Floral leaf*, *Melia azedarach*, *Lycium* seed, *Paederia foetida*, *Urtica fissa*, *Houttuynia cordata*, *Eleutherococcus senticosus senticosus* and *Portulaca oleracea*.

1.2 Test nematodes
Caenorhabditis elegans is provided by Caenorhabditis genetics Center (CGC).

1.3 Microbial strains
OP50 *Escherichia coli*

1.4 Main reagents
M9 buffer, tryptone, NaCl, agar powder, calcium chloride, magnesium sulfate, cholesterol, phosphate buffer, and purified water.

1.5 Main instruments
Microscope (Shanghai Batuo Instrument Co., Ltd., BTS-300V), 1/10000 electronic balance (Sedoris Scientific Instruments Co., Ltd., BSA224S-CW), Ultrasonic cleaner (Shanghai Kedao Ultrasonic Instrument Co., Ltd., SK3210LHC), Rotating evaporator (Shanghai Yarong Biochemical Instrument Factory, RE-52AA), Ultra clean workbench (Suzhou Purification Equipment Co., Ltd., SW-CJ-1F); Thermostatic incubator (Shanghai Yiheng Scientific Instrument Co., Ltd., BPH-9162).

2 Method
2.1 Preparation of extracts
18 medicinal plants were extracted using Soxhlet extraction method, with a material to liquid ratio of 1:15 and 70% ethanol added. After heating and refluxing for 6 hours, the solvent was evaporated to obtain the extract.

2.2 Preparation of test samples
Prepare 18 medicinal plant extracts into 20 mg/mL sample solutions for future use.

2.3 Activation and cultivation of nematodes
Take activated *Escherichia coli* (OP50) 80 μL was coated onto NGM medium and incubated at 22 ℃ for 24 hours. Use the tweezers after burning and cooling to draw square blocks of 1×1 cm from the purchased culture medium containing a large amount of *Caenorhabditis elegans* and transfer it to NGM medium containing *Escherichia coli* (OP50). Place the square block tightly between the surface containing the nematodes and the E. coli surface of the NGM medium. Incubate at 22 ℃ and regularly observe the growth of nematodes.

2.4 Preliminary screening for resistance to nematodes

Select a culture medium with fewer eggs and a more uniform distribution of nematodes in the larval and adult stages. Use a pipette to absorb a small amount of M9 buffer and gently rinse the nematodes on the culture medium into a beaker. Gently shake the beaker to evenly distribute the nematodes, then absorb 50 μL and place it in a 96 well plate, observe under a microscope whether the number of nematodes is between 20 and 50. Dilute the nematode solution according to the situation. Add 50 μL nematode solution to the 96 well plate and record the number of live worms, then add 50 μL the sample solution of medicinal plant extract (after double dilution, the concentration becomes 10 mg/mL). At 50 μL M9 buffer was used as a blank control, and each group was repeated three times. Incubate in the 22 ℃ incubator for 24 hours, observe and record the number of dead nematodes, calculate the fatality rate of nematodes and the corrected mortality rate of nematodes, and screen out the extracts of medicinal plants with a mortality rate of more than 90%.

$$\text{Nematode mortality} = \frac{\text{Nematode death}}{\textit{The total nematodes}} \times 100\%$$

$$\text{Nematode corrected mortality} = \frac{\text{Handing nematode mortality} - \text{Control nematode mortality}}{1 - \textit{Control nematode mortality}} \times 100\%$$

2.5 Determination of fatality rate of nematode larvae and adults

Add the corresponding groups of 50 μL M9 buffer solution to the 96 well plate based on the quantity of medicinal plant extracts obtained after initial screening. Select the medium with a small number of eggs and a large number of nematodes in the larval stage (or adult stage), use the inoculation ring after being burned and cooled by Alcohol burner to pick the larvae (or adults) under the microscope, put them into a 96 hole plate and record the number of live worms (each hole contains 20~50 nematodes), and then add 50 μL sample solution (10 mg/mL after double dilution). At 50 μL M9 buffer was used as a blank control, and each group was repeated three times. Incubate in the 22 ℃ incubator for 24 hours, observe and record the number of dead nematodes, and calculate the fatality rate of larvae (or adults). The calculation formula is the same as 1.2.4.

