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Activity of the Ethanolic Extract Obtained from *Citrus microcarpa* Pericarps against *Meloidogyne enterolobii*, and Chemical Composition Analysis

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***Abstract.* Citrus microcarpa is a popular nutritious fruit that is widely cultivated in China. In recent years, many compounds with significant pharmacological activities have been isolated successfully from the pericarp of *C. microcarpa*. However, to date, there are no reports on the activity of *C. microcarpa* pericarp against root-knot nematodes. This study used the ethanolic extract from the pericarp of Hainan *C. microcarpa* and the impregnation method to determine its activity on J2 *Meloidogyne enterolobii* specimens and on single-egg hatching. The results showed that when J2 individuals were treated with 50 mg·mL^{-1} of the extract, the lethal concentration 50 values after 24 and 48 hours were 17.124 and 8.858 mg·mL^{-1}, respectively. The mortality rate of nematodes after 48 hours of treatment was 100%, and the inhibition rate of single-egg hatching after 24 hours was 89.29%. The ethanolic extract of *C. microcarpa* peels showed high inhibitory and lethal activity against the *M. enterolobii*. The analysis of the chemical composition of the extract revealed 28 substances with insecticidal and antibacterial effects, including lignans, flavonoids, fatty acids, organic acids, terpenoids, and imidazole. The formulas of the chemical structures and pharmacological effects of these potential insecticidal and antibacterial substances were elucidated to provide a scientific basis and a theoretical reference for the use of *C. microcarpa* pericarps as a raw material for the development of new, natural plant nematicides.**

Meloidogyne enterolobii is one of the most harmful root-knot nematodes (Kiewnick et al. 2009). It was first reported on elephant ear bean trees in Danzhou City, Hainan Province, China, in 1983, and is currently found in various regions of the world, including the Americas, Africa, and Europe (Lian et al. 2015). This nematode has a wide host range that includes most monocotyledonous crop species, dicotyledonous grasses, and woody plants (Liu et al. 2024). Even crops such as tomatoes (*Mi-1* gene), chili peppers (*N* gene), and cowpeas (*Rk* gene) carrying disease resistance genes can be parasitized, resulting in heavy yield losses that can reach more than 65% (Long et al. 2015). Recent investigations have reported that, except for Wuzhishan City, all crops in 18 cities and counties in Hainan Province, China, were affected by the root-knot nematode of elephant ear bean, *Meloidogyne enterolobii*, which has thus become a dominant pathogenic nematode population in crops grown in Hainan Province, where prevention and control of *M. enterolobii* has become of paramount importance for the rescue of agricultural production (Li et al. 2020; Yang and Eisenback 1983).

Citrus peels are rich in essential volatile oils and have a special aroma. The multicomponent, natural flavoring-oil extracted from these peels is highly valued for its numerous applications in the field of insecticidal and bactericidal compound development (Zhang et al. 2024). Thus, for example, the *Citrus* ethanolic extract shows inhibitory activity on Lepidoptera (*Spodoptera frugiperda*) and Diptera (*Bactrocera oleae*, *Ceratitis capitata* adults, and *Aedes aegypti*) (Sarma et al. 2019; Siskos et al. 2009; Villafañe et al. 2011). Similarly, lemon peel extract shows inhibitory activity against gram-positive bacteria (Ekwenye and Edeha 2010; Harfouch et al. 2019). In turn, *Citrus* extract shows excellent antibacterial activity against *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli*, and *Salmonella* (Lin et al. 2021; Meng 2013; Roanisca and Mahardika 2020). *Citrus microcarpa* is an evergreen shrub of the *Citrus* L. genus in the Rutaceae family. A native to China, it is mainly distributed in Hainan Province (Duan et al. 2015). Unlike other *Citrus* species, *C. microcarpa* has very high nutritional and medicinal values, such that it is known as the "king of plants." In ancient times, the unique medicinal value of *C. microcarpa* was recorded in the *Compendium of Materia Medica*, which states that it has strong effects, including generating fluids, quenching thirst, strengthening the stomach, and reducing digestion. The *C. microcarpa* pericarp contains abundant bioactive substances such as flavonoids, coumarins, alkaloids, and volatile oils with various effects, including antibacterial, insecticidal, and antiviral effects (Cao and Pan 2022). Indeed, in recent years, the variety of pharmacological activities of *C. microcarpa* have been reported often. Thus, for example, using gas chromatography (GC)–mass spectrometry (MS) technology, Su et al. (2020) identified the essential oil component limonene, the greatest content of which was observed in the pericarp of *C. macrocarpa*. Limonene shows biological activity against crop diseases and pests, as well as against weeds (Liu 2019). Furthermore, its lethal effects on rhizobial nematodes have been confirmed in Indian soils (Saxena et al. 1987). Thus, *C. microcarpa* pericarps seemingly contain substances with an inhibitory effect on *M. enterolobii*.

