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Altaf Khan
College of Pharmacy

Manar Fawzi Bani Mfarrej
Zayed University

Fohad Mabood Husain
King Saud University

Qamar Zia
Aligarh Muslim University

Mohd Adil
Dalhousie University

See next page for additional authors

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Acalypha indica aqueous leaf extract as potential nematicide against the root-knot nematode, *Meloidogyne incognita*: in vitro and molecular docking studies

Altaf Khan[a], Manar Fawzi Bani Mfarrej[b], Fohad Mabood Husain[c], Qamar Zia[d], Mohd Adil[e], Mohammed Arshad[f], Khalid Umar[g], Niraj Kumar Jha[h,i,j], Pieter Malan[k], Soumya Pandit[l] and Faheem Ahmad[m]

[a]Department of Pharmacology, College of Pharmacy, King Saud University, Riyadh, KSA; [b]Department of Life and Environmental Sciences, College of Natural and Health Sciences, Zayed University, United Arab Emirates; [c]Department of Food Science and Nutrition, King Saud University, Riyadh, KSA; [d]Interdisciplinary Biotechnology Unit, Aligarh Muslim University, Aligarh, India; [e]Department of Environmental Sciences, Dalhousie University, Truro, Nova Scotia, Canada; [f]Dental Health Department, College of Applied Medical Sciences, King Saud University, Riyadh, KSA; [g]School of Chemical Sciences, Universiti Sains Malaysia, Gelugor, Pulau Pinang, Malaysia; [h]Centre for Global Health Research, Saveetha Medical College, Saveetha Institute of Medical and Technical Sciences, Saveetha University, Chennai, India; [i]Department of Biotechnology Engineering and Food Technology, Chandigarh University, Mohali, India; [j]School of Bioengineering & Biosciences, Lovely Professional University, Phagwara, India; [k]Unit for Environmental Sciences and Management, Mafikeng Campus, North-West University, Mmabatho, South Africa; [l]Department of Biotechnology, Graphic Era University, Dehradun, Uttarakhand, India; [m]Department of Botany, Aligarh Muslim University, Aligarh, India

ABSTRACT

Root-knot nematode (RKN) (*Meloidogyne incognita*) is a major plant parasitic nematode that severely damages crops, leading to significant yield losses and substantial economic impact globally. This study aims to investigate an environmentally sustainable biological strategy for mitigating parasitic populations of the root-knot nematode, *M. incognita*. Specifically, the research focuses on assessing the nematicidal efficacy of *Acalypha indica* against *M. incognita* mortality and second-stage juveniles' (J2) hatching under controlled in vitro conditions. *A. indica* leaf aqueous extract was applied at varying concentrations (250, 500, 750, and 1000 ppm) to J2s and egg masses of *M. incognita*. Notably, at 1000 ppm, a significant increase in J2 mortality and hatching inhibition was observed, while 250 ppm concentration showed the least favorable outcome; with mortality rates ranging from 22–82%. Chemical analysis via gas chromatography-mass spectroscopy (GC-MS) identified Benzoic acid, Cyclooctasiloxane, and 3-Isopropoxy-1,1,1,7,7,7-hexamethyl-3,5,5-tris (trimethylsiloxy) tetrasiloxane as predominant compounds. The nematicidal activity of *A. indica* leaf extract was further validated through in silico molecular docking, revealing that benzoic acid, Cyclooctasiloxane, and 3-Isopropoxy-1,1,1,7,7,7-hexamethyl-3,5,5-tris (trimethylsiloxy) tetrasiloxane bind to the ODR 3 protein of *M. incognita* with binding energies of −15.72, −8.91, and −7.35 kJ/mol, respectively. These findings hold promise for environmentally benign root-knot nematode management, contributing to improved soil health.

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1. Introduction

Plant parasitic nematodes (PPNs) are microscopic obligate bio-trophic pathogens that feed on plant roots. Nematodes can completely kill crops and plants in cases of severe infestation (Escobar et al., 2015; Pandey et al., 2002). Examples of PPNs include root-knot nematodes (RKNs). Jones et al. (2013) state that RKN, *Meloidogyne incognita*, seriously damages economically significant food and crops. When their economic threshold crosses, endo-parasite *M. incognita* might restrict agricultural productivity (Ibrahim, 2011). The nematode triggers root galling, inhibiting root development and turning leaves yellow. Because *M. incognita* directly affects crop production, it is a significant species of RKNs globally (Janati et al.,

CONTACT Qamar Zia qamarbiotech@gmail.com Interdisciplinary Biotechnology Unit, Aligarh Muslim University, Aligarh, India; Faheem Ahmad faheem.bt@amu.ac.in Department of Botany, Aligarh Muslim University, Aligarh, India.

2018; Onkendi et al., 2014). *M. incognita* infestation can specifically result in an estimated 5%–43% yield loss for vegetable crops grown in tropical and subtropical areas (Seid et al., 2015) and an estimated $100 billion loss annually globally (Mukhtar et al., 2014).

