VOLUME 10 ISSUE 1 2024

ISSN 2454 – 3055

INTERNATIONAL JOURNAL OF ZOOLOGICAL INVESTIGATIONS

Forum for Biological and Environmental Sciences

Published by Saran Publications, India

International Journal of Zoological Investigations Vol. 10, No. 1, 791-802 (2024)

International Journal of Zoological Investigations
Contents available at Journals Home Page: www.ijzi.net
Editor-in-Chief: Prof. Ajai Kumar Srivastav
Published by: Saran Publications, Gorakhpur, India

ISSN: 2454-3055

Nematicidal and Molecular Docking Investigation of Secondary Metabolites Derived from *Embelia tsjerium-cottam* Fruit Extract Against *Meloidogyne incognita*

Trambadiya Krishna[1]*, Kanabar Riddhi[1], Poriya Paresh[1], Visavadia Manishkumar[2] and George Linz-Buoy[3]

[1]Department of Zoology, Bahauddin Government Science College, Bhakta Kavi Narsinh Mehta University, Junagadh, Gujarat, India
[2]Department of Zoology, Government Science College, Gandhinagar, Gujarat, India
[3]Department of Zoology, Biomedical Technology, Human Genetics and Wildlife, Gujarat University, Ahmedabad, Gujarat, India

**Corresponding Author*

Received: 9th November, 2023; **Accepted:** 22nd February, 2024; **Published online:** 15th May, 2024

https://doi.org/10.33745/ijzi.2024.v10i01.086

Abstract: Root-knot nematode, *Meloidogyne incognita* is a notorious plant-parasitic nematode that causes significant agricultural damage. The search for sustainable and eco-friendly alternatives to chemical nematicides has led to an exploration of natural resources of nematode control agents. In this study, we investigated the nematicidal efficacy of *Embelia tsjerium-cottam* fruit extract against second-stage juvenile (J2) of *M. incognita* in an *in vitro* condition. J2s of *M. incognita* were treated with different concentrations (0.5-8%) of the extracts and observations were taken 24, 48 and 72 h after exposure. These results indicate that all concentrations had highly toxic effects on J2s and EC_{50} value was 1.0825 mg/ml. Using liquid chromatography-mass spectroscopy (LCMS), we identified three secondary metabolites viz., quercetin 3-galactoside, catechin 3-glucoside and potassium embelate. Molecular docking analysis affirmed the efficacy of *E. tsjerium-cottam* fruit extract on the binding interactions of its secondary metabolites with the targeted protein, acetylcholine esterase (AChE) of *M. incognita.* Remarkably, the computed values of binding free energy revealed a hierarchy of potency with -11.5, -11.2 and -8.0 kcal/mol observed for quercetin 3-galactoside, catechin 3-glucoside and potassium embelate. Among all of these ligands, quercetin 3-galactoside binds with the receptor AChE more efficiently than the other two ligands. Furthermore, molecular docking studies provide valuable insights into the mechanisms underlying their nematicidal activity, paving the way for the development and novel nematode control strategies that are both effective and environment friendly.

Keywords: *Embelia tsjerium-cottam* fruit extract, *Meloidogyne incognita*, Scanning electron micrographs, Liquid chromatography-mass spectroscopy, Molecular docking

Citation: Trambadiya Krishna, Kanabar Riddhi, Poriya Paresh, Visavadia Manishkumar and George Linz-Buoy: Nematicidal and molecular docking investigation of secondary metabolites derived from *Embelia tsjerium-cottam* fruit extract against *Meloidogyne incognita.* Intern. J. Zool. Invest. 10(1): 791-802, 2024.
https://doi.org/10.33745/ijzi.2024.v10i01.086

Introduction

Nematodes are microscopically unsegmented, bilaterally symmetric roundworms. Plant-parasitic nematodes (PPNs) are obligate parasites that feed mainly on plant roots (Briar *et al.*, 2016; Kumar and Yadav, 2020). Plant-parasitic nematodes specially root-knot nematodes belonging to the *Meloidogyne* genus cause significant decreases in crop production for many horticultural crops worldwide in tropical and subtropical areas (Jones *et al.,* 2013; Ahmad *et al.*, 2021; Almutairi *et al.,* 2022). The genus *Meloidogyne* comprises more than 90 species with *M. incognita, M. javanica, M. arenaria* and *M. hapla* being recognized as significantly reducing agriculture crops (Hunt and Handoo, 2009; Radwan *et al.*, 2012). Notably, *M. incognita* alone has been reported to induce approximately 30% crop yield reductions under field conditions (Moens *et al.*, 2009). Root-knot nematodes cause nutritional deficiency as well as galling, stunting and yellowing (Jindapunnapat *et al.,* 2013; Kumar and Yadav, 2020). Root-knot nematode obstructs the development of host plants, disturbing nodulation processes and interfering with nitrogen fixation as they employ their stylets to feed on the roots (Trudgill and Block, 2001; Perry *et al*., 2009; Escobar *et al.*, 2015; Tsunoda *et al*., 2017; Trambadiya *et al.*, 2023).

Chemical nematicides are used to suppress root-knot nematodes but their adverse effects on human health and the environment have raised significant concerns (Diyapoglu *et al.*, 2022). Hence, it is imperative to explore alternative root-knot nematode management strategies in agro ecosystems that prioritize the preservation of both human health and the environment. Global meta-analyses have demonstrated the positive impact of organic farming practices known for their sustainability on soil biota (Reganold *et al.*, 2016; Lori *et al.*, 2017). Medicinal plants have nematicidal or nematostatic efficacy against plant-parasitic nematodes (Moosavi, 2012; Chetia *et al.*, 2019). Medicinal plants are rich reservoirs of diverse phytochemicals like alkaloids, phenols, sesquiterpenes, diterpenes and polyacetylenes (Alkari and Chaturvedi, 2014; Benit *et al.*, 2022). The efficacy of plant extracts in nematode control varies with extract concentration and the duration of nematode exposure (Haroon *et al*., 2018; Khan *et al.*, 2019).

