Research Article

Nematocidal Constituents from the Ethanol Extract of Evodia rutaecarpa Hort Unripe Fruits

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The ethanol extract of Chinese medicinal herb, Evodia rutaecarpa Hort unripe fruits, was found to possess nematocidal activity against the root-knot nematodes, Meloidogyne incognita, during the screening program for new agrochemicals from local wild plants and Chinese medicinal herbs. Bioactivity-guided chromatographic separation of the ethanol extract of E. rutaecarpa on repeated silica gel columns led to isolate five constituent components (two limonoids, evodol and limonin; three alkaloids, evodiamine, rutaecarpine, and wuchuyuamide I). Evodiamine (LC50 = 73.55 μg/mL) and rutaecarpine (LC50 = 120.85 μg/mL) exhibited stronger nematocidal activity against M. incognita than the crude ethanol extract of E. rutaecarpa (LC50 = 131.54 μg/mL). Wuchuyuamide I, evodol, and limonin also possessed nematocidal activity against M. incognita with LC50 values of 147.87 μg/mL, 155.02 μg/mL, and 197.37 μg/mL, respectively, but weaker than the crude ethanol extract of E. rutaecarpa.

1. Introduction

Root-knot nematodes are important agricultural pests worldwide. Possible control strategies include chemical nematicides, crop rotation, and use of resistant cultivars, when available. The broad host range of Meloidogyne species, however, makes crop rotation difficult, and the use of nematicides, in spite of their efficacy for controlling nematodes, has negative side effects that have led to banning or restricting their use [1]. On the other hand, resistance-breaking populations of Meloidogyne are challenging the use of resistant cultivars [2]. Those are now resulting in a strong interest in nematicides of natural origin [3]. One alternative is to screen naturally occurring compounds in plants, which are known as plant secondary compounds. Many plant constituents and metabolites have been investigated for activity against plant-parasitic nematodes [4–7]. A series of nematicidal substances of plant origin such as triglycerides, sesquiterpenes, alkaloids, steroids, diterpenes, and flavonoids have been identified [3]. During our screening program for new agrochemicals from local wild plants and Chinese medicinal herbs, ethanol extract of Evodia rutaecarpa Hort unripe fruits (Family: Rutaceae) was found to possess strong nematocidal activity against the root-knot nematodes, Meloidogyne incognita (Kofoid and White) Chitwood. M. incognita is the most economically important and widely distributed nematode throughout China, and a considerable crop loss is caused by this nematode [7].

E. rutaecarpa unripe fruit is a commonly used Chinese medicinal herb and has been recommended for the treatment of abdominal pain, acid regurgitation, nausea, diarrhea, hernia, and dysmenorrhea and is also used externally for treatment of aphthous stomatitis [8]. E. rutaecarpa is also documented as insecticidal/antiparasitic in the literature [9]. The essential oil of E. rutaecarpa unripe fruits possessed strong fumigant toxicity against two grain storage insects, Tribolium castaneum and Sitophilus zeamais, and three active constituent compounds were isolated from the essential oil of E. rutaecarpa [10, 11]. Two quinolone alkaloids isolated from the herb exhibited toxicity to brine shrimp (Artemia salina) [12]. Moreover, three alkaloids, evodiamine, rutaecarpine, and rhetsinine, isolated from E. rutaecarpa by bioassay-guided fractionation showed insecticidal activity against fruit
flies (Drosophila melanogaster) [13]. The ethanol extract of E. rutaecarpa also possessed strong antifeedant activity against the two stored product insect pests (T. castaneum and S. zeamais) [14]. However, as far as we know, there are no reports about isolation of active components against nematodes from this Chinese medicinal herb. In this paper, the ethanol extract of E. rutaecarpa was evaluated for toxicity against M. incognita and five active constituent compounds (Figure 1) were isolated and identified from the ethanol extract of E. rutaecarpa by bioassay-directed fractionation.

2. Experimental

$^1$H nuclear magnetic resonance (NMR) spectra were recorded on Bruker ACF300 (300 MHz ($^1$H)) and AMX500 (500 MHz ($^1$H)) instruments using deuterochloroform (CDCl$_3$) as the solvent with tetramethylsilane (TMS) as the internal standard. Electron impact mass spectra (EIMS) were determined on a Micromass VG7035 mass spectrometer at 70 eV (probe).

2.1. Chinese Medicinal Herb and Extraction. E. rutaecarpa (5 kg, dried unripe fruits), purchased from Anguo Chinese Herbs Market, Hebei province, China, were ground to a powder and extracted with 95% ethanol ($3 \times 10$ L) at room temperature over a period of three weeks. The extracts were concentrated using a vacuum rotary evaporator to afford a syrupy gum (454 g). This syrup was partitioned between methanol water and n-hexane ($3 \times 1,000$ mL). The n-hexane extracts were evaporated off to give a residue (232 g). The aqueous layer was repartitioned with chloroform ($3 \times 1,000$ mL) to provide a residue (44 g) after evaporation of chloroform. Further partitioning with ethyl acetate ($3 \times 1,000$ mL) gave a residue (65 g) after evaporation of the solvent.