To date, chemical control remains the prevailing tool for root-knot nematode control, with the concomitant threat of the environmentally dangerous residual effects of chemical control agents. Alternatively, plant-derived pesticides are characterized by low toxicity, easy degradation in natural environments, no residual effects, and no accumulation in the environment. Given all these advantages, these plant-derived compounds are safe to use and protect the environment. Therefore, developing and using plant-based pesticides to reduce chemical control of crop diseases, pests, and weeds is in line with current needs of human society and nature (Liang 2019). Hence the efforts involved in the search for plant-based pesticides have become a research hot spot (Dan et al. 2011; Liu 2019). However, to date, neither the activity associated with root-knot nematodes, the chemical structural formulas, nor the pharmacological effects of these compounds have been reported. We study explored the inhibitory activity of the ethanolic extract from *C. microcarpa* pericarps on *M. enterolobii* and screened potential active substances preliminarily. The chemical structural formulas and pharmacological effects of the active substances were elucidated, thus providing a scientific basis and a theoretical reference for the development of *C. microcarpa* pericarps as a new, natural plant-derived raw material for the development of environmentally friendly effective nematicides.

Materials and Methods

Test plants

The test plant material, *C. macrocarpa* fruit, was purchased in Jun 2023 at a fruit market in the Qiongshan District, Haikou City, Hainan Province, China. Fruit with lime-green, fresh, and smooth skin were selected and confirmed to be *C. microcarpa* by Chen Xuyu (Hainan Branch of the Institute of Medicinal Plant Development, Chinese Academy of Medicinal Sciences & Peking Union Medical College, Haikou City, Hainan Province, China).

Test nematode

Second-stage juvenile (J2) specimens of the root-knot nematode *M. enterolobii* were collected from pepper crop plants in Honghua Village, Jiyang District, Sanya City, Hainan Province, China (lat. 18.339471°N, long. 109.569134°E) and were reared in the laboratory of the Institute of Plant Protection, Hainan Academy of Agricultural Sciences (Hainan Province, Haikou, China).

Instrumentation and reagents

Reagents. The reagents used in the experiments described included pure ethanol, analytical grade (Hengshun Chemical Co., Ltd., Wenzhou, China); dimethyl sulfoxide (DMSO; Shanghai McLean Biochemical Technology Co., Ltd., China), 90% abamectin original powder (Aino Pharmaceutical Co., Ltd., North China); 2-octanol, methoxamine salt (Tokyo Chemical Industry Co., Ltd., Japan); chloroform and pyridine (Shanghai Adamas Reagent Co., Ltd., China); ribol (Shima Co., Ltd., Japan); BSTFA (with 1% trimethylchlorosilane, v/v; REGIS Technologies, IL, Morton Grove, USA); FAMEs (Dr. Ehrenstorfer GBH, Augsburg, Germany), and double distilled (ddH_2O).

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Data from this study are presented in the figures and tables in this article, and in the supplemental material.
W.H. conceptualized the study and designed the research proposal. D.X. implemented the research process, collected and organized the data, and wrote the article. Y.W. and X.Y. searched for relevant literature and purchased the experimental materials. L.L. obtained the research funding and designed the framework of the article. M.F. and X.Z. revised the article. All authors read and agreed to the published version of the manuscript.
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Instrumentation. The instruments used in the experiments included a rotating evaporator (Heidelberg, Schwarzbach, Germany); a 7890A gas chromatograph, a 7890B gas chromatograph, a 5977B mass spectrometer, a DB-5MS (30 m × 250 μM × 0.25 μm) and a DB Wax (30 m × 250 μM × 0.25 μm) chromatographic column (Agilent, Santa Clara, CA, USA); a PEGASUS HT mass spectrometer (Lico, San Jose, USA); a Heraeus Fresco17 centrifuge and a Forma 900 series ultra-low temperature refrigerator (Thermo Fisher Scientific, Waltham, MA, USA); a BSA124S-CW analytical balance (Sedolis Scientific Instruments Co., Ltd., Beijing, China); a JXFSTPRP-24 grinder (Jingxin Technology Co., Ltd., Shanghai, China); a YM-080S ultrasonic instrument (Fangao Microelectronics Co., Ltd., Shenzhen, China); a DHG-9023A oven (Yiheng Scientific Instrument Co., Ltd., Shanghai, China), and a LNG-T98 vacuum dryer (Huamei Biochemical Instrument Factory, Taicang City, China).

Root-knot nematode species validation

Nematode DNA was extracted as described by Wang et al. (2011). The molecular identification of root-knot nematode referred to the method of Long et al. (2006), using sequence characterized amplified region (SCAR)-labeled primers Me-F/Me-R (5′-AA CTTTTGTGAAAGTGCCGCTG-3′/5′-TCA GTTCAGGCAGGATCAACC-3′) of the root-knot nematode to amplify specific bands. The primers used in this experiment were synthesized by Sangong Bioengineering Technology Service Co., Shanghai, China.

