Chemical Composition, Antioxidant Property, and Nematicidal Activity of *Cucumis metuliferus*

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Abstract [**Objectives**] To investigate the chemical composition, antioxidant property, and nematicidal activity of *Cucumis metuliferus* and establish a theoretical foundation for the development of botanical nematicides. [**Methods**] The compounds were isolated and purified using silica gel, reversed-phase medium, low-pressure gel Sephadex LH-20 column chromatography, and preparative high-performance liquid chromatography. Their identification was based on physicochemical properties, various spectral analyses, and data reported in the literature. Subsequently, the nematicidal and antioxidant activities of the isolated compounds were evaluated. [**Results**] A total of four compounds were isolated from *C. metuliferus*, which were identified as isovitexin-2″-O-glucoside (compound 1), 9E,11E-(13-oxo) tridecadienoic acid (compound 2), 2E,4E-tridecadienedioic acid (compound 3), and 9E,11E-(13-hydroxy) tridecadienoic acid (compound 4). Compound 1 demonstrated a notable degree of antioxidant activity, achieving a DPPH scavenging rate of 49.40% at a mass concentration of 1 mg/mL. Additionally, compounds 1, 3, and 4 exhibited varying levels of nematicidal activity. Specifically, compound 1 displayed strong nematicidal activity at a concentration of 2.0 mg/mL, resulting in a mortality rate of 90.5% against nematodes. Furthermore, compounds 3 and 4 achieved a mortality rate of 100% against nematodes at a concentration of 0.25 mg/mL. [**Conclusions**] This study reports the first isolation of four compounds: isovitexin-2″-O-glucoside, 9E,11E-(13-oxo) tridecadienoic acid, 2E,4E-tridecadienedioic acid, and 9E,11E-(13-hydroxy) tridecadienoic acid from *C. metuliferus*. Notably, 2E,4E-tridecadienedioic acid and 9E,11E-(13-oxo) tridecadienoic acid are identified as novel natural products. Among these compounds, isovitexin-2″-O-glucoside, 9E,11E-(13-oxo) tridecadienoic acid, and 9E,11E-(13-hydroxy) tridecadienoic acid exhibit nematicidal activity, whereas isovitexin-2″-O-glucoside demonstrates antioxidant properties.

Key words *Cucumis metuliferus*, Botanical pesticide, Chemical composition, Root knot nematode, Antioxidant property, Nematicidal activity

1 Introduction

Approximately 3 000 species of nematodes have been identified as detrimental to plant health, with the majority residing in the soil and a subset exhibiting parasitic behavior on plants. Nematodes are disseminated through soil or seeds and possess the capability to inflict damage on plant roots or invade aerial organs, thereby negatively impacting the growth and development of crops. Furthermore, nematodes serve as indirect vectors for diseases caused by various microorganisms, resulting in significant economic losses in agricultural production. The management of root-knot nematodes in China predominantly relies on chemical control methods. Practices such as soil fumigation and broadcasting of various agents have resulted in significant detrimental effects on soil health, including the accumulation of pesticide residues that are challenging to decompose, soil compaction, and excess of soil nutrients. Consequently, there is a growing demand for the adoption of biological and botanical pesticides as alternative control strategies[1]. *Cucumis metuliferus* is a characteristic plant species within the genus *Cucumis*, belonging to the family Cucurbitaceae. This species exhibits resistance to root-knot nematodes and can serve as a rootstock for melon and cucumber varieties that are also resistant to *Meloidogyne incognita* and blight. Additionally, *C. metuliferus* demonstrates high levels of resistance to powdery mildew, blight, and various other pests and diseases[2]. The investigation of the chemical composition, as well as the antioxidant and nematicidal activities of *C. metuliferus*, holds substantial importance for the development and utilization of botanical pesticides. This research is also critical for the environmentally friendly and safe management of root-knot nematodes in melons and other agricultural crops.

Globally, there exist over 30 nematicides classified within single dose category, with more than 10 of these being widely utilized. Nematicides currently employed, including bromomethane, carbofuran, fosthiazate, and abamectin, exhibit high levels of toxicity and significant soil residues. Furthermore, the majority of these nematicides are highly toxic to non-target animals, thereby presenting substantial risks to both biosafety and the environment. In comparison to chemically derived nematicides, botanical nematicides offer several advantages, including rapid decomposition, low toxicity, minimal residue, reduced environmental pollution, and decreased resistance among pests[3]. The development of botanical nematicides through the extraction of nematicidal active

