Research Article

Nematicidal effect of Methanolic Plant Crude Extracts against the Root-Knot Nematode, *Meloidogyne incognita*

Saima Maher[1*], Salma javed[2*], Gul mina[1], Rizwana Salam[1], Farah Mukhttar[1], Noureen Khan[3] and Fakhra Batool[3]

[1]Department of Chemistry, Sardar Bahadur Khan Women University Quetta, Pakistan; [2]National Nematological Research Center, University of Karachi, Pakistan; [3]Department of Pharmacy, The Women University Multan, Pakistan.

Abstract | This research focuses on Six Pakistani medicinal plants, from which five different extracts methanol, ethanol, ethyl acetate, hexane, and chloroform were prepared from various plant. Preliminary phytochemical analysis of these extracts revealed the existence of main phytochemical constitutes, including alkaloids, glycosides, flavonoids, tannins and phenolic compounds. These compounds were found to contribute to the observed anti-nematicidal activity of the plant extracts. The study concluded that the plant extracts possess significant potential as nematicidal agents based on their phytochemical composition and demonstrated activity against nematodes. The effectiveness of medicinal plant extracts against *M. incognita*, the research also emphasizes the need for further investigations to isolate active components, optimize concentrations, and develop practical application methods for field use. The research emphasizes the need for further investigations to isolate active components, optimize concentrations, and develop practical application methods for field use. These findings contribute to ongoing efforts aimed at developing environmentally sustainable nematode management solutions.

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***Correspondence** | Salma javed, National Nematological Research Center, University of Karachi, Pakistan; **Email:** salmajaved202@gmail.com, Saima Maher, Department of Chemistry, Sardar Bahadur Khan Women University Quetta, Pakistan; **Email:** saimamaher@yahoo.com
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Introduction

Natural product chemistry certainly delves into a fascinating array of organic compounds sourced from nature, encompassing a broad spectrum of compounds with diverse structures and properties (Newman and Cragg, 2016). These compounds are not only significant for their intrinsic biological activities but also hold immense potential for various applications in medicine and the pharmaceutical industry. (Harvey *et al*., 2015). Bioactive compounds derived from natural sources serve as valuable reservoirs of both simple and complex molecules possessing a broad range of biological properties. (Atanasov *et al*., 2015). They are utilized extensively in the development of therapeutics to combat a plethora of diseases due to their anti-bacterial, anti-inflammatory, antifungal, anti-diabetic, and anti-cancer properties. (Gibbons,

2008). Terpene glycosidase NF-KB is one such example, exhibiting notable anti-inflammatory and anti-cancer effects. These compounds are often found in fruits, vegetables, and spices, where they contribute to the characteristic flavors and aromas. (Procopio *et al*., 2012). Phenols and terpenoids, in particular, are noteworthy chemical constituents abundant in these natural sources, further underscoring the significance of natural product chemistry in understanding and harnessing the potential of bioactive compounds for various applications. (Wagner *et al*., 2003). Similarly, natural product bioactive compound work against nematode (Burow *et al*., 2007). *Meloidogyne incognita* (root-knot nematode) is a plant parasite that reasons of crop losses yearly. Its reproductive mechanism is unique, relying on mitotic parthenogenesis, and it expresses immune suppressor genes to successfully colonize the host plant. Understanding the biology and reproductive mechanism of this nematode is essential for developing effective control strategies. Over 28000 species of nematode and *meloidogyne incognita* wide ranging species plant parasite nematode is plant parasite which causes the loss of yield. (Chitwood, 2003). Plant naturally having the pesticides which are non-dangerous as compare chemical pesticides. (Ujvary, 2002). (Augusto *et al*., 2021). Plant extract having the essential oils volatile chemical. (Brown and Morra, 1997). Which shown properties of anti-bacterial and nematicidal properties. (Digrak *et al*., 1999; Okoko *et al*.,1999). The sample extract of plant evaluated for nematicidal assay against (RKN) root knot-nematode. (Faizi *et al*., 2011; Samina *et al*., 2020; Zareena *et al*., 2020; Choudhary *et al*., 2010). It has catastrophic effects on root knot of plant because of environment is favorable for the flourishing growth of meloidogyne nematode. (Latif *et al*., 2014). (Murslain *et al*., 2013) The previous research that has focused on the nematicidal potential of some medicinal plant extracts in managing RKN *in vitro* and *in vivo*. Extracts of solidago and periwinkle leaves have shown promising nematicidal activity, and more studies are necessary to isolate and evaluate the potential compounds as environmentally friendly and synthesis the plant based biopesticides. Plant extracts is used as a substitute to synthetic pesticides for the controlling of RKNs due to the harmful effects of synthetic nematicides and the lack of resource of resistant plant cultivars. The research study aims to collect various medicinal plants from SBKWU botanical garden, prepare various extracts from different organic solvents, and evaluate the most clearly active fractions. The study focuses on the *in vitro* screening of nematocidal efficiency against cultured *M. incognita*. Plant parasite nematode that causes significant yield loss. The purpose is to study the prospects of plant-based nematicidal products and cure RKN diseases aggressively using natural products. The selected medicinal plants for the research include *Punica Granatum, Camellia sinensis, Cassia fistula*, Grapes, *Morus alba,* and *Ocimum bacilicum*. The study will prepare various extracts from the entire part of the plants extract of hexane, chloroform, ethyl acetate, ethanol, methanol.

