

Nematicidal Potential of Green Chiretta Extracts Against *Meloidogyne enterolobii* and *M. incognita*: *In vitro* Assessment for Agricultural Application

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Abstract

Root-knot nematodes pose a persistent threat to various economically important crops in Thailand, with limited available management strategies for farmers. This study aimed to assess the efficacy of aqueous (AE), methanolic (ME), ethanolic (EE), ethyl acetate (EAE), and chloroform (CE) crude extracts derived from green chiretta in controlling *Meloidogyne enterolobii* and *M. incognita* under laboratory conditions. The results demonstrated significant inhibitory effects of all crude extracts, except for CE, against both nematode species. Notably, AE (10 and 20 mg/mL), ME (20 mg/mL), and EE (20 mg/mL) treatments exhibited maximum inhibition of hatching, leading to a considerable reduction in nematode hatch rates by 87.5 - 96.8 % for *M. enterolobii* and 88.5 - 96.5 % for *M. incognita*, compared with distilled water (DW). In terms of second-stage juveniles (J2s) mortality, AE (10 and 20 mg/mL), ME (20 mg/mL), and EAE (20 mg/mL) demonstrated the highest nematicidal activity, resulting in mortality rates of 94.2 - 100 % for *M. enterolobii* and 92.2 - 99.2 % for *M. incognita*. Chemotaxis assays revealed a repellent effect of all extracts on both nematode species, except for the ethyl acetate extract (EAE), which attracted *M. incognita* J2s without affecting *M. enterolobii* J2s. The observed efficacy was attributed to the higher concentrations of bioactive compounds, including flavonoids, phenolics, and terpenoids, in the extracts compared to CE. Based on these *in vitro* findings, it is suggested that AE, ME, EE, and EAE of green chiretta exhibit significant nematicidal activity against *M. enterolobii* and *M. incognita*. Further evaluation under greenhouse and field conditions is warranted to ascertain the practical applicability and effectiveness of these extracts in a real-world agricultural setting.

Keywords: Crude extracts, Green chiretta, Medicinal plants, Nematicidal activity, Root-knot nematodes

Introduction

The root-knot nematodes (RKNs), *Meloidogyne* spp., have been identified as the most significant plant-parasitic nematodes affecting numerous economic crops [1]. In Asia, 45 species of the genus *Meloidogyne* have been documented [2], and over 3,000 cultivated plant species are reported to be

susceptible hosts for *Meloidogyne* spp. [3]. RKN infestations can lead to substantial crop losses across a variety of plants, with *M. incognita* causing, for example, 40 % losses in herbage yield, 46 % losses in mental mint, and 12 - 60 % losses in cucumber [4,5]. Additionally, *Meloidogyne* spp. infestation has been linked to a 74 % reduction in ginger rhizome weight [6], and *M. enterolobii* has caused significant yield reductions in okra, reaching up to 37 % [7]. In Thailand, 9 *Meloidogyne* species have been identified [8], with *M. enterolobii* and *M. incognita* being common species frequently found in economic crops, particularly in guava and chili [9,10]. The economic impact of *Meloidogyne* spp. on chili yields in Thailand is substantial, estimated at 1 - 2.5 million USD [11]. Despite the availability of various management methods for RKNs, their effectiveness is contingent on specific conditions and circumstances [12,13]. Consequently, there is a need to continually study and update effective strategies tailored to each condition/location for successful RKN control [14].

The utilization of extracts derived from medicinal plants has gained significant attention as a promising approach for the management of RKNs [15,16]. Notably, various studies have highlighted the efficacy of these plant extracts in mitigating RKN infestations. For instance, ginger aqueous extracts at a concentration of 100 % demonstrated remarkable capabilities in reducing egg hatch and increasing the mortality of second-stage juveniles (J2) of *M. javanica* by 93 and 85 %, respectively [17]. Additionally, studies involving periwinkle aqueous extracts and thyme ethanolic extracts reported complete mortality (100 %) of *M. incognita* J2 [16]. Furthermore, investigations into the nematicidal properties of garlic methanolic extracts and *Tanacetum balsitanicum* ethyl acetate extracts at a 1 % concentration exhibited substantial activity against *M. incognita* J2, resulting in mortality rates of 95 and 80 %, respectively [15]. These findings underscore the potential of medicinal plant extracts as effective agents in the control of RKNs, offering insights into their diverse nematicidal activities against different nematode species. The exploration of such natural alternatives contributes to the ongoing pursuit of sustainable and eco-friendly strategies for nematode management.

