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Nematotoxic Phenolic Compounds from *Melia azedarach* Against *Meloidogyne incognita*

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ABSTRACT: In the present study, evaluated was the paralysis activity of whole Italian and Algerian *Melia azedarach*, commonly known as chinaberry, fruits and parts (seeds, wood, and kernels) against *Meloidogyne incognita* second stage juveniles (J_2). The paralysis activity was evaluated in vitro after 1 h and 1 day immersion periods of nematodes in test solutions. Phenolic constituent components of the extracts were identified and quantified by high-performance liquid chromatography−mass spectrometry, while confirmation was performed by high-performance liquid chromatography−diode array. The water extract of the Italian *M. azedarach* fruit pulp (IPWE) showed significant nematicidal activity ($EC_{50/48h}$ = 955 μg/mL) and among its active ingredient components were *p*-coumaric acid and *p*-hydroxybenzoic acid ($EC_{50/48h}$ = 840 and 871 μg/mL, respectively). This is the first report of the nematicidal activity of *M. azedarach* pulp water extract and phenolic acids against the root knot nematode *M. incognita*.

KEYWORDS: *HPLC-DAD, HPLC-MS/MS, chinaberry, nematotoxic, paralysis, phenolic acids*

INTRODUCTION

At the present, there is an urgent need for new field control measures of nematodes among which the most promising involves the use of secondary metabolites or semiochemicals produced by plants, exhibiting nematicidal activities.[1] There is a great variety of botanical species possessing nematicidal ingredients, and *Melia azedarach* L. (Meliaceae), or commonly Paraiso, Chinaberry, or Persian Lilac, is one of the most important.[2,3] In our recent studies, we have proved that the pulverized lyophilized *M. azedarach* fruits (PMF) showed nematicidal activity against juveniles (J2) of *Meloidogyne incognita*, and the EC_{50} value established in pot experiments was 3400 mg/kg. The melia methanol extract (MME) produced from PMF tested at concentrations higher than 800 mg/kg against J2 was proved nematicidal, whereas lower concentrations were nematostatic with reversible paralysis. When MME was tested in pot experiments at doses higher than 2.5% w/w, it caused 100% nematode control, while the EC_{50} value was calculated at 0.916% w/w.[2] Additionally, in our previous laboratory studies, we showed that *M. azedarach* enhances plant deference mechanisms, when applied on *M. incognita* infecting cucumber plants, since it decreases the activities of catalase (CAT) and peroxidase (POX) involved in host H_2O_2 detoxification. At the same time, the crushed fruits of *M. azedarach*, tested in the soil at rates of 30 and 60 g/kg, exhibited nematicidal activity similar to the one of fenamiphos (0.02 g a.i./kg).[3] Plant secondary metabolites of *M. azedarach* fruits methanol extract (MME), principally responsible for *M. azedarach* efficacy against *M.incognita*, were reported to be hexadecanoic, acetic, and hexanoic acids as well as furfural, 5-hydroxymethylfurfural, 5-methylfurfural, and furfurol, and the EC_{50} values were calculated at 38.3, 41.1, 8.5, 45.7, and 41.2 μg/mL, respectively.[4] Interestingly, furfural representing the most active bionematicidal compound of *M. azedarach* exhibited significant fumigant activity against *M. incognita* with a calculated EC_{50} value of 24 μg/mL.[4]