Preparation of plant extracts

Using an ultrasonic extraction method, *C. microcarpa* pericarps were peeled off and cleaned of impurities on the surface with sterile water. Pericarps were then placed in a constant-temperature oven at 60 °C and dried to a constant mass. A wall crusher was used to crush fruit peels, which were then passed through a 50-μm mesh sieve. Dried pericarp powder samples (10 g each) were placed in 500-mL triangular flasks, to which was added 100 mL of 95% ethanol solution in a 1:10 ratio. Ultrasound extraction was performed at 35 °C at 80 W of power for 2 h before filtration. The obtained filtrate was evaporated at 40 °C using a rotary evaporator to form a paste, which was then stored at 4 °C until use.

Determination of nematicidal activity

Using the maceration method, a nematode suspension with 100 nematodes in it was added to each well on a 24-well cell culture plate with 500 μL of the sample solution, which was brought to 1 mL with sterile water to yield sample concentrations of 50, 25, 12.5, 6.25, and 3.125 $mg \cdot mL^{-1}$. Because the ethanolic extract from *C. microcarpa* pericarp turned into a paste after evaporation and required the use of organic solvents for solubilization, we tested using acetone and DMSO for such solubilization of the extract. We found that acetone, but not DMSO, caused J2 juveniles to die; thus, DMSO was ultimately selected as the cosolvent. To eliminate the influence of the cosolvent, DMSO was set as a blank control, 90% abamectin original powder as a pharmaceutical control, and sterile water as a negative control. Each concentration was tested in triplicate. After placing the processed cell culture plate at 25 °C for 24 and 48 h, treated nematodes were transferred to a centrifuge tube for centrifugation, after which the supernatants were aspirated carefully. Nematodes were washed twice with sterile water and placed into a 3-cm culture dish, and sterile water was added to resuscitate them. After 48 h of resuscitation, nematodes were observed using a stereomicroscope. Living nematodes show a curved body shape and move by peristaltic coiling and extending, whereas dead nematodes appear stiff. Using acupuncture, if nematodes remain stiff and do not move, it is judged they are dead (Wang et al. 2013). The mortality rate and the corrected mortality rate of nematodes were calculated per Eqs. [1] and [2], respectively:

$$\text{Mortality rate} = \left(\frac{\text{No. of dead nematodes}}{\text{No. of nematodes in the treatment}}\right) \times 100\% \quad [1]$$

and

$$\text{Corrected mortality rate} = \left(\frac{\text{Treatment nematode mortality rate} - \text{Control nematode mortality rate}}{1 - \text{Control nematode mortality rate}}\right) \times 100\%. \quad [2]$$

Effects of plant extracts on single-egg hatching of nematodes

Fresh, plump yellow-brown egg sacs were selected for incubation experiments. Egg sacs were placed in a 1.0% sodium hypochlorite solution and shaken for 1 min before centrifugation. The supernatant was discarded and the bottom solution was washed three times with sterilized water. Subsequently, water was added and the mixture was shaken to collect the supernatant to obtain a single egg suspension (Haseeb et al. 2005; Lu et al. 2006). A single-egg suspension (100 particles/well) was added to each well in a 12-well cell culture plate; then, 500 μL of sample solution was added to each well, and sterile water was used to bring the volume in each well to 1 mL for a final sample concentration of 50, 25, 12.5, 6.25, or 3.125 $mg \cdot mL^{-1}$. Sterile water and DMSO were used as controls, and each treatment was repeated three times. After treatment at 25 °C for 4, 8, 16, 24, and 48 h, the extraction solution adhered to the surface of the egg was centrifuged and washed with sterile water. The egg was then placed in sterile water for further incubation at 25 °C in an incubator. After 4 d, egg hatching was observed using a stereomicroscope. The hatching and incubation inhibition rates were calculated according to Qi et al. (2011), using Eqs. [3] and [4], respectively:

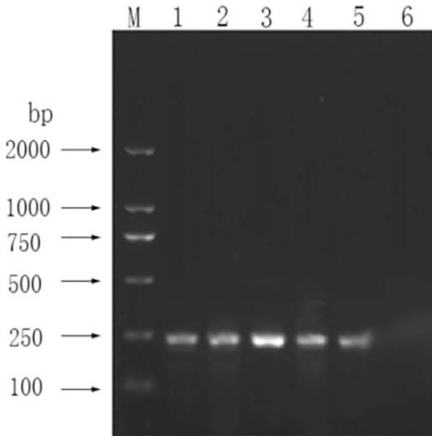

Fig. 1. Amplification results of sequence characterized amplified region marker-specific primers for root-knot nematodes. M = DNA marker D2000; 1–5 = root-knot nematode *Meloidogyne enterolobii*; 6 = blank control.

