Research Article

The Isolation and Screening of the Bioactive Compound of *Viscum album* against *Meloidogyne incognita*

Saima Maher[1,3*], Shahina Fayyaz[2], Nadra Naheed[3] and Zarbakth Dar[1]

[1]Sardar Bahadur Khan Women University, Chemistry Department, Quetta, Balochistan, Pakistan; [2]University of Karachi, National Nematological Research Centre, Pakistan; [3]HEJ Research Institute of Chemical and Biological Sciences.

Abstract | The pure compounds of mistletoe (*Viscum album*) were evaluated in order to determine their nematicidal activity against *Meloidogyne incognita* (the root-knot nematode). The seven pure bioactive compounds have been isolated and examined, from which VA 4 and VA7 had a potential effect against *M. incognita* by showing 90% mortality at 0.1 g/mL after 48 hours of exposure. The LC_{50} 0.0149, 0.1575 µg/ml of (VA4 and VA7), respectively found to be significantly effective than compounds VA5 (LC_{50} = 0.1575 µg/ml), VA 6 (LC_{50} =0.1575 µg/ml), VA2 (LC_{50} = 0.6269µg/ml and VA3 (LC_{50} = 0.25) VA1(LC_{50} = 0.3969µg/ml). The findings indicated that the constituents of *V. album* have a natural potential to use as nematicides and introduction in eco-friendly management strategies.

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***Correspondence** | Saima Maher, Sardar Bahadur Khan Women University, Chemistry Department, Quetta, Balochistan, Pakistan; **Email:** saimamaher@yahoo.com
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Introduction

In view of the wide-ranging and economic aspects of *Meloidogyne incognita*, i.e. Kofoid and White Chitwood, over 28,000 species of nematode are identified. Almost 16,000 of them should, nonetheless, be parasitic (Liu *et al.*, 2013). Plant parasite nematodes are one of the lead devastator world-wide and responsible for yield losses of $ 125 billion equal to 12.3% perennial harvest per annum (Chitwood, 2003). Root-knot nematodes are largely controlled by continuous use of synthesized pesticides or soil fumigants (Nicol and Rivoal, 2007). However, the chemical nematicides are imparting the environmental troubles by effect on well beings.

Such as the chemical nematicides are ethylene dibromide (1, 2-Dibromoethane), DBCP (1, 2-Dibromo-3-chloropropane), and highly toxic aldicarb (2-Methyl-2-(methylthio) propanal O-(Nmethylcarbamoyl) oxime), have been banned from the world market (Anonymous, 2010), These methods have negative consequences for humans, the environment, and other beneficial organisms. There is a need for the development of alternative control methods that are both safe for humans and beneficial to crop production (Sultana *et al.*, 2010). Natural sources of nematode control, such as plants and plant products, are promising. Natural occurring compounds are inexpensive, simple to apply, and pose no pollution risks (Burow *et al.*, 2007) the axenic components of plant extracts were tested for nematicidal activity against root-knot nematodes (Faizi *et al.*, 2007; Samina *et al.*, 2020; Zareena *et al.*, 2020; Choudhary *et al.*, 2010). Galore flora infusion and biogenic lipoid have been tested for their nematicidal activity

against plant-parasitic nematodes (Hooper, 1986; Elbadri *et al*., 2008; Sabira *et al*., 2008). The *V. album* is widely distributed in the tropical and subtropical areas of the Indian subcontinent (Arndt, 2000). It is found as a hemi-parasite on the ligneous plant *Juglans regia* (Walnut) in Pakistan's Neelam valley. The biological activities have been reported such as immune stimulatory, antitumor, antiglycation activity (Arndt, 2000). It also used for the treatment of several diseases (Hooper, 1986). *V. album*'s bioactive potential crude have shown effective nemiticidal activity against *Meloidogyne incognita*.

The results of this research are based on the isolation of constituents from VA 1 to VA7 that had anti-nematicidal activity. The compounds VA4 and VA7 demonstrated that nematicidal components are highly competitive. Flavanone glychoside and phenyl-propoanoid were the major isolated components of *Viscum album*. Spectroscopic techniques such as H-NMR, C^{13}-NMR, UV, and IR were used to evaluate the composition of these isolated compounds.

Materials and Methods

Plant extracts

The *Viscum album* was collected from walnut (*Juglans regia*) trees. It is located in Neelum Valley, Azad Kashmir. The identification of flora takes place by Prof. Shafiq-ur-Rehman (A voucher specimen (No. Azbuherb 231) was deposited in the Herbarium of the University of Azad Jammu and Kashmir, Muzaffarabad.

Chemicals

The chemicals included all organic solvent, such as *n*-Hexane, $CHCl_3$, EtOAc, MeOH and BuOH, were analytical grade. Merck, Germany commercially provides these chemicals. Furadan 5G were purchased from registered pesticide shop, Karachi.

