



The Antibacterial, Insecticidal and Nematocidal Activities and Toxicity Studies of *Tanacetum falconeri* Hook. f.

Tanacetum falconeri Hook. f.'nin Antibakteriyel, İnsektisidal ve Nematosidal Aktiviteleri ve Toksisite Çalışmaları

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ABSTRACT

Objectives: Secondary bioactive metabolites from plants may be realistic alternatives to conventional synthetic chemicals. The aim of the present research was to test the *Tanacetum falconeri* Hook. f. in different bioassays to evaluate its potential as nematocidal, insecticidal, antibacterial, cytotoxic, and phytotoxic agent. The plant *T. falconeri* was further studied for its chemical constituents.

Materials and Methods: The methanolic extract from *T. falconeri* was fractionated into various solvent fractions. All solvent fractions were further subjected to different bioassays. Antibacterial activity and cytotoxicity were determined by broth micro-dilution method and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay, respectively. Nematocidal activity was evaluated by using second-stage juvenile of *Meloidogyne incognita*. Insecticidal activity and phytotoxicity were measured with the help of *Rhyzopertha dominica* and *Tribolium castaneum* and fronds of *Lemna minor* L., respectively. Compound-1 was isolated by using liquid chromatography and its structure was determined by spectroscopic data as *cis*-dehydromatricaria ester.

Results: The excellent nematocidal activity (reduction in 100% motility) of the compound-1 against a root-knot nematode was obtained at 1% concentration after 72 h incubation time, whereas the reductions in motilities were 95% and 75% at concentrations 0.5% (w/v) and 0.125% (w/v), respectively. Similarly, the mortalities were 90% and 82% at 1% and 0.5% concentrations, respectively, after 24 h of the treatment. Compound-1 also exhibited the excellent insecticidal activity against *Sitophilus oryzae* at 1% concentration (providing 100% mortality) with effective dose 50 (EC₅₀) of 0.08 mg/L. The EC₅₀ of phosphine, which was used as standard, was 0.07 mg/L. Two fractions of *T. falconeri* showed a moderate cytotoxic activity against 3T3 cells lines at the concentration of 30 mg/mL with inhibitory concentration 50 values of 22.4 and 25.8 mg/L corresponding to TfP and TfM, respectively.

Conclusion: This study demonstrates that *cis*-dehydromatricaria ester-1 provides nematocidal activity (100% mortality) against the root-knot nematodes. Its insecticidal activity against *S. oryzae* may enable its use as marketable insecticide.

Key words: Pesticides, *Tanacetum falconeri*, secondary metabolites, root-knot nematodes, toxicity

ÖZ

Amaç: Bitkilerden elde edilen ikincil biyoaktif metabolitler, geleneksel sentetik kimyasallara gerçekçi alternatifler olabilir. Bu araştırmanın amacı farklı biyoyöntemlerle *Tanacetum falconeri* Hook. f.'nin nematosidal, insektisidal, antibakteriyel, sitotoksik ve fitotoksik ajan olarak potansiyelini test etmektir. *T. falconeri* bitkisi, kimyasal bileşenleri için daha ileri incelemelere de tabi tutulmuştur.

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Gereç ve Yöntemler: *T. falconeri*'den elde edilen metanolik ekstre çeşitli çözücü fraksiyonlarına bölünmüştür. Tüm çözücü fraksiyonları ayrıca farklı biyoyöntemlere tabi tutulmuştur. Antibakteriyel aktivite ve sitotoksosite, sırasıyla broth mikro seyreltme yöntemi ve 3-(4,5-dimetiltiazol-2-il)-2,5-difeniltetrazolyum bromür yöntemi ile belirlenmiştir. Nematosit aktivitesi, *Meloidogyne incognita*'nın ikinci evre yavrusu kullanılarak değerlendirilmiştir. İnsektisidal aktivitesi ve fitotoksitesisi, sırasıyla *Rhyzopertha dominica* ve *Tribolium castaneum* ve *Lemna minor* L.'nin yaprakları yardımıyla ölçülmüştür. Bileşik-1, sıvı kromatografi kullanılarak izole edilmiş ve yapısı cis-dehidromatricaria ester olarak spektroskopik verilerle belirlenmiştir.