The current research work were to collect various medicinal plants, prepare various extracts, and evaluate the most active fractions for their possible antiemetic activity against cultured *Meloidogyne incognita* species. The research aims to study the prospects of plant-based nematicidal products and cure RKN diseases aggressively using natural products. The selected medicinal plants have shown promising nematicidal activity, and further studies are required to isolate and analyze the active compounds for use as environmentally friendly biopesticides against RKN.

Materials and Methods

Collection of plants materials

Plants materials were collected from the botanical garden at SBKW University and then washed with water for removed latex and extra dirt's and then shade dried. Plant taxonomist Miss Yasmeen from SBKWU Quetta botany department identified the all plants material. The plant materials were examined for the phytochemical content and nematicidal assay.

Chemicals

The different chemicals were utilized for the research purpose such as ethanol (99.9%) and methanol (99.9%) purchased from Sigma Aldrich.

Extraction and Fractionation of Bioactive Compounds from Plant Material

Plant material undergoes systematic washing and drying before being ground into a powder. This powder is then subjected to extraction with methanol, yielding a crude gummy material after evaporation. Fractionation is achieved through liquid-liquid extraction using solvents on the polarity bases hexane, chloroform, acetone, and ethyl acetate. Each solvent selectively extracts different organic compounds based on polarity and density. The resulting fractions were stored separately for further analysis.

Phytochemical analysis

Phytochemical analysis is a crucial step in identifying the bioactive chemical constituents present in plants. The phytochemical screening of various organic soluble fractions has detected the existence of several compounds, counting phenols, alkaloids, tannins, steroids, flavonoids, and saponins, these major components have been retain various biological properties. The plant materials were selected on the basis of their indigenous therapeutic uses. The primary objective of the current research work involving medicinal plants was to evaluate the nematicidal potency of six Pakistani medicinal plants by preparing five different extracts (methanol, ethanol, ethyl acetate, hexane, and chloroform) and analyzing their phytochemical constituents. The research aimed to determine the effectiveness of these extracts against *Meloidogyne incognita* and to identify the key phytochemical compounds contributing to their nematicidal activity. The study also sought to highlight the potential of these plant extracts as environmentally sustainable nematode management solutions and to emphasize the need for further investigations to optimize their use in practical applications.

Extraction of root-knot nematodes

Root-knot nematodes were extrated from roots and soil and then were properly mixed with water in a plastic bucket within a few minutes, all clods were broken down. The water was put over a 36 mm mesh screen. The rootlets from the 36 mm mesh were collected in a beaker for the examination of root-knot nematodes.