Nematode culture

The egg-masses were obtained by means of Sterilized forceps from the excessively infected tomato roots. The collected egg-masses were then washed by means of NaOCl and distilled water. They were then placed in 15 mesh sieves that are approximately 8 cm in diameters. They were incorporated with the crossed layers of tissue paper at 25 °C in order to extract the root-knot larvae (J_2). The larvae that were collected within 24 for the accomplishment of the nematicidal activity (Eisenback *et al*., 1981).

Nematicidal toxicity

The nematicidal assay was performed at 25°C in NNRC Laboratory, University of Karachi.

The methanol extract of *Viscum album* were diluted with 10 ml ethanol and then finally concentrated in 1 mg/ml with (10 ml) distilled water. Approximately 100 juveniles (J_2) were then transferred to vial to freshly prepared solution of the methanol extracts. Observation was taken after 30 minutes, 1hr, 2hrs, 3hrs, 4hrs, 24hrs and 48hrs. Number of dead nematodes was noted in order to calculate the mortality percentage of larvae. Control was kept in distilled H_2O.

Extraction and isolation of pure constituents of V. album

The approximately 1 kg of the floral material was dried and soaked in 80% methanol-water (3 × 3 L). At room temperature, the floral content was extracted over a period of three weeks. In order to get a brown gummy material, the solvent was evaporated under attenuated the pressure using a vacuum rotary evaporator.

This gummy material partitioned between methanol-water and hexane. Approximately 38 g residue was obtained on the evaporation of the hexane extracts. About (173 g) residues after evaporation of chloroform takes place by the the aqueous layer was re-partitioned with chloroform. Around 76 g of a crude gummy extract incur after phase transition of the solvent. Then the column chromatography was introduced that the crude extract was loaded on its silica gel and were then eluted with $CHCl_3$(1.2 g) and MeOH in a gradient mode. By using the solvent system $CHCl_3$ and MeOH (8%, 500 ml) yielded compounds VA5, VA6 (7 and 5 mg) and VA7 (6 mg). On silica gel, the fraction VA -2 was loaded. The fraction which is 60 mg was measured in order to interact with flash silica gel of solvent system 16% MeOH-$CHCl_3$ (500 ml) to yield 4 (8 mg) takes place in column chromatography.

The fraction VA 4 i.e. 30 mg and VA 5i.e.50 mg were conjunct and then subjected to polyamide column chromatography. On eluted with 100% $CHCl_3$ which was proceed to the fractional increment of polarity with MeOH and then were eluted to the seven main sub-fractions (VA 1-7). Amongst all of the fractions, subjected to silica gel column chromatography by using 20% MeOH in $CHCl_3$ (600 ml) as obtained

compounds VA 3 (12 mg) and VA 1, 2 (6, 5 mg).

Table 1: *Mortality (%) of Meloidogyne incognita against $CHCl_3$ compounds of Viscum album.*

Treatment	Dose (mg/mL)	½	1	2	3	4	24	48
Compound VA 1	1	0	0	0	10 a	10 a	20 a	40 a
	0.5	0	0	0	10 b	10 b	10 b	20 b
	0.25	0	0	0	10 c	20 b	20 c	50 c
	0.125	0	0	0	10 c	10 c	20 d	40 d
Compound VA2	1	0	0	0	10 a	20 a	10 a	40 a
	0.5	0	0	0	10 b	10 b	20 b	40 b
	0.25	0	0	0	10 c	10 c	20 c	40 b
	0.125	0	0	0	10 c	10 d	20 d	40 c
Compound VA3	1	0	0	0	20 a	20 a	20 a	60 a
	0.5	0	0	0	20 b	20 b	20 b	60 b
	0.25	0	0	0	10 c	20 c	20 c	50 c
	0.125	0	0	0	10 d	10 d	20 d	40 d
Compound VA4	1	0	0	0	20 a	30 a	40 a	90 a
	0.5	0	0	0	20 b	30b	40b	70 b
	0.25	0	0	0	20 c	30c	30 c	80 c
	0.125	0	0	0	20 d	30 d	20 d	70 d
Compound VA5	1	0	0	0	20 a	20 a	20 a	60 a
	0.5	0	0	0	20 b	20 b	20 b	60 b
	0.25	0	0	0	10 c	10 c	30 c	50 c
	0.125	0	0	0	10 d	10 d	30 d	50 d
Compound VA6	1	0	0	0	20 a	20 a	30 a	70 a
	0.5	0	0	0	20 b	20 b	30 b	70 a
	0.25	0	0	0	20 c	10 c	20 c	50 b
	0.125	0	0	0	20 d	10 d	10 d	40 c
Compound VA7	1	0	0	0	20 a	30 a	40 a	90 a
	0.5	0	0	0	20b	30 b	40ab	90 b
	0.25	0	0	0	20 c	20bc	30 c	80 c
	0.125	0	0	0	10 d	20bc	40 d	70 d
Furadan	1	0	0	0	0	50 a	100 a	-
	0.5	0	0	0	0	30 b	100 a	-
	0.25	0	0	0	0	0 c	100 a	-
	0.125	0	0	0	0	0 c	100 a	-
Control	1.0	0	0	0	0	0	0	0
	0.5	0	0	0	0	0	0	0
	0.25	0	0	0	0	0	0	0
	0.125	0	0	0	0	0	0	0

Same letters in column are not significantly different.