Green chiretta (*Andrographis paniculata* (Burm.f.) Nees) stands out as a noteworthy medicinal plant with a rich history of traditional use for treating various ailments, including sore throat, cold, fever, laryngitis, and several infectious diseases [18,19]. The leaves of green chiretta have been extensively studied, revealing the presence of diverse bioactive compounds such as deoxy-andrographolide, andrographolide, neoandrographolide, 14-deox-11, 12-didehydroandrographolide, and homoandrographolide [19,20]. These compounds exhibit a range of therapeutic properties, including antibacterial, antioxidant, anti-inflammatory, anticancer, and immune-stimulating effects [19,20]. Interestingly, Nisha *et al.* [21] reported a significant reduction (88 - 92 %) in the population density of *M. incognita* in soil and pepper roots achieved through soil amendment with dried leaves of green chiretta, providing initial evidence of its potential as a nematode management tool. However, beyond this report, there is a dearth of information on the utilization of green chiretta for the management of plant-parasitic nematodes. Therefore, the present study aims to fill this knowledge gap by assessing the efficacy of green chiretta extracts in suppressing the eggs and second-stage juveniles (J2s) of *M. enterolobii* and *M. incognita*, within controlled laboratory conditions. In addition to evaluating the nematode-suppressive properties of green chiretta, this study seeks to quantitatively measure the levels of nematicidal bioactive compounds present in the extracts. Specifically, we will focus on the assessment of flavonoids, phenolics, and terpenoids, previously identified in green chiretta and known for their potential nematicidal activities [14, 19,20]. The quantification of these bioactive compounds is anticipated to provide valuable insights into the underlying mechanisms contributing to the observed nematode-suppressive effects. This investigation carries the potential to significantly enhance our understanding of green chiretta's viability as a sustainable and natural solution for controlling plant-parasitic nematodes. The findings of this study may pave the way

for the development of eco-friendly and effective nematode management strategies, promoting the broader adoption of green chiretta in agricultural practices.

Materials and methods

Root-knot nematode species verification

Meloidogyne enterolobii and *M. incognita* specimens were procured from the Department of Plant Pathology, Faculty of Agriculture, Kasetsart University. The identification and validation of nematode species were conducted through a combination of perineal pattern analyses and Polymerase Chain Reaction (PCR) techniques based on DNA amplification with species-specific primers. The species-specific primers employed were MK7F/MK7R for *M. enterolobii* [22] and Inc14F/Inc14R for *M. incognita* [23]. These methods ensured accurate and reliable confirmation of the RKN species under investigation.

To conduct perineal pattern observations, females of each *Meloidogyne* species were carefully extracted from okra roots and placed into a droplet of 45 % lactic acid on a glass slide. Subsequently, a female specimen was sectioned at one-third posterior, and the cuticle around the perineal pattern was trimmed and cleaned with lactic acid. The isolated perineal patterns were then mounted in a droplet of 4 % glycerin on a new glass slide, covered with a slip, and sealed with nail polish. Using a compound microscope (Olympus CX33) coupled with a digital camera (Canon EOS750D), the perineal patterns were meticulously observed and captured for detailed documentation.

For molecular characterization, DNA extraction was carried out following the procedure outlined by Beesa *et al.* [24]. The Polymerase Chain Reaction (PCR) was then conducted using the extracted nematode DNA as the template. The 15- μ L PCR mixture comprised 3 μ L of DNA template, 4 μ L of sterilized distilled water, 0.5 μ L of each 10 μ M forward and reverse primers, and 7 μ L of 2X PCR master mix (Intron biotechnology, Korea). The PCR conditions were set as follows: initial denaturation at 94 °C for 5 min, followed by 35 cycles of denaturation at 94 °C for 30 s, annealing at 62 °C (MK7F/MK7R) or 64 °C (Inc14F/Inc14R) for 30 s, extension at 72 °C for 1 min, and a final extension at 72 °C for 7 min. The resulting PCR products' band sizes were compared against a 100 bp DNA ladder (Biotechrabbit, Germany). The PCR products were electrophoretically detected for 20 min at 100 V in 1xTAE buffer on a standard 1.5 % agarose gel and visualized using a UV transilluminator.

Nematode preparation

To obtain eggs and second-stage juveniles (J2s) of the nematodes, 2-month-old okra roots infected with RKNs were carefully uprooted and thoroughly cleaned under running tap water to remove any soil particles or debris. Subsequently, the roots were sliced into small pieces (1 - 2 cm in length) and agitated in a 0.6 % Sodium hypochlorite (NaOCl) solution for a duration of 2 min [34]. The resulting nematode suspension was then filtered through nested sieves with apertures of 150 and 25 μ m. The nematode eggs, separated and collected using the 25 μ m aperture sieve, were subjected to a 7-day incubation period following the Baermann funnel method. The eggs and J2s obtained through this process were utilized in subsequent experiments.