Phenolic acids are known to exhibit nematicidal properties,[1] while the nematode infection, in turn, influences the levels of phenolic compounds in tomato plants as a result to the induction of abiotic stress.[5] Phenolic compounds with electron-donating substituents in general and chloro substituents in particular, as well as the corresponding substituted phenoxyacetic acid esters, show good nematicidal activity, while the hydrazides are largely inactive.[6] It was shown that increased concentrations of phenols and frequently of amino acids, proteins, and carbohydrates in soil to which oilcakes of mahua, castor, neem/margosa, mustard, and groundnut were added decreased *M. incognita* infection.[7] Additionally, 2,6-dimethoxy-1,4-benzoquinone, 3,3′-di-*O*-methylellagic acid, 1,3,8,9-tetrahydroxydibenzopyran-6-one, and yangambin, isolated from the methanol extract of the bark of *Eucalyptus exserta*, exhibited nematicidal activity against *M. incognita*.[8] Similarly, the ethyl acetate fraction of *Viola betonicifolia* (VB) whole plant, rich in flavonoid and phenolic contents, was highly effective against *M. incognita*,[9] while treating cucumber seedlings with β-amino-butyric acid significantly reduced *M. javanica* infection and, specifically, the nematode galls, the number of egg masses per plant and the number of eggs per individual egg mass.[10] Finally, the potential of *Haplophyllum tuberculatum* and *Plectranthus cylindraceus* oils used to control *M. javanica* in vitro and in a greenhouse were proved to be based on C10 phenolic compound contents.[11]

M. azedarach has been already reported to contain phenolic compounds, but their nematicidal activity was yet to be studied. Chinaberry phenolics are mainly reported as antioxidants, tested by 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging, ferrous ion chelation, and ferric-reducing antioxidant power (FRAP)

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assays.[12] Interestingly, the extract of *M. azedarach* was found to contain higher amounts of phenolic compounds in comparison to the species *Azadirachta indica* and, thus, to exhibit significant scavenging property mainly due to the presence of constituents bearing hydroxyl groups.[13] Caffeic acid, (+)-epicatechin, and kaempferol[14] as well as flavonoids[15] were identified among the antioxidant phenolic compounds of *M. azedarach*, while five minor ferulic acid esters were identified in an antineoplastic petrol extract of its stem bark, namely, hexasosylferulate, tetracosylferulate, pentacosylferulate, heptacosylferulate, and octacosylferulate.[16] *M. azedarach* extracts are reported to exhibit antimicrobial, insecticidal, and genotoxic effects due to their vanillin,[17] phenol and flavonoid contents.[18] Finally, the ethanolic extract of in vitro grown *M. azedarach* was found to exhibit significant antioxidant activity, and the IC_{50} value ranged from 55 to 200 (μg/mg), while ascorbic acid was used as standard with an IC_{50} value of 5700 mg/kg.[19]

The aim of this study was to evaluate (1) the in vitro nematicidal activity of *M. azedarach* water extracts (pulp, stone, kernel, and seed) against *M. incognita*, (2) the identification and quantification of phenolic acids in the aqueous nematicidal extracts by means of high-performance liquid chromatography−mass spectrometry (HPLC-MS/MS) and confirmation by high-performance liquid chromatography−diode array (HPLC-DAD), and (3) the in vitro paralysis activities of phenolic compounds found in the *M. azedarach* water extract. This is the first report on the nematicidal properties of *M. azedarach* phenolic compounds and their chemical characterization by the means of HPLC-MS/MS.

MATERIALS AND METHODS

Plant Material. Ripe fruits (4 kg) of *M. azedarach* were collected at Cagliari, Sardinia, Italy, and Targa ouzemour, Bejaia, Algeria. The fruits were kept in the dark at room temperature for 30 days. Fruits were then separated in parts (pulp, seeds, stone, and kernel) and immediately analyzed (Figure 1). Voucher specimens were deposited in the Department of Life and Environmental Sciences (Botany and Botanical Garden Division, Herbarium CAG, Sardinian Section, University of Cagliari, Italy) for species identification.