Embelia tsjerium-cottam (Primulaceae) commonly known as "Vidang" in Ayurveda, is a red-listed medicinal plant species; particularly renowned for its anthelmintic properties of embelin (Pandey and Ojha, 2011; Mohapatra and Basak, 2015). Additionally, *E. tsjeriam-cottam* has been investigated for its antioxidant and anti-inflammatory potential, with studies suggesting its ability to combat oxidative stress and reduce inflammatory responses. Its traditional use in ayurvedic medicine also indicates its potential in treating gastrointestinal disorders and promoting digestive health (Patil and Patil, 2011; Poojari, 2014; Triantafillidis *et al.*, 2016). *E. tsjeriam-cottam* fruit also contains a unique composition of potassium embelate, embelin, embolic acid, rapanone, sitosterol, daucosterol, embelinol, embeliaribyl ester, quercitol, tannin, christembine, embelic acid and vilangin (Nayak *et al.*, 2009; Mhaskar *et al.*, 2011).

Presently, molecular docking stands as a highly efficient method for elucidating interactions between protein and ligand. Moreover, it aids in understanding protein-ligand interactions, enabling systematic learning through non-covalent access to the active sites of targeted proteins and ensuring binding for each ligand (Sheik *et al*., 2020; Keerthiraj *et al*., 2021). Almutairi *et al.* (2022) revealed that multi-modal inhibitory effects of 9, 12-octadecadienoic acid, n-hexadecanoic acid, and tetradecanoic acid elucidating distinct binding mechanisms involving pi-sigma, pi-alkyl, van der waals and hydrogen bonds.

This study investigated *E. tsjerium-cottam* as an eco-friendly approach for combating root-knot nematodes, identifying nematicidal compounds through LC-MS analysis and evaluating their potential through *in silico* molecular docking

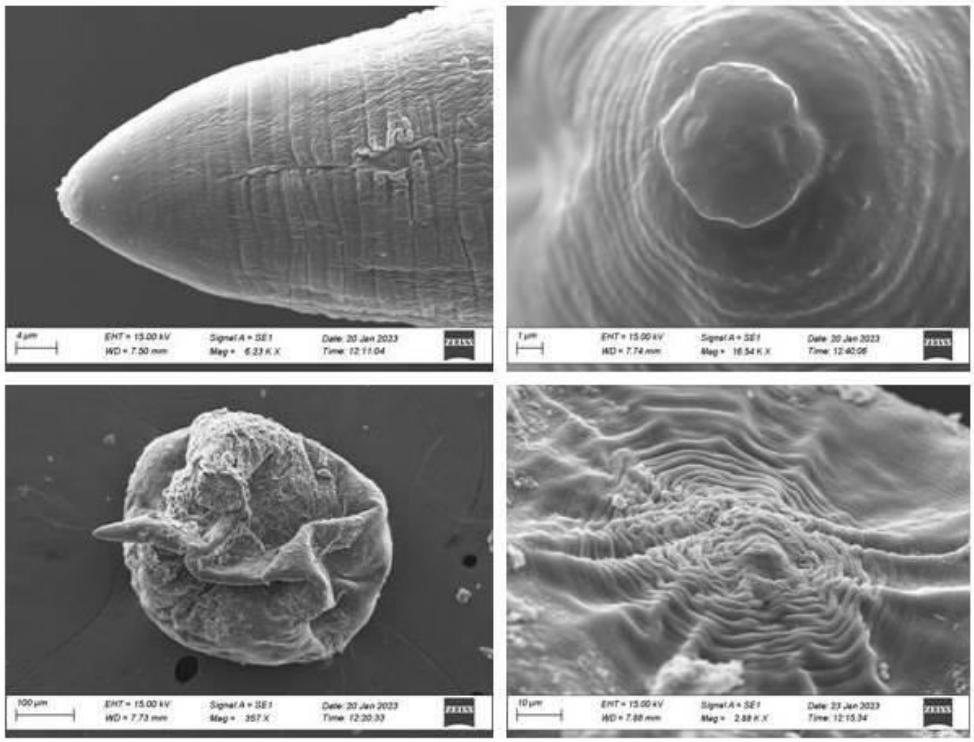

Fig. 1: Scanning electron micrographs of *M. incognita* (a) Mouth with esophageal bulb, (b) Anterior end in face view, (c) Whole body and (d) Perineal pattern.

analysis.

Materials and Methods

Collection and multiplication of inoculums (J2s) of root-knot nematodes:

Tomato is the most common host of root-knot nematodes. Tomato roots infected with *Meloidogyne spp*. were taken from Bahauddin Government Science College (21°30'30" N, 70°27'33" E), Junagadh, Gujarat, India. The identification of *M. incognita* were observed through perineal pattern characteristics (Eisenback and Hunt, 2009; Hunt and Handoo, 2009). The J2s of *M. incognita* was multiplied on separate tomato plants and maintained it in a greenhouse condition. Tomato plants were carefully uprooted with egg masses. They were thoroughly washed with distilled water to remove soil debris. Egg masses were collected from the infected roots using sterilized forceps, transferred in petri dishes with distilled water and placed in an incubator for 25-28 °C for J2s hatching. Freshly hatched J2s concentration was standardized by specifications and preserved for future research.

Identification of M. incognita through scanning electron microscopy (SEM):

M. incognita has been identified by the application of SEM analysis (Fig. 1). An adult female of *M. incognita* was identified through perineal pattern analysis (Isabel and Santos, 1989). The perineal pattern of nematodes were coated with gold-palladium.

Collection and preparation of Embelia tsjerium-cottam fruit extract:

E. tsjerium-cottam fruits was collected from Ahwa (20°48'29" N, 73°53'25" E), Dang, Gujarat, India. The voucher specimen of *E. tsjerium-cottam* (BSC/BOT/A/A20) was identified by Dr. Manish Jani and it has been deposited at the Bahauddin Government Science College, Junagadh, Gujarat, India. *E. tsjerium-cottam* fruits were shade dried for 3-5 days and subsequently powdered using a grinder. Plant extract was prepared with a soxhlet extractor using hydro methanolic solvent system 1:20 w/v. The extract was concentrated and dried

and stored at 4 ˚C for further use. Dilution of the 100% stock solutions was prepared with distilled water to achieve concentrations of 0.5% (5 mg/ml), 1% (10 mg/ml), 2% (20 mg/ml), 4% (40 mg/ml) and 8% (80 mg/ml) for further experiments. A control experiment was also performed using distilled water without any plant extracts.