Plant extract preparation

Green chiretta (*Andrographis paniculata* (Burm.f.) Nees) plants were cultivated in 15-cm-diameter pots filled with 400 cc of sterilized soil and nurtured in a greenhouse at the Department of Plant Pathology, Faculty of Agriculture, Kasetsart University. A fertilizer blend (N-P-K, 16-16-16) was applied at a rate of 5 g/pot twice, 1 and 2 months after transplantation. Upon reaching a maturity of 3 months, the aerial parts

of the green chiretta plants were carefully harvested, washed with tap water, and subsequently air-dried in the shade for 10 days. The dried plant materials were finely ground using a blender (DXM-1000, DXFILL machine).

For the preparation of crude extracts, 5 g of the finely ground plant material was soaked in 50 mL of various solvents, including distilled water, methanol, ethanol, chloroform, and ethyl acetate, for a duration of 2 days. Subsequently, the mixtures were filtered through double layers of muslin cloth and Whatman No.1 filter paper (Global Life Sciences Solutions Operations, UK). The resulted filtrates were concentrated at 45 °C under reduced pressure using a rotary evaporator (Buchi Rotavapor R-300) to yield crude extracts. The weights of the crude extracts were recorded to calculate the extraction yield, and the extracts were stored at 4 °C until further use.

Second-stage juvenile hatching and mortality tests

The evaluation of green chiretta extracts against eggs and J2s of *M. enterolobii* and *M. incognita* was conducted in 96-well polystyrene plates following the procedures outlined by Beesa *et al.* [14]. In each well, a 20 µL nematode suspension, containing approximately 20 ± 5 eggs or J2s, was added to 180 µL of each extract diluted in 10 % Dimethyl sulfoxide (DMSO) (at concentrations of 5, 10, and 20 mg/mL). Distilled water and 2 % DMSO served as controls in this study. The experiments were organized in a Completely Randomized Design (CRD) with 5 replications and repeated once for validation. For hatching tests, the number of hatched J2s was recorded at 7 days after incubation. In the case of J2 mortality, inactive J2s were counted at 2 days after incubation. Following the Day 2 count, the nematodes underwent 3 rinses with distilled water, and dead J2s were counted again on Day 3 (24 h after the rinse). The percent inhibition of hatching and the percent J2 mortality were then calculated [25].

Repellent assays

Chemotaxis assays were conducted following the methods outlined by Jindapunnapat *et al.* [35]. In each 55-mm diameter plastic plate, 10 mL of 1.4 % water agar was poured and left undisturbed for 1 h. The plate was divided into 3 distinct areas (refer to **Figure 1**): The starting area, the treatment area, and the control area. Extract treatments at a concentration of 20 mg/mL (10 µL) were gently applied to the agar at the treatment point, 15 µL of live J2s (approximately 30 J2s) were introduced onto the starting point, and 10 µL of water was added to the control point. Distilled water and 2 % Dimethyl sulfoxide (DMSO) were employed as controls. Each treatment was replicated with 3 plates in each of the 2 trials. The number of J2s in each area was counted, and the chemotaxis index (CI) was calculated using the formula: $CI = (\text{number of nematodes in the treatment area} - \text{number of nematodes in the control area}) / \text{total number of nematodes in the assay}$. Interpretations regarding CI values for the treatments were categorized as follows: ≥ 0.2 indicated an attractant; between 0.2 and 0.1, a weak attractant; from 0.1 to -0.1 , without effect; between -0.1 and -0.2 , a weak repellent; and ≤ -0.2 , a repellent.

Phytochemical analyses

The quantification of total flavonoid (TFC), phenol (TPC), and terpenoid (TTC) contents was conducted following the methodology outlined by Beesa *et al.* [14]. Specifically, 0.14 g of green chiretta extracts were individually dissolved in 1.4 mL of 70 % ethanol. The resulting crude extracts were then employed for the assessment of total flavonoid, phenol, and terpenoid contents. All phytochemical analyses were performed in triplicate to ensure accuracy and reliability of the results.

The flavonoid content was determined employing the aluminum chloride ($AlCl_3$) colorimetric assay as outlined by Pavun *et al.* [43]. In brief, 300 µL of the obtained extract was mixed with 900 µL of distilled

water, and 200 μL of 5 % (w/v) NaNO_2 was added. The mixture was then incubated in the dark for 6 min. Following this, 2 mL of 1 M NaOH was introduced, and the solution was left in the dark for an additional 15 min. The total flavonoid content was assessed by measuring the absorbance at 510 nm using a UV spectrophotometer (Biochrom). Quercetin (QE) at various concentrations (0 - 100 $\mu\text{g}/\text{mL}$) served as a standard ($y = 0.0009x + 0.0779$, $R^2 = 0.9949$). The flavonoid content was calculated and expressed as milligrams of quercetin equivalent per gram of crude extract (mg QE/g).