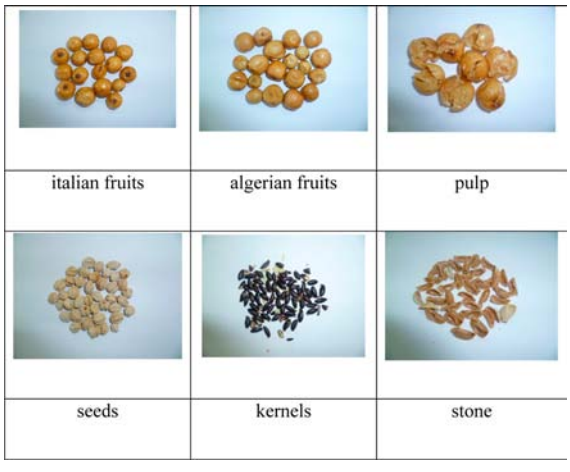

Figure 1. *M. azedarach* fruit and fruit parts.

Chemicals. Analytical standards of caffeic acid, ferulic acid, gallic acid, protocatechin, vanillic acid, chlorogenic acid, β-naphtoic acid, nicotinic acid, salicylic acid, benzoic acid, *p*-hydroxybenzoic acid, *p*-coumaric acid, malic acid, epicatechin, rutin, and fosthiazate were purchased from Sigma-Aldrich, Italy (purity ≥98−99%). The standards were used for component identification analysis and for nematodes bioassays. All solvents and reagents were of analytical grade.

Melia Extracts Preparation. Dried powdered fruit parts (pulp, seeds, stone, and kernel) of the Algerian and Italian *M. azedarach* specimens were extracted with water (1:5, w/v) and sonicated for 30 min, filtered through a Whatman no. 40, and finally centrifuged for 10 min at 11000 rpm. Plant materials were also dried at 105 °C for 24 h, and the moisture content was calculated. The extraction yield was determined on average over three replicates.

Apparatus and Chromatography. *HPLC-MS/MS Analysis.* For the identification and quantitation of the phenolic compounds in the nematicidal extracts, a Varian 1200 L triple-quadrupole tandem mass spectrometer (Palo Alto, CA) coupled with a ProStar 410 autosampler and two ProStar 210 pumps and a 1200 L triple-quadrupole mass spectrometer was used with an ESI source. The Varian MS workstation version 6.7 software was used for data acquisition and processing. Chromatographic separation was performed on a Zorbax Column Synergi 4u MAX-RP 80A (150 mm × 4.60 mm, 4 μm) (Phenomenex). The mobile phase consisted of (A) double-distilled water and (B) methanol containing 0.1% formic acid. The solvent gradient started at 10% of B reaching 100% in 20 min and 100% in 5 min followed by post-time isocratic conditions for 5 min at 10% of B before the next injection. The mobile phase, previously degassed with high-purity helium, was pumped at a flow rate of 0.3 mL/min, and the injection volume was 10 μL. ESI was operated in the positive and negative ion mode. The electrospray capillary potential was set to 65 V, the needle was set at 5850 V, and the shield was set at 750 V. Nitrogen at 48 mTorr and 375 °C was used as a drying gas for solvent evaporation. The atmospheric pressure ionization (API) housing was kept at 50 °C. Parent compounds were subjected to collision-induced dissociation using argon at 2.40 mTorr in the multiple reaction monitoring (MRM) positive and negative mode. The scan time was 1 s, and the detector multiplier voltage was set to 2000 V, with an isolation width of m/z 1.2 for quadrupole 1 and m/z 2.0 for quadrupole 3. Analysis was performed three times (n = 3).

HPLC/DAD Analysis. For confirmation, an Agilent Technologies (Waldbronn, Germany) model 1100 high performance liquid chromatograph was used fitted with a diode array detector (DAD) mode. An analytical column Waters Spherisorb 5 μm ODS2 (4.6 mm × 250 mm Analytical Cartridge) (Milford, MA) was employed. For HPLC analysis, an aliquot (100 μL) was injected into the column and eluted at 40 °C. For the analytical separation, the gradient profile of the mobile phase consisting of (A) acetonitrile and (B) 0.22 M H_3PO_4 (10:90, v/v) at the constant flow of 1 mL/min was as follows: initial start at 10% of A reaching 100% in 20 min and hold to 20 min. Before the next injection, the HPLC system had to be stabilized for 10 min with acetonitrile/aqueous 0.22 M H_3PO_4 (10:90, v/v). Detection was carried out at wavelengths of 210, 280, 313, and 360 nm. Analysis was performed three times (n = 3).