2.2. Chromatography. Based on bioassay, the strongest nematocidal extract, the CHCl$_3$ residue (20 g) was applied to a silica gel column (600 mm in height and 100 mm in diameter, 160–200 mesh, Qingdao Marine Chemical Plant, Shandong province, China), eluting with petroleum ether containing increasing accounts of ethyl acetate (from 100:1 to 0:100) to give 27 combined fractions according to thin-layer chromatography (TLC) detection. Based on bioassay, fractions 2 (232 mg), 3 (283 mg), 6 (194 mg), 13–14 (503 mg), and 22–24 (632 mg) were chosen for further
fractionation. Evodol (14.3 mg) was isolated from fraction 2 after being repeatedly purified on silica and PTLC (precoated GF254 plates, Qingdao Marine Chemical Plant). Fraction 3 was further chromatographed on silica gel column and repeated PTLC to provide the bioactive compound, which was recrystallized and determined to be limonin (21.0 mg). Wuchuyuamide I (10.4 mg) was obtained from further chromatographed on silica gel column and silica gel TLC to obtain rutaecarpine (26.4 mg) after recrystallization. Evodiamine (27.9 mg) was obtained from further chromatographed on silica gel TLC and recrystallized from fraction 22–24. Evodiamine has been shown to reduce fat uptake in mouse [20]. Moreover, evodiamine shows the same level of inhibiting proliferation, invasion, and metastasis, inducing apoptosis of a variety of tumor cell lines [20]. Moreover, evodiamine has been shown to reduce fat uptake in mouse studies. It is suspected that its mechanism of action is similar to that of capsaicin [21]. As such, it has been included in some dietary supplements. Rutaecarpine has shown a variety of intriguing biological properties such as antithrombotic, anticancer, anti-inflammatory and analgesic, antiobesity and thermoregulatory, vasorelaxing activity, as well as effects on the cardiovascular and endocrine systems [22]. Moreover, the two isolated alkaloids, evodiamine and rutaecarpine, exhibited insecticidal activity against the fruit flies (D. melanogaster) [13]. However, the two isolated constituent compounds were first time to be evaluated for nematocidal activity against the root-knot nematodes. Five isolated constituent compounds and the crude ethanol extract of *E. rutaecarpa* unripe fruits exhibited nematocidal toxicity against the root-knot nematodes, *M. incognita* (Table 1). The two alkaloids, evodiamine (LC$_{50} = 73.55$ μg/mL) and rutaecarpine (LC$_{50} = 120.85$ μg/mL), exhibited stronger nematocidal activity against *M. incognita* than the crude ethanol extract (LC$_{50} = 131.54$ μg/mL). It indicated that the nematocidal activity of ethanol extract of *E. rutaecarpa* unripe fruits may be attributed to the present of the two alkaloids. Moreover, evodiamine shows the same level of nematocidal activity against *M. incognita* as the positive control, carbofuran (LC$_{50} = 72.29$ μg/mL), while the ethanol extract of *E. rutaecarpa* unripe fruits and rutaecarpine exhibit only half level of nematocidal activity, compared with carbofuran. In the previous studies, evodiamine was demonstrated to possess anticancer activities both in vitro and in vivo by inhibiting proliferation, invasion, and metastasis, inducing apoptosis of a variety of tumor cell lines [20]. Moreover, evodiamine has been shown to reduce fat uptake in mouse studies. It is suspected that its mechanism of action is similar to that of capsaicin [21]. As such, it has been included in some dietary supplements. Rutaecarpine has shown a variety of intriguing biological properties such as antithrombotic, anticancer, anti-inflammatory and analgesic, antiobesity and thermoregulatory, vasorelaxing activity, as well as effects on the cardiovascular and endocrine systems [22]. Moreover, the two isolated alkaloids, evodiamine and rutaecarpine, exhibited insecticidal activity against the fruit flies (D. melanogaster) [13]. However, the two isolated constituent compounds were first time to be evaluated for nematocidal activity against the root-knot nematodes.