$$\text{Hatching rate} = \left(\frac{\text{Total no. of incubations}}{\text{Total no. of eggs}}\right) \times 100\% \quad [3]$$

and

$$\text{Incubation inhibition rate} = \left(\frac{\text{Control hatching rate} - \text{Treatment hatching rate}}{\text{Control hatching rate}}\right) \times 100\%. \quad [4]$$

Analysis of the chemical composition of *C. microcarpa* peel extracts

To identify the bioactive substances in *C. microcarpa* pericarps, ultrahigh-performance liquid chromatography (UHPLC)–MS was used to separate compounds that are difficult to volatilize and are thermally unstable. GC-MS was used to separate volatile and thermally stable compounds, and GC-MS-volatile organic compounds (VOCs) was used to separate flavor-volatiles and characterize the active compounds comprehensively.

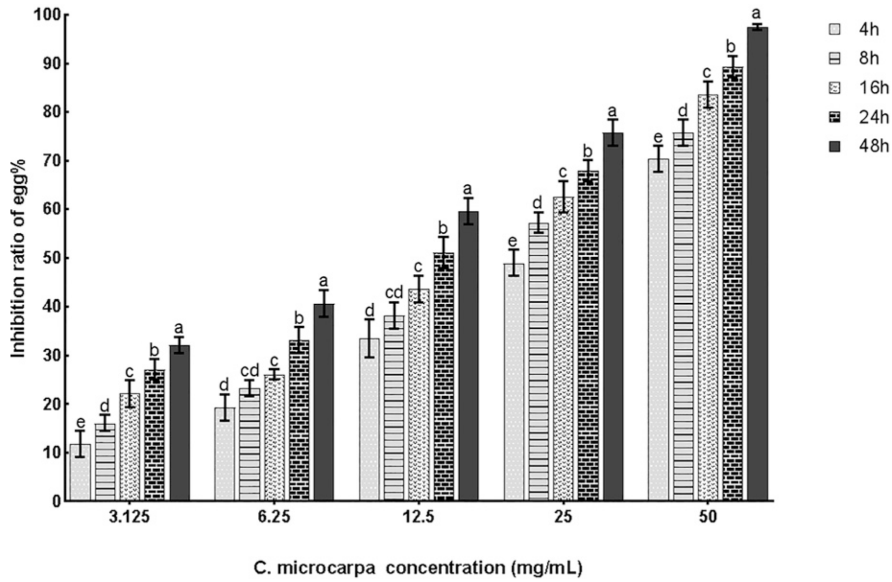

Fig. 2. Effects of *Citrus microcarpa* pericarp extract on the hatching of nematode eggs. The data in the figure are mean ± the standard error. Different letters within the same column group show significant differences at $P < 0.05$.

UHPLC-MS analysis. A total of 100 μL of the supernatant of the ethanolic pericarp extract was placed in a feed vial and analyzed by UHPLC-MS.

GC–time-of-flight-MS analysis. Analysis by GC–time-of-flight (TOF)–MS was performed using an Agilent 7890 gas chromatograph coupled with a TOF mass spectrometer. The system used a DB-5MS capillary column. A 1-μL aliquot of sample was injected in splitless mode. Helium was used as the carrier gas. The front inlet purge flow was 3 $mL \cdot min^{-1}$, and the gas flow rate through the column was 1 $mL \cdot min^{-1}$. The initial temperature was kept at 50 °C for 1 min, raised to 310 °C at a rate of 10 $°C \cdot min^{-1}$, and kept at that point for 8 min. The injection, transfer line, and ion source temperatures were 280, 280, and 250 °C, respectively. The energy used was –70 eV in the electron impact mode. MS data were acquired in full-scan mode with an m/z range of 50 to 500 at a rate of 12.5 spectra per second after a solvent delay of 6.4 min.

Analysis of GC-MS-VOCs. The solid-phase microextraction (SPME) cycle of the PAL rail system (a liquid automatic sampler system) was as follows: the incubation temperature was 60 °C, the preheat time was 15 min, the incubation time was 30 min, and the desorption time was 4 min. GC-MS analysis was performed using an Agilent 7890 gas chromatograph system coupled with a 5977B mass spectrometer. The system used DB-Wax, and the injection was in the splitless mode. Helium was used as the carrier gas, the front inlet purge flow was 3 $mL \cdot min^{-1}$, and the gas flow rate through the column was 1 $mL \cdot min^{-1}$. The initial temperature was kept at 40 °C for 4 min, then was raised to 245 °C at a rate of 5 $°C \cdot min^{-1}$, and was kept at that point for 5 min. The injection, transfer line, ion source, and quad temperatures were 250, 250, 230, and 150 °C, respectively. The energy was –70 eV in electron impact mode. MS data were acquired in scan mode with an m/z range of 20 to 400 and a solvent delay of 2.13 min.