J2 Paralysis. The extracts and pure compounds were tested on *M. incognita* at the test concentration ranges of 100−2500 and 10−1000 μg/mL, respectively, for EC_{50} values calculation. The melia compounds used for the paralysis bioassays were nicotinic acid, salicylic acid, malic acid, vanillic acid, chlorogenic acid, *p*-coumaric acid, gallic acid, caffeic acid, ferulic acid, protocatechin, *p*-hydroxybenzoic acid, and rutin. As part of a study on the nematicidal activity of phenolic acids, benzoic acid, β-naphtoic acid, and epicatechin were also tested in the bioassay in the concentration interval 1−20 μg/ml. Freshly hatched J2 (24 h) were extracted from tomato roots according to the method of Hussey and Barker[20] and were used for paralysis experiments. Fosthiazate and tap water as well as solvent carriers were used as controls for paralysis correction. The bioassays were performed in Cellstar 96-well cell culture plates (Greiner bio-one), and each treatment was represented by 25 J2 per well. Plates were covered with plastic lids and were maintained in the dark at 28 °C. Juveniles were observed after 24 and 48 h with the aid of an inverted microscope (Euromex, The Netherlands) at 40× and were ranked into two distinct categories: motile or paralyzed. After the last assessment (1 day), J2 were washed with tap water through a 20 μm pore screen, to remove the excess of test substance, and were moved to plain water. Motility regain was studied by transferring J2 to tap water after the last assessment

and observing again after 24 h. Paralysis results presented herein correspond to data before rinsing, because J2 never regained activity after moving to plain water. The paralysis experiments were performed twice, and every treatment was replicated six times.

Statistical Analysis. Paralysis data were replicated five times, and each experiment was performed twice. The percentages of paralyzed J2 in the microwell assays were corrected by elimination of the natural death/paralysis in the water control according to the Schneider Orelli formula:[21] corrected % = [(mortality % in treatment − mortality % in control)/(100 − mortality % in control)}] × 100, and they were analyzed (ANOVA) combined over time. Because ANOVA indicated no significant treatment by time interaction, means were averaged over experiments. Corrected percentages of paralyzed J2 treated with test substance(s) were subjected to nonlinear regression analysis using the log−logistic equation proposed by Seefeldt et al.:[22] $Y = C + (D - C)/\{1 + \exp[b\ (\log(x) - \log(EC_{50})]\}$, where C = the lower limit, D = the upper limit, b = the slope at the EC_{50}, and EC_{50} = test substance(s) concentration required for 50% death/paralysis of nematodes after elimination of the control (natural death/paralysis). In the regression equation, the test substance(s) concentration (% w/v) was the independent variable (x), and the paralyzed J2 (percentage increase over water control) was the dependent variable (y). The mean value of the five replicates per test concentration and immersion period was used to calculate the EC_{50} value. The efficacy results were corrected according to moisture contents of the pulp, seeds, stone, and kernel being 51.18, 88.46, 79.12, and 79.60%, respectively.

RESULTS AND DISCUSSION

HPLC-DAD and triple quadrupole mass spectrometry detectors are the elective techniques for the chemical characterization and quantitation of phenolic compounds in botanical extracts.