Bulgular: Bileşik-1'in bir kök-düğüm nematoduna karşı mükemmel nematosit aktivitesi (%100 motilitede azalma), 72 saatlik inkübasyon süresinden sonra %1 konsantrasyonda elde edilirken, motilitelerdeki azalmalar %0,5 ve %0,125 (a/h) konsantrasyonlarda sırasıyla; ka %95 ve %75 olarak belirlenmiştir. Benzer şekilde, uygulamadan 24 saat sonra ölümler sırasıyla %1 ve %0,5 konsantrasyonlarda %90 ve %82 olarak belirlenmiştir. Bileşik-1 ayrıca, 0,08 mg/L'lik etkin doz 50 (EC₅₀) ile %1 konsantrasyonda (%100 ölüm sağlar). *Sitophilus oryzae*'ye karşı mükemmel insektisidal aktivite sergilemiştir. Standart olarak kullanılan fosfinin EC₅₀'si 0,07 mg/L olarak belirlenmiştir. *T. falconeri*'nin iki fraksiyonu, sırasıyla T1p ve T1m'ye karşılık gelen 22,4 ve 25,8 mg/L'lik inhibitör konsantrasyon 50 değerleri ile 30 mg/mL konsantrasyonda 3T3 hücre hatlarına karşı orta derecede sitotoksik aktivite göstermiştir.

Sonuç: Bu çalışma, cis-dehidromatricaria ester-1'in kök-düğüm nematodlarına karşı nematosit aktivite (%100 ölüm) sağladığını göstermektedir. *S. oryzae*'ye karşı insektisidal aktivitesi, pazarlanabilir insektisit olarak kullanılmasını sağlayabilir.

Anahtar kelimeler: Pestisitler, *Tanacetum falconeri*, sekonder metabolitler, kök-düğüm nematodları, toksisite

INTRODUCTION

Asteraceae family belongs to the flowering plants having 23,000 species of 12 sub-families and 1,620 genera.¹ The family is quite diverse group of vascular plants containing shrubs, trees, and vines widely grown in sub-tropical and lower temperature latitude regions.² Worldwide, this family is used in medicines, cosmetics, and pesticidal products.^{2,3} The genus *Tanacetum* commonly known as tansy belongs to the family *Asteraceae* and contains 200 species across the globe and abundantly found in Europe and Western Asia.^{4,5} These species contain several biologically active compounds that are extensively used in herbal medication and in cosmetics.⁶ *Tanacetum* species have vast medicinal importance and used to cure different diseases for many centuries. Many species of *Tanacetum* are used as an edible vegetable as well as medicinal plants. Different classes of secondary metabolites, including flavonoids, phenolic acids, sesquiterpene lactone, monoterpene, diterpene, glycosides, alkaloid, phytosterol, heterocyclic compounds, and polyacetylenes, have been reported from the different species of *Tanacetum*. Biologically, plants of genus *Tanacetum* have shown a variety of activities including insect anti-feedant and antimicrobial properties.⁷ In addition to this, different species of genus *Tanacetum* also have biological activities like antimicrobial, cytotoxicity, growth regulating, phytotoxic, anti-ulcer, anthelmintic, anti-fungal, and antioxidant activities.² *Tanacetum chiliophyllum* has shown a promising insecticidal activity against the stored granary products pest *S. granaries*.⁸ Similarly, compounds isolated from *T. chiliophyllum* have been reported in the literature to possess cytotoxic, antimicrobial activities, and acetylcholinesterase, butyrylcholinesterase inhibitory effects.⁹ Many sesquiterpene lactones and flavonoids as anti-inflammatory agents have been isolated from *Tanacetum sinaicum*.¹⁰ Pyrethroids are commercial insecticidal compounds isolated from *Chrysanthemum cinerariaefolium*.¹¹

In this paper, we report cis-dehydromatricaria ester (compound-1) for the first time from *T. falconeri*, which has been further tested for its nematocidal and insecticidal activities. In addition to this, different fractions of *T. falconeri* extract have

been screened for their potential nematocidal, insecticidal, antibacterial, cytotoxic, and phytotoxic activities.