Table 1. Toxicity of different treatments against J2 *Meloidogyne enterolobii*.

Treatment	Concn ($mg \cdot mL^{-1}$)	Avg mortality rate at 24 h (%)[ii]	Avg mortality rate at 48 h (%)[ii]
Citrus microcarpa pericarp	50	90.33 ± 1.527 a[i]	1 ± 0.00 a
	25	64.33 ± 4.041 b	73.67 ± 6.027 b
	12.5	51.33 ± 1.154 c	62.33 ± 2.516 c
	6.25	37.67 ± 2.516 d	47.33 ± 6.806 d
	3.125	21.33 ± 2.081 e	34.33 ± 6.027 e
90% Abamectin original powder	0.1	63.33 ± 1.527 b	68.33 ± 1.527 bc
Dimethyl sulfoxide	—	1.33 ± 0.577	1.33 ± 0.577
Sterile water	—	0.67	0.67

[i] Different lowercase letters within columns represent significant differences at $P < 0.05$.
[ii] The maximum corrected mortality rate is defined as a.

Table 2. Toxicity of *Citrus microcarpa* pericarp extract on *Meloidogyne enterolobii*.

Extract	Period of treatment (h)	Linear equation ($y = ax + b$)	LC_{50} ($mg \cdot mL^{-1}$)[i]	LC_{90} ($mg \cdot mL^{-1}$)	Correlation coefficient
Orange peel extract	24	$y = 1.331x + 27.207$	17.124	47.177	0.909
	48	$y = 1.286x + 38.608$	8.858	39.96	0.924

[i] LC = lethal concentration.

Data processing and analysis

Data processing for root-knot nematode bioactivity assay. The data were analyzed using Excel (WPS Office 12.1.0.16729) and IBM SPSS Statistics 27 statistical analysis software to calculate the mortality rate in each treatment and to determine the lethal concentration 50 (LC_{50}) value of the *C. microcarpa* peel extract.

Qualitative analysis of the chemical composition of the pericarp. The peak extraction, baseline correction, deconvolution, peak integration, peak alignment, and mass spectral matching were performed to analyze the mass spectral data using ChromaTOF software v. 4.3x (LECO) (Dunn et al. 2011; Kind

Table 3. Ultrahigh performance liquid chromatography–mass spectrometry analysis of some portions of the ethanolic extract of *C. microcarpa* pericarps.

No.	Compound name	Retention time (min)	m/z	Molecular formula	Ion	Biotree class
1	Isoacteoside	306.773	623.20	$C_{29}H_{36}O_{15}$	M-H	Lignan
2	o-Methoxycinnamaldehyde	568.24	163.08	$C_{10}H_{10}O_2$	[M+H]+	Lignan
3	p-Coumaric acid	70.4794	165.05	$C_9H_8O_3$	[M+H]+	Simple phenylpropanoids
4	Ferulate	252.5945	177.05	$C_{10}H_{10}O_4$	[M-H$_2$O+H]+	Phenylpropanoids
5	Cinnamaldehyde	252.413	133.06	C_9H_8O	[M+H]+	Lignan
6	Piplartine	229.897	356.09	$C_{17}H_{19}NO_5$	[M-H$_2$O+H]+	Lignan
7	Luteolin	368.729	285.04	$C_{15}H_{10}O_6$	[M-H]-	Flavone
8	Phloretic acid	146.073	165.05	$C_9H_{10}O_3$	M-H	Flavone