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compounds from plants such as *C. metuliferus* aligns with contemporary principles of green production. Research on the chemical screening and acute toxicity of *C. metuliferus* has demonstrated that this species is abundant in saponins, alkaloids, carbohydrates, flavonoids, tannins, cardiac glycosides, steroids, terpenoids, and various other bioactive compounds with pharmacological properties[4]. These compounds are frequently utilized in the treatment of numerous chronic conditions, including gastric ulcers, diabetes mellitus, hypertension, and even acquired immunodeficiency syndrome[5]. According to medical studies, the extract derived from the fruit pulp of *C. metuliferus* exhibits a restorative effect on the gastric mucosa in rats and significantly influences the secretion of gastric acid, thereby reducing the risk of gastric mucosal lesions[6]. Additionally, the alkaloids present in *C. metuliferus* demonstrate notable anti-ulcer activity, indicating their potential utility in the treatment of peptic ulcer disease[7]. Furthermore, the aqueous extract of *C. metuliferus* has been shown to possess hypoglycemic properties, effectively lowering blood glucose levels in rats with alloxan-induced hyperglycemia[8]. It has been demonstrated that *C. metuliferus* enhances the hemogram of leukemic rats[5] and exhibits resistance properties against African trypanosomiasis[9], *Salmonella gallinarum*[10], malaria[11], and various viruses[12]. The prior investigation conducted by our research group revealed that the ethanolic crude extracts derived from the roots, stems, and leaves of *C. metuliferus* exhibit varying levels of toxic activity against *M. incognita*[13]. These findings suggest the potential application of these extracts as a botanical nematicide. The primary compositions of botanical pesticides are naturally occurring compounds, predominantly composed of carbon (C), hydrogen (H), and oxygen (O) elements. Through an extensive evolutionary process, plants have developed a stable metabolic pathway characterized by a fixed cycle of energy and materials[3]. The application of botanical pesticides results in rapid degradation and is associated with several advantageous properties, including low residue levels, low toxicity, high target selectivity, and safety for non-target organisms and the environment[14]. Currently, chemical control methods predominantly serve as the primary means of managing nematodes in agricultural production in China. In contrast, the variety of botanical nematicides available remains limited. While some achievements have been made regarding the nematicidal properties of *C. metuliferus*, there is a notable scarcity of reports concerning the isolation and identification of its nematicidal compounds, as well as studies investigating their biological activity. Consequently, it is essential to clarify the structures of the isolated compounds and the antioxidant and nematicidal activities exhibited by the ethanolic extracts of *C. metuliferus*, and to develop and implement botanical nematicides derived from *C. metuliferus* extracts. Utilizing silica gel, reversed-phase medium, low-pressure gel Sephadex LH-20 column chromatography, and preparative high-performance liquid chromatography, the rhizome extracts of *C. metuliferus* were isolated, purified, and identified. Furthermore, the antioxidant and nematicidal activities of the compounds were elucidated, thereby providing a theoretical foundation for the separation and enrichment of nematicidal substances from *C. metuliferus* for the development of botanical nematicidal agents.

2 Materials and methods

2.1 Materials

2.1.1 Test materials. The rhizome of *C. metuliferus* was obtained from Zhengzhou Fruit Research Institute, Chinese Academy of Agricultural Sciences (No. PI292190).

2.1.2 Source of test insects. Root-knot nematodes were cultured in the laboratory at Huazhong Agricultural University. The specific isolation and culture methods employed were as follows: the root systems of plants infected with *M. incognita* were collected, thoroughly washed with water, and subsequently cut into small pieces. These pieces were then placed into a soybean milk machine, to which aseptic water and an appropriate amount of sodium hypochlorite (approximately 5 mL) were added. The root tissues were homogenized for 3 – 5 sec. The resulting mixture was then passed through a series of sieves, specifically a 35-mesh, 200-mesh, and 500-mesh sieve. This process allowed for the isolation of egg masses and larvae in the early parasitic stage from the 500-mesh sieve. A homemade nematode incubator was prepared, consisting of a petri dish at the bottom, followed by a 20-mesh sieve, and two layers of handkerchief paper placed on the sieve. The collected egg masses of root-knot nematodes were transferred onto the handkerchief paper, with water added to the interface. The larvae were incubated at a temperature range of 25 – 28 ℃, and the second instar larvae were subsequently collected after 4 d. The collected nematodes were allowed to settle naturally, after which the surface water was aspirated using a glue-tipped burette[13], thereby retaining the nematode suspension for subsequent use.

2.1.3 Test instruments and reagents. The instruments utilized in the experiment comprised the following: a rotary evaporator from Shanghai Ailang Instrument Company; a DLSB-10/30 low-temperature cooling circulating pump manufactured by Zhengzhou Greatwall Scientific Industrial and Trade Co., Ltd.; a MAT95XP high-resolution mass spectrometer; a Bruker AVANCE AV 600 MHz superconducting pulsed Fourier transform nuclear magnetic resonance spectrometer from Switzerland; an ultra-cleaning bench provided by Shanghai Boxun Industrial Co., Ltd.; an autoclave sterilizer from HIRAYAMA, Japan; an Agilent Infinity 1260 analytical high-performance liquid chromatograph (Agilent, USA); an Agilent Infinity 1260 II semi-preparative high-performance liquid chromatograph (Agilent, USA); semi-preparative chromatographic columns (Cs, 250 mm × 100 mm, 5 μm; COSMOSIL, Japan); a Bruker AmaZon SL mass spectrometer (Bruker, Germany); TMS utilized as an internal standard; an autoclave from HIRAYAMA, Japan; an ELX-800 microplate reader from Bio-Tek, USA; column chromatography silica gel (60 – 80, 200 – 300 mesh), thin-layer chromatography silica gel plates, and silica gel H from Qingdao Haiyang Chemical Group Company; gel Sephadex LH-20

from Merck, USA; and RP-18 filler (20 – 45 μm) from Fuji Silysia Chemical Ltd., Japan. The organic reagents utilized in this study included domestic analytical reagent (AR) grade chemicals sourced from Tianjin Concord Technology Co., Ltd.; methanol of high-performance liquid chromatography (HPLC) grade from Merck; dimethyl sulfoxide (DMSO) from Qingdao Tenglong Weibo Technology Co., Ltd.; lipopolysaccharide, Griess reagent, and methyl thiazolyl tetrazolium (MTT) from Sigma, USA; Dulbecco's Modified Eagle Medium (DMEM) and fetal bovine serum from Thermo Fisher Scientific; phosphate-buffered saline (PBS) from Beijing Xinjingke Biotechnology Co., Ltd.; and sodium bicarbonate and DMSO from Xilong Chemical Co., Ltd.

2.2 Methods

2.2.1 Extraction and separation. 5 kg of rhizome from *C. metuliferus* was placed in a 50 L stainless steel extraction tank, which also served as an ultrasonic extraction reactor. The rhizome was immersed in a 40 L solution composed of a 50 ∶ 50 volumetric ratio of ethanol to water for 7 d at room temperature. Following this period, the mixture was filtered using filtering cloth to obtain the filtrate. Subsequently, the filtrate was subjected to vaporization to eliminate acetonitrile, ensuring that the concentration of acetonitrile residue in the final extract was maintained at less than 10%. The resulting medicinal extract of *C. metuliferus* was then prepared for subsequent use.