In the past, chemical nematicides were an effective way to reduce the population of RKNs; however, because of their toxic effects, these are bad for the environment and human health (Patel & Patel, 1999). Thus, the necessity for innovative and alternate biological management approaches remains critical due to the detrimental effects of nematicides and the potential for large economic losses (Sikora, 1992). As a result, using plant extracts to reduce the PPN population may offer a different approach to sustainable management. Using plants as bio-fumigants is a less expensive and more ecologically friendly way that nematologists have investigated to manage PPNs (Li et al., 2017). Nematicidal secondary plant metabolites are typically likewise ecologically benign, according to field studies (Ntalli & Caboni, 2012).

According to Abdeldaym et al. (2014), soil additives improve the soil's physico-chemical properties, including total and organic nitrogen. Soil microorganisms' release of fatty acids, ammonia, and polyphenols from nitrogen sources is primarily responsible for nematicidal activity (Cayuela et al., 2008; Renčo et al., 2010). Secondary metabolites produced by plant extracts are nematicidal when added to the soil (Ntalli et al., 2020; Taniwiryono et al., 2009). These amendments are well-known for their capacity to increase soil fertility and structure as well as for their biocidal and antibacterial potential (Oka, 2010; Thoden et al., 2011). In literature, the nematicidal action of plant extracts or their metabolites has already been demonstrated (Andrés et al., 2012; Chitwood, 2002). *M. incognita* dies at a high rate when exposed to aqueous extracts of *Xanthium strumarium* (Akhter & Khan, 2018), *Taxus baccata* (Zaidat et al., 2020), and *Azadirachta indica* (Mojumder et al., 2002; Panpatte et al., 2021). *M. javanica* is adversely affected by aqueous extracts of *Crambeabyssinica*, *Cuminum cyminum*, *Curcuma longa*, *Nigella sativa*, and *Piper nigrum* (Abbas et al., 2009; Tarini et al., 2020). High activity against *M. incognita* and/or *M. javanica* was demonstrated by aqueous extracts of *Caleaurticifolia*'s leaves and roots and ethanolic extract of *Eugenia winzerlingii*'s leaves (Cruz-Estrada et al., 2019; Herrera-Parra et al., 2009). *Acalypha indica*, generally called 'Indian Mercury' or 'Indian Nettle', is a member of the Euphorbiaceae family. Despite being considered a weed, *A. indica* is documented as a valuable resource of botanicals with medicinal properties (Zahidin et al., 2017).

Currently, molecular docking is a fantastic technique for producing and providing data on interactions between ligands and receptors, which helps forecast the ligands' orientation to their target proteins or DNA (Akram et al., 2022; Lee & Kim, 2019). Furthermore, this approach facilitates systemic knowledge by delivering a molecule to the binding site of the targeted macromolecule in a non-covalent manner, ensuring precise attachment at each ligand's energetic pocket (Bharathi et al., 2014).

The objectives of our study were to (1) examine the fatality of second-stage juveniles (J2s) and suppression of egg inlaying in *M. incognita* when subjected to various dosages of *A. indica* leaf extract; (2) detect major chemicals in leaf extract of *A. indica* by Gas Chromatography-Mass Spectrometry (GC-MS) technique; (3) validate the nematicidal capability of the extract through *in silico* molecular docking. An effective and cost-efficient method for controlling RKNs is the use of plant extracts. Therefore, our tactic of utilizing botanicals in agrarian technologies could improve the physiological makeup of the soil and offer a way to manage RKNs.

2. Materials and methods

2.1. Chemicals

For the preparation of the leaf extract, Fisher Scientific (Illkirch, France) supplied methanol (HPLC grade).

2.2. Collection and extraction of A. indica leaves extract

Plant material has been collected according to institution guidelines. The collection of this specimen was done for non-commercial scientific purposes only. The plant material was authenticated by the authors from the Lovely Professional University and Graphic Era University. The voucher specimen no. (BT_1501) of the plant was deposited in the Department of Biotechnology, Graphic Era University, Dehradun, Uttarakhand. *A. indica* fresh leaves were picked and dried out in the air at their normal state for four weeks. To make a fine powder, the dried material was crushed using a mortar and pestle. About 0.5 g of leaf residue was dissolved into 20 mL of HPLC-methanol for 8 hours. After filtration using Whatman No. 1 filter paper, the leaf extract was analyzed using GC–MS. To perform the bioassays, Whatman filter paper was used to filter 1.0 g of

powdered *A. indica* leaf suspended in one litre of double distilled water (DDW). This suspension was termed the stock solution (1000 ppm), while other concentrations (250, 500, and 750 ppm) were also prepared accordingly (Abdullah et al., 2023).

2.3. Maintenance of inoculum of M. incognita

The pure culture of *M. incognita* was initiated and sustained from a single egg mass of an adult female nematode using the methodology described by Hussey and Barker (1973). From the closest field, two-month-old brinjal 'BR-112' roots infested with *Meloidogyne* spp. were isolated. However, *M. incognita* was identified by looking at features of the perineal pattern (Eisenback & Hunt, 2009). Further, *M. incognita* J2s were reproduced and kept alive on brinjal. The brinjal plants were carefully pulled to prevent the egg masses from getting detached from the roots. Afterward, they were thoroughly cleaned under running tap water to eliminate any remaining soil grains (Gowda et al., 2022). The infested brinjal roots were then immersed in 1% bleach or sodium hypochlorite (NaClO) for 5 min. All the collected eggs were passed through a 25 µm pore size sieve and placed in Baermann trays. The hatched J2s were gathered after every 2 hrs, and fresh tap water was added; repeating the process for up to 3 days. The collected J2s were utilised as the inoculum for future research.