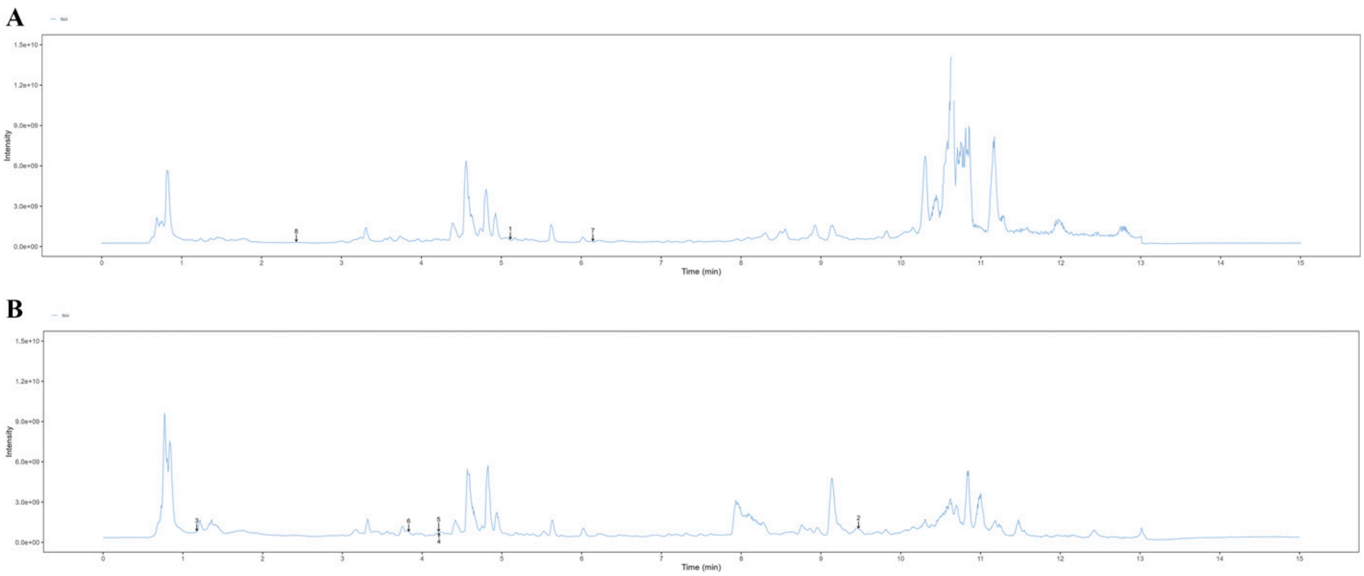

Fig. 3. Total ion flow diagram of ultrahigh performance liquid chromatography–mass spectrometry of the ethanolic extract of *Citrus microcarpa* pericarps. (**A**) Positive ion mode. (**B**) Negative ion mode.

et al. 2009). Using the LECO-Fiehn Rtx5 database, mass spectral and retention time index matching were performed, combined, and compared with the compounds in the relevant databases (KEGG and HMDB, as well as the self-constructed database of Shanghai Baiyi Biomedical Technology Co. Ltd.) to characterize the substances present in the ethanolic extract of the pericarps.

Results

Confirmation of root-knot nematode species identity

Using the SCAR–polymerase chain reaction identification method, a single band of 236 bp was identified using Me-F/Me-R–specific primers for the root-knot nematode *M. enterolobii*. No other bands were observed (Fig. 1).

Nematocidal effect of *C. microcarpa* pericarp extracts

The bioactivity assay results showed that the ethanolic extract of the pericarps exhibited strong bioactivity against J2 *M. enterolobii*, which was significantly greater than those of the blank control (DMSO) and the negative control (sterile water). Furthermore, the inhibitory activity tended to increase with time. The LC_{50} value was 17.124 and 8.858 mg·mL^{-1} after 24 and 48 h of treatment, respectively, and the nematode mortality rate reached 100% after 48 h at 50 mg·mL^{-1} of pericarp extract (Tables 1 and 2).

Effects of *C. microcarpa* pericarp extract on the hatching of nematode eggs

The ethanol extract of *C. microcarpa* pericarps at different concentrations has inhibitory effects on the hatching of nematode eggs in *M. enterolobii*. With the extension of treatment time and the increase of concentration, the inhibition rate of egg hatching also increases. The concentration of 50 mg·mL^{-1} has the best effect on nematode egg hatching, and the inhibition rate reaches 89.29% after 24 h of treatment (Fig. 2).

Chemical composition analysis of *C. microcarpa* pericarps

UHPLC-MS analysis. UHPLC-MS analysis yielded 611 peaks that were analyzed by reviewing the literature. Eight lignans and flavonoids with insecticidal and bacteriostatic effects were screened from the extract preliminarily (Table 3; Fig. 3).

GC-TOF-MS analysis. The GC-TOF-MS analysis revealed 671 peaks that were analyzed through a review of the literature. Preliminary screening of the extract revealed 10 organic

Table 4. Gas chromatography–time-of-flight mass spectrometry analysis of some chemical constituents of the ethanolic extract of *C. microcarpa* pericarps.

No.	Compound name	Unique mass (g/mol)	Retention time (min)	Molecular formula	Biotree class[i]
1	L-alanine	187	7.997	$C_3H_7NO_2$	Amino acid
2	Citric acid	273	17.176	$C_6H_8O_7$	Organic acid
3	Ethyl cinnamate	485	13.439	$C_{11}H_{12}O_2$	sth or sb else
4	Myo-inositol	217	19.872	$C_6H_{12}O_6$	Glycitols
5	Nonanoic acid methyl ester	68	10.093	$C_{10}H_{20}O_2$	Organic acid
6	Oxalic acid	73	8.387	$C_2H_2O_4$	Organic acid
7	Palmitic acid	117	19.319	$C_{16}H_{32}O_2$	Fatty acid
8	Shikimic acid	204	17.068	$C_7H_{10}O_5$	Organic acid
9	Stearic acid	117	21.315	$C_{18}H_{36}O_2$	Fatty acid
10	Xylose 2	103	15.23	$C_5H_{10}O_5$	sth or sb else

[i] sb = somebody; sth = something.