Table 1. Retention Time and UV max Absorbances of the Phenolic Compounds Identified in the APWE and the IPWE by HPLC Analyses

compd	t_R (min)	UV max absorbance
nicotinic acid	3.639	210, 280
malic acid		
p-coumaric acid	9.119	210, 280, 313
vanillic acid	7.273	210
gallic acid	3.219	210
caffeic acid	6.659	313
ferulic acid	10.527	313
Protocatechin	4.437	210
p-hydroxybenzoic acid	6.269	210
chlorogenic acid	3.921	313
rutin	12.519	210

In the frame of our continuous search for nematicidal components of plant origin, we studied the water extracts of chinaberry. The activity of *M. azedarach* extracts against *M. incognita* was found only in the pulp; therefore, this was the only fruit part that we decided to study for the chemical composition. Specifically, we performed the chemical characterization of the Algerian pulp water extract (APWE) and Italian pulp water extract (IPWE) by means of HPLC-DAD and HPLC-MS/MS techniques. The most abundant phenolic compounds present in the IPWE were *p*-coumaric, malic, nicotinic, vanillic, and ferulic acids, while in the APWE extracts, we found *p*-coumaric, vanillic, malic, and chlorogenic acids. In Table 1, we report the UV elution time of phenolic compounds present in the extracts together with their UV max absorbances, while Table 2 reports the observed mass transitions and collision energy used for quantitation of different phenolics.

The only extract exhibiting substantial nematicidal activity was the water pulp extract (IPWE) of the Italian *M. azedarach*, probably due to the difference in the chemical composition based on geographical distribution. Specifically, the IPWE EC_{50} value calculated after 48 h of nematodes immersion in test solutions was 955 μg/mL (±SD 300.1), while J2 immersion in 1540 μg/mL for 1 day induced 100% paralysis. Contrary to the Italian *M. azedarach*, the Algerian was not found nematicidal at the dose range of 100−2500 μg/mL (data not shown).

When pure phenolic acids were tested on J2, it was shown that the most nematicidal was salicylic acid followed by benzoic, *p*-coumaric, *p*-hydroxybenzoic, and nicotinic acid ($EC_{50/1d}$ = 379, 501, 840, 871, and 1732 μg/mL, respectively) (Table 3). Caffeic, ferulic, gallic, vanillic, chlorogenic, b-naphtoic, malic acid, protocatechin, epicatechin, and rutin were not active at the doses 100−1000 μg/mL. *p*-Coumaric was present in IPWE and APWE extracts at the concentrations of 79.9 and 0.52 μg/g, respectively, while *p*-hydroxybenzoic was found only in IPME (0.24 μg/g) (Table 1). *p*-Coumaric and *p*-hydroxybenzoic EC_{50} values after 24 h of J2 immersion in test solutions were 840 and 871 μg/mL, respectively (Table 3). Interestingly, salicylic acid paralyzed all nematodes after 1 h of immersion in 1000 μg/mL (data not shown). Also, previously, it has been reported the strong nematicidal activity of salicylic acid as component of other nematicidal botanicals. Specifically, salicylic acid in nematodes infested tobacco cultivars, as a result to *Pseudomonas fluorescens* and *Trichoderma harzianum* applications in *M. incognita*, was found to decrease root galls and egg masses/root system.[23] According to Wuyts and co-workers, salicylic acid is a strong attractant for *M. incognita*, but it is also nematicidal (LC_{50} of