2.4. Hatching bioassay

Using the egg mass dipping scheme, the inhibitory potential of increasing doses of *A. indica* extract on J2s hatching was examined. Carefully selected fresh egg masses from the brinjal plant's galled roots were rinsed three or four times in DDW. Five recently harvested egg masses were subsequently positioned in petri dishes with 10 mL of previously mentioned leaf extract concentrations. To stop evaporation, parafilm was placed over each petri dish, and the whole setup was then incubated at 28 °C. DDW-dipped eggs were used as the controls. Five duplicates of every experiment exist, not counting the control. Twice the work was done to confirm the findings. After four days, the total J2s hatchlings were counted in replicates using a binocular microscope. Then, using the formula given below, the mean value was used to compute the percent inhibition in J2s hatching (Abdullah et al., 2023).

$$\text{Percent hatching inhibition} = \left(\frac{C_0 - T_\infty}{C_0}\right) \times 100$$

where C_0 = Quantity of hatched J2s in distilled water
T_α = Quantity of hatched J2s in different dosages of leaf extract

2.5. Mortality assay

In order to assess the nematicidal potential, 9 mL of the extract at increasing dosages (250, 500, 750, and 1000 ppm) was combined with 1 mL of *M. incognita* suspension (holding ~75 J2s) in petri plates. The petri plates containing distilled water was selected as control. To reduce being dried, the dishes were wrapped in parafilm and maintained at 28 °C for stipulated time. Using a microscope, the amount of live and dead J2s was calculated after 12, 24, 48, and 72 hours of exposure. J2s displaying any motion or those with a snaky shape thought to be live (El-Rokiek & El-Nagdi, 2011), while J2s with no motion and a streamlined form and appearance were thought to be lifeless (Aissani et al., 2015). The experiment was repeated five times with the same setup and 50% Lethal Concentration (LC_{50}) estimates was determined (Behreus & Karbeur, 1953; Sakuma, 1998). The J2s percentage of mortality was estimated as described:

$$\%\text{Mortality of J2s} = \left(\frac{\text{Total J2s} - \text{Dead J2s}}{\text{Total J2s}}\right) \times 100$$

2.6. GC–MS analysis of leaf extract of A. indica

To identify substances found in *A. indica* methanolic leaf extract, we performed GC–MS on a Shimadzu QP2010 Plus instrument outfitted with a Rtx–5MS capillary column (30 m length, 0.25 mm diameter) with helium as a carrier gas (Gomathi et al., 2015; Thamer & Thamer, 2023). Next, we loaded ~250 mL of the extract onto the syringe, and 1 mL was introduced into the machine at the rate of 1.21 mL/min. After maintaining the temperature at 100 °C, it was raised to 250 °C at a rate of 5 °C per minute, and at last, to 280 °C at a rate of 10 °C per minute. Employing an MS spectrometer in full scan setting, distinctive peak disintegration trends in intermediates were discovered. Data analysis, peak integration and deconvolution of chromatograms was done using the LabSolutions GCMS software. Metabolites were identified using retention times, and their molecular weights were calculated. The chemicals were validated by matching their spectral peaks with reference chromatograms of three datasets (National Institute of Standards and Technology, NIST 14, NIST 14, and Wiley 8).

2.7. Molecular docking (MD) studies

The docking investigation was carried out utilizing Autodock 4.2 and Autodock tools (ADT) with the Lamarckian genetic (LGA) method. The target protein Odorant Response Gene-3 (ODR 3) protein was chosen as the target receptor. The crystal structure of Benzoic acid (CID: 243), Cyclooctasiloxane (CID: 18993663), and 3-Isopropoxy-1,1,1,7,7,7-hexamethyl-3,5,5-tris (trimethylsiloxy) tetrasiloxane (CID: 553025) were obtained from Pub Chem. After selecting protein chain A, the hydrogen elements were introduced and water molecules as well as ions were eliminated. The partial Kollman charges are then applied to ODR 3. The polypeptide was made stiff, and no solvent molecules were taken into account during the process. Throughout the X, Y, and Z axes, the grid dimension was configured to 120, 120, and 120 alongside a grid spacing of 0.546. The GA population size was set to 100, and the highest possible energy modifications were chosen as 2.5 million. For additional study, ten top options centered on docking scores were marked. Discovery Studio Visualizer 3.5 was utilized for the visualization of binding residues (Amir et al., 2021; Amir & Javed, 2023).

2.8. Statistical analysis

The R software (version 2.14.1) was used to perform a statistical analysis of the collected data. Duncan's Multiple Range Test (DMRT) was used to evaluate whether the studied characteristics predict noteworthy differences ($p = 0.05$). ANOVA and LC_{50} values for each treatment were then determined (Sheoran et al., 1998).