LC-MS analysis of Embelia tsjerium-cottam fruit extract:

The HPLC system (Shimadzu Corporation, Japan) comprising an LC-20AD solvent delivery system, DGU-20A5R vacuum degasser, CTO-20 AC thermostated column compartment, SIL20AC autosampler, and SPD-M20A PDA detector was employed for chromatographic analysis. Data acquisition and processing were performed using Lab Solution 5.99 SP2 software from Shimadzu Corporation, Japan. Separation was performed on a Phenomienx Gemini C18 column (250 mm × 4.6 mm, 5 µm) placed in a column oven at 40 ˚C. The mobile phase consisted of methanol (A) and water (B), with a gradient elution sequence: 20% → 80% B (0.0-0.01 min), 40% → 60% B (0.01-5.0 min); 90% → 10% B (5.0-25.0 min), 20% → 80% B (25.0-40.0 min). The flow rate was 1.00 ml/min and a 20 µl sample injection volume was used throughout the 40 min analytical run. Desolvation line temperature and heat block temperature were set at 250 °C and 450 °C for precise and reliable analysis.

Screening of anti-nematode potential:

To assess *E. tsjerium-cottam* fruit extract nematicidal potential against *M. incognita*, 250 µl of J2s suspension (Approx 50 nematodes) was added to each well of a 24-well plate along with 1 ml hydromethanolic *E. tsjerium-cottam* fruit extract at concentrations of 0.5%, 1%, 2%, 4% and 8%. Incubation occurred at 25-28 ˚C for 24, 48 and 72 h with five replicates per treatment. We recorded the number of live nematodes and evaluated the anti-nematode percentage of active second-stage juveniles at specific intervals (Fatima *et al.,* 2022).

$$\text{Per cent Mortality (\%)} = \frac{C0 - T\alpha}{C0} \times 100$$

Where C0= Number of J2s alive in control; Tα= Number of J2s alive after an exposure period of 24, 48, and 72 h in different concentrations of *E. tsjerium-cottam* fruit extract. A log-logistic nonlinear regression analysis was conducted using following equation (Caboni *et al.,* 2012):

$$y = C + (D - C)/\{1+\exp[b(\log(x)-\log(EC_{50}))]\}$$

Where C= the lower limit, D = the upper limit, b = the slope at the EC_{50}, and EC_{50} = the test compound concentration required for 50% paralyzed nematodes after control elimination (natural death/paralysis). Plant extract concentration (%w/v) served as the independent variable (x), while the paralyzed J2 nematodes were dependent variable (y). The EC_{50} value was determined using the mean values derived from five replicates for plant extract concentrations and immersion time. The EC_{50} values were determined using an online half-max graphing calculator (https://www.aatbio.com/tools/ec50-calculator) (Liu *et al.,* 2021).

In silico molecular docking:

Protein preparation:

Hypothetical protein sequence of *M.incognita* was obtained from NCBI and UNIPROT databases. NCBI BLAST with PDB database was employed for sequence analysis and functional annotation. Subsequently, homology modeling of AChE protein was conducted using Modeler version 10.4.

Ligand preparation:

The chemical structures of quercetin 3-galactoside (CID: 5281643), catechin 3-glucoside (CID: 14104302) and potassium embelate (CID: 23677950) were acquired from the PUBCHEM database as sdf files. Before the molecular docking study, all ligand files were converted into pdb files using OpenBabelGUI Software.

Molecular docking:

In this study, molecular docking was executed using the Autodock Vina 1.5.7 program on an

Table 1: List of constituents compounds present in hydro-methanolic fruit extract of *E. tsjerium-cottam* determined by LC-MS analysis

No.	RT (min)	Molecular weight (g/mol)	m/z ratio	Tentative identification	Formula	Pubchem ID
1	11.240	464.4	463	Quercetin 3-galactoside	$C_{21}H_{20}O_{12}$	5281643
2	18.039	452.4	451.1	(+)-Catechin 3-glucoside	$C_{21}H_{24}O_{11}$	14104302
3	20.183	332.5	331.2	Potassium embelate	$C_{17}H_{25}KO_4$	23677950

Intel(R) Core(TM) i5-8250U CPU-1.60GHz, 64-bit processor. The interactions between docked receptors and ligands were analyzed using Discovery Studio (DS) 2021 Client, with a focus on AChE and its three ligands (Quercetin 3-galactoside, (+)-Catechin 3-glucoside and potassium embelate) based on docking score and interacting residues. All interactions, identification of active site residues and 2D poses were prepared with BIOVIA Discovery Studio Visualizer 2021 Client.

Statistical analysis:

Data were expressed as mean ± standard error (S.E.). Regression analysis, ANOVA and student's t-test were carried out using Microsoft Excel 2010. The EC_{50} values were determined using an online half-max graphing calculator (https://www.aatbio.com/tools/ec50-calculator). To compare treatment means, Fisher's protected least significant difference test (LSD) was employed between two variables viz., exposure time (factor A), concentration (factor B) and their interaction (A×B) at 1% and 5% probability levels. All experiments included five replicates and appropriate control groups.

Results and Discussion

Liquid chromatography-mass spectroscopy (*LC-MS*) *analysis*:

E. tsjerium-cottam hydro-methanolic fruit extract LCMS analysis was conducted and identified three compounds along with their respective retention time, m/z ratio, area, and percentage area and ms spectrum (Table 1). LC-MS chromatogram and ms spectrum of three secondary metabolites depicted the peaks observed in the *E. tsjerium-cottam* fruit extract (Fig. 2).

The identified compounds are quercetin 3-galactoside, catechin 3-glucoside and potassium embelate. Our LC-MS results aligned with established literature in PUBCHEM database, confirming the presence of quercetin 3-galactoside ([M-H]- 463), (+)-catechin 3-glucoside ([M-H]- 451.1), potassium embelate ([M-H]- 331.2) extract of embelia fruits and their characterization employed diverse spectroscopic tools (Garcia, 2020; Araujo *et al.*, 2020).