Extraction of crude product: A chromatographic column measuring 50 mm × 500 mm was packed with HP-20 macroporous absorbent resin (Mitsubishi Chemical). The resin underwent a preliminary washing with ethanol followed by pure water. Subsequently, the medicinal extract was introduced into the column and washed with pure water until a lighter color was observed. The elution process was conducted using a solution composed of 60% ethanol and 40% water (*v*/*v*) until the color of the eluate lightened. Finally, the eluate was concentrated and dried. The elution time for compounds 1 – 4 were recorded as 7.9 – 11.8, 21.1 – 22.5, 19.8 – 20.5, and 20.51 – 21 min, respectively. The specification of HPLC column utilized was DAC 100 mm × 250 mm, YMC AQC$_{18}$, 10 μm. The detection wavelengths employed for HPLC analysis were 214 and 254 nm, with a flow rate set at 300 mL/min. The mobile phase consisted of two compositions: mobile phase A was acetonitrile, and mobile phase B was a 0.1% aqueous acetic acid solution. The ratios of acetonitrile to 0.1% aqueous acetic acid for elution were 1 ∶ 9, 1 ∶ 9, and 3 ∶ 2, respectively. The total elution time required to obtain the crude product was 65 min.

Fine separation and purification: 1/3 of the final crude product from elution was weighed and subsequently dissolved in 500 mL of a 0.1% acetic acid aqueous solution. The resulting solution was then filtered using a 0.45 μm membrane, with 10 mL aliquots taken at a time (equivalent to 2 g per aliquot), resulting in a total of 50 separations of the crude product. The liquid collected was analyzed using HPLC, and the target impurities were sequentially combined, concentrated to a reduced volume through rotary evaporation, and subsequently re-purified to achieve a purity exceeding 95%. The conditions employed for the HPLC analysis comprised mobile phase A, which was acetonitrile, and mobile phase B, which was a 0.1% aqueous solution of acetic acid. The chromatographic column utilized was a DAC 100 mm × 250 mm, YMC AQ C$_{18}$, 10 μm. The wavelengths employed for the analysis were 214 and 254 nm, and the flow rate was set at 300 mL/min.

2.2.2 DPPH scavenging test. 2.0 mL of the sample solution at various concentrations was transferred into stoppered test tubes. Subsequently, 3.0 mL of the DPPH ethanol solution with a concentration of 0.004 mg/mL was added, and the mixture was shaken thoroughly. The test tube was then left in the dark for 30 min to prevent light exposure, after which the absorbance (*Ai*) was measured at a wavelength of 514 nm. Following the same procedure, an equivalent volume of anhydrous ethanol was used to replace the DPPH ethanol solution, and the absorbance (*Aj*) was measured at a wavelength of 517 nm. Additionally, an equal volume of anhydrous ethanol was used to replace the sample solution, and the absorbance (*Ac*) was measured at the same wavelength of 517 nm. All measurements were conducted in triplicate, and the average values were calculated. The scavenging rate of DPPH (*K*) was subsequently determined.

$$K = [1 - (Ai - Aj)/Ac] \times 100\% \quad (1)$$

2.2.3 Determination of contact toxicity of compounds against nematodes. A suspension of nematodes (200 – 300 individuals) was prepared by combining 95 μL of sterile water with 5 μL of the sample solution, which was subsequently added to each well of a 96-well plate. The final concentrations of the samples tested were 0.25, 0.50, 1.00, 2.00, and 4.00 mg/mL. A blank control was established by replacing the sample solution with an equivalent volume of sterile water, while a positive control was created by substituting the sample solution with an equal volume of 1 mg/mL abamectin. This procedure was conducted in triplicate. The wells around the 96-well plate were moistened with 200 μL of sterile water, thoroughly mixed, and incubated at room temperature for 24 h. The mortality of nematodes was assessed using a fluorescent digital biomicroscope, ensuring that a minimum of 100 nematodes were counted. The nematode mortality rate and the corrected mortality rate were calculated independently.

Nematode mortality rate (%) = Number of dead nematodes/Total number of nematodes supplied × 100% (2)

Corrected nematode mortality rate (%) = (Nematode mortality rate in treatment group – Nematode mortality rate in control group)/(1 – Nematode mortality rate in control group) × 100% (3)

2.3 Data statistics and analysis The data were subjected to statistical and analytical analysis utilizing LD_{50} data processing software 1.01 and SPSS 19.0, developed by Landzo Studio.

3 Results and analysis

3.1 Structural identification of compounds Compound 1: yellow powder, ESI-MS *m/z* 617.5[M + Na]$^+$; molecular formula: $C_{27}H_{30}O_{15}$; ^{1}H-NMR (600 MHz, DMSO-d_6) δ: 13.6 (1H,

5-OH), 7.93 (2H, d, J = 8.8 Hz, H-2′, 6′), 6.93 (2H, d, J = 8.8 Hz, H-3′, 5′), 6.77 (1H, s, H-3), 6.48 (1H, s, H-8), 4.66 (1H, d, J = 10.0 Hz, H-1″), 4.42 (1H, m, H-2″), 4.18 (1H, d, J = 8.4 Hz, H-1‴), 3.69 (1H, dd, J = 11.8, 5.0 Hz, H-6″a), 3.43 (1H, m, H-3″), 3.39 (1H, m, H-6″b), 3.18 (1H, m, H-6‴a), 3.17 (1H, m, H-5″), 3.16 (1H, m, H-4″), 3.05 (1H, m, H-3‴), 3.01 (1H, m, H-4‴), 2.94 (1H, m, H-6‴b), 2.86 (1H, m, H-2‴), 2.65 (1H, m, H-5‴); ^{13}C-NMR (125 MHz, DMSO-d_6) δ: 164.0 (C-2), 103.3 (C-3), 182.7 (C-4), 162.1 (C-5), 108.4 (C-6), 163.5 (C-7), 94.0 (C-8), 157.0 (C-9), 103.9 (C-10), 121.7 (C-1′), 129.0 (C-2′, 6′), 161.7 (C-4′), 116.5 (C-3′, 5′), 71.7 (C-1″), 81.5 (C-2″), 78.9 (C-3″), 70.9 (C-4‴), 82.2 (C-5″), 62.0 (C-6″), 106.0 (C-1‴), 75.2 (C-2‴), 77.0 (C-3‴), 69.9 (C-4‴), 77.0 (C-5‴), 60.9 (C-6‴). The data presented above are largely consistent with the findings documented in the literature [15-19], leading to the identification of the compound as isovitexin 2″-O-β-glucoside.