3. Results and discussion

3.1. J2s mortality analysis

It was discovered that every tested concentration showed some degree of toxicity toward J2s, with death rates varying from 22% to 82%. The individual harmful effects of different *A. indica* leaf extract concentrations (250, 500, 750, and 1000 ppm) on J2 mortality are displayed in Table 1. In general, J2 mortality increases as exposure duration and extract concentration increase. The anti-nematode activity of *A. indica* leaf extract was directly correlated with the quantities of extract and the length of exposure (Khan et al., 2019). No J2s fatality was noted in control experiments. There was an upsurge in J2 mortality upon increasing the dosage from 250 to 1000 ppm. At a concentration of 1000 ppm, 62% mortality of J2s was observed after 72 hours of incubation. The incubation period had a substantial impact on the percent mortality of J2s as well, reaching its maxima after 72 hours. The toxicity in descending order was as follows: toxicity after 72 h of treatment with LC_{50} 282.66 ppm > toxicity after 48 h of treatment with LC_{50} 394.5 ppm, > toxicity after 24 h of treatment with LC_{50} 685.17 ppm > toxicity after 12 h of treatment with LC_{50} 1686.12 ppm (Table 2).

3.2. J2s hatching inhibition bioassay

In our investigation, *A. indica* leaf extract at all tested concentrations significantly reduced J2 hatching and, to varied degrees, increased J2 mortality of *M. incognita*. Significant differences were found between different concentrations of extract used. In comparison

Table 1. Susceptibility of J2 of *M. incognita* to different concentrations of aqueous leaf extract of *A. indica* over different inoculation periods.

Treatment	Concentrations (ppm)	Number of dead J2s (Mean ± SE) at different time intervals (hours)			
		12	24	48	72
A. indica extract	250	17 ± 1.52 (22.66%)	23 ± 1.52 (30.66%)	31 ± 1.73 (41.33%)	37 ± 1.52 (49.33%)
	500	22 ± 1.52 (29.33%)	31 ± 1.52 (41.33%)	39 ± 2.08 (52%)	45 ± 1.73 (60%)
	750	26 ± 1.54 (34.66%)	38 ± 1.73 (50.66%)	47 ± 2.08 (62.66%)	55 ± 2.08 (73.33%)
	1000	33 ± 1.52 (44%)	45 ± 2.08 (60%)	57 ± 1.73 (76%)	62 ± 1.52 (82.66%)
	DW	0 ± 0 (0%)	0 ± 0 (0%)	0 ± 0 (0%)	0 ± 0 (0%)
Degrees of freedom		3	3	3	3
Sum of squares		411	800.25	1133	1088.25
Mean squares		137	266.75	371	362.75
F		21.92	29.63	33.72	40.30
P		0.00033	0.00011	0.00007	0.00004

Values given in brackets represent J2s percent mortality over control.
Values given without brackets represent the number of dead J2s in different concentrations of leaf extract of *A. indica*.
DW = Double Water (control); SE = Standard Error; J2s = Second-stage juveniles.

Table 2. Nematicidal activity of aqueous leaf extract of *A. indica* against J2s of *M. incognita*.

Treatment	Exposure period (hours)	LC_{50} value in ppm (95% CL)
A. indica extract	12	1,686.12
	24	685.17
	48	394.50
	72	282.66

LC_{50} - Lethal concentration caused 50% mortality after 12, 24, 48, and 72 hours at 95% confidence limits.
CL = Confidence limit.

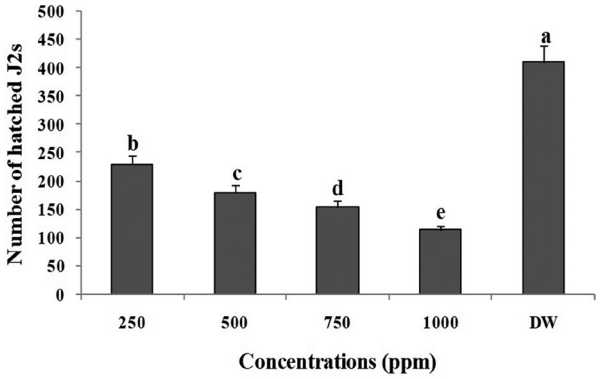

Figure 1. Effect of several concentrations of leaves extract of A. indica on J2s hatching of M. incognita over 4 days of incubation period. Each value is an average of five replicate. Each bar followed by same letter is not significantly different according to Duncan's multiple-range test ($p \leq 0.05$). [DW-Distilled water (Control); J2s-Second stage juveniles; ppm-parts per million].

with the control, J2s emergence was effectively reduced at all concentrations. Enhancement in inhibiting activities of J2s hatching was observed to be in tandem with an increment in concentrations of extract from 250 to 1000 ppm. Comparing 250 ppm to control, however, there was also a noticeable restriction of J2s hatching. Figure 1 shows the inhibitory activities of different doses of the extract on J2s hatching.

The process of nematode suppression could involve protein denaturation and degradation, enzyme activity that inhibits the electron transport chain, or ADP phosphorylation mechanism (Konstantopoulou et al., 1992). In the last ten years, research on RKN control has mostly concerned methods for producing secondary metabolites (Westcott & Kluepfel, 1993) or substances that impede egg hatching (El-Habashy et al., 2020; Sousa et al., 2020). Plant extracts' processes are poorly known, yet secondary metabolites found in leaf extracts, which are found in botanical extracts as alkaloids, flavonoids, phenolics, and saponins, act antagonistically against *M. incognita*'s J2s and egg masses (Chitwood, 2002; Mousa et al., 2011). To create a secure substitute for the chemical nematicides that are currently employed for the treatment of RKNs, a new study concentrated on plant-derived materials (Marron, 2019). Many plant-based products are being used for the sustainable management of RKNs, including seed cakes, dried powders, and crude plant extracts (Dutta et al., 2021; Khan et al., 2017).