Juvenile mortality:

The mortality of J2s in different concentrations like 0.5%, 1%, 2%, 4% and 8% was determined through *in vitro* analysis. With increasing concentration 0.5 - 8 % and exposure from 24 to 72 h, there was increased J2 mortality on a successive basis. This result indicates that all concentrations were highly toxic to J2s (Table 2).

Linear regression was used to illustrate the relative significance of *E. tsjerium-cottam* fruit extract concentrations and their dependent effects on juvenile (J2) mortality. The two factors that make up the present research's base are mortality and concentration. The "best fit" regression line has been used to evaluate the association between these variables. According to the coefficient of determination (R^2), the independent variable (X) explains how far the dependent variable (y)

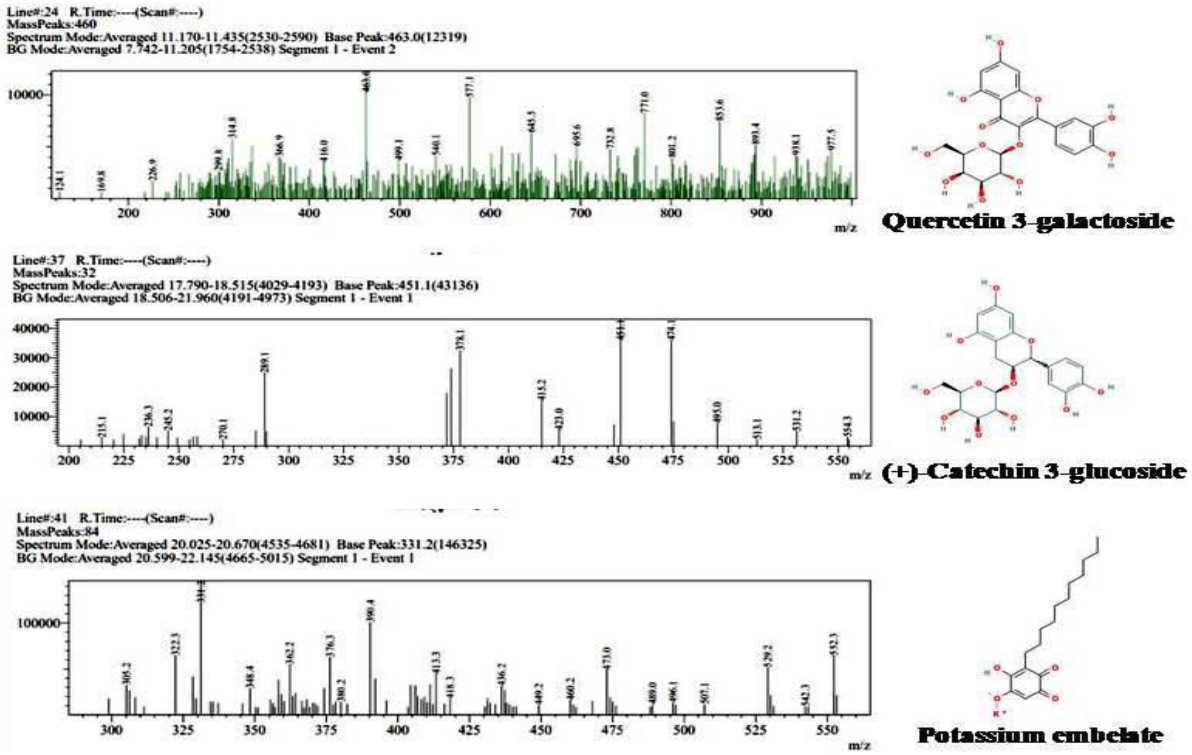

Fig. 2: LC-MS analysis of PDA chromatogram and MS spectrum of secondary metabolites present in Table 1.

Table 2: Effect of different concentrations of *E. tsjerium-cottam* fruit extract on the mortality of J2s of *M. incognita*

Plant extract (Concentration)	**Number of live J2s (Mean ± SE) at different time intervals (Hours)**		
	24 h	**48 h**	**72 h**
0.5%	26.41 ± 0.63	11.48 ± 0.83	7.46 ± 0.58
1%	21.10 ± 0.33	9.78 ± 0.56	6.06 ± 0.41
2%	16.18 ± 0.21	5.92 ± 0.41	3.95 ± 0.28
4%	16.79 ± 0.03	4.42 ± 0.3	2.23 ± 0.15
8%	19.51 ± 0.35	3.78 ± 0.24	1.54 ± 0.11
Control	50	50	50
Significance	0.001	0.0006	0.0002
EC_{50} Value	1.0825 mg/ml		

LSD (0.05)	A	42.5813	**LSD (0.01)**	A	98.2151
	B	59.6153		B	93.4924
	A×B	5.8069		A×B	8.2595

Each value is an average of five replicates. DW = Double distill water (control). SE-Standard Error. Parentheses indicate percentages of J2 mortality compared to control. The numbers presented without parentheses represent the number of *M. incognita* J2s that have dead at different concentrations.

deviates from the mean (Fig. 3).

Mortality was regarded as a dependent variable in this study, whereas concentration is regarded as an independent variable. At 1%, 2% and 4% concentrations, *E. tsjerium-cottam* fruit extract showed anti-nematode activity, with an EC_{50} value of 1.0825 mg/ml. This study aligns with prior research by Mojumder and Mishra (1990) against root-knot nematode (*Meloidogyne incognita*) with 90% mortality after 48 h exposure (at 100 ppm concentration level). *Embelia schimperi* ethanolic crude extract inhibited eggs hatchability and juvenile mortality of *M. incognita* (Waweru *et al.*, 2022). Prominent nematicidal properties were observed in coumaric, benzoic, p-hydroxybenzoic, flavonoid glycosides, benzoquinones and nicotinic acids against *M. incognita* (Du *et al.*, 2011; Tarini *et al.,* 2020).