2.6 Determination of hatching inhibition rate of nematode eggs

The nematodes were synchronized and cultured using lysis method to obtain their eggs.Add the corresponding groups of 50 μL sample solution to the 96 well plate based on the quantity of medicinal plant extracts obtained after initial screening.Join 20 μL egg solution (approximately 20-50 eggs) and 30 μL.Cultivate in purified water at 22 ℃ for 24 hours, using sterile water as the control, and repeat three times.Record the number of nematode hatching and calculate the nematode hatching inhibition rate.

$$\text{Nematode hatching inhibition rate} = \frac{1 - \text{Treatment of nematode hatchability}}{\textit{Control nematode hatchability}} \times 100\%$$

2.7 Determination of fatality rate of different concentrations of extracts to nematode at different development stages

Rescreen the extracts of medicinal plants with a fatality rate rate or an incubation inhibition rate of more than 90%, prepare them into different concentrations, and measure the fatality rate rate of nematodes or the incubation inhibition rate of nematode eggs with different concentrations.

Results and Analysis

1. Preliminary screening of nematode resistance activity of medicinal plant extracts

Eight medicinal plant extracts with good nematode resistance were screened out through preliminary screening, including *Stellera chamaejasme*, *Thladiantha dubia*, *Coptis chinensis Franch*, *Houpoea officinalis* seed, *Melia azedarach*, *Atractylodes macrocephala*, *Cortex fraxini* and *Chaenomeles speciosa* extracts. At the concentration of 10 mg/mL, their fatality rate to nematode is more than 90%, and the fatality rate of extracts from *Stellera chamaejasme*, *Thladiantha dubia*, *Coptis chinensis Franch* and *Houpoea officinalis* seed to nematode is 100%.

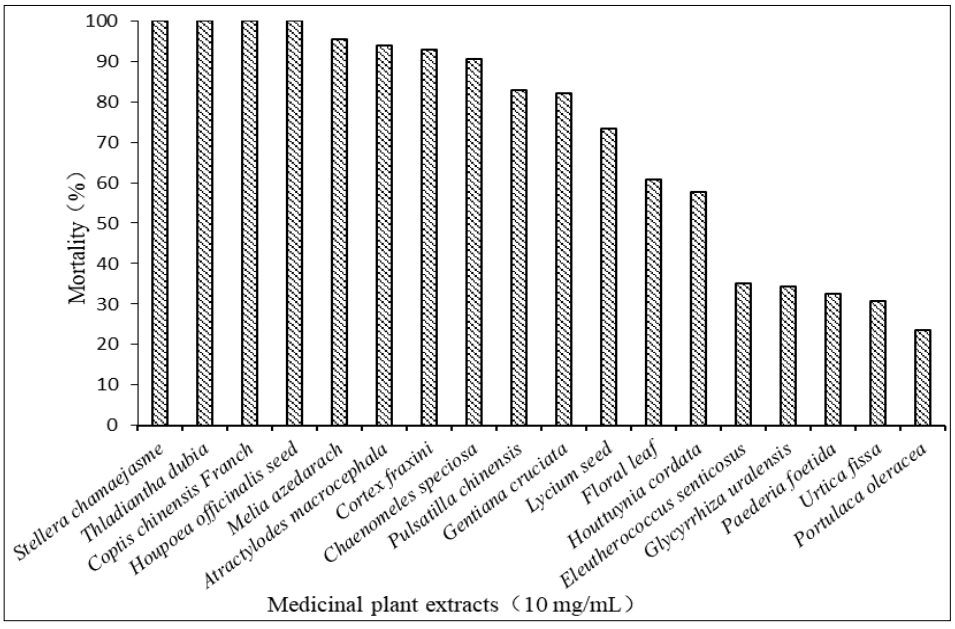

Fig 1: Fatality rate of Nematode Extracts from 18 Medicinal Plants

2. Lethal activity of medicinal plant extracts against nematodes at different growth stages

The lethal effects of 8 medicinal plant extracts obtained from the initial screening on nematodes at different stages are shown in Fig 2. The extracts of *Stellera chamaejasme*, *Thladiantha dubia* and *Houpoea officinalis* seed have strong inhibitory effects on adult nematode, and the fatality rate is more than 90%. The lethal effect on larvae and nematode eggs is generally relatively low.