3.3. GC-MS investigation

GC-MS is indispensable for the examination of active compounds and chemotaxonomic research on organic materials with physiologically active constituents. Following a GC-MS examination of the methanol extract, nine compounds were acknowledged (Table 3) along with their retention duration, MW, and formula, as well as percent area. Figure 2 displays a chromatogram of the methanolic extract. The recognized compounds are 2-chloro- Benzaldehyde, Decamethyl-cyclopentasiloxane, Cyclooctasiloxane, Benzoic acid, Pentasiloxane, 3-Isopropoxy-1,1,1,7,7,7-hexamethyl-3,5,5-tris (trimethylsiloxy) tetrasiloxane, 1,1,1,5,7,7,7-Heptamethyl-3,3-bis(trimethylsiloxy) tetrasiloxane, Tetracosamethyl-cyclo dodecasiloxane, and Hexasiloxane. Of these nine compounds, Cyclooctasiloxane, 3-Isopropoxy-1,1,1,7,7,7-hexamethyl-3,5,5-tris(trimethylsiloxy) tetrasiloxane, and Benzoic acid were observed as chief compounds. Therefore, we used the ODR 3 protein, which is present in *M. incognita*, to perform molecular docking of these two molecules. The existence of the aforementioned metabolically active chemicals, which had nematicidal action against *M. incognita*, was detected by GC-MS analysis. Many phytochemicals with anti-nematode properties against different pests, including *Meloidogyne* spp., have been reported (Atolani & Fabiyi, 2020; Lu et al., 2020). Consistent with our findings, esters like methyl stearate and methyl palmitate are known to inhibit gall formation, reduce the hatching of eggs, and repel larvae and J2s (Lu et al., 2020). Botanicals present in aqueous extracts of fresh and dried *Eucalyptus citriodora* leaves show nematicidal properties (El-Rokiek & El-Nagdi, 2011). According to Ntalli et al. (2009) and Nguyen et al. (2013), phenylpropanoids, flavonoids, isoflavonoids, tannins, vanillic acid, caffeic acid, ferulic acid, cinnamic acid, and gallic acid exhibit nematicidal efficacy.

3.4. Molecular docking analysis

Docking analysis is a unique way to logic-driven natural product selection and biopesticidal lead detection, allowing one to comprehend the bioactivity of

Table 3. Compounds detected in methanolic leaf extract of *A. indica* by GC-MS analysis.

Peak number	Retention time	Height	Area	Area %	Name of compound	MW (g/mol)	Molecular formula
1	7.340	17,614,582	1,647,450.4	1.670	Benzaldehyde, 2-chloro-	140.567	C_7H_5ClO
2	7.928	12,384,097	1,033,446.6	1.048	Decamethylcyclopentasiloxane	370.77	$C_{10}H_{30}O_5Si_5$
3	11.833	14,516,173	2,320,576.5	2.352	Benzoic acid	122.12	$C_7H_6O_2$
4	13.424	42,177,808	1,512,630.5	1.533	Pentasiloxane	204.42	O_4Si_5
5	13.504	59,671,924	4,567,111.0	4.629	3-Isopropoxy-1,1,1,7,7,7-hexamethyl-3,5,5-tris (trimethylsiloxy) tetrasiloxane	577.2	$C_{18}H_{52}O_7Si_7$
6	15.482	78,668,408	2,787,523.5	2.825	Cyclooctasiloxane	352.77	$C_{10}H_{30}O_8Si_8$
7	17.153	54,168,708	1,377,761.4	1.397	1,1,1,5,7,7,7-Heptamethyl-3,3-bis(trimethylsiloxy)tetrasiloxane	443.96	$C_{13}H_{39}O_5Si_6$
8	28.794	24,434,122	970,156.8	0.983	Tetracosamethyl-cyclododecasiloxane	889.8	$C_{24}H_{72}O_{12}Si_{12}$
9	29.975	21,968,244	1,090,607.0	1.105	Hexasiloxane	248.51	O_5Si_6

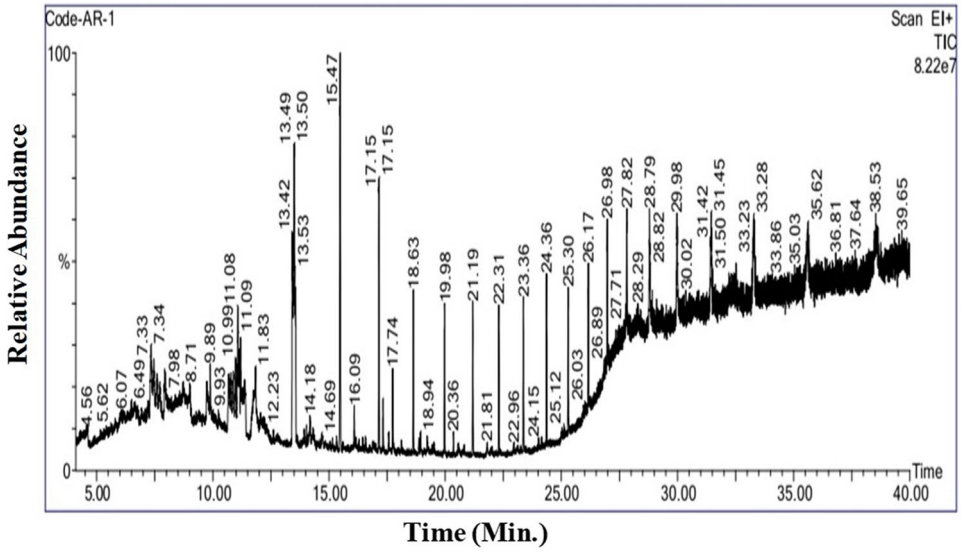

Figure 2. GC–MS chromatograms of methanolic leaf extract of A. indica.