Recent research has uncovered various mechanisms, including protein degradation and enzyme function disruption that contribute to the suppression of root-knot nematodes. Additionally, research efforts have been dedicated to identifying factors that inhibit egg hatch or exploring the production of secondary metabolites as means to manage root-knot nematodes for innovative and sustainable strategies for managing these agricultural pests.

Consequently, our investigation encompassed the molecular docking analysis of three compounds in association with AChE protein found in *M. incognita*. All of these three compounds in the *E. tsjerium-cottam* fruit extract alone or in combination with other secondary metabolites showed toxicity against J2s of *M. incognita.* They are proven to possess nematicidal properties, which may contribute to managing root-knot nematodes for sustainable agriculture.

Molecular docking analysis:

Molecular docking analysis was carried out to evaluate the binding affinities of three compounds (Quercetin 3-galactoside, Catechin 3-glucoside and Potassium embelate) present in *E. tsjerium-cottam* fruit extract with the target protein (AChE) of *M. incognita*. The docking parameters including binding free energy (Table 3) illustrate the molecular docking poses, showcasing the best binding conformations for the studied ligands with the target protein (Figs. 4, 5).

Quercetin 3-galactoside, (+)-catechin 3-glucoside and potassium embelate exhibited high affinity with aromatic and basic amino acids present in the active site of AChE. The components containing aromatic rings exhibited notable attraction owing to their propensity for π-π interactions with organic residues situated within the binding region of the AChE. The most common amino acids involved in binding with ligands are Glu, Gln, Gly, Asp, Tyr and Ser. The amino groups and hydroxyl groups of amino acids are involved in making hydrogen bonds with the -COOH group of these ligands.

The 2D representation of interactions between ligands and receptors are depicted (Figs. 4, 5) and other binding details are tabulated (Table 3). It can be seen that quercetin 3-galactoside with amino acids residues Glu241, Gln110, Tyr173, Gly160, Asp113; catechin 3-glucoside with amino acid residues Tyr380, Asp113, Ser165, Ser111, Ser242, Glu241, Tyr173, Gly160 and potassium embelate with amino acid residues Tyr173, Gln110 established conventional hydrogen bonds. Additionally, Tyr380, Tyr384, Trp164 (π-π bond); Gln385, Phe381, His499, Ser242, Gly161, Asn126, Ser165, Tyr159, Trp125, Gly166, Leu170, Thr167, Tyr115 (van der waals); Gly500 (C-H bond) interaction could be seen in case of quercetin 3-galactoside. Whereas, catechin 3-glucoside interacted with different residues: Trp125, Gly500, His499, Tyr159, Leu170, Thr167, Gly161, Gly166, Phe381, Tyr384, Gln385, Asn126, Pro112, Gln110(van der waals); Trp164 (π-π bond). Similarly, the residues His499, Asp113, Trp125, Ser165, Asn126, Gly166, Leu170, Gly160, Gly161, Trp164 (van der waals); Tyr380, Phe381 (π sigma); Tyr384 (π alkyl) interacted with potassium embelate. The computed values of binding free energy revealed a hierarchy of potency with -11.5, -11.2 and -8.0 kcal/mol

Table 3: The interactions of selected ligands with the target protein (AChE) of *M. incognita*

Ligands	Binding free energy (kcal/mol)	Interactions	
		Hydrogen bonding	Others
Quercetin 3-galactoside	-11.5	Glu241, Gln110, Tyr173, Gly160, Asp113	Gln385, Tyr384, Phe381, Trp164, Tyr380, His499, Gly500, Ser242, Gly161, Asn126, Ser165, Trp125, Gly166, Leu170, Thr167, Tyr159, Tyr115
(+)-Catechin 3-glucoside	-11.2	Tyr380, Asp113, Ser165, Ser111, Ser242, Glu241, Tyr173, Gly160	Trp125, Gly500, His499, Tyr159, Leu170, Thr167, Gly161, Gly166, Trp164, Phe381, Tyr384, Gln385, Asn126, Pro112, Gln110
Potassium embelate	-8.0	Tyr173, Gln110	His499, Asp113, Trp125, Ser165, Asn126, Gly166, Leu170, Gly160, Gly161, Tyr380, Trp164, Tyr384, Phe381

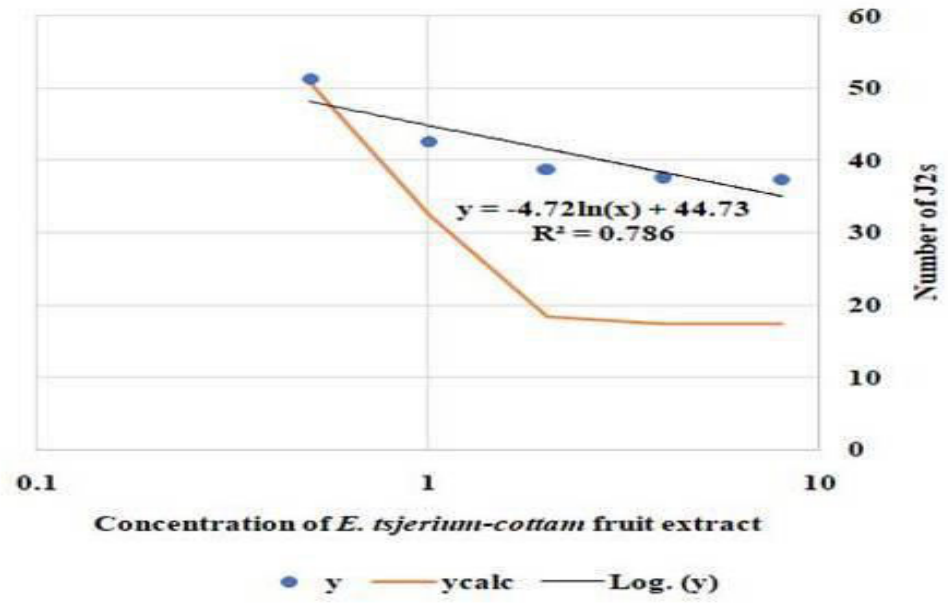

Fig. 3: Regression analysis of *E. tsjerium-cottam* fruit extract on J2s of *M. incognita*.