Bioassay-directed fractionation

The research was performed from the consequents of preliminary nematicidal assay of all crude extracts (hexane, ethyl acetate, chloroform and butanol) of *Viscum album* the crude extracts were directed through active fraction of the plant.

Statistical analysis

Completely randomized design was used for this experiment with three replicates using BioStat. Treatment difference was measured by means of Duncan's multiple range test (DMRT) ($P \leq 0.05$). The survival analysis is used for the Probit analysis under for LC_{50} values (Kleine, 2010).

Experimental material

^{13}C-NMR (100 MHz) the spectra were registered in CD_3OD solution on a Bruker. The 2D NMR spectra were recorded on 500 MHz NMR spectrometer. The 70 eV on a Finnigan MAT-112 or MAT-312 mass spectrometers (EI-MS) were registered. By using CD_3OD, the Chemical shifts are according in ppm (δ), The HPLC (JAI, LC-908W, Japan Analytical Industry Co. Ltd.) was utilized for terminal refinement with ODS H-80 or L-80 columns (YMC, Japan).

Results and Discussion

In vitro assay

The *Viscum album* crude extracts were evaluated for the nematicidal activity. The assorted crude extracts of *V. album* were assessed for parturition inhibition effects on *M. incognita* to deduce the convincing outcomes against the nematodes but before that it had been screened for nematicidal activity. The CHCl3 crude extract seems to have nematicidal activity. As, the fresh simple nematicides to control plant-parasitic nematodes i.e. the CHCl3 extracts (80% mortality) may have potential for devolution, the conclusion deduced it to be as. The CHCl3 floral extracts of *V. album* killed at least half of *M. incognita* juveniles (J2) (Table 2).

Table 2: *Median lethal concentrations (LC_{50}) and R^2 of $CHCl_3$ compounds from V. album against Meloidogyne incognita.*

Compounds	48h	LC_{50} (µg/ml) R^2	R^2
1	60	0.3969	0.57
2	60	0.6269	0.89
3	60	0.25	0.96
4	90	0.0149	0.89
5	60	0.1575	0.89
6	70	0.1575	0.89
7	90	0.0487	0.96
Carbofuran		72.3	

Its concentration is 0.1 mg/mL after 48 h post exposure. The compound VA 4 and VA 7 expose brawny nematicidal activity against the root-knot nematode with LC50 values of µg/ml the chloroform extract of *V. album* had a LC50 value of µg/mL.

While encoding with the positive control, carbofuran (LC50= 72.3 µg/ml), compound VA 4 showed strong nematicidal activeness and compound VA 7 had also present the assonant level of morbidity against the *M. incognita* (RKN) nematode. It is advisable that the activity of the crude chloroform extract of *V. album* against the root-knot nematode was mainly attributed to compound 4 and 7. The other five organic constituents, compound VA 2 (LC50=0.6269 µg/mL), compound VA 3 (LC50 = 0.25µg/mL) and compound VA 6 (LC50=0.1575µg/mL) compound VA 5 (LC50 = 0.1575µg/mL), and compound VA 1(LC50=0.3969µg/mL), have shown less effectiveness the *M. incognita* juvenile (J2). By considering the five isolated compounds, compound 3 showed the least effect against the (RKN) with at LC50 value of µg/ml (Table 2). The *V. album* was not reported so far regarding the nematicidal activity of their isolated compounds against nematodes. The results suggest that nematicidal activity of the CHC13 infusion of *V. album* and the sporadic compounds, markedly flavanone compound VA 4 and VA 7 are promising and they own potential for devolution as natural nematicide for the administration of nematodes by considering the presently used nematicides are synthetic and ordinarily have toxicity against nematodes. The two phenol derivative and one tritepene, the nematicidal action against the root-knot nematode as mentioned (Figure 1). This is due to the fact that the nematicidal diterpenoid, compound 3 was almost 17 times less active than compound VA 4. Furthermore, the existing phenolic group 17-hydroxy i.e. in (Figure 1) appear to boast nematicidal activity because compound VA 5 appears to display the stronger activity than compound VA 2 and compound 6 also has potential as stronger nematicidal activity than compound VA 3.