Table 4. Molecular interaction details of docking of ODR 3 protein with three different ligands.

Target protein	Ligand	Binding energy (kJ/mol)	Bonds involved
Odorant Response Gene-3 (ODR 3)	Benzoic acid	−15.72	Van der Waals, ConventionalHydrogen, Pi-Anion, Pi-Sigma, Pi-Sulfur, Pi-Alkyl
	Cyclooctasiloxane	−8.91	Conventional Hydrogen, Carbon Hydrogen, Alkyl hydrophobic
	3-Isopropoxy-1,1,1,7,7,7-hexamethyl-3,5,5-tris(trimethylsiloxy) tetrasiloxane	−7.35	Van der Waals, Pi-Alkyl

compounds (Almutairi et al., 2022; Bharathi et al., 2014; Kundu et al., 2020). Table 4 summarizes the findings of the best energy ranking. Figure 3 shows that Benzoic acid binds to ODR 3 with a binding energy of -15.72 kJ/mol, while Figure 4 depicts that Cyclooctasiloxane interacts with ODR 3 with a binding energy of −8.91 kJ/mol. Figure 5 shows that 3-Isopropoxy-1,1,1,7,7,7-hexamethyl-3,5,5-tris(trimethylsiloxy) tetrasiloxane binds with ODR3 with a binding energy of −7.35 kJ/mol. Benzoic acid interacts with ODR 3 via hydrogen bonding with Asp 274, Cys 327, and Ala 328. ODR 3-Benzoic acid composite also stabilized by hydrophobic interaction between Asp 152, Tyr 178, Asn 271, Lys 272, Leu 275, and Thr 329. On the other hand, Cyclooctasiloxane forms only two hydrogen bonds with ODR 3 via Lys 319 and Thr 320, and results in the formation of stabilized ODR 3-Cyclooctasiloxane complex. Additionally, 3-Isopropoxy-1,1,1,7,7,7-hexamethyl-3,5,5-tris(trimethylsiloxy)tetrasiloxane interacts with ODR3 involving Van der Waals and Pi-Alkyl bonds.

Benzoic acid displayed a higher affinity towards the ODR 3 polypeptide than other botanicals, with a binding energy of −15.72 kJ/mol. The outcomes of this study are in accordance with that of Keerthiraj et al. (2021), who showed through ligand binding studies

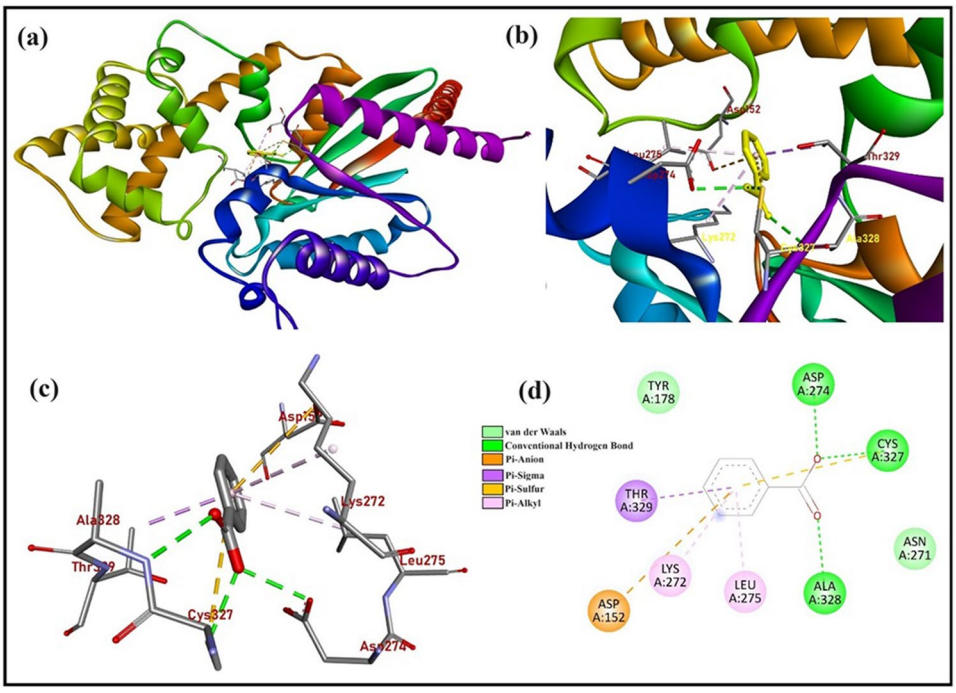

Figure 3. In-silico molecular docking results. (a) The binding poses and (b) binding interaction of ODR 3 with Benzoic acid. (c) Amino acids involved in interaction (d) 2-D structure showing bonds involved.