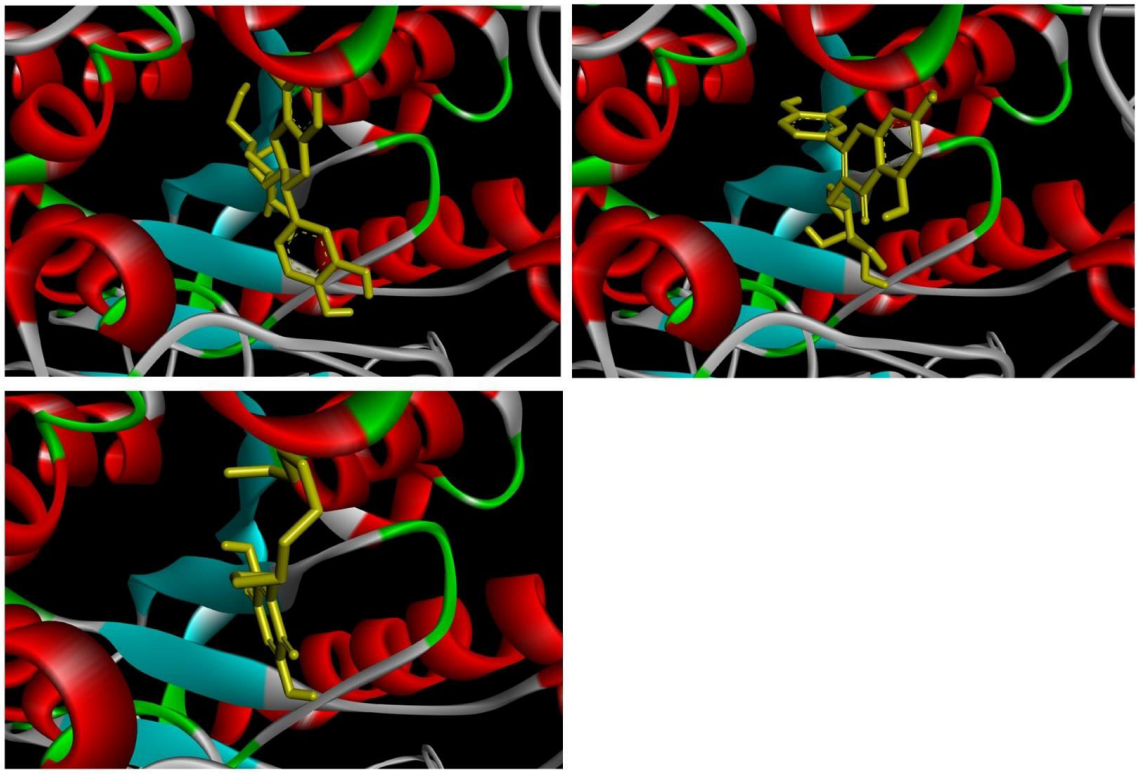

Fig. 4: The best docked poses of AChE receptor with (a) Quercetin 3-galactoside, (b) (+)-Catechin 3-glucoside and (c) Potassium embelate. A yellow stick represents the ligand. The colors present in protein structure are based on different amino acids, which exhibit in the protein.

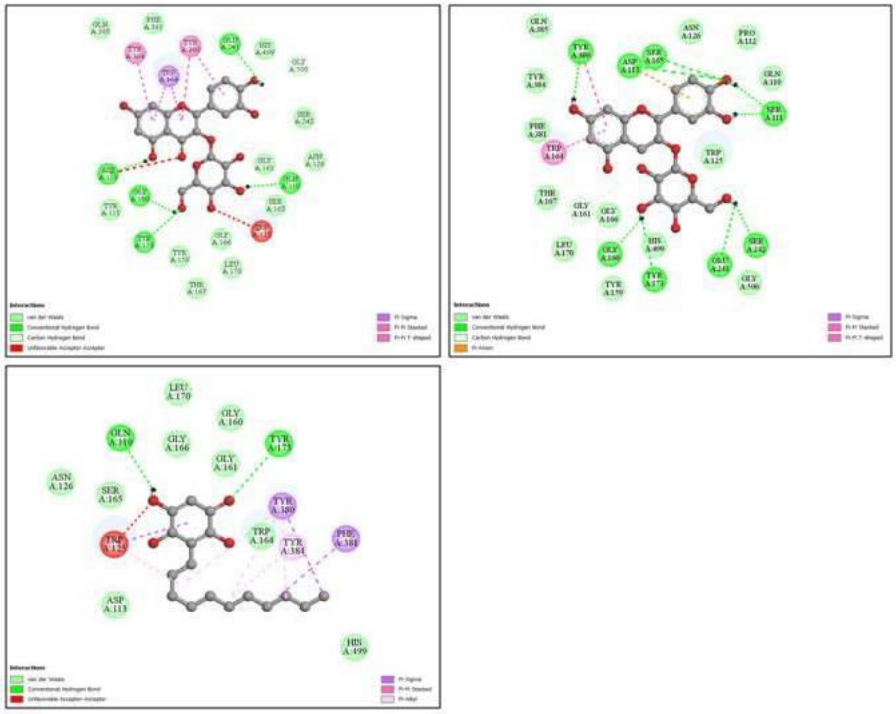

Fig. 5: The 2D representation of interactions for receptor (AChE) docked with with (a) Quercetin 3-galactoside, (b) (+)-Catechin 3-glucoside and (c) Potassium embelate.

observed for quercetin 3-galactoside, catechin 3-glucoside and potassium embelate. Among all of these ligands, quercetin 3-galactoside binds with the receptor AChE more efficiently than the other two ligands.

Our findings align with the study of Keerthiraj *et al.* (2021), investigating the multi-modal inhibitory potential of molecular docking and *in silico* analysis of α-bulnesene and α-guaiene on three target proteins (AChE, ODR1, ODR3) with emphasis on unique binding mechanisms involving pi-alkyl, pi-sigma, and hydrophobic interactions. Molecular docking and *in silico* analysis study binding affinity of 9, 12-octadecadienoic acid (-5.3 kcal/mol), n-hexadecanoic acid (-4.5 kcal/mol), and tetradecanoic acid (-4.9 kcal/mol), respectively, with the AChE receptor of *M. incognita* (Almutairi *et al.*, 2022).