The determination of phenol content was carried out using the Folin–Ciocalteu method [43]. In a concise procedure, 2.5 mL of 0.2 N Folin–Ciocalteu reagent was added to 60 μL of the extract and left at room temperature in the dark for 2 min. Subsequently, 2 mL of Na_2CO_3 was introduced, and the solution was subjected to further 15 min incubation in a water bath at 50 $^\circ\text{C}$. The absorbance of the resulting mixture was measured at 760 nm using a UV spectrophotometer. Gallic acid (GAE) at various concentrations (0 - 250 $\mu\text{g}/\text{mL}$) was employed as a standard ($y = 0.0007x + 0.0672$, $R^2 = 0.9917$). The total phenol content (TPC) was calculated and expressed as milligrams of gallic acid equivalent per gram of crude extract (mg GAE/g).

The quantification of terpenoid content was achieved through the colorimetric method [44]. In this process, 1.5 mL chloroform and 200 μL 95 % ethanol were added to 200 μL of crude extract, and the mixture was thoroughly mixed using a vortex for 1 min. The resulting blend was left undisturbed for 3 min before introducing 100 μL concentrated H_2SO_4 , followed by a further 60 min incubation at room temperature in the dark. The supernatant was gently removed, leaving the reddish-brown precipitate at the bottom. Subsequently, 500 μL of 95 % ethanol was added and mixed using a vortex to dissolve the precipitate. The mixtures were then centrifuged at 1,500 $\times g$ for 2 min, and the terpenoid content (TPC) was estimated from a standard curve plotted using 0 - 500 $\mu\text{g}/\text{mL}$ linalool (LE) ($y = 0.0001x - 0.0252$, $R^2 = 0.9924$). Terpenoid content was calculated and expressed as milligrams of linalool acid equivalent per gram of crude extract (mg LE/g).

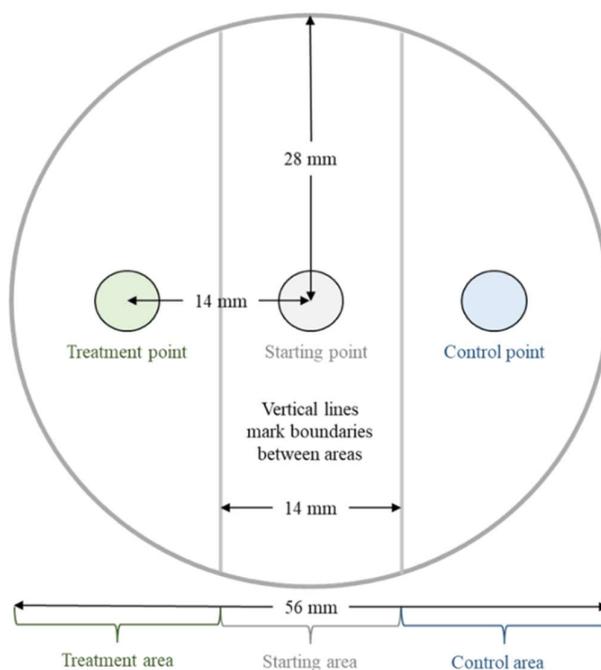


Figure 1 Schematic representation of a chemotaxis assay adapted from Jindapunapat *et al.* [35].

Statistical analysis

Statistical analysis was conducted using the SPSS software (version 16.0; SPSS). Differences among means were assessed utilizing Duncan's Multiple Range Test with a significance level set at $p \leq 0.05$.

Results and discussion

Verification of root-knot nematode species

Perineal patterns were examined, and the identified characteristics were aligned with the descriptions of *M. enterolobii* and *M. incognita* as reported by Jindapunnapat *et al.* [8] and Aydinli and Mennan [45]. The perineal pattern of *M. enterolobii* displayed an oval shape with moderate to high elevation, a squarish dorsal arch, smooth striae in the ventral arch, and a faint lateral line. In contrast, the perineal pattern of *M. incognita* exhibited an oval shape with high elevation, a squared-off dorsal arch, forked striae in the ventral arch, and the absence of a lateral line was noted (**Figure 2**).