Culture of Nematodes

The nematode *Meloidogyne incognita* was obtained at the greenhouse of the National Nematological Research Centre (NNRC) by isolating single egg masses from tomato plants (*Solanum lycopersicum*). The pure population of root-knot nematodes was confirmed through perineal pattern analysis. Infested plants with root galls were carefully washed to remove soil and egg masses were collected using forceps. Fifty even sized egg were hatched in spring water at 28±2°C, and the freshly hatched second-stage juveniles (J2) and it were used for further studies. This process ensured the availability of a controlled and pure population of *Meloidogyne incognita* for research purposes.

Evaluation of Nematicidal Activity

The nematicidal activity of six selected plants was assessed using crude methanolic extracts. The extracts, prepared at a concentration of (50 mg/mL^{-1}) and further dissolved in 5% DMSO (dimethyl sulfoxide). Different concentrations (0.25%, 0.5%, or 1%) of the stock solution were prepared for the assay. For each dose, 100 J2 root-knot nematodes were handpicked and kept in Petri dishes (5 cm dia). The Petri dishes were then maintained at 25°C, and all handlings were replicated three times. Interpretations were conducted at different time intervals 24, 48, or 72 hours. a stereoscopic microscope is used for determine the efficiency of the extracts against the nematodes. 5% DMSO was used as a control. Six plants different extract were utilized to assess their nematicidal toxicity against *Meloidogyne incognita* second-stage juveniles (J2). The nematodes were obtained from tomato plants (*Solanum lycopersicum*) cultured in a greenhouse. Egg masses were extracted and incubated in glass cavity blocks with fresh water for egg hatching at ambient temperature over 72 hours. 100 freshly hatched juveniles (J2) were placed in separate 3 × 3 glass cavity blocks for all concentration of compounds, with three replicates for the whole experiment. A stock solution of the plant extract was prepared at a concentration of 10 mg/ml with 5% Dimethyl sulfoxide (DMSO). The experiment was conducted at concentrations of 1%, 0.5%, 0.25%, and 0.125%, with 1 ml added to all cavity block. 5% (DMSO) and water used as controls. The percentage % death ratio was determined by a stereoscopic microscope after each interval of 24, 48, and 72 hrs.

Data analysis

The data underwent multifactor study of variance ANOVA by SAS (version 9.1, SAS Institute, Cary, NC). Significant interactions were elucidated and interpreted accordingly. When interactions were notable, Least Squares Means analysis was employed, followed by Tukey's test for mean separation. Furthermore, MS Excel (Microsoft, Redmond, WA, USA) was utilized to construct line graphs, perform regression analysis, and develop regression equations to explore the data relationships in depth.

Results and Discussion

The research proposes addressing root-knot nematode (RKN) diseases using natural products by identifying and utilizing the nematicidal properties of extracts from six Pakistani medicinal plants. These extracts, rich in bioactive phytochemicals such as

Table 1: *Phytochemical Analysis of selected plant* **.**

s.no	Phytochemical Test	indication	Green tea	Ponicag Rantum	Mint	Cassisn fistula	Grapes	Morus alba	Ocimumbe-silicum
1.Carbohydrates									
1	Molish test	Volite colour ring	+	+	+	+	+	+	+
2.Protein									
1	Xanthoprotic	Yellow colour	+	+	+	+	+	+	+
3.Lipid									
1	Extract+pressed between filter paper	Oil stain on filter paper	+	+	+	+	+	+	+
4.Phenol									
5.Tannin.									
1	Extract+1%leadacetate	Yellowish colour ppt	+	+	+	+	+	+	+
6. alkaloid									
1	2ml extract+ 2 to 3 drops of mayar reagent	Yellowish colour ppt	V	Negative	positive	V	Negative	+	+
7.Phytosteroid.									
1	2ml extract+chloroform conc sulfuric acid +shakr	Golden colour	+	+	positive	+	+	+	+
8.Saponin									
1	Foam teat	Formation of foam	negative	+	positive	+	Negative	+	+
9.Quinones									
1	2ml extract+1ml con sulfu-ric acid	Red colour appear	+	+	positive	+	+	+	+
10. Steroid									
1	Salkowsk test	Redish brown junction	+	+	Positive	+	+	+	+
11.Glycoside test									
1	Borntrager test	Blue greenish colour	+	+	positive	+	Negative	+	+
12.Phlobatannin									
1	2ml extract+2ml HCL 1% heat	Raddish ppt	negative	Negative	negative	+	Negative	negative	+