The unique mechanism of action associated with secondary metabolites from medicinal plants involves altering nematode cell membrane permeability and neuro-sensitive receptor proteins like AChE, disrupting nerve impulses effectively (Mills *et al.*, 2004; Almutairi *et al.*, 2022). Utilizing molecular modeling, interactions between receptors and ligands, including hydrophobic, covalent and non-covalent interactions were determined. Ligand efficiency values measured the blocking potential and the prediction of strong binding between the ligand and nematode protein. One strategy to reduce the accumulation of organic waste in the environment is to use organic resources in agricultural practices. We can lessen environmental harm while managing nematode numbers successfully by utilizing these techniques.

Conclusion

In summation, the research focused on the nematicidal properties and molecular docking interaction of secondary metabolites derived from *E. tsjerium-cottam* fruit extract against *M. incognita* which offers valuable insights into the potential agricultural applications of this natural resource. In both individual and combined forms, quercetin 3-galactoside, catechin 3-glucoside and potassium embelate being the predominant active compounds exhibited significant toxicity towards *M. incognita* based on *in vitro* investigations. The findings highlight promising nematicidal properties of the *E. tsjerium-cottam* fruit extract suggesting its potential as an eco-friendly alternative for managing nematode infestations in crop cultivation. Furthermore, molecular docking analysis provides a deeper understanding of the bioactive compounds interactions with the nematode proteins for further research and development of novel nematicides. Overall, this study underscores the unique contribution of plant-derived secondary metabolites to the realm of nematode control while unraveling the distinctive molecular mechanisms underlying their effectiveness.

Acknowledgements

Authors are thankful to Dr. R. P. Bhatt, Principal, Bahauddin Government Science College, Junagadh for his support throughout the research. The authors also acknowledge Shailesh Dodiya, Dr. Manish Jani, Dr. Rajkumar Yadav, Vinesh Gamit and Zankhana Pandit for their valuable help in plant sample collection and data analysis. The authors acknowledge Dr. Trupesh Pethani, Department of Pharmaceutical Sciences, Saurashtra University, Rajkot for providing help in LC-MS analysis. Trambadiya Krishna and Kanabar Riddhi are thankful to Government of Gujarat for providing SHODH scholarship.

References

Ahmad G, Khan A, Khan AA, Ali A and Mohhamad HI. (2021) Biological control: a novel strategy for the control of the plant parasitic nematodes. Antonie Van Leeuwenhoek 114(7): 885-912.

Alkari S and Chaturvedi A. (2014) Phytochemical basis of antiinflammatory and ant-microbial activity of *Achyranthes aspera*. Biol. 1(4): 128-38.

Almutairi FM, Khan A, Ajmal MR, Khan RH, Khan MF, Lal H, Ullah MF, Ahmad F, Ahamad L, Khan A, Arif H and

Ayaz Ahmad M. (2022) Phytochemical analysis and binding interaction of cotton seed cake derived compounds with target protein of *Meloidogyne incognita* for nematicidal evaluation. Life 12(12): 2109.

Araujo NMP, Arruda HS, Dos Santos FN, de Morais DR, Pereira GA and Pastore GM. (2020) LC-MS/MS screening and identification of bioactive compounds in leaves, pulp and seed from *Eugenia calycina* Cambess. Food Res Int. 137: 109556.

Benit N, Kumar TSJ, Almaary KS, Elshikh MS, Rasheed RA and Antonisamy P. (2022) Phytopathogenic bacterial and nematicidal activity of extracts and powder of *Adhatoda vasica* on *Meloidogyne incognita*. Physiol Mol Plant Pathol. 119: 101816.

Briar SS, Wichman D and Reddy GV. (2016) Plant-parasitic nematode problems in organic agriculture. In: Organic farming for sustainable agriculture. Springer, Switzerland, pp. 107-122.

Caboni P, Ntalli NG, Aissani N, Cavoski I and Angioni A. (2012) Nematicidal activity of (E, E)-2, 4-decadienal and (E)-2-decenal from *Ailanthus altissima* against *Meloidogyne javanica*. J Agric Food Chem. 60(4): 1146-1151.

Chetia A, Gogoi P, Roy P and Goswani M. (2019) Antagonistic plants as a tool of nematode management. J Med Plants Stud. 7(6): 113-116.

Diyapoglu A, Oner M and Meng M. (2022) Application potential of bacterial volatile organic compounds in the control of root-knot nematodes. Molecules 27(14): 4355.

Du SS, Zhang HM, Bai CQ, Wang CF, Liu QZ, Liu ZL, Wang YY and Deng ZW. (2011) Nematocidal flavone-C-glycosides against the root-knot nematode (*Meloidogyne incognita*) from *Arisaema erubescens* tubers. Molecules 16(6): 5079-5086.

Eisenback JD and Hunt DJ. (2009) General morphology. In: Root-knot nematodes, CABI, Wallingford UK, pp. 18-54.

Escobar C, Barcala M, Cabrera J and Fenoll C. (2015) Overview of root-knot nematodes and giant cells. In: Advances in botanical research, Academic Press, Spain, pp. 1-32.

Fatima S, Khan F, Asif M, Alotaibi SS, Islam K, Shariq M, Khan A, Ikram M, Ahmad F, Khan TA, Meena R and Siddiqui MA. (2022) Root-knot disease suppression in eggplant based on three growth ages of *Ganoderma lucidum*. Microorganisms 10(5): 1068.

Garcia BM. (2020) Revalorization of food by-products as sources of bioactive compounds. Ph. D. thesis, Departamento de Química Analítica, University of Granada.

Haroon SA, Hassan BA, Hamad FM and Rady MM. (2018) The efficiency of some natural alternatives in root-knot nematode control. Adv Plants Agric Res. 8(4): 355-362.

Hunt DJ and Handoo ZA. (2009) Taxonomy, identification and principal species. In: Root-knot nematodes, CABI, Wallingford UK, pp. 55-97.