MATERIALS AND METHODS

General experimental procedure

Analytical and laboratory grade of different chemicals including reagents and solvents were purchased from the trustworthy chemical companies (e.g., E. Merck, Fisher Scientific). For soaking and extraction, commercial grade of different solvents (methanol, *n*-hexane, ethyl acetate, and dichloromethane) were utilized. While chromatography and purification of the isolated compound was carried out by using distilled solvents. Silica gel was used as adsorbents for liquid column chromatography (E. Merck: 100-380 µm mesh). For determining samples purity pre-coated silica gel plates (GF 254, TLC) were used. Bruker AM-400 and AMX-500 and 125 MHz instrument was utilized for ¹H- and ¹³C-NMR (1D and 2D spectra), respectively. By using the reference solvent proton signal of CDCl₃, chemical shifts values were represented in ppm and coupling constant (J) were represented in Hz. To determine the exact mass of the pure compounds, at 70 eV Electron-impact mass spectra was noted by using Finnigan MAT-112 instrument.

Collection of plant material

The collection of plant *Tanacetum falconeri* Hook. f. (5.2 kg) was carried out in July 2017 from Astore (Daosai). The identification of the plant was done in Karakoram International University Gilgit Baltistan, by the taxonomist Dr. Sher Wali Khan. The specimen (voucher no. 145/17) has been stored and protected at the herbarium of the department of biological sciences, KIU for future reference.

Preparation of sample extracts

The air-dried aerial parts of *T. falconeri* Hook. f. (2.18 kg) was extracted three times (3×2 L) with 95% methanol at 20°C by soaking for three days each time. Following filtration, the combined methanol extracts were evaporated by using a rotary evaporator at 40°C to dryness, to obtain the *T. falconeri* whole

plant extract (TfP) (105 g, TfP). The combined and concentrated TfP was further dissolved in water (500 mL) and extracted with *n*-hexane (3×500 mL) first, and water was evaporated by using a rotary evaporator to get the concentrated MeOH extract [91 g, *T. falconeri* methanolic (TfM)]. The methanolic extract was further fractionated through the successive solvent-solvent extractions with ethyl acetate (3×300 mL), and *n*-butanol saturated with H₂O (3×250 mL) in a separatory funnel. Each extract, as well as its remaining aqueous phase (R-H₂O) after solvent extractions were evaporated to dryness under a reduced pressure to yield an *n*-hexane fraction [14 g, *T. falconeri* hexane (TfH)], EtOAc fraction [37 g, *T. falconeri* ethyl acetate (TfE)], *n*-BuOH fraction [13 g, *T. falconeri* butanolic (TfB)], and R-H₂O fraction [25 g, *T. falconeri* aqueous (TfA)], respectively.

Isolation of compound-1

The EtOAc extract (37 g) obtained from the methanolic extract of *Tanacetum falconeri* Hook. f. was subjected to silica gel column chromatography. At first, system was eluted with 100% *n*-hexane, and then by using respective solvent system of *n*-hexane: EtOAc (98:2, 95:5, 93:7, 90:10, 88:12, 85:15, 80:20, 70:30, 50:50); and finally washed with 100% ethyl acetate; and then with 50:50 ethyl acetate; and methanol (1L with each polarity). At the end 64 fractions (TF1-TF64) were obtained from the column. At gradient of *n*-hexane: Ethyl acetate (98:2) pure compound-1 was obtained.

Physical state: Colorless needle crystals. ESI-MS (+Ve) *m/z*: 173.0589[M+H]⁺. Molecular formula: C₁₁H₈O₂. ¹H-NMR: (CDCl₃, 600 MHz) δ ppm: 6.25 (1H, d, J=11.4 Hz, H-2), 6.14 (1H, d, J=11.4 Hz, H-3), 3.75 (3H, s, H-1'), 1.99 (3H, s, H-10). ¹³C-NMR: (CDCl₃, 150 MHz) δ ppm: 164.5 (C-1), 132.53 (C-2), 121.55 (C-3), 86.14, 80.82, 72.19, 70.97, 64.82, 58.39 (C-4-C-9, acetylenic carbons), 51.7 (C-1'), 4.80 (C-10).

Biological assays

All the extract fractions and pure compound-1 of *Tanacetum falconeri* were subjected to different biological assays including nematocidal, insecticidal, cytotoxic, antibacterial, and phytotoxic activities.