### 3. Results and Discussion

Five isolated constituent compounds and the crude ethanol extract of *E. rutaecarpa* unripe fruits exhibited nematocidal toxicity against the root-knot nematodes, *M. incognita* (Table 1). The two alkaloids, evodiamine (LC$_{50} = 73.55$ μg/mL) and rutaecarpine (LC$_{50} = 120.85$ μg/mL), exhibited stronger nematocidal activity against *M. incognita* than the crude ethanol extract (LC$_{50} = 131.54$ μg/mL). It indicated that the nematocidal activity of ethanol extract of *E. rutaecarpa* unripe fruits may be attributed to the present of the two alkaloids. Moreover, evodiamine shows the same level of nematocidal activity against *M. incognita* as the positive control, carbofuran (LC$_{50} = 72.29$ μg/mL), while the ethanol extract of *E. rutaecarpa* unripe fruits and rutaecarpine exhibit only half level of nematocidal activity, compared with carbofuran. In the previous studies, evodiamine was demonstrated to possess anticancer activities both in vitro and in vivo by inhibiting proliferation, invasion, and metastasis, inducing apoptosis of a variety of tumor cell lines [20]. Moreover, evodiamine has been shown to reduce fat uptake in mouse studies. It is suspected that its mechanism of action is similar to that of capsaicin [21]. As such, it has been included in some dietary supplements. Rutaecarpine has shown a variety of intriguing biological properties such as antithrombotic, anticancer, anti-inflammatory and analgesic, antiobesity and thermoregulatory, vasorelaxing activity, as well as effects on the cardiovascular and endocrine systems [22]. Moreover, the two isolated alkaloids, evodiamine and rutaecarpine, exhibited insecticidal activity against the fruit flies (D. melanogaster) [13]. However, the two isolated constituent compounds were first time to be evaluated for nematocidal activity against the root-knot nematodes.

### Table 1: Nematocidal toxicity of the ethanol extract of *E. rutaecarpa* and isolated compounds against *Meloidogyne incognita*.

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Concentration</th>
<th>LC$_{50}$ (μg/mL) (95% FL)</th>
<th>Slope ± SD</th>
<th>Chi square ($\chi^2$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Evodiamine</td>
<td>12.5–200.0</td>
<td>73.55 (45.62–140.36)</td>
<td>1.08 ± 0.08</td>
<td>20.34</td>
</tr>
<tr>
<td>Evodol</td>
<td>25.0–400.0</td>
<td>155.02 (82.29–273.23)</td>
<td>0.85 ± 0.07</td>
<td>16.81</td>
</tr>
<tr>
<td>Limonin</td>
<td>25.0–400.0</td>
<td>197.35 (91.82–381.60)</td>
<td>0.81 ± 0.06</td>
<td>18.44</td>
</tr>
<tr>
<td>Rutaecarpine</td>
<td>12.5–200.0</td>
<td>120.85 (76.16–203.07)</td>
<td>0.67 ± 0.05</td>
<td>16.25</td>
</tr>
<tr>
<td>Wuchuyuamide I</td>
<td>25.0–400.0</td>
<td>147.87 (47.95–458.70)</td>
<td>0.58 ± 0.09</td>
<td>15.89</td>
</tr>
<tr>
<td>Ethanol extract</td>
<td>25.0–400.0</td>
<td>131.54 (51.12–238.98)</td>
<td>0.69 ± 0.11</td>
<td>19.12</td>
</tr>
<tr>
<td>Carbofuran</td>
<td>25.0–400.0</td>
<td>72.29 (37.86–117.97)</td>
<td>1.21 ± 0.14</td>
<td>13.57</td>
</tr>
</tbody>
</table>
activity against Aedes albopictus and A. aegypti [26, 27]. Several tirucallane triterpenoids derived from Melia azedarach fruits, for example, 3-α-tigloylmelanol, melianone, 21-β-acetoxy-melianone, and methyl kulanote as well as limonin were evaluated for their nematicidal activity against M. incognita, and no significant effect on M. incognita was observed at a dose range of 31.2–500 μg/mL [28]. However, the two other isolated constituent compounds were first time to be evaluated for nematicidal activity against the root-knot nematodes.

Considering the currently used nematicicides are synthetic and usually possess high toxic to nontarget organisms, nematicidal activity of the ethanol extract of E. rutacearpa unripe fruits and the five isolated constituent compounds are quite promising and they show potential to be developed as possible natural nematicides for control of the root-knot nematodes. However, little has been done on mechanisms of action of these five compounds (two limonoids and three indole alkaloids) against nematodes. In addition, further testing is necessary to evaluate the spectrum of nematicidal activity against other plant parasitic and free-living nematodes and their phytotoxicity to crops and to develop formulations to improve the efficacy and stability and to reduce cost. Moreover, for the practical use of the ethanol extract of E. rutacearpa unripe fruits and its constituents as novel nematicides to proceed, further research is needed to establish their human safety and environmental safety. However, in traditional Chinese medicine, the unripe fruits of E. rutacearpa were commonly used for the treatment of abdominal pain, acid regurgitation, nausea, diarrhea, hernia, and dysmenorrhea and were also used externally for treatment of aphthous stomatitis [8]. However, no experimental data about the safety of ethanol extract of this medicinal herb and the four other isolated constituents is available so far. Therefore, any attempt to develop an alkaloid-derived agrochemical must be carefully evaluated for harmful effects.

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