(+) means chemical compounds is present in these plants

alkaloids, glycosides, flavonoids, tannins, and phenolic compounds, demonstrated significant effectiveness against *Meloidogyne incognita*. The study highlights the potential for developing environmentally sustainable nematode management solutions through further investigation, isolation of active components, and optimization of application methods. In this study, we assessed the activity of various plant extracts obtained using different solvents and doses, focusing on their potential to contribute to environmentally safe pest and pathogen management strategies. The plants studied included M. alba, Z G.M, SM, J-M-M, M-Chloroform, O. B, PG(M), PG acetone, P.G E. acetate, U-A-M, and G.T, with extracts tested across various doses and time intervals. Our findings align with those reported in the literature, including those from (Chitwood, 2002), which highlight the environmental safety and broad spectrum of activity of bioactive natural products against diverse pests and pathogens. These products also have significant potential in reducing nematode density, supporting their role in sustainable agriculture practices. The effectiveness of various solvents in extracting bioactive compounds confirms the impact of chemical characteristics on the efficacy of the extracts. Chloroform has been shown to be particularly effective, consistent with previous research emphasizing the significance of solvent properties in maximizing bioactive phytochemical yields. (Nikoletta and Pierluigi, 2012). Integrating new insights from the research on

Table 2: *Nematicidal Activity of Selected medicinal Plants*.

Sample code	Dose	24hr	48hr	72hr
M. alba Hexane	1	0	0	65 ± 0.2a
	0.5	0	0	60 ± 1.0b
	0.125	0	0	50 ± 1.2c
M. alba Methanol	1	0	25 ± 2.0a	30± 0.2a
	0.5	0	20± 1.0b	25 ± 0.9b
	0.125	0	05 ± 1.5c	10 ± 1.5c
M. alba Chloroform	1	48 ± 0.1a	62 ± 1.0a	82 ± 1.0a
	0.5	42 ± 0.5b	50 ± 1.0b	75 ± 0.1b
	0.125	37 ± 1.0c	45 ± 2.2c	70 ± 1.2c
Z G.M	1	03 ± 0.5a	50 ± 0.5a	70 ± 1.0a
	0.5	03 ± 1.2a	25 ± 0.2b	40 ± 1.0b
	0.125	01 ± 0.2a	25 ± 1.0b	35 ± 1.0c
SM	1	15 ± 0.2a	70± 1.0a	98 ± 0.2a
	0.5	10 ± 1.5b	25 ± 0.2b	40 ± 0.5b
	0.125	05 ± 1.0c	10 ± 0.2c	20 ± 1.2c
J-M-M	1	70± 0.5a	100± 0.0a	100± 0.0a
	0.5	20± 1.1b	80 ± 0.1b	100± 0.0a
	0.125	05± 1.0c	20 ± 0.5c	50 ± 2.0c
M- Chloroform	1	0	0	48 ± 1.5a
	0.5	0	0	47 ± 0.2b
	0.125	0	0	40 ± 2.5c
O. B Methanol	1	15 ± 0.5a	32 ± 1.2a	50 ± 1.2a
	0.5	10 ± 0.1b	15 ± 1.2b	45 ± 0.1b
	0.125	05 ± 2.0c	12 ± 2.5c	35 ± 1.0c
PG(M)	1	0	0	60 ± 0.5a
	0.5	0	0	52 ± 1.2b
	0.125	0	0	45 ± 0.2c
PG acetone	1	90 ± 1.0a	100 ± 0.0a	100 ± 1.2a
	0.5	50 ± 1.2b	65 ± 0.5b	74 ± 1.5 b
	0.125	10 ± 0.2c	30 ± 1.0c	60 ± 1.0c
P.G E. acetate	1	5 ± 1.0a	30 ± 0.5a	74 ± 0.5a
	0.5	0	10± 1.2b	25± 1.0b
	0.125	0	05 ± 1.5c	10 ± 1.0c
U-A-M	1	40 ± 0.2a	100 ± 0.0 a	100 ±0.0 a
	0.5	05 ± 1.2b	60 ±1.5 b	75 ± 1.0b
	0.125	0 ± 0.5c	30 ±0.5 c	45 ±1.0c
G.T Hexane	1	22 ± 1.0a	50 ± 0.5a	72 ± 0.2a
	0.5	18 ± 2.0b	28± 1.2b	28± 1.2b
	0.125	15 ± 1.5c	32 ± 1.5c	12 ± 1.2c
G.T Chloroform	1	42 ± 0.2a	82 ± 1.0a	92 ± 1.5a
	0.5	32 ± 0.1b	70 ± 1.2b	85 ± 0.2b
	0.125	37 ± 1.5c	65 ± 1.2c	80 ± 1.0c
G.T Chloroform	1	42 ± 0.2a	82 ± 1.0a	92 ± 1.5a
	0.5	32 ± 0.1b	70 ± 1.2b	85 ± 0.2b
	0.125	37 ± 1.5c	65 ± 1.2c	80 ± 1.0c