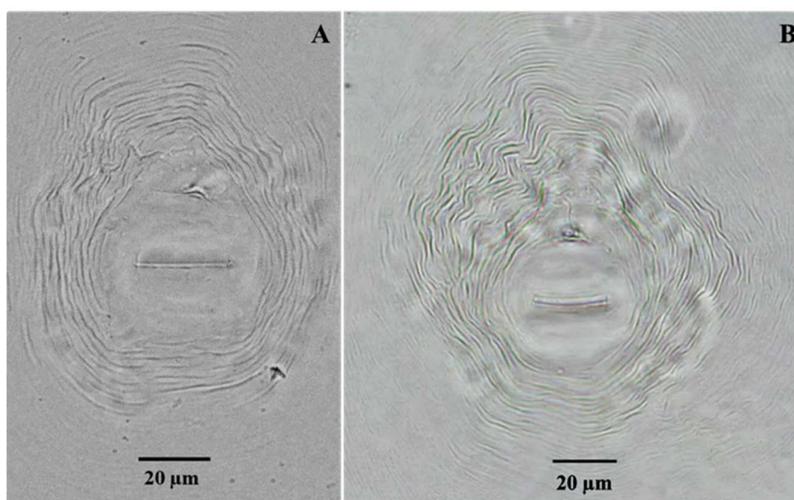


Figure 2 Perineal patterns of *Meloidogyne enterolobii* (A) and *M. incognita* (B) as utilized in the current study.

The DNA amplification of nematodes utilizing species-specific primers, MK7F/MK7R for *M. enterolobii* and Inc14F/Inc14R for *M. incognita*, resulted in amplicons of 520 and 400 bp, respectively (see **Figure 3**). This outcome is consistent with the findings of previous studies [8, 10, 26], where DNA amplification of *M. enterolobii* isolated from the rain tree and chili using primer MK7F/MK7R, and *M. incognita* extracted from tomato using primer Inc14F/Inc14R, yielded a similar amplicon size. Hence, the molecular characterization confirms that the RKNs utilized in this study were indeed *M. enterolobii* and *M. incognita*.

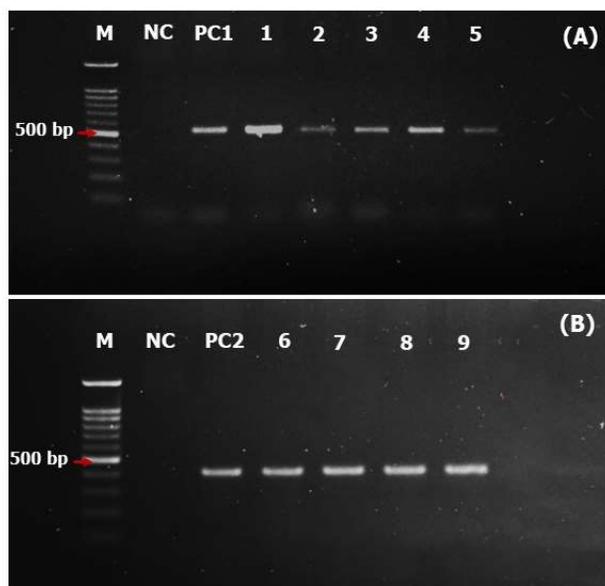


Figure 3 Agarose gel electrophoresis of PCR-amplified DNA of *Meloidogyne enterolobii* using Inc14F/Inc14R primer (A) and *M. incognita* using MK7F/MK7R primer (B). M: 100 bp DNA ladder; NC: Negative control; PC: Positive control (PC1: Genomic DNA of *M. enterolobii* and PC2: Genomic DNA of *M. incognita*); Lane 1 - 5: DNA template of *M. enterolobii*; Lane 6 - 9: DNA template of *M. incognita*.

Extraction yields

The extraction yields of phytochemicals derived from the aerial parts of green chiretta in each solvent were determined. The highest crude yield was observed in the aqueous extract (14.6 %), followed by the methanolic extract (9.4 %) and the ethanolic extract (6 %), while the ethyl acetate and chloroform extracts showed the lowest yields in this study (**Table 1**).

In contrast, a study conducted by Banji *et al.* [27] reported a much higher extraction yield in the ethyl acetate extract (37.7 %) compared to aqueous (34.2 %) and methanolic (21.1%) extracts of green chiretta. Additionally, Kumoro *et al.* [28] determined the quantity of diterpenoid lactone in *A. paniculata* leaves and found that the highest yields of andrographolide were obtained in ethanolic (33.1 %) and methanolic (32.1 %) extracts, followed by water extract (21.54 %). This discrepancy may be attributed to the presence of andrographolide, which contains large and polar hydrocarbon molecules and exhibits better solubility in methanol and ethanol compared to ethyl acetate and chloroform [29].

Table 1 Extraction yields derived from 5 g dried materials of green chiretta.

Solvent	Extraction weight (g)	Yield (%)
Aqueous	0.73 ± 0.03 ^a	14.6
Methanol	0.47 ± 0.03 ^b	9.4
Ethanol	0.37 ± 0.03 ^{bc}	6.6
Ethyl acetate	0.30 ± 0.06 ^{bd}	6.0
Chloroform	0.23 ± 0.03 ^d	4.6

Values are means of 3 replicates ± SE. Values in each column followed by the same letter are not significantly different according to Duncan's Multiple Range Test at 0.05 level.