Nematocidal activity

The impact of six extracts and compound-1 of *T. falconeri* were used to study the larval mortality of root-knot nematode. Inhabitants of second-stage juvenile (J2) of *Meloidogyne incognita* was obtained from culture on tomato plants in microplot of a screen room. From the infected tomato plant, the egg masses were collected and placed in a cavity block with water. The cavity block was placed under conditions that promote the development of egg hatching at an ambient temperature for 72 h. In the next stage, 100 larvae were counted in a chamber for each dose and replicated thrice to introduce in 3×3 glass cavity block. The stock solution was prepared by using 10 mg/mL plant extracts in 5% dimethyl sulfoxide (DMSO). Three concentrations of 1%, 0.5%, and 0.125% were applied at a rate of 1 mL at each cavity block. Furadan was chosen as standard drug, while 5% DMSO as a control treatment. Stereoscopic microscope was used to observe the percentage death rate (mortality) after an

interval of 24, 48, and 72 h. Nematodes were considered dead when no movement was detected after a mechanical nudge. Then the nematode bodies were transferred into distilled water for conformation of irreversible mobility.

Insecticidal activity

By using the impregnated filter paper, *Rhyzopertha dominica* and *Tribolium castaneum* (insect species) were subjected to the methanolic extract and all solvent fractions.¹²⁻¹⁴ Stock solution was made by mixing the sample (200 mg) in methanol (3 mL). On Petri-plates with the help of micropipette samples (1019.10 µg/cm²), were applied to filter paper of size 9 cm or 90 mm. The solvent evaporated after 24 h. From each species, ten insects were set in each plate for test and control. For positive and negative control, 239.5 µg/cm² of Permethrin and methanol were used, respectively. After maintaining the ambient temperature and 50% humidity in growth chamber, the test plates were incubated in the chamber for 24 h. The next day, from each species the number of survivals was calculated, and percentage mortality (%M) was determined by applying the formula given below:

$$\%M = \frac{100 - \text{No. of insects alive in test}}{\text{No. of insects alive in control}} \times 100$$

Insecticidal activity of compound-1

In laboratory conditions, compound was diluted in 5% DMSO to acquire the different concentrations (1%, 0.5%, and 0.125%). Ten active adults of rice weevil were collected from rearing cage and commence in Petri dishes (90 mm diameter) bottomed with filter paper disk (whatman no. 1), applied 1 mL each compound and concentration separately. The Petri dishes were sealed by parafilm (PM-996) and kept at 28±2°C. Pesticide phosphine was chosen as a standard drug. Mortality was recorded after 24, 48, and 72 h of intervals with 5% DMSO as a control treatment. Each treatment was replicated three times.

Statistical analysis

To analyze the treatment differences multifactor analysis of variance was used. By using SPSS statistical software, the obtained data was further submitted to Duncans' multiple range test (p<0.05). Probit analysis was done under survival analysis for effective dose 50 (EC₅₀) values by SAS, 2000 software.

Antibacterial activity

With the help of broth microdilution method, minimum inhibition concentration was recorded. The required tests were carried out by application of *Tween-80*, and a final concentration of 0.5% (v/v) in Mueller Hinton Broth. For preparation of serial doubling dilutions of the extract 96-well microtiter plate (200-25 ppm) were used. A concentration of 10 µL of indicator solution and 10 µL of Mueller Hinton Broth were mixed. Then, 10 µL of bacterial suspension (10⁶ CFU/mL) was mixed to each well to attain a concentration of 10⁴ CFU/mL. Each plate was covered with cling film to avoid water loss. The plates were prepared in triplicates to calculate the average of three values. The sample plates were then set in an incubator for 24 h at 37°C. The lowest concentration was obtained by observing the color change with

naked eye. The growth of microorganism was indicated by turbidity.^{15,16}

Cytotoxicity (3-(4,5-dimethylthiazole-2-yl)-2,5-diphenyl-tetrazolium bromide) (MTT)

The cytotoxicity of different extracts of *T. falconeri* was studied by applying the standard MTT colorimetric assay on 96-well micro plates.¹⁷ In this process, mouse fibroblast cells (3T3) were cultured in Dulbecco's modified eagle medium and mixed with 5% of fetal bovine serum, 100 IU/mL of penicillin and 100 µg/mL of streptomycin in 75-cm² flasks. The flasks were placed in incubator at 37°C having 5% CO₂. Hemocytometer was used to calculate the growing cells, and the dilution of cells with medium was also done. Cell suspension (5×10⁴ cells/mL) was prepared and added (100 µL/well) into 96-well plates. After 12 h of incubation, the medium was removed and 200 µL of fresh medium containing different concentrations of samples (1-30 mg/L) was added. After 48 h, the medium was removed and 200 µL MTT (0.5 mg/mL) was added to each well after 48 h and was kept for further 4 h incubation. Later, 100 µL of DMSO was mixed to each well. The amount of MTT decrease to formazan within cells was obtained with the help of a micro plate reader by determining the absorbance at 540 nm. The cytotoxic activity was noted as inhibitory concentration 50 (IC₅₀) for 3T3 cell. At last, % inhibition of cells was determined by applying the formula which is given below:

$$\% \text{ inhibition} = \frac{100 - (\text{mean of O.D of test compound} - \text{mean of O.D of -ve control})}{(\text{mean of O.D of +ve control} - \text{mean of O.D of -ve control})} \times 100$$