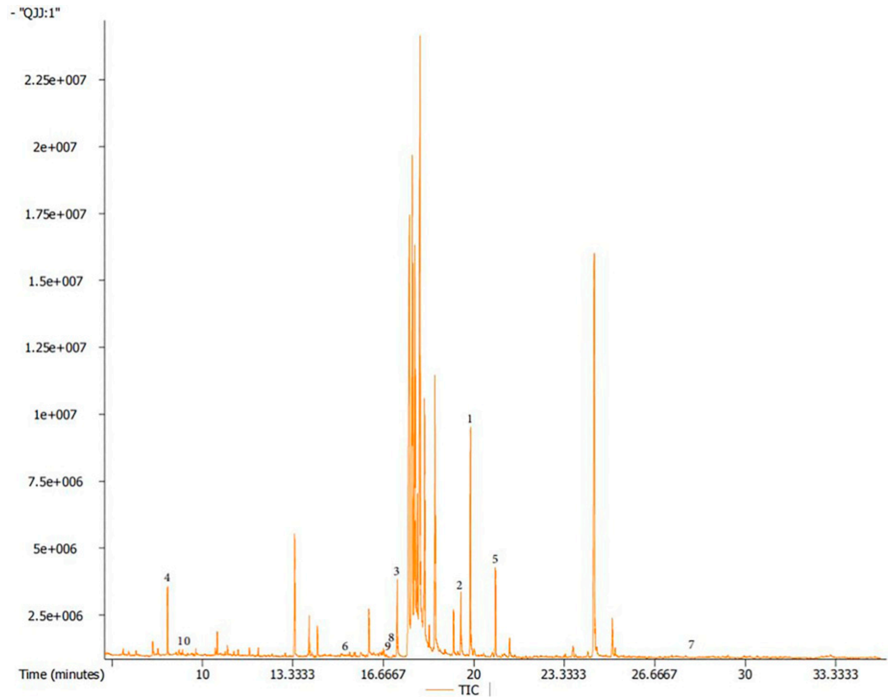

Fig. 4. Total ion flow diagram of gas chromatography–time-of-flight mass spectrometry of the ethanolic extract of *Citrus microcarpa* pericarps. TIC = total ion current.

acids and fatty acids with insecticidal and bacteriostatic effects (Table 4; Fig. 4).

GC-MS-VOC analysis. The GC-MS-VOC analysis yielded 1427 peaks, and preliminary screening of 10 terpenoids and imidazoles with insecticidal and bacteriostatic effects from the extract was achieved by review of the literature (Table 5; Fig. 5).

Discussion

There are many types of citrus plants that have both edible and pharmacological value. Furthermore, to date, numerous studies have reported on the insecticidal and antibacterial activities of extracts from citrus medicinal plants. Our study found that the ethanolic extract of the pericarp of the local plant resource *C. macrocarpa*, grown in Hainan, China, has strong biological activity against J2 *M. enterolobii.* and hatching of its eggs. Specifically, using UHPLC-MS, GC-TOF-MS, and GC-MS-VOCs, we detected 2979 active substances through chemical composition analysis of ethanolic extracts. In addition, we organized and analyzed 28 of these substances with insecticidal and antibacterial effects, including lignans, flavonoids, fatty acids, organic acids, terpenes, and imidazoles (Frota et al. 2023; Liu et al. 2002; Ma and Gu 2003). In recent years, researchers have isolated more than 100 monomeric components from medicinal plants, including flavonoids, coumarins, alkaloids, organic acids, and terpenoids that exhibit strong nematicidal activity. Furthermore, there are studies reporting that flavonoids such as luteolin and quercetin can inhibit plant parasitic nematodes (Bano et al. 2020; Chin et al. 2018); terpene compounds, such as carvacrol and the aromatic compound eugenol, which can effectively kill *Caenorhabditis elegans* (Tsao and Yu 2000); monoterpenoid compounds, such as geraniol, aromatic alcohol, citral, and so on (Abdel-Rahman et al. 2013), which have strong lethal effects on threads; organic acid compounds, such as oxalic and citric acids, which have high inhibitory activity on southern root-knot nematodes (Liu et al. 2011); and the aldehyde cinnamaldehyde, which has high inhibitory activity against southern root-knot nematodes, Poaceae root-knot nematodes, and pine wood nematodes (D'Addabbo et al. 2020). The results of our study further indicated that the ethanolic extract of *C. microcarpa* pericarp contains substances with strong inhibitory and lethal effects on *M. enterolobii*, thus providing a sound basis for the development of plant-based nematicides from *C. macrocarpa* extracts. However, we investigated only the nematicidal activity of ethanolic extracts from *C. macrocarpa*. Further research is needed to establish unequivocally the identity of the components that play a major role, and the underlying mechanism of action.