Second-stage juvenile hatching and mortality tests

Following the bioassay tests, all concentrations of green chiretta crude extracts exhibited effective potency in reducing both *M. enterolobii* and *M. incognita* hatch, with the exception of 5 mg/mL of ethanolic and chloroform extracts, which did not impact the hatching of *M. enterolobii* compared to the control (distilled water). The most significant inhibitory effects on hatching were observed for methanolic (20 mg/mL), ethanolic (20 mg/mL), and aqueous (10 and 20 mg/mL) extracts for both *M. enterolobii* and *M. incognita*, reducing hatching by 87.5 - 96.8 and 88.5 - 96.5 %, respectively (**Figure 4**). However, ethyl acetate and chloroform crude extracts exhibited lower nematicidal activity against the nematode eggs compared to the other extracts. Regarding second-stage juvenile (J2) mortality, all concentrations of crude extracts resulted in a significantly higher number of inactive J2s than those of the distilled water and 2 % DMSO controls at 48 h after exposure. Nevertheless, at 72 h, over 60 % of the J2s from ethanolic and chloroform crude extracts resumed activity from their inactive state. In the current study, the most effective suppression of *M. enterolobii* and *M. incognita* J2s was observed for 20 mg/mL of methanolic and ethyl acetate extracts and 10 - 20 mg/mL of aqueous extract, achieving 94.2 - 100 and 92.2 - 99.2 % mortality, respectively (**Table 2**). These findings align with those of Nisha *et al.* [21], who successfully used aqueous and methanol extracts of green chiretta to control *M. incognita*, resulting in 75 - 94 % J2 mortality at 24 h after treatment. Additionally, Banerjee *et al.* [30] reported that ethanol and methanol extracts of green chiretta exhibited the highest inhibitory effects on *Ancylostoma duodenale* hatch, while the most activity against larval motility was recorded for ethyl acetate extract. Various bioactive compounds, including flavonoids, polyphenols, and diterpenoids, have been detected in every part of green chiretta [31]. Andrographolide, one of the major diterpenoids, possesses potential antibacterial, anticancer, and anti-inflammatory properties [19,20] and has shown efficacy against hookworms [30]. The presence of these flavonoids, polyphenols, and diterpenoids in the extracts may contribute to their effectiveness in controlling *Meloidogyne* spp., as supported by [32,33].

Repellent assays

Table 3 illustrates the repellent effects of extracts from green chiretta on *M. enterolobii* and *M. incognita* J2s. The results demonstrate that J2s of both nematodes were repelled by aqueous, methanolic, ethanolic, and chloroform extracts. However, the ethyl acetate extract was attractive only to *M. incognita* J2s and not to *M. enterolobii*. This aligns with the findings of Jindapunnapat *et al.* [35], who reported that crude aqueous extracts from vetiver shoots and its roots act as repellents to *M. incognita*. A similar result was observed with oak, walnut, maple, and sycamore leaf aqueous extracts, which repelled *M. incognita* J2s by 59 - 75 % [36]. To date, there is no documented information on the repellent or attractive effects of green chiretta extracts on plant-parasitic nematodes. However, the plant has been reported to exhibit strong spatial repellency against *Aedes albopictus* (96.7 % escape) and *A. aegypti* (71.7 % escape) at concentrations of 2.5 and 0.5 %, respectively [37]. Methanol leaf extracts of green chiretta provided 100 % repellency against *Culex quinquefasciatus* [38]. Despite the effectiveness of green chiretta in repelling mosquitoes, the specific mode of action regarding its activity has not been well-described.