Compound 2: colorless oil, ESI-MS m/z 263.1[M + Na]$^+$; molecular formula: $C_{13}H_{20}O_4$; ^{1}H-NMR (600 MHz, DMSO-d_6) δ: 7.12 (1H, dd, J = 15.3, 10.4 Hz, H-11), 6.23 (1H, dd, J = 15.1, 10.4 Hz, H-10), 6.17 (1H, dd, J = 15.1, 6.7 Hz, H-9), 5.77 (1H, d, J = 15.3 Hz, H-12), 2.17 (2H, t, J = 7.4 Hz, H-2), 2.12 (2H, m, H-8), 1.47 (2H, m, H-3), 1.37 (2H, m, H-7), 1.25 (6H, m, H-4, H-5, H-6); ^{13}C-NMR (125 MHz, DMSO-d_6) δ: 175.1 (C-1), 34.3 (C-2), 25.0 (C-3), 29.0 (C-4), 29.0 (C-5), 29.0 (C-6), 28.6 (C-7), 32.8 (C-8), 144.4 (C-9), 128.9 (C-10), 144.7 (C-11), 121.1 (C-12), 168.5 (C-13). The aforementioned data were not found in the database; therefore, this compound remains unidentified and has been initially designated as 9E, 11E-(13-oxo) tridecadienoic acid.

Compound 3: colorless oil, ESI-MS m/z 249.2[M + Na]$^+$; molecular formula: $C_{13}H_{22}O_3$; ^{1}H-NMR (600 MHz, DMSO-d_6) δ: 6.12 (1H, dd, J = 15.0, 10.3 Hz H-11), 6.02 (1H, dd, J = 15.0,10.7 Hz, H-10), 5.65 (1H, m, H-12), 5.62 (1H, m, H-9), 3.95 (2H, dd, J = 5.4, 1.5 Hz, H-13), 2.17 (2H, t, J = 7.4 Hz, H-2), 2.03 (2H, m, H-8), 1.47 (2H, m, H-3), 1.33 (2H, m, H-7), 1.25 (6H, m, H-4, H-5, H-6); ^{13}C-NMR (125 MHz, DMSO-d_6) δ: 175.0 (C-1), 34.2 (C-2), 25.0 (C-3), 28.9 (C-4), 29.0 (C-5), 29.1 (C-6), 29.2 (C-7), 32.4 (C-8), 134.0 (C-9), 130.4 (C-10), 129.9 (C-11), 132.4 (C-12), 61.7 (C-13). The aforementioned data were not found in the database; therefore, this compound remains unidentified and has been initially designated as 2E, 4E-tridecadienedioic acid.

Compound 4: colorless oil, ESI-MS m/z 263.0[M + K]$^+$; molecular formula: $C_{13}H_{20}O_3$; 1H-NMR (600 MHz, DMSO-d_6) δ: 9.49 (1H, d, J = 8.1 Hz, H-13), 7.31 (1H, dd, J = 15.2, 9.4 Hz, H-11), 6.40 (1H, m, H-10), 6.39 (1H, m, H-9), 6.09 (1H, dd, J = 15.2, 8.1 Hz, H-12), 2.19 (2H, m, H-8), 2.18 (2H, m, H-2), 1.48 (2H, m, H-3), 1.41 (2H, m, H-7), 1.27 (6H, m, H-4, H-5, H-6); ^{13}C-NMR (125 MHz, DMSO-d_6) δ: 175.0 (C-1), 34.2 (C-2), 25.0 (C-3), 29.0 (C-4), 29.0 (C-5), 29.0 (C-6), 28.4 (C-7), 33.0 (C-8), 147.7 (C-9), 129.3 (C-10), 153.9 (C-11), 130.4 (C-12), 194.8 (C-13). The data presented above are largely consistent with the findings documented in the literature [20-22], leading to the identification of the compound as 9E, 11E-(13 hydroxy) tridecadienoic acid.

The chemical structures of the isolated compounds from *C. metuliferus*, specifically isovitexin 2″-O-β-glucoside, 9E, 11E-(13-oxo) tridecadienoic acid, 2E, 4E-tridecadienedioic acid, and 9E, 11E-(13-hydroxy) tridecadienoic acid, are illustrated in Fig. 1.