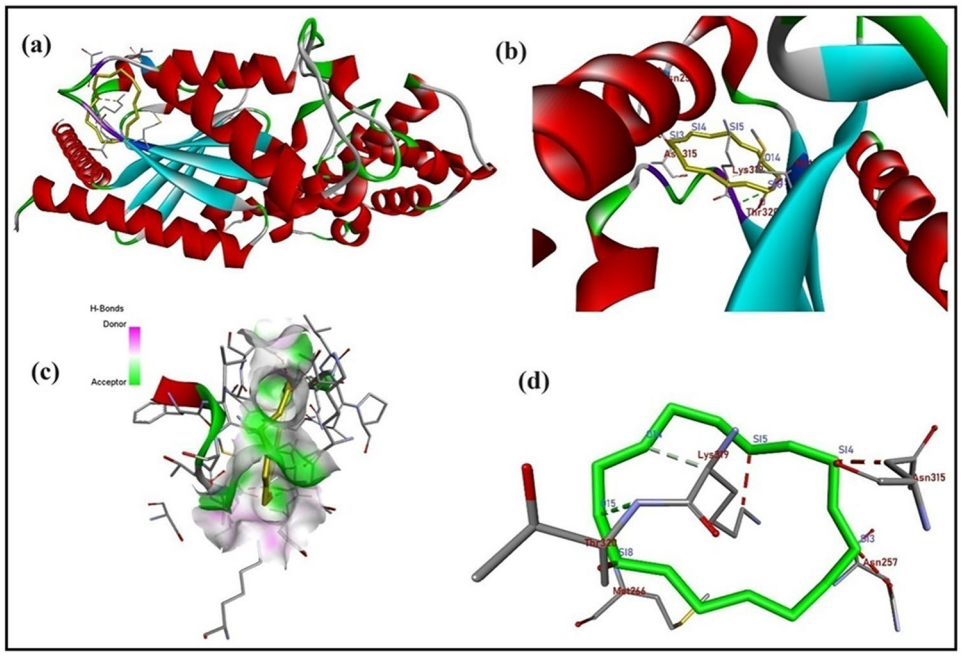

Figure 4. In-silico molecular docking results (a) The binding poses (b) Binding interaction of ODR 3 with Cyclopentasiloxane (c) H-bond receptor surfaces in the interaction between protein and ligand (d) Amino acids involved in the interaction.

that plant-based compounds possess a multi-modal antagonistic effect on many *M. incognita* target proteins. The potential for ligand-receptor interaction with hydrophobic, covalent, and/or non-covalent bonding was shown by molecular modeling, and ligand efficiency values indicated a molecule's capacity to block a target site (Keerthiraj et al., 2021).

4. Conclusions

Utilizing plant extracts presents an eco-friendly, cost-effective, and safe approach to managing root-knot nematodes (RKNs) within an integrated pest management (IPM) framework. With this in mind, we investigated the nematotoxic capability of

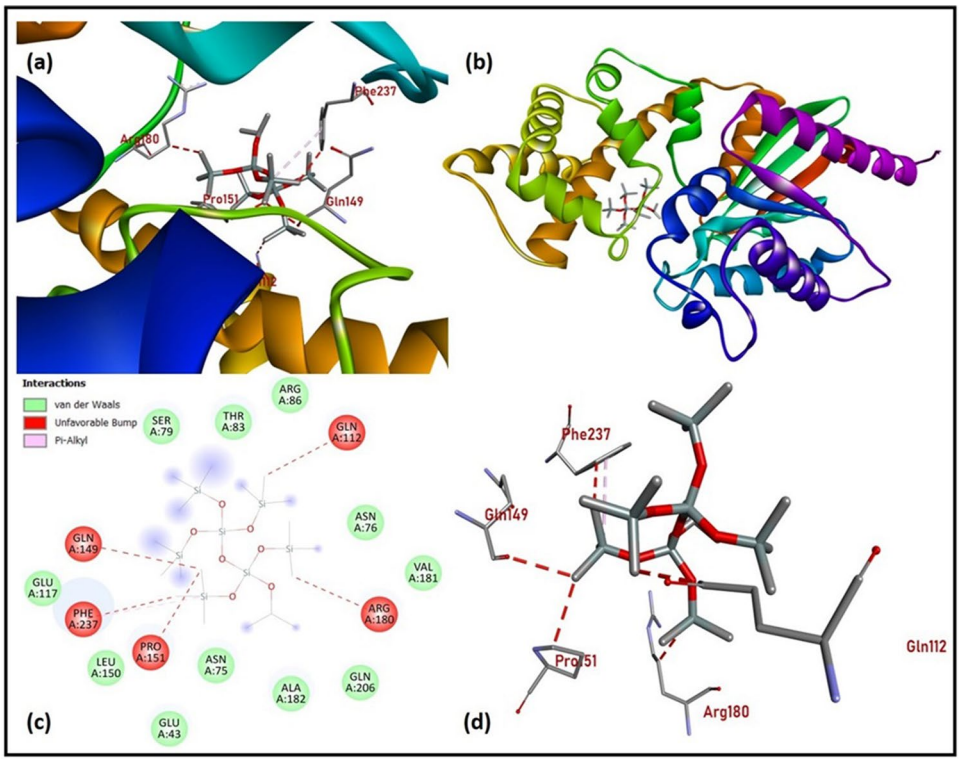

Figure 5. In-silico molecular docking results. (a) The binding poses and (b) binding interaction of ODR3 with 3-Isopropoxy-1,1,1,7,7,7-hexamethyl-3,5,5-tris(trimethylsiloxy)tetrasiloxane. (c) Type of bonds in the interaction between protein and ligand, and (d) Amino acids involved in the interaction.