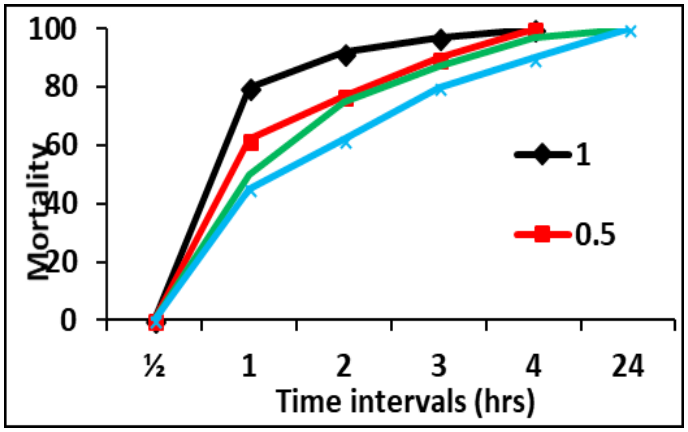

Figure 1: *Mortality (%) of Meloidogyne incognita against $CHCl_3$ extracts.*

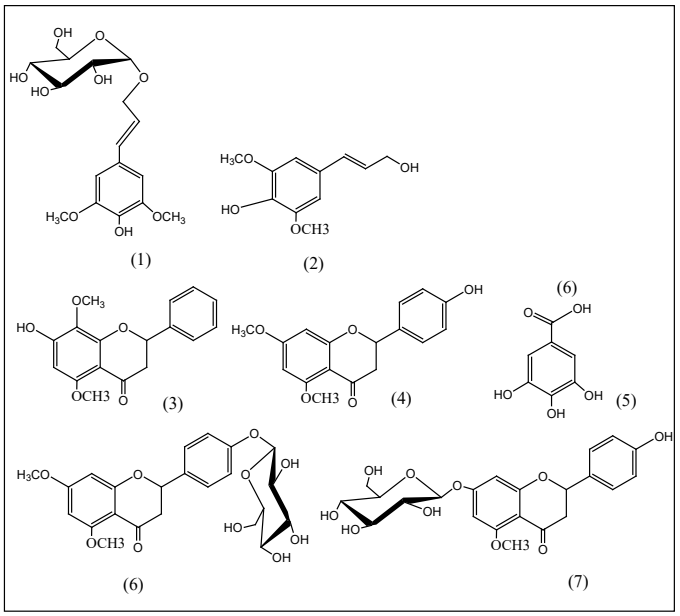

Figure 2: *Structure of pure isolated compounds of Viscum album. (1)-3-(4-hydroxy-3,5-dimethoxy)-phenyl-2E-propenylβ-D-glucopyranoside; (2) (7) 3-(4-hydroxy-3,5-dimethoxy)-phenyl-2Epropenol; (3) 5-dimethoxy-7-hydroxy 8-dimethoxy f lavanone; (4) 4,5-dimethoxy-4'-hydroxy flavanone; (5) Gallic acid; (6) 5, 7-dimethoxy-4-O-β-D-glucopyranoside flavanone; (7) 5-methoxy-7-O-β-D-glucopyranoside flavanone.*

The advance acquisition will be protracted to appraise the manner of activeness of the nematicidal generalization along with the practical challenge of their aggregation in phytonematode management application. Furthermore, the more investigation requisite on these isolated constituents for the agriculture aspects for the development and necessary formulations is required. In order to meliorate the efficiency and stability and regulatory value reduction.

Conclusions and Recommendations

The nematicidal activity against *M. incognita* has been determined for the *Viscum albums* extracts. The screening of the potential extract has shown effective nematicidal activity. All seven active organic constituents have different level of expression in comparison with standard carbofuran, chloroform crude extract. The compound VA 4 and VA 7 have exhibited the maximum toxicity against the *M. incognata* (RKN). The isolated compounds have potency for the novel nematicides for the criterion of the root-knot nematodes.

Our results suggest that these isolated compounds could be used as botanical nematicides for the control of RKN. Further, more research is required to determine the refine or pure compounds of this plant

which would be more capable and highly targeted.

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Novelty Statement

The pure compounds of mistletoe (Viscum album) were evaluated in order to determine their nematicidal activity against Meloidogyne incognita (the root-knot nematode). The seven pure bioactive compounds have been isolated and examined, from which VA 4 and VA7 had a potential effect against M. incognita by showing 90% mortality at 0.1 g/mL after 48 hours of exposure.

Author's Contribution

Conceptualization: Dr. Saima Mehar, formal nematicidal activity analysis: Dr. Shahina Fayyaz writing original drafts Zarbakth Dar, authors have read and agreed to the published version of the manuscript.

Conflict of interest

The authors have declared no conflicts of interest.

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