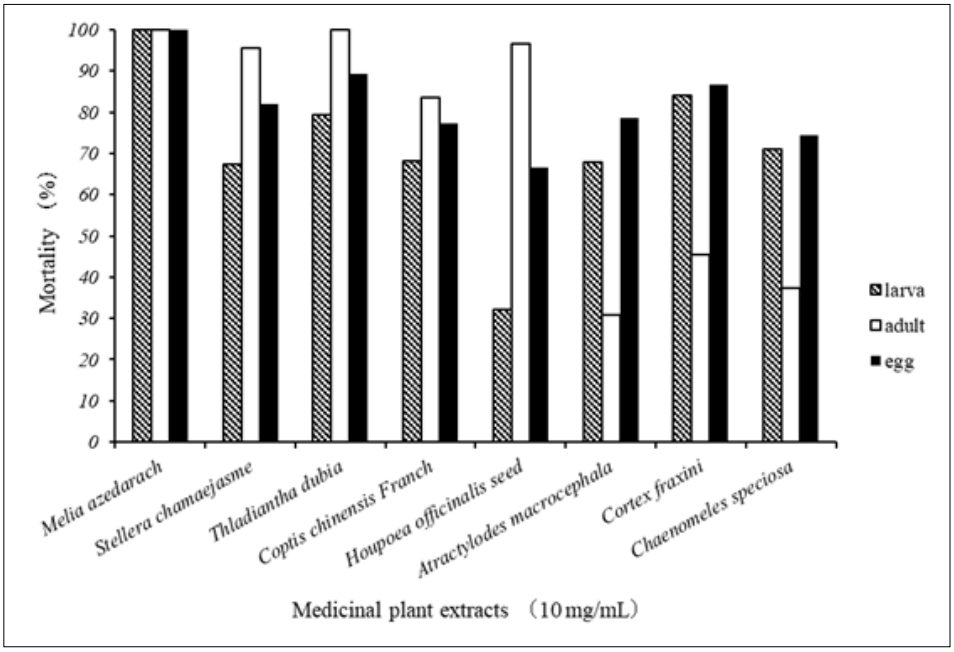

Fig 2: Fatality rate of nematodes extracted from 8 medicinal plants in different periods

3 Lethal activity of re screened samples treated with different concentrations against nematodes at different growth stages

The anti-nematode activity of rescreened samples (the fatality rate and hatching inhibition rate of nematode at different stages are more than 90%) at different concentrations was determined at different stages. As shown in Fig 3, the effect of *Melia azedarach* extract on nematodes at different stages is positively correlated with concentration. The lethal effect on adult nematodes is relatively highest, while the lethal effect on larval nematodes is lowest. The lethal effect of *Melia azedarach* extract at different concentrations on nematodes at different stages was compared as adults>eggs>larvaes. This indicates that nematodes have the strongest drug resistance during the larval stage, while adults are the weakest.