Syzygium cumini, it is noted that extracts of this plant have been found to reduce the penetration of infective J2 nematodes, further underscoring the nematicidal potential of natural extracts (Salim *et al.*, 2009). In addition to their nematicidal properties, phytochemicals as a management strategy enhances their utility as alternatives to synthetic chemicals, especially in organic agriculture where bio-based pesticides are preferred. (Javid *et al.*, 2021) Given the promising results, further research should explore the specific mechanisms by which these extracts impact pests and pathogens. Investigations into the bioavailability, environmental degradation, and persistence of these compounds are crucial for their effective implementation in field conditions. In conclusion, our study supports the efficacy of plant-derived extracts in pest and pathogen management and extends previous research by providing empirical evidence of their effectiveness. This approach not only aligns with sustainable agricultural principles but also opens avenues for developing safer and more effective pest management solutions.

Conclusions and Recommendations

Plant-based biopesticides offer significant environmental benefits in agricultural pest management. Unlike synthetic chemical pesticides, these biopesticides typically degrade more quickly, reducing their persistence in the environment and minimizing residual contamination of soil, water, and non-target organisms. They pose lower risks to beneficial insects, birds, and mammals, thereby supporting biodiversity and ecological balance in agricultural ecosystems. Moreover, plant-based biopesticides often exhibit multiple modes of action, which can help mitigate the development of resistance in pest populations over time—a common issue with conventional pesticides. Emphasizing sustainable agriculture practices, these biopesticides align with organic farming principles and integrated pest management strategies. Furthermore, some plant-derived biopesticides contribute organic matter to the soil, enhancing soil health and promoting better plant growth and productivity. Overall, the adoption of plant-based biopesticides represents a promising approach to pest management that not only reduces environmental impact but also supports long-term agricultural sustainability.

Novelty Statement

Our study directly supports the development of en-

vironmentally friendly pest management strategies suitable for organic agriculture, emphasizing the economic and ecological benefits of replacing synthetic chemicals with plant-derived alternatives.

Author's Contribution

Saima mehar: idea of this project and supervisor of BS thesis research work
Salma Javed: Done nematicidal activity and statistical analysis.
Rizwana Salam: Performed research work for BS thesis project
Gul mina: Performed research work for BS thesis project
Farah Mukhttar: All plant material is collected and provided.
Saleha Suleman Khan: critical view of this manuscript

Conflict of interest
The authors have declared no conflict of interest.

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