Conclusion

In our study ethanol was used as a solvent to analyze the chemical components in the ethanolic extracts from *C. microcarpa* pericarps. Twenty-eight potential active substances were identified along with their corresponding chemical structural formulas. The compounds that have been reported to show strong nematode inhibitory activity in plants were isolated from the ethanolic extract obtained from *C. microcarpa* pericarps, indicating that such extract contains more nematicidal bioactive substances, and that 95% ethanol is effective for the extraction of bioactive substances from the pericarp. The chemical composition and pharmacological effects of plant extracts are the foundation and core of the development of plant-based pesticides. The composition and content of substances extracted by different solvents may vary, and the biological activities exhibited may also vary. Thus, for example, Peng (2014) pointed out that different extracts have the following effects on larvae upon contact: killing, growth inhibition, and delayed molting time. Solvent efficacy with respect to these effects ranked in the following order: ethanol extract > chloroform extract > acetone extract, which confirmed that ethanol has the greatest biological activity as an extraction solvent. Therefore, in future research, other solvents can be used for the extraction of bioactive compounds from plant tissues to compare their biological activities, such as to obtain other effective compounds and provide a basis for the development of plant-derived

Table 5. Gas chromatography–mass spectrometry–volatile organic compound analysis of some chemical constituents of the ethanolic extract of *Citrus microcarpa* pericarps.

No.	Compound name	Unique mass	Retention time (min)	Molecular formula	Biotree class[i]
1	2-Methylbutanal	57	2.722	$C_5H_{10}O$	sth or sb else
2	Caryophyllene	148	20.3539	$C_{15}H_{24}$	Sesquiterpene (chemistry)
3	D-Limonene	59	10.305	$C_{10}H_{16}$	Monoterpene
4	Geraniol	69	25.99	$C_{10}H_{18}O$	Noncyclic monoterpene alcohols
5	Lilac aldehyde A	111	18.923	$C_{10}H_{16}O_2$	sth or sb else
6	Limonene oxide, trans	57	16.822	$C_{10}H_{16}O$	Epoxy compound
7	Linalool	69	19.353	$C_{10}H_{18}O$	Chained terpene alcohols
8	Paraldehyde	161	46.192	$C_6H_{12}O_3$	sth or sb else
9	Pentanal	44	3.704	$C_5H_{10}O$	sth or sb else
10	Tioconazole	145	23.551	$C_{16}H_{13}Cl_3N_2OS$	Imidazole

[i] sb = somebody; sth = something.

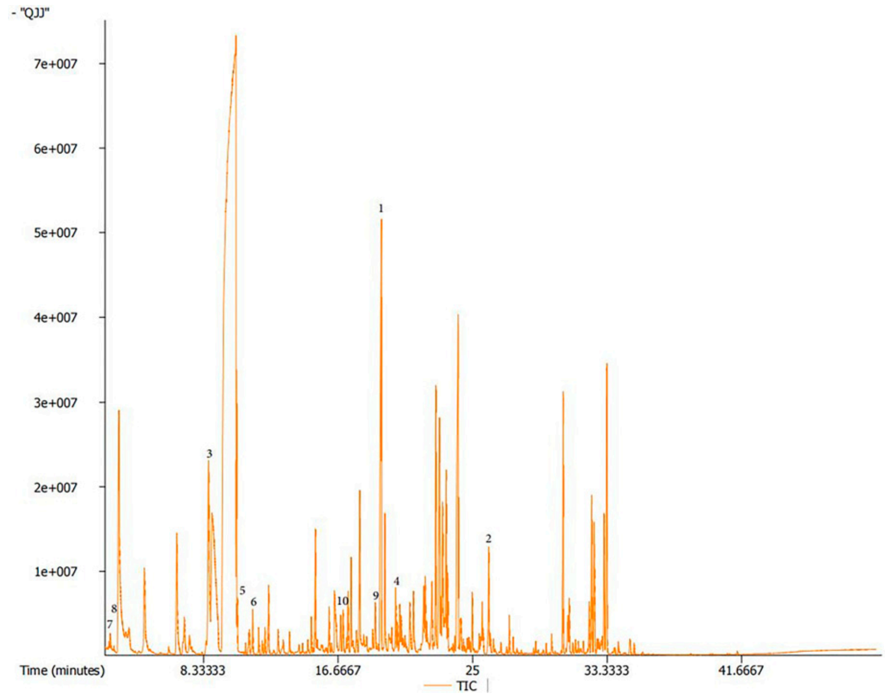

Fig. 5. *Citrus microcarpa* pericarp headspace volatility gas chromatography–mass spectrometry–volatile organic compounds total ion flow map. TIC = total ion current.

nematicides and other insecticidal and antibacterial bioactive substances.

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