A. indica leaf extract against *M. incognita*. Hatching and mortality assays depict their potent anti-nematicidal activity. Further, *A. indica* leaf extract includes many active components, identified using the GC-MS method that could be further investigated for the synthesis of natural nematicides. The nematotoxic potential of the main bioactive chemicals was investigated using a molecular docking method. These substances appear to have a higher binding affinity to the ODR 3 protein of *M. incognita*. Thus, our methods of applying *A. indica* leaf extract to agricultural practices may improve the physiochemical qualities of the soil and offer an affordable and environmentally beneficial way to control RKNs.

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Authors' contributions

AK: Conceptualization, Data curation, Formal analysis, Methodology, Validation, Writing - original draft; MFBM: Formal analysis, Funding acquisition, Methodology, Resources, Software, Writing - review & editing; FMH: Conceptualization, Supervision, Formal analysis, Methodology, Funding acquisition, Resources, Software, Writing - original draft; QZ: Conceptualization, Supervision, Data curation, Methodology, Project administration, Resources, Visualization, Writing - review & editing; MA: Project administration, Formal analysis, Resources, Software, Writing - original draft; MAr: Data curation, Investigation, Methodology, Software, Validation, Writing - review & editing; KU: Project administration, Supervision, Visualization, Writing - review & editing; NKJ: Supervision, Writing - review & editing; PM: Project administration, Writing - review & editing; SP: Data curation, Writing - review & editing; FA: Conceptualization, Supervision, Methodology, Project administration, Resources, Software, Formal analysis, Writing - review & editing. All authors have approved the final version of the manuscript.

Disclosure statement

There are no relevant financial or non-financial competing interests to report.

About the authors

Altaf Khan obtained his PhD from the Department of Biochemistry at Jamia Hamdard, India. His research primarily focuses on the utilization of natural compounds and plant-derived products for the treatment of plant diseases, employing computational methodologies in his investigations.

Manar Fawzi Bani Mfarrej is currently working as an Assistant Professor in the College of Natural and Health Sciences at Zayed University, United Arab Emirates. Her research interests include environmental sustainability, plant protection, pesticide residues, environmental pollution, and waste management.

Fohad Mabood Husain is a distinguished academician who specializes in Applied Microbiology and Food Safety. He is a faculty member at the College of Food Science and Nutrition, King Saud University, Saudi Arabia. He has made significant strides in researching antimicrobial strategies, mainly focusing on using plant-based materials and nanomaterials.

Qamar Zia earned his PhD in Biotechnology from Aligarh Muslim University, India. His research interests involve the development of nanomaterial or plant-based natural bioproducts for anti-microbial, anti-cancer, and anti-pathogenic activities.

Mohd Adil completed his PhD in Biotechnology at Aligarh Muslim University, India, where he explored the antimicrobial potential of agents derived from medicinal plants. Currently, Dr. Adil is advancing his research at the Dalhousie University, Canada, where he is dedicated to identifying and characterizing bioactive compounds produced through seaweed fermentation.

Mohammed Arshad works as a Research Scientist at King Saud University, KSA. His research spans antimicrobial studies, protein binding, and anticancer investigations, with a particular focus on natural products and nanomaterials. In addition, he engages in bioinformatics studies to further enhance his findings.

Khalid Umar is a Senior Lecturer at the School of Chemical Sciences, Universiti Sains Malaysia (USM), Penang, Malaysia. His research focuses on the synthesis of nanoparticles/botanicals and their diverse applications, including their biological activities.

Niraj Kumar Jha is an Assistant Professor at Lovely Professional University, India and holds a PhD in Biotechnology from Delhi Technological University, New Delhi, India. His work focuses on the therapeutic potential of small molecules, both natural and synthetic, aiming to uncover innovative solutions for disease prevention and treatment.

Pieter Malan is an Associate Professor at the North West University, Mafikeng Campus, South Africa. An expert in plant protection, he has published over 20 research articles in peer-reviewed journals and supervised several master's and Ph.D. students.

Soumya Pandit is an Associate Professor at Graphic Era University, Dehradun, India. With expertise in environmental biotechnology, he has edited 8 books, written over 60 book chapters, and holds 24 Indian patents.

Faheem Ahmad is a Senior Assistant Professor at Aligarh Muslim University, India in the Department of Botany and author of over 60 peer-reviewed publications. His research interests include plant-nematode interaction, agricultural biotechnology, and nematode management based on nematicidal bioagents and plant natural product repertoire.

ORCID

Manar Fawzi Bani Mfarrej http://orcid.org/0000-0003-1144-3125
Qamar Zia http://orcid.org/0000-0002-0284-4974
Khalid Umar http://orcid.org/0000-0002-2081-316X
Faheem Ahmad http://orcid.org/0000-0002-7450-0900

Data availability statement

The authors confirm that the data supporting the findings of this study is available within the article.

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