Phytotoxicity

The crude methanolic and remaining solvent fractions of *T. falconeri* were subjected to the phytotoxicity assay.^{18,19} For this purpose, a medium was prepared at a pH of 6.0-7.0 by adding distilled water (1000 mL) to KOH pellets. The extract (30 mg) was mixed with methanol (1.5 mL) to a prepared stock solution. Three types of concentrations (10, 100, and 1000 µg/mL) were obtained after a dilution of stock solution of the extract. A total of nine flasks were obtained, among which three were prepared for each dilution. Under sterilized conditions, the solvent was kept overnight to evaporate the solvent. In next stage, to each flask, 20 mL medium and 10 plants were added, each one containing a rosette of two fronds of *Lemna minor* L. A flask with a medium used as a positive control and Paraquate (reference plant growth inhibitor) as a negative control. The sample flasks were placed in the growth cabinet of the incubator for one week at 30°C. After incubation, the number of fronds in each sample flask was measured, and by using the following formula, growth regulation as a percent regulation was calculated.

$$\% \text{ regulation} = \frac{100 - \text{No. of fronds in test}}{\text{No. of fronds in -ve control}} \times 100$$

RESULTS

From the EtOAc fraction of *T. falconeri*, *cis*-dehydromatricaria ester-1 has been obtained (Figure 1). Column chromatography

was used for the purification of the compound, and the structure was identified with the help of modern spectroscopic techniques. The structure of compound obtained from *T. falconeri* (EtOAc fraction) was confirmed as *cis*-dehydromatricaria ester by the analysis of spectral data of NMR (1D and 2D), whereas the molecular mass was obtained by using EI-MS. The compound's data (¹H-NMR and ¹³C-NMR) were compared and matched with the reported data in the literature.²⁰

In the current study, we evaluated the crude methanolic extract (95%) and different fractions of *T. falconeri* for bioassays like nematocidal, cytotoxic, insecticidal, and phytotoxic activities, whereas the methanolic extract and all the solvent fractions of *T. falconeri* were also analyzed for their nematocidal activities.

Nematocidal activity

The nematocidal activity of *T. falconeri* extracts on larval mortality of *Meloidogyne incognita* (root-knot nematode) was studied at various concentrations after 24, 48, 72 hours incubation. The nematocidal activities of TfA, TfB, TfE, TfH, TfM, and TfP of *T. falconeri* were more consistent at the concentration of 1%, 0.5%, and 0.125% with the total larval mortality rate of 50-68% after 72 h incubation time. The results presented in Table 1 indicate that all the fractions of *T. falconeri* showed moderate activities of 60%. The active fractions of *T. falconeri* were comparable to each other showing the activity against root-knot nematodes. We recommend *in vivo* testing of active extracts, which have been never reported yet to promote the green practices for sustainable agriculture and protection of the environment.

In addition to this, compound-1 was also tested for its nematocidal activity against root-knot nematode (*M. incognita*). Compound-1 showed excellent and exceptional activity of 100%, 95%, and 75% mortality at 1%, 0.5%, and 0.125% after 72 h of the treatment, respectively. Whereas the activity at 1%, 0.5%, and 0.125% after 48 h of treatment was recorded as 90%, 82%, and 37% mortality of root-knot nematodes (Table 1).

Insecticidal activity

The insecticidal effect of *T. falconeri* TfP and all the solvent fractions were studied against *R. dominica* and *S. oryzae*. The insecticidal activity of all six fractions of the extract of *T. falconeri* was carried out using 1019.10 µg/cm² with a reference to the standard drug permethrin (239.5 µg/cm²). The results revealed that activity of all fractions remain insignificant at all concentration. Therefore, the data results have not been