Table 2. MRM Transitions and Content of Phenolic Compounds in *M. azedarach*

			first transition		second transition			concn (μg/g ± SD; n = 3)	
compd	MW	precursor mass (m/z)	mass (m/z)	CE (V)	mass (m/z)	CE (V)	R_t (min)	APWE	IPWE
nicotinic acid	123	$[M - H]^-$	121.0 → 77.9	13.0			8.47	1.57 ± 0.51	
malic acid	134	$[M - H]^-$	133.0 → 71.0	13.5	133.0 → 114.8	9.0	10.86	91.1 ± 33.7	1170 ± 345
p-coumaric acid	164	$[M - H]^-$	163.0 → 92.9	34.0	163.0 → 118.9	16.0	19.28	0.52 ± 0.22	79.9 ± 41.7
vanillic acid	168	$[M - H]^-$	167.0 → 107.8	24.0	167.0 → 122.8	14.0	18.04	17.0 ± 5.9	56.2 ± 20.0
gallic acid	170	$[M - H]^-$	169.0 → 124.8	12.5			15.66	0.53 ± 0.21	0.26 ± 0.16
caffeic acid	180	$[M - H]^-$	179.0 → 133.7	26.0	179.0 → 134.8	16.0	19.09	29.5 ± 7.89	36.6 ± 8.4
ferulic acid	194	$[M - H]^-$	193.0 → 177.7	16.0	193.0 → 133.8	18.0	19.51	87.4 ± 22.0	278 ± 76
3,4-dihydroxybenzoic acid	154	$[M - H]^-$	153.0 → 107.8	26.0	153.0 → 108.9	16.0	16.26	1.16 ± 0.58	3.70 ± 1.49
p-hydroxybenzoic acid	138	$[M - H]^-$	137.0 → 92.9	13.5			20.30		0.24 ± 0.10
salicylic acid	138	$[M - H]^-$	137.0 → 92.9	14.5			20.20		0.91 ± 0.55
rutin	610	$[M - H]^-$	609.0 → 270.6	46.5	609.0 → 299.6	35.5	21.72	8.53 ± 3.99	136 ± 33
isoquercitin	464	$[M - H]^-$	463.0 → 270.7	41.5	463.0 → 300.9	19.0	21.71		1.81 ± 0.46

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46 μg/mL) and an irreversible inhibitor of hatch.[24] Salicylic acid is determined as an elicitor of systemic acquired resistance, inhibiting CAT activity. Reproduction of the root knot nematodes in plants of the susceptible cv. Moneymaker treated by root dip and soil-drench in salicylic acid showed 20−25% reduction, as compared with untreated plants.[25] Salicylic acid together with isonicotinamide, 2-chloronicotinic acid, 5-nitrosalicylic acid, 4-chlorosalicylic acid, DL-2 aminobutyric acid, 2-aminobutyric acid, *O*-acetylsalicylic acid, and 4-amino salicylic acid, used as soil drench induced reduction in root-knot severity, suggesting a strong possibility of the use of these activators in integrated management.[26] Moreover, salicylic acid was found to increase the terpenoid aldehyde content of cotton roots, while in plants inoculated with root knot nematodes and treated with salicylic acid, additional amounts of root gossypol and hemigossypolone were induced.[27] Similarly, coumaric and *p*-hydroxybenzoic acids have already been reported of significant nematicidal activity against *Meloidogyne* spp. Specifically, caffeic, ferulic, coumaric, benzoic, vanillic, chlorogenic, and hydroxybenzoic acid found in the aqueous extracts of dry and fresh leaves of *Eucalyptus citriodora* extracts were found to exhibit significant nematicidal activities.[28] *p*-Hydroxybenzoic acid, vanillic acid, caffeic acid, ferulic acid, and a quercetin glycoside, 7-glucoside, were found of considerable nematicidal activity against as contained in root leachate of *Lantana camara*.[29] Coumaric, ferulic, salicylic, and benzoic acids, used as soil drench in propolis extract, decreased the juvenile *Meloidogyne* sp. population density and the number of root galls in bean plants when tested at 1000 mg/L.[30]

Table 3. EC_{50} and SD Values of Individual Compounds against *M. incognita* Calculated at 24 h of Immersion in Test Solutions[a]

compd	24 h of J2 immersion in test solutions: EC_{50} μg/mL (SD)	compd not present in *M. azedarach*	24 h of J2 immersion in test solutions: EC_{50} μg/mL (SD)
nicotinic acid	1732 ± 730	benzoic acid	501 ± 158
salicylic acid	379 ± 96	malic acid	>1000
p-hydroxybenzoic acid	871 ± 222	vanillic acid	>1000
p-coumaric acid	840 ± 196	gallic acid	>1000
caffeic acid	>1000	*β*-naphtoic acid	>1000
protocatechin/ 3,4-dihydroxybenzoic acid	>1000	ferulic acid	>1000
epicatechin	>1000	chlorogenic acid	>1000
rutin	>1000	fosthiazate	0.4 ± 0.93
furfurale[b]	8.5 ± 2.29	acetic acid[b]	38.3 ± 22.0
5-hydroxymethyl-furfurale[b]	45.7 ± 11.8	butyric acid[b]	40.7 ± 21.3
furfurol[b]	41.2 ± 10.1	hexanoic acid[b]	41.1 ± 20.0