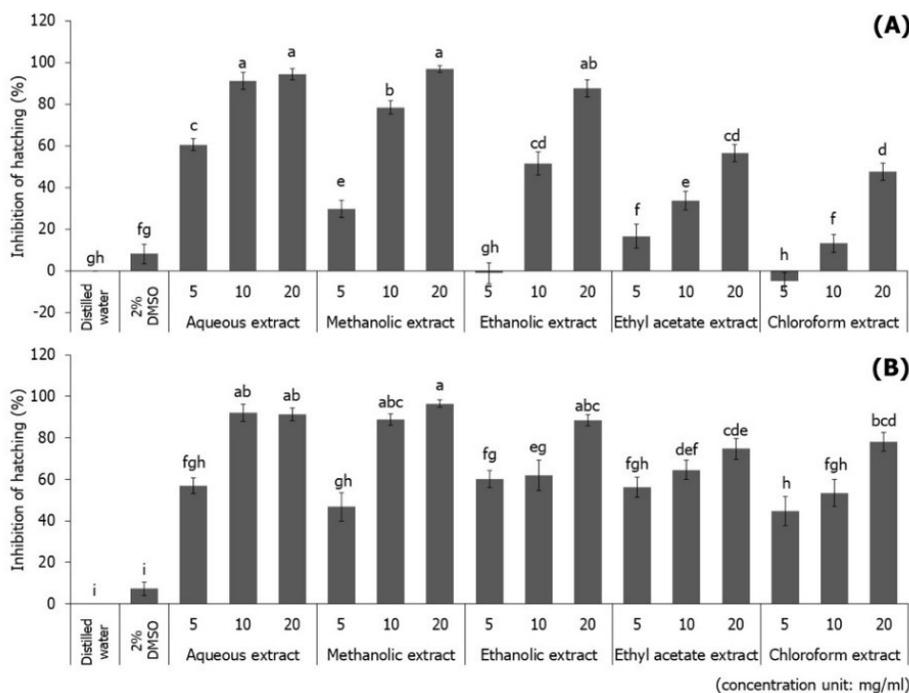


Figure 4 Effect of green chiretta extracts on percentage inhibition of *Meloidogyne enterolobii* (A) and *M. incognita* (B) hatch. Bars refer to standard error of the mean from 2 trials (n = 10). Similar lower-case letters in each value indicated that means are not significantly different according to Duncan’s Multiple Range Test at 0.05 level.

Table 2 Percent inactive and mortality of *Meloidogyne enterolobii* and *M. incognita* J2s after incubation with aqueous (AE), methanolic (ME), ethanolic (EE), ethyl acetate (EAE), and chloroform (CE) extract of green chiretta at 48 and 72 h, respectively.

Treatment	Conc. (mg/mL)	<i>Meloidogyne enterolobii</i>			<i>Meloidogyne incognita</i>		
		Inactive J2 (%)	Dead J2 (%)	Recovery (%)	Inactive J2 (%)	Dead J2 (%)	Recovery (%)
Distilled water	-	0.0 ⁱ	0.0 ^f	-	0.0 ^g	0.0 ^e	-
2% DMSO	-	0.0 ⁱ	3.1 ± 1.3 ^{ef}	-	0.0 ^g	1.0 ± 1.0 ^e	-
AE	5	27.5 ± 2.6 ^{gh}	25.1 ± 2.3 ^c	8.7	24.3 ± 2.6 ^f	19.5 ± 4.0 ^d	19.8
	10	30.7 ± 5.1 ^{gh}	99.1 ± 0.9 ^a	-	63.9 ± 9.5 ^{bc}	92.2 ± 1.9 ^a	-
	20	42.0 ± 4.6 ^{ef}	100.0 ^a	-	77.0 ± 7.6 ^{ab}	98.3 ± 1.2 ^a	-
ME	5	26.1 ± 5.6 ^{gh}	11.3 ± 2.4 ^d	56.7	45.4 ± 3.7 ^{de}	5.5 ± 1.8 ^e	87.9
	10	53.5 ± 4.6 ^{de}	34.1 ± 4.0 ^b	36.3	62.5 ± 9.2 ^{bc}	47.4 ± 4.3 ^c	24.2
	20	85.2 ± 8.4 ^{ab}	100 ^a	-	67.3 ± 6.3 ^{bc}	99.2 ± 0.8 ^a	-
EE	5	19.2 ± 5.8 ^h	3.4 ± 1.6 ^{ef}	82.3	32.5 ± 3.2 ^{ef}	1.7 ± 0.7 ^e	94.8
	10	72.6 ± 3.6 ^{bc}	24.6 ± 2.4 ^c	66.1	62.6 ± 5.5 ^{bc}	5.4 ± 1.7 ^e	91.4

Treatment	Conc. (mg/mL)	<i>Meloidogyne enterolobii</i>			<i>Meloidogyne incognita</i>		
		Inactive J2 (%)	Dead J2 (%)	Recovery (%)	Inactive J2 (%)	Dead J2 (%)	Recovery (%)
	20	94.9 ± 2.0 ^a	35.9 ± 5.1 ^b	62.2	65.9 ± 4.5 ^{bc}	17.9 ± 2.7 ^d	72.8
EAE	5	25.2 ± 5.0 ^{gh}	2.1 ± 1.1 ^{ef}	91.7	52.2 ± 6.7 ^{cd}	5.4 ± 1.7 ^e	89.7
	10	37.6 ± 3.9 ^{fg}	6.9 ± 1.8 ^{def}	81.6	54.7 ± 4.4 ^{cd}	58.5 ± 6.1 ^b	-
	20	81.9 ± 8.6 ^{ab}	94.2 ± 3.9 ^a	-	55.6 ± 4.0 ^{cd}	97.5 ± 2.5 ^a	-
CE	5	62.2 ± 4.6 ^{cd}	0.5 ± 0.5 ^f	99.2	66.9 ± 6.7 ^{bc}	1.8 ± 1.7 ^e	97.3
	10	78.6 ± 2.3 ^b	2.8 ± 1.1 ^{ef}	96.4	86.6 ± 2.2 ^a	2.6 ± 1.1 ^e	94.8
	20	82.4 ± 2.9 ^{ab}	9.2 ± 3.0 ^{de}	88.8	78.2 ± 3.7 ^{ab}	6.6 ± 2.0 ^e	91.6

Values are means of 10 replicates from 2 trials ± SE. Values in each column followed by the same letter are not significantly different according to Duncan's Multiple Range Test at 0.05 level.