Isabel MDO and Santos MSNDA. (1989) A technique for preparing perineal patterns of root-knot nematodes for scanning electron microscopy. J Nematol. 21(1): 138.

Jindapunnapat K, Chinnasri B and Kwankuae S. (2013) Biological control of root-knot nematodes (*Meloidogyne enterolobii*) in guava by the fungus *Trichoderma harzianum*. J Dev Sustain Agric. 8(2): 110-118.

Jones JT, Haegeman A, Danchin EG, Gaur HS, Helder J, Jones MG, Kikuchi T, Manzanilla-lopez R, Palomares-Rius JE, Wesemael WML and Perry RN. (2013) Top 10 plant-parasitic nematodes in molecular plant pathology. Mol Plant Pathol. 14(9): 946-961.

Keerthiraj M, Mandal A, Dutta TK, Saha S, Dutta A, Singh A and Kundu A. (2021) Nematicidal and molecular docking investigation of essential oils from *Pogostemon cablin* ecotypes against *Meloidogyne incognita*. Chem Biodivers. 18(9): e2100320.

Khan F, Asif M, Khan A, Tariq M, Ansari T, Shariq M and Siddiqui MA. (2019) Evaluation of the nematicidal potential of some botanicals against root-knot nematode, *Meloidogyne incognita* infected carrot: In vitro and greenhouse study. Curr Plant Biol. 20: 100115.

Kumar Y and Yadav BC. (2020) Plant-parasitic nematodes: Nature's most successful plant parasite. Int J Res Rev. 7(3): 379-386.

Liu C, Fan H, Guan L, Ge RL and Ma L. (2021) *In vivo* and *in vitro* efficacy of crocin against *Echinococcus multilocularis*. Parasites Vectors 14(1): 1-17.

Lori M, Symnaczik S, Mäder P, De Deyn G and Gattinger A. (2017) Organic farming enhances soil microbial abundance and activity—A meta-analysis and meta-regression. PLoS One 12(7): 180442.

Mhaskar M, Joshi S, Chavan B, Joglekar A, Barve N and Patwardhan A. (2011) Status of *Embelia ribes* Burm f. (Vidanga), an important medicinal species of commerce from northern Western Ghats of India. Curr Sci. 100(5): 547-552.

Mills C, Cleary BV, Walsh JJ and Gilmer JF. (2004) Inhibition of acetylcholinesterase by tea tree oil. J Pharm Pharmacol. 56(3): 375-379.

Moens M, Perry RN and Starr JL. (2009) *Meloidogyne* species-a diverse group of novel and important plant

parasites. In: Root-knot nematodes, CABI, Wallingford UK, pp. 1-17.

Mohapatra M and Basak UC. (2015) Quantitative reckoning of embelin from fruits of *Embelia tsjeriam-cottam* using water bath process as an alternate method of extraction. Indian J Pharm Biol Res. 3(3): 15.

Mojumder V and Mishra SD. (1990) *In vitro* studies on toxicity of embelin and its haloderivatives to *Meloidogyne incognita* juveniles. Curr Nematol. 1: 47-48.

Moosavi MR. (2012) Nematicidal effect of some herbal powders and their aqueous extracts against *Meloidogyne javanica*. Nematropica 42(1): 48-56.

Nayak SU, Joshi VK, Maurya S and Singh UP. (2009) Analysis of phenolic acids in different market samples of vidanga (False black pepper). AYU 30(2): 181-187.

Pandey AK and Ojha V. (2011) Estimation of embelin in *Embelia tsjeriam-cottam* fruits by HPLC to standardize harvesting time. Indian J Pharm Sci. 73(2): 216.

Patil PA and Patil SA. (2011) Anti-inflammatory effect of the *Embelia tsjeriam-cottam* fruit extracts. Res J Pahrmacol Pharmacodyn. 3(5): 246-249.

Perry RN, Moens M and Starr JL. (2009) Root-knot nematodes. CABI, Wallingford UK.

Poojari R. (2014) Embelin–a drug of antiquity: shifting the paradigm towards modern medicine. Expert Opin Invest Drugs 23(3): 427-444.

Radwan MA, Farrag SAA, Abu-Elamayem MM and Ahmed NS. (2012) Biological control of the root-knot nematode, *Meloidogyne incognita* on tomato using bioproducts of microbial origin. Appl Soil Ecol. 56: 58-62.

Reganold JP and Wachter JM. (2016) Organic agriculture in the twenty-first century. Nat Plants 2(2): 1-8.

Sheik Amamuddy O, Veldman W, Manyumwa C, Khairallah A, Agajanian S, Oluyemi O, Verkhivker GM. and Tastan Bishop Ö. (2020) Integrated computational approaches and tools for allosteric drug discovery. Int J Mol Sci. 21(3): 847.

Tarini G, Melo AS, Fontana LF, da Silva Júnior E, Bolanho BC, Moreno BP, Saragiotto MH and Dias-Arieira CR. (2020) Aqueous extracts of *Crambe abyssinica* seed cake: Chemical composition and potential for nematode control. Ind Crops Prod. 156: 112860.

Trambadiya K, Kanabar R and Visavadia M. (2023) Nematicidal properties of medicinal plants against root-knot nematode-a systematic review. European J Theoretical Appl Sci. 1(4): 501-509.

Triantafillidis JK, Triantafyllidi A, Vagianos C and Papalois A. (2016) Favorable results from the use of herbal and plant products in inflammatory bowel disease: evidence from experimental animal studies. Ann Gastroenterol. 29(3): 268.

Trudgill DL and Blok VC. (2001) Apomictic, polyphagous root-knot nematodes: exceptionally successful and damaging biotrophic root pathogens. Annu Rev Phytopathol. 39(1): 53-77.

Tsunoda T and van Dam NM. (2017) Root chemical traits and their roles in belowground biotic interactions. Pedobiologia 65: 58-67.

Waweru BW, Pili NN, Wetiba MW, Lusweti D, Koske MC, Ramkat RC and Kiprop AK. (2022) Control of *Meloidogyne incognita* and *Pratylenchus zeae* using *Embelia schimperi* extracts. Trop Subtrop Agroecosystems 25(2): 1-11.