[a]If SD values are not presented and the EC_{50} values are reported >1000, they are outside the test concentration range and are estimated higher than the upper concentration level (1000 mg/L). [b]Data reported in Ntalli et al. (2010).[4]

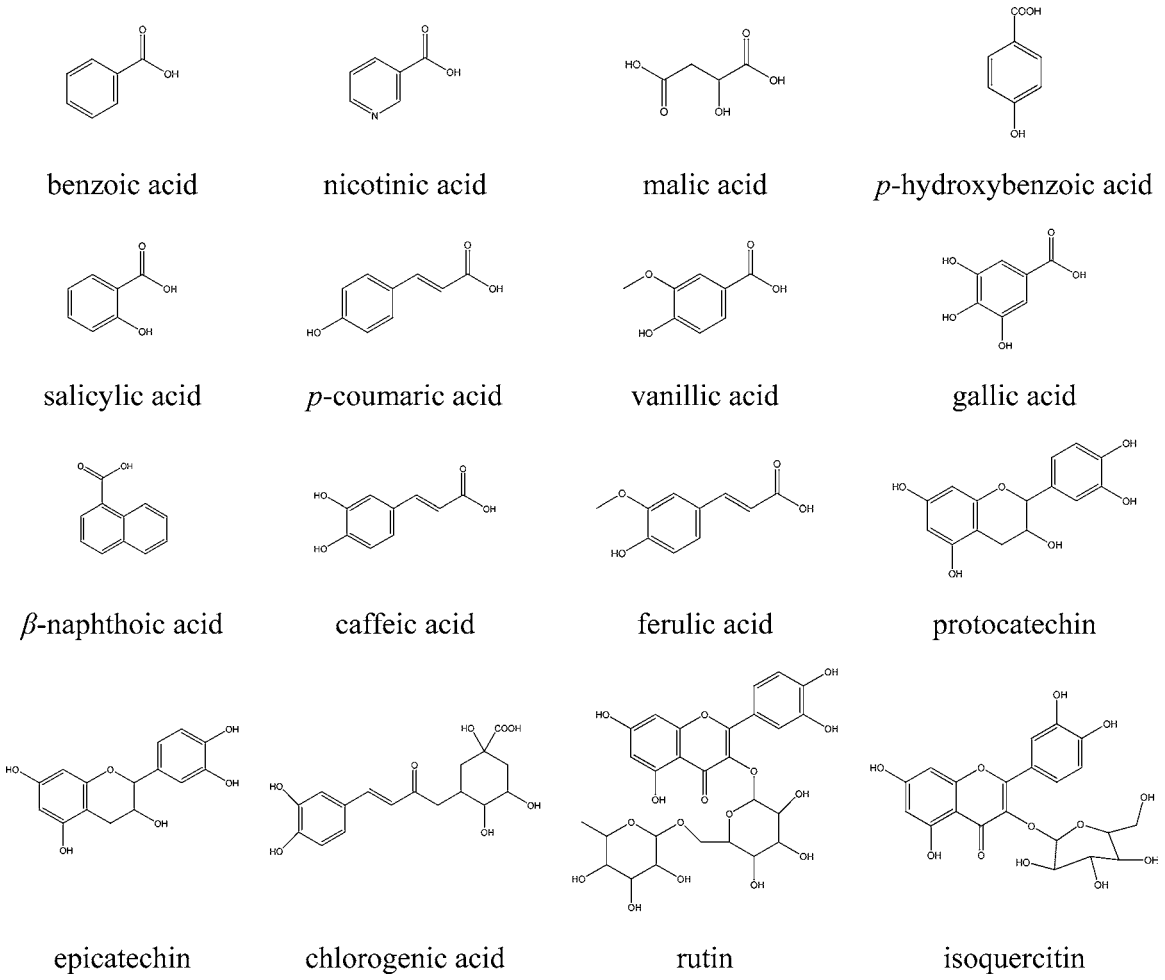

Figure 2. Chemical structures of phenolic acids used for bioassays against *M. incognita* J2.