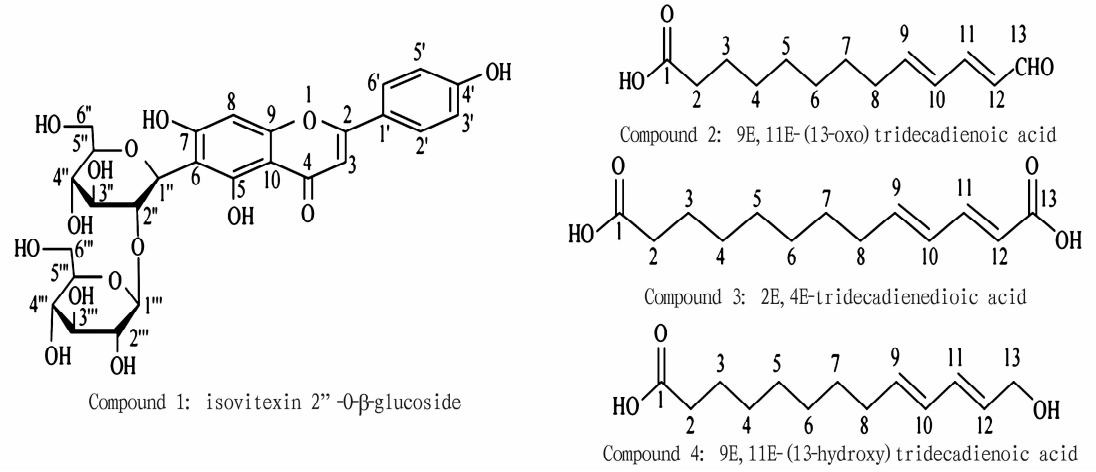


Fig. 1 Chemical structures of compounds 1, 2, 3 and 4

3.2 Activity of isolated compounds from *C. metuliferus*

3.2.1 DPPH scavenging effect. Compounds 2, 3, and 4 exhibited no antioxidant activity within the concentration range of 0.4 to 1.0 mg/mL, as evidenced by a scavenging rate of zero. In contrast, Compound 1 (isovitexin 2″-O-β-glucoside) demonstrated a scavenging effect on DPPH, which was positively correlated with the increasing mass concentration of the compound. Notably, the scavenging rate achieved 49.40% at a mass concentration of 1 mg/mL.

The antioxidant activity of compound 1 exhibited an increase with rising mass concentration, but it remained lower than that of Vc (Fig. 2).

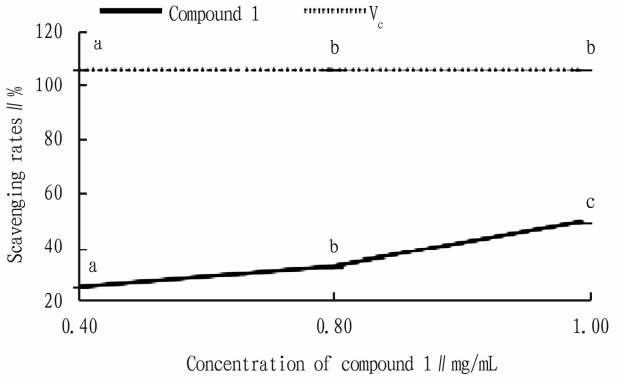
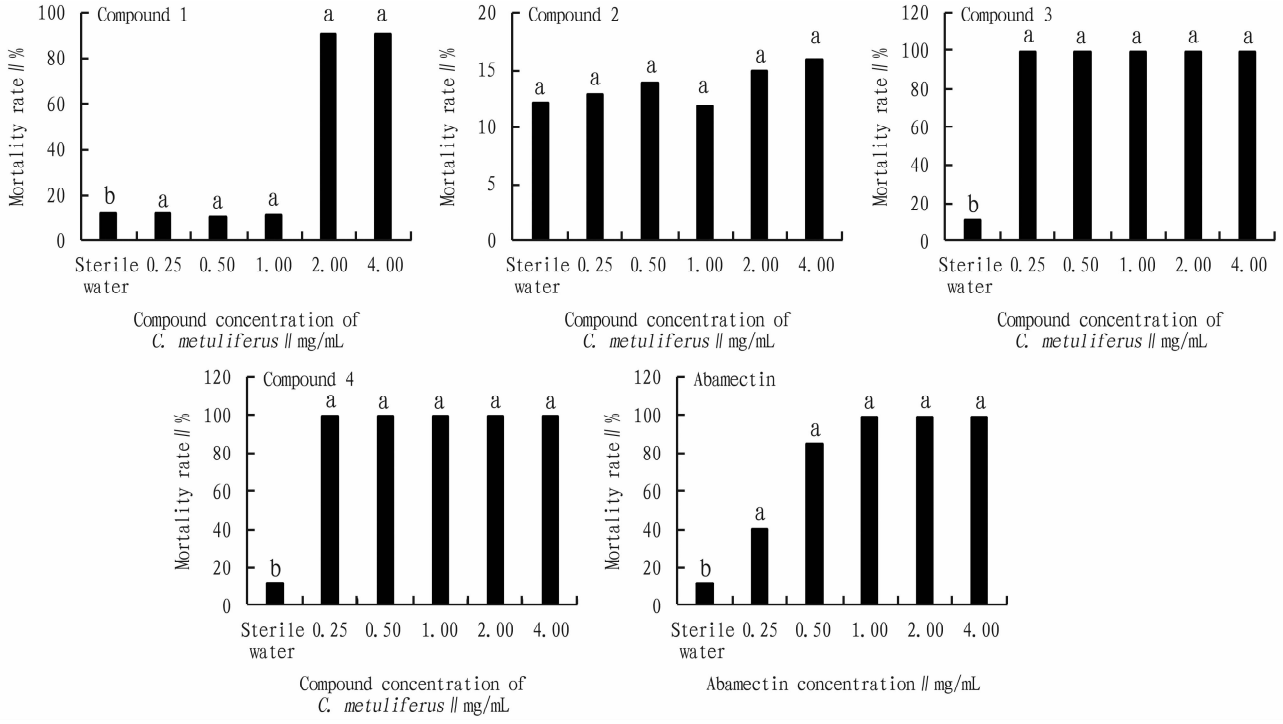


Fig. 2 Scavenging effect of isovitexin-2″-O-glucoside on DPPH

3.2.2 Nematicidal effect. As illustrated in Fig. 3, compound 1 at concentrations of 2.0 and 4.0 mg/mL exhibited strong contact toxicity against *M. incognita*, with mortality rates of 90.5% and 90.6%, respectively. These rates were significantly different from those observed at other concentrations. Additionally, compounds 3 and 4, at concentrations of 0.25 mg/mL and higher, demonstrated mortality rates of 100%, which were significantly different from the control group. In contrast, the mortality rate of compound 2 did not differ significantly from that of the control sterile water, indicating a lack of nematicidal activity. The mortality rate of abamectin against *M. incognita* was observed to be 100% at a concentration of 1 mg/mL, 40% at 0.25 mg/mL, and 85% at 0.5 mg/mL. In comparison, the contact toxicities of compound 1 at these concentrations were lower than that of abamectin, suggesting that the contact toxicity of compound 1 against *M. incognita* is relatively weak. Conversely, the contact toxicities of compounds 3 and 4 at the same concentrations exceeded that of abamectin, indicating that the contact toxicities of compounds 3 and 4 against *M. incognita* are comparatively stronger.