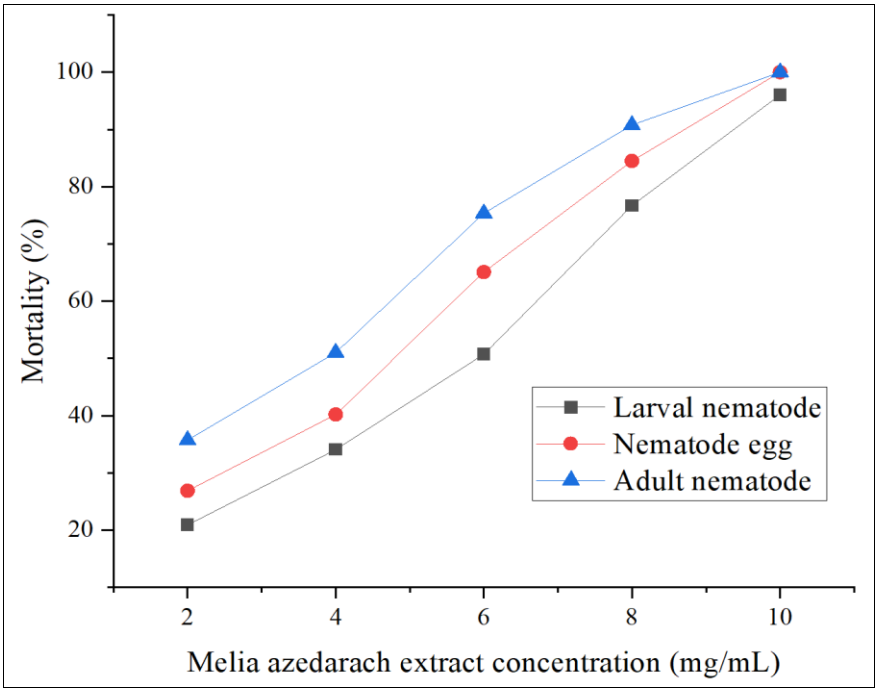

Fig 3: Effects of Different Concentrations of *Melia azedarach* Samples on Different Stages of Nematodes

Discussion

According to current reports, there are over 40 families and nearly 100 species of plants with nematicidal activity, among which more than 10 categories and over 100 plant compounds have nematicidal activity. It has been found that there are 28 plant extracts from 15 families that have toxic effects on Bursaphelenchus xylophilus, including *Melia azedarach* [4]. However, there is currently limited research on screening the activity of medicinal plants against nematodes. Wang Weixuan and others have studied the nematode resistance of *Pinellia ternata*, *Sophora alopecuroides* alkaloids [1] and essential oils of *Cymbopogon citratus* and *Tithonia diversifolia* leaves [5] and found that they all have certain nematode resistance activities. And this experiment also screened and studied the anti-nematode activity, and found that the extracts of *Stellera chamaejasme*, *Thladiantha dubia*, *Coptis chinensis Franch*, *Houpoea officinalis* seed, *Melia azedarach*, *Atractylodes macrocephala*, *Cortex fraxini*, and *Chaenomeles speciosa* have good killing effects on nematodes. Among them, the extract of *Melia azedarach* has the best anti-nematode activity. However, there are certain differences and errors in the lethal effects of extracts from *Melia azedarach* at different concentrations on larvaes, adults, and eggs. It has the highest lethal effect on adult nematodes and the lowest lethal effect on larval nematodes. It may be caused by individual differences in nematodes, differences in stress resistance at different stages [6], interference caused by stronger larval vitality at the Dauer stage [7], nematode damage during operation, and 96 well plate dead corner zone.

Conclusion

Eighteen medicinal plants (10mg/mL) were screened for nematode resistance, and eight extracts with fatality rate of more than 90% were screened. Among them, four extracts of *Stellera chamaejasme*, *Thladiantha dubia*, *Coptis chinensis Franch* and *Houpoea officinalis* seed had the highest fatality rate of 100% against nematodes. Through further research on different stages of nematodes, it was concluded that the extract of Melia azedarach has the best anti-nematode activity. At the concentration of 10 mg/mL, the inhibition rate of egg hatching of nematode and the fatality rate rate of larval nematode and adult nematode were 100%. However, extracts from *Stellera chamaejasme*, *Thladiantha dubia* and *Houpoea officinalis seed* only have higher fatality rate to adult nematodes. Further exploration of the effects of different concentrations of *Melia azedarach* extract on different stages of nematodes revealed that it had the strongest inhibitory effect on adult nematodes and the weakest inhibitory effect on larval nematodes. The research results provide a basis for the development and development of plant-based insecticides. Moreover, the four medicinal plants, namely *Melia azedarach*, *Stellera chamaejasme*, *Thladiantha dubia*, and *Houpoea officinalis seed*, are worth conducting in-depth research on their nematode resistance activities.

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