Vanillic, *trans*-cinnamic, *p*-coumaric, and syringic acids exhibited great in vitro nematicidal effects ($P \leq 0.05$) against *Nacobbus aberrans*.[31] According to our results, the EC_{50} values of benzoic and salicylic acid were comparable (501 and 379 μg/mL, respectively), while the introduction of an hydroxyl group in the *para* position (Figure 2) reduced by a half the activity against J2. *p*-Coumaric acid showed an EC_{50} value of 840 μg/mL, and when caffeic acid was tested for comparison, there was not observed any activity at 1000 μg/mL (Table 1). The difference in the chemical structure of the nematicidal phenolic compounds probably implies differences in the modes of action against nematodes, resulting in decreased probability of resistance development. Although volatile constituent components of *M. azedarach* were found active at lower concentrations,[4] the present study's verification of the nematicidal action of phenolic constituent components completes the nematicidal potency of *M. azedarach* profile against *M. incognita*. Specifically, furfural was found to exhibit an EC_{50} value of 8.5 μg/mL after 24 h of J2 immersion in test solutions, while organic acids EC_{50} values ranged from 38.3 to 45.7 μg/mL, with acetic acid being the most active (Table 3). In Figure 3 are presented the damages

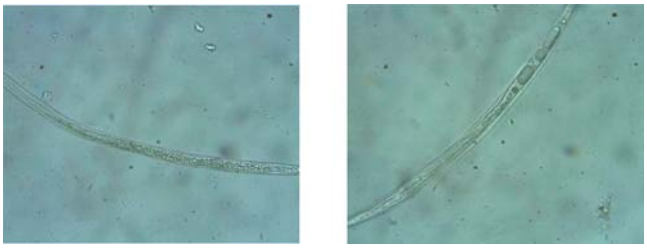

Figure 3. Microscope images from immersion treatment of J2 in (left) water and (right) acetic acid at 100 μg/mL.

on J2 body after immersion in acetic acid solutions. It is evident the removal of the cuticle from the internal organs of the nematode's body, probably due to the osmoregulation disruption and subsequent fluid accumulation. All other experimental treatments, including phenolics treatments, paralyzed J2 but did not induce such damages on juveniles body, apart for furfural.[32]

Even though the phenolic compounds nematicidal activity is fair with regard to volatile components' of *M. azedarach*, there is an interesting advantage of water extract use, as compared to the methanolic one, concerning field application. Specifically, the preparation of IPME is cost effective, it does not necessitate sophisticated equipment, and it is environmentally friendly since it does not involve the extraction with toxic solvents. It is thus of significant practical importance for countries where environmental conditions easily allow the growth of *M. azedarach*, as well as for developing countries in cases where cost effectiveness is mandatory. For these reasons, we are currently studying the efficacy of IPME under field conditions as well as the fate of the *M. azedarach* nematicidal ingredients in soil.

Finally, we can conclude that *M. azedarach* fruits contain different chemical compounds that exhibit significant nematicidal activity against the root knot nematodes, such as phenols, aldehydes, and carboxylic acids. Most interestingly, these substances were identified and quantified in the aqueous extracts of *M. azedarach*. In that sense, *M. azedarach* extracts might be used under field conditions for the control of *Meloidogyne* sp. as an alternative, cost-effective, and environmentally sustainable method.

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Notes

The authors declare no competing financial interest.

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