NOTE Different lowercase letters indicate significant differences among treatments ($P < 0.05$).

Fig. 3 Contact toxicity of compounds 1 – 4 on *Meloidogyne incognita*

4 Discussion

Previous research has demonstrated the antioxidant properties of *C. metuliferus*[11, 23-24]. Four compounds were isolated from the rhizome of PI292190 *C. metuliferus*, comprising one flavonoid (compound 1) and three terpenoids (compounds 2, 3, and 4). Notably, this marks the first instance of these compounds being isolated from *C. metuliferus*. Compound 1 is classified as a flavonoid, comprising over 90% of the total isolated compounds. It exhibited certain antioxidant properties; however, its antioxidant capacity was significantly lower than that of Vc. The remaining three compounds did not demonstrate any antioxidant properties, which may be attributed to their distinctive benzene ring structures. Compounds 3 and 4 exhibited significant nematicidal activity. The contact toxicity of compound 1 was found to be lower than that of avermectin at equivalent concentrations during production. Conversely, the contact toxicities of compounds 3 and 4 at the same concentrations surpassed that of avermectin in production. Based on the structural analysis of compounds 2, 3, and 4, it was observed that these compounds exhibit a high degree of similarity. However, the most significant differences are attributed to the

presence of hydroxyl, carboxyl, and aldehyde functional groups located on the 12th carbon. These variations may be the primary factors contributing to the observed contact toxicity against *M. incognita*.

Nematocidal or inhibitory activity has been documented in 316 species across 226 genera within 102 families, with Asteraceae and Leguminosae identified as the most prevalent families of nematicidal plants[25-26]. Currently, alkaloids, terpenoids, flavonoids, isothiocyanates, glucosinolates, phenols, steroids, polyacetylenes, and lipids represent some of the primary chemical classes of nematicidal active compositions[27]. Flavonoid compounds derived from various crops exhibit varying degrees of contact toxicity against *M. incognita*. Specifically, a concentration of 1 mg/mL of myricetin resulted in a 52.7% inhibition of egg hatching in *M. incognita*. Additionally, the flavonoid glycosides linaroside and lantanoside, extracted from *Lantana camara*, demonstrated lethal effects on *M. incognita* at a concentration of 1.0%. Furthermore, kaempferol was found to inhibit egg hatching of *M. incognita* by 58.3% at a concentration of 1 mg/mL[28]. The flavonoids derived from *C. metuliferus* exhibited activity comparable to that of myricetin and kaempferol. This similarity can be attributed to their high structural resemblance, although notable differences in the benzene rings were observed. These structural variations may account for the comparatively lower nematicidal activity of the flavonoids in *C. metuliferus* relative to those found in *L. camara*. The representative terpenoids, specifically terpenoid aldehyde and acetylantanolic acid, have been documented to exhibit significant nematicidal activity. For instance terpenoid aldehyde at a concentration of 50 mg/L was found to inhibit the activity of *M. incognita* larvae, while a 1.0% solution of acetylantanolic acid demonstrated the capability to lethally affect *M. incognita* larvae[29-34]. Compounds 3 and 4, which were isolated from *C. metuliferus*, exhibited lower nematicidal activity compared to terpenoid aldehyde and acetylantanolic acid. Furthermore, their nematicidal efficacy against *M. incognita* was inferior to that of alkaloids such as Waltherione A and 3,4-dihydroxybenzoic acid, while demonstrating higher activity than that of pyrrolizidine alkaloids, (Z)-3-(4-hydroxybenzylidene)-4-(4-hydroxyphenyl)-1-methylpyrrolidin-2-one, *etc.*

In the current study, while nematicidal compounds have been isolated from *C. metuliferus*, the specific targets of these compounds on nematodes require further investigation. At present, the nematicidal activity of extracts from *C. metuliferus* has not been thoroughly examined and lacks a systematic approach, thereby hindering the identification of the critical factors contributing to nematode lethality. Botanical pesticides offer numerous advantages and hold significant potential for application in agricultural practices. The investigation and application of plant secondary metabolites are poised to become a primary approach for the development of novel pesticides aimed at controlling root-knot nematodes[35-36]. Research on botanical pesticides derived from Cucurbitaceae species, such as *C. metuliferus*, plays a crucial role in the management of root-knot nematodes and is expected to contribute substantially to environmentally friendly and safe methods for the control of agricultural pests and diseases.

5 Conclusions

Four compounds were isolated from *C. metuliferus* and identified as isovitexin 2″-O-β-glucoside, 9E, 11E-(13-oxo) tridecadienoic acid, 2E, 4E-tridecadienedioic acid, and 9E, 11E-(13-hydroxy) tridecadienoic acid, respectively. The three compounds, isovitexin 2″-O-β-glucoside, 9E, 11E-(13-oxo) tridecadienoic acid, and 9E, 11E-(13-hydroxy) tridecadienoic acid, exhibited nematicidal activity, while isovitexin 2″-O-β-glucoside also demonstrated antioxidant activity. The scavenging rate of isovitexin 2″-O-β-glucoside on DPPH was observed to be 49.40% at a concentration of 1 mg/mL. Furthermore, isovitexin 2″-O-β-glucoside demonstrated significant nematicidal activity at a concentration of 2.0 mg/mL, resulting in a mortality rate of 90.5%. In comparison, the mortality rates of 9E, 11E-(13-oxo) tridecadienoic acid and 9E, 11E-(13-hydroxy) tridecadienoic acid at a concentration of 0.25 mg/mL reached 100%. All compounds were isolated for the first time from the rhizome of *C. metuliferus*, including 2E, 4E-tridecadienedioic acid and 9E, 11E-(13-oxo) tridecadienoic acid, which are identified as novel natural products.