Table 3 Repellent activity of extracts of green chiretta at a concentration of 20 mg/mL on *Meloidogyne enterolobii* and *M. incognita* second-stage juveniles. The chemotaxis index (CI) was recorded after 24 h. The values are means ± standard error.

Treatment	CI value	
	<i>M. enterolobii</i>	<i>M. incognita</i>
Distilled water	0.03 ± 0.03	-0.02 ± 0.02
2 % DMSO	-0.01 ± 0.06	-0.02 ± 0.01
Aqueous extract	-0.24 ± 0.05	-0.37 ± 0.03
Methanolic extract	-0.11 ± 0.03	-0.28 ± 0.06
Ethanol extract	-0.11 ± 0.02	-0.31 ± 0.06
Ethyl acetate extract	0.05 ± 0.02	0.13 ± 0.04
Chloroform extract	-0.08 ± 0.04	-0.16 ± 0.05

Phytochemical analyses

Quantitative analysis of phytochemicals in 5 crude extracts from green chiretta revealed notable variations. The methanolic extract exhibited the highest total flavonoid content (16.53 mg QE/g crude extract), while the aqueous extract had the highest total phenolic (6.5 mg GA/g crude extract) and terpenoid (106.99 mg LE/g crude extract) contents. Terpenoids were identified as the predominant compounds in all crude extracts, surpassing flavonoids and phenolics in quantity (**Table 4**). This finding is consistent with reports by Nagajothi *et al.* [40], who observed higher terpenoid content in aqueous extract compared to ethanolic extract, while flavonoids were enriched in methanolic and ethanolic extracts more than in the aqueous extract [41]. The primary bioactive compounds in the 50 % methanol fraction of green chiretta belong to the diterpenoid lactone group, including andrographolide and its derivatives (14-deoxy-11,12-didehydroandrographolide, neoandrographolide, and andrograpanin) [39]. Additionally, flavonoid derivatives (quercetin and kaempferol) were identified in green chiretta by Nguyen *et al.* [42]. The remarkable potential of the aqueous, methanolic, and ethanolic extracts of green chiretta against *M. enterolobii* and *M. incognita* may be attributed to the higher amounts of bioactive compounds, such as terpenoids, phenolics, and flavonoids.

Table 4 Quantification of flavonoid, phenol and terpenoid contents in the extracts of green chiretta.

Treatment	Total flavonoids (mg QE/ g)	Total phenolics (mg GA/ g)	Total terpenoids (mg LE/ g)
Aqueous extract	6.23 ± 0.12 ^{dB}	6.50 ± 0.07 ^{aB}	106.99 ± 1.45 ^{aA}
Methanol extract	16.53 ± 0.14 ^{aB}	4.79 ± 0.09 ^{bC}	54.13 ± 4.04 ^{cA}
Ethanol extract	15.37 ± 0.24 ^{bB}	4.50 ± 0.05 ^{cC}	67.50 ± 3.68 ^{bA}
Ethyl acetate extract	10.12 ± 0.22 ^{cB}	4.14 ± 0.04 ^{dB}	45.35 ± 4.85 ^{cA}
Chloroform extract	1.66 ± 0.02 ^{eB}	0.71 ± 0.05 ^{eB}	15.66 ± 1.96 ^{dA}

Values are means of 3 replicates ± SE. Differences among treatments were determined by analysis of variance (ANOVA) and means were compared using Duncan's Multiple Range Test at 0.05 level. Similar lower case letters indicated that means are not significantly different within a column; similar upper case letters indicated that means are not significantly different within a row.

Conclusions

In summary, our investigation highlights the efficacy of green chiretta extracts, excluding the chloroform extract, in suppressing the eggs and second-stage juveniles (J2s) of *M. enterolobii* and *M. incognita*. The effectiveness of root-knot nematode (RKN) suppression correlates with higher extract concentrations. Additionally, all extracts exhibit a repellent effect on both nematode species, except for the ethyl acetate extract (EAE), which attracts *M. incognita* J2s while having no impact on *M. enterolobii* J2s. Importantly, these findings align with the observed phytochemical contents, emphasizing the significance of terpenoids, phenolics, and flavonoids. Notably, the chloroform extract, with the lowest phytochemical content, proves ineffective in controlling both nematode species.

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