References

[1] DONG DF, CAO ZP, WANG XH, *et al.* The effect of root knot nematode resistant rootstocks on tomato growth and yield[J]. Journal of Horticulture, 2007(5): 1305-1308. (in Chinese).

[2] LAI CW, WANG JH, CHENG ZY, *et al.* Molecular identification and sequence analysis of the pathogen causing kiwano melon (*Cucumis metuliferus*) virus disease in Fujian[J]. Journal of Fruit Science, 2021, 38(11): 1868-1876. (in Chinese).

[3] YAN X, LIU HQ, ZOU YQ, *et al.* Research progress in the physiological activity and synthesis of flavonoids[J]. Organic Chemistay, 2008, 48(9): 1534-1544. (in Chinese).

[4] DU XF, XU JM, WANG WZ, *et al.* Research progress in plant-based pesticides[J]. Pesticides, 2000, 39(11): 8-10. (in Chinese).

[5] WANNANG NN, JIMAM NS, OMALE S, *et al.* Effects of *Cucumis metuliferus* (Cucurbitaceae) fruits on enzymes and haematological parameters in albino rats[J]. African Journal of Biotechnology, 2007, 6(22): 2515-2518.

[6] WANG NN, GYANG SS, OMALE S, *et al.* The effect of *Cucumis metuliferus* E Meye (Cucurbitaceae) on rat gastric functions and mucosa intergrity[J]. Nigerian Journal of Natural Products and Medicine. 2008, 12(1). DOI:10. 4314/NJNPM. V12I1. 45663.

[7] OMALE S, WUYEP NN, AUTA A, *et al.* Anti-ulcer properties of alkaloids isolated from the fruit pulp of *Cucumis metuliferous* (Curcurbitacece)[J]. International Journal of Pharmaceutical Sciences and Research, 2011, 10(2): 2586-2588.

[8] JIMAM NS, OMALE S, WANNANG NN, *et al.* Evaluation of the hypoglycemic activity of *Cucumis metuliferus* (Cucurbitaceae) fruit pulp extract in normoglycemic alloxan induced hyperglycemic rats[J]. Journal of Young Pharmacists, 2010, 4(2): 384-387.

[9] ALIERO AA, GUMI AM. Studies on the germination, chemical composition and antimicrobial properties of *Cucumis metuliferus*[J]. Annals of Biological Research, 2012, 3(8): 4059-4064.

[10] USMAN JG, SODIPO OA, SAND ABE UK. In vitro antimicrobial activity of *Cucumis metuliferus* E. Mey. ex. Naudin fruit extracts against *Salmonella gallinarum*[J]. International Journal of Hytomedicine, 2014, 6(2): 268-274.

[11] WANG JM, SHANG JL, LI N, *et al.* Current situation and suggestions on the research and utilization of new crop in Cucumis-horned melon[J]. China Cucurbits and Vegetables, 2020, 33(4): 1-6. (in Chinese).

[12] AMAGON KI, WANNANG NN, ILIYA HA, *et al.* Flavonoids extracted from fruit pulp of *Cucumis metuliferus* have antiviral properties [J]. British Journal of Pharmaceutical Research, 2012, 2(4): 249 – 258.
[13] GAO Q, ZHANG H, REN HL, *et al.* The contact activity of extracts from *Cucumis metuliferus* against *Meloidogyne incognita* [J]. Anhui Agricultural Science, 2022, 50(8): 122 – 125. (in Chinese).
[14] ZHU YJ, XIE H. Study on the control effect of several plant derived pesticides on wheat sheath blight [J]. Henan Agriculture, 2023, 33(22): 49 – 50. (in Chinese).
[15] MZENA T, SWAI H, CHACHA M. Antimalarial activity of *Cucumis metuliferus* and *Lippia kituiensis* against *Plasmodium berghei* infection in mice [J]. Research & Reports in Tropical Medicine, 2018(9): 81 – 88.
[16] NISHIYAMA T, HAGIWARA Y, HAGIWARA H, *et al.* Inhibition of malonaldehyde formation from lipids by an isoflavonoid isolated from young green barley leaves [J]. Journal of the American Oil Chemists' Society, 1993, 70(8): 811 – 813.
[17] CHENG G, BAI Y, ZHAO Y, *et al.* Flavonoids from *Ziziphus jujuba* Mill var. *spinos* [J]. Tetrahedron, 2000, 56(45): 8915 – 8920.
[18] KITTA K, HAGIWARA Y, SHIBAMOTO T. Antioxidative activity of an isoflavonoid, 2"-O-glycosylisovitexin isolated from green barley leaves [J]. Journal of Agricultural & Food Chemistry, 1992, 40(10): 1843 – 1845.
[19] NISHIYAMA T, HAGIWARA Y, HAGIWARA H, *et al.* Formation and inhibition of genotoxic glyoxal and malonaldehyde from phospholipids and fish liver oil upon lipid peroxidation [J]. Journal of Agricultural & Food Chemistry, 1994, 42(8): 1728 – 1731.
[20] KONDO Y, HASHIDOKO Y, MIZUTANI J. An enzymatic formation of 13-oxo-trideca-9, 11-dienoic acid from 13-hydroperoxylinolenic acid by a homolytic hydroperoxide lyase in elicitor-treated soybean cotyledons [J]. Biochimica et Biophysica Acta, 1995(1): 9 – 15.
[21] VAZ AD, ROBERTS ES, COON MJ. Reductive betascission of the hydroperoxides of fatty acids and xenobiotics: role of alcohol-inducible cytochrome P-450 [J]. Proceedings of the National Academy of Sciences of the United States of America, 1990, 87(14): 5499 – 5503.
[22] SUN M, DENG Y, BATYREVA E, *et al.* Novel bioactive phospholipids: Practical total syntheses of products from the oxidation of arachidonic and linoleic esters of 2-lysophosphatidylcholine(1) [J]. Journal of Organic Chemistry, 2002, 67(11): 3575 – 3584.
[23] XIE HQ, HE YJ, YANG T, *et al.* Analysis and evaluation of nutrient composition and antioxidant activity of *Cucumis metuliferus* [J]. Chinese Vegetable Journal, 2017, 42(9): 26 – 31. (in Chinese).
[24] GU JF, DU SF, GUO CJ. Modern clinical nutrition [M]. Beijing: Science Press, 2009: 148. (in Chinese).
[25] AN YX, SUN DL, ZHOU LJ, *et al.* Research and application of nematicidal activity in Asteraceae plants [J]. Chinese Journal of Agronomy, 2009, 25(23): 364 – 369. (in Chinese).
[26] JI YR, YANG QL, DONG Y, *et al.* Research and application progress on nematicidal activity of leguminous plants [J]. Anhui Agricultural Science, 2014, 42(26): 8884 – 8889.
[27] CHITWOOD DJ. Phytochemical based strategies for nematode control [J]. Annual Review of Phytopathology, 2002, 40(1): 221 – 249. (in Chinese).
[28] WU FZ, TIAN GM, BAI JZ, *et al.* Analysis of flavonoids in tillering onion bulbs and their relationship with the occurrence of tomato root knot nematode disease [J]. Journal of Northeast Agricultural University, 2021, 52(9): 19 – 28. (in Chinese).
[29] VEECH JA. Histochemical localization and nematotoxicity of teerpenoid aldehydes in cotton [J]. Journal of Nematology, 1979, 11: 240 – 246.
[30] JANG JY, LE DQ, CHOI GJ, *et al.* Control of root-knot nematodes using *Waltheria indica* producing 4-quinolone alkaloids [J]. Pest Management Science, 2019, 75(8): 2264 – 2270.
[31] LIU XC, LAI IO, LIU QZ, *et al.* Bioactivities of a new pyrrolidine alkaloid from the root barks of *Orixa japonica* [J]. Molecules, 2016, 21(12): 1665.
[32] CHEN JX, SONG BA. Natural nematicidal active compounds: Recent research progress and outlook [J]. Journalof Integrative Agriculture, 2021, 20(8): 2015 – 2031.
[33] LI GH, ZHANG KQ. Natural nematicidal metabolites and advances in their biocontrol capacity on plant parasitic nematodes [J]. Natural Product Reports, 2023, 12(7): 87 – 94.
[34] CHEN KY. Chemical composition and anti southern root knot nematode activity of Shu Ma [D]. Kunming: Kunming University of Science and Technology, 2023. (in Chinese).
[35] BEGUM S, WAHAB A, SIDDIQUI BS, *et al.* Nematicidal constituents of the aerial parts of *Lantana camara* [J]. Journal of Natural Products, 2000, 63: 765 – 767.
[36] LIU JQ, HAN XJ, XIE SL. Research progresses on the prevention and control of Meloidogyne spp. by botanical nematocides [J]. Agrochemicals, 2011, 50(6): 395 – 398, 410. (in Chinese).

(From page 6)

[17] GUO WW, ZHUO MC, FANG JP, *et al.* Anatomical characteristics and environmental adaptability of *Rhododendron aganniphum* var. *schizopeplum* leaf in Sejila Mountain, southeastern Tibet [J]. Acta Botanica Boreali – Occidentalia Sinica, 2020, 40(5): 811 – 818. (in Chinese).
[18] GONZALEZ-PALEO L, RAVETTA DA. Relationship between photosynthetic rate, water use and leaf structure in desert annual and perennial forbs differing in their growth [J]. Photosynthetica, 2018, 56: 1177 – 1187.
[19] NISHIMURA E, SUZAKI E, IRIE M, *et al.* Architecture and growth of an annual plant *Chenopodium album* in different light climates [J]. Ecological Research, 2010, 25(2): 383 – 393.
[20] MA GF, MANSUER S, ZHANG XQ. Relationship between soil properties in different vegetation types and altitudes on the south slope of Mt. Tuomuer [J]. Pratacultural Science, 2017, 34(6): 1149 – 1158. (in Chinese).
[21] CHEN HY, CHEN YT, HUANG BX, *et al.* Variation of Soil Physicochemical Properties in the Root System of Forsythia at Different Altitudes [J]. Northern Horticulture, 2023 (13): 69 – 76. (in Chinese).
[22] TIAN J, WANG QW, YU DP, *et al.* Air temperature variation along altitudinal gradient on the northern slope of Mt., Changbai, China [J]. Journal of Arid Land Resources and Environment, 2013, 27(4): 65 – 69. (in Chinese).
[23] WANG DX, WANG JM, JIANG ZC, *et al.* Effects of soil physicochemical properties on vegetation distribution in the Qilian Mountains [J]. Forest Science and Technology, 2024(2): 84 – 86. (in Chinese).
[24] BRADFORD MA, BERG B, MAYNARD DS, *et al.* Understanding the dominant controls on litter decomposition [J]. Journal of Ecology, 2016, 104: 229 – 238.
[25] ZHANG DY, WEN H, WANG W, *et al.* Study on influencing factors of active ingredient contents of of *Magnolia officinalis* grown in Sichuan large-scale cultivation areas [J]. Journal of Chinese Medicinal Materials, 2017, 40(6): 1280 – 1283. (in Chinese).
[26] ZHANG CC, SUN JH, WANG YH, *et al.* Research progress in microevolutionary process of excellent traits and quality of Dao-di herbs [J]. China Journal of Chinese Materia Medica, 2023, 48(22): 6021 – 6029. (in Chinese).