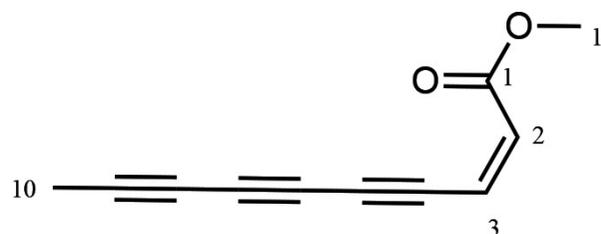


Figure 1. Structure of *cis*-dehydromatricaria ester (1) isolated from *T. falconeri*

presented in the tabular form. The *cis*-dehydrometracaria ester (1) isolated from *T. falconeri* showed the strong insecticidal activity against stored grain pest (*S. oryzae*). In general, the mortality rate was increased with an increase in the concentration of compounds and exposure time. Compound-1 showed 100% mortality at 1% concentration comparable to a standard pesticide (phosphine). The EC_{50} values of compound-1 against *S. oryzae* were 0.0897 mg/L at 1% concentration after 72 h of incubation (Table 2).

Cytotoxicity

The cytotoxicity of *T. falconeri* (extract and all fractions) was examined at concentrations of 30 mg/mL with a reference to the standard drug cyclohexamide. The extracts of *T. falconeri* (methanol and whole plant) showed low inhibition with IC_{50} values of 22.4 and 25.8 mg/L against the 3T3 normal cell

lines, respectively (Table 3). The rest of the extracts including *n*-hexane, ethyl acetate, *n*-butanol and aqueous fractions were found inactive in MTT assay with inhibition <20%. Therefore, the insignificant data results have been not presented in the tabular form.

Antibacterial activity

The antibacterial susceptibility of all six extracts TfA, TfB, TfE, TfH, TfM, and TfP of *T. falconeri* were tested against *E. coli*, *B. subtilis*, *Staphylococcus aureus*, *P. aeruginosa*, and *Salmonella typhi*. The bacterial isolates were revived on nutrient agar. The bacterial cultures were preserved at 28°C prior to use, and periodic sub-culturing was done to maintain these cultures. The antibacterial activity of all the extract of *T. falconeri* with a standard drug ofloxacin presented in Table 4. All extracts have low inhibitory activity against tested bacterial strains, whereas,

Table 1. Nematocidal activity of different fractions from *T. falconeri* against root-knot nematode

Sample	Time (h)	Concentration in % (w/v)			* EC_{50} (\pm SE)
		0.125*	0.5*	1*	
Compound-1	24	22 \pm 2.0c	35 \pm 2.5b	42 \pm 1.3a	3.4 \pm 0.6
	48	37 \pm 1.2c	82 \pm 1.0b	90 \pm 1.5a	0.18 \pm 0.04
	72	75 \pm 1.0c	95 \pm 1.4b	100 \pm 0.0a	0.04 \pm 0.1
TfA	24	10 \pm 1.0c	15 \pm 1.0b	25 \pm 0.5a	2.1 \pm 0.1
	48	45 \pm 1.5b	45 \pm 1.5b	50 \pm 2.0a	1.09 \pm 0.5
	72	55 \pm 1.1Cb	60 \pm 1.5Ba	60 \pm 0.5a	0.01 \pm 0.5
TfB	24	10 \pm 2.0c	10 \pm 1.0b	15 \pm 1.0a	4.5 \pm 0.2
	48	35 \pm 1.0a	35 \pm 1.1a	35 \pm 1.5a	4.5 \pm 0.2
	72	60 \pm 1.0b	60 \pm 1.1b	65 \pm 1.0a	4.5 \pm 0.2
TfE	24	15 \pm 1.5b	15 \pm 1.0b	25 \pm 1.1a	2.6 \pm 1.8
	48	40 \pm 1.0b	40 \pm 1.5b	45 \pm 2.0a	1.92 \pm 0.5
	72	62 \pm 1.0b	62 \pm 1.0b	68 \pm 1.5a	0.001 \pm 1.0
TfH	24	15 \pm 0.5bc	18 \pm 1.0b	20 \pm 1.0a	4.8 \pm 0.3
	48	30 \pm 1.0c	30 \pm 2.0b	35 \pm 1.5a	3.4 \pm 0.4
	72	60 \pm 1.0bc	62 \pm 0.2b	65 \pm 1.5a	2.9 \pm 0.8
TfM	24	12 \pm 1.0c	15 \pm 2.0b	18 \pm 1.0a	4.1 \pm 0.2
	48	32 \pm 1.0b	32 \pm 1.5b	35 \pm 1.0a	5.0 \pm 0.8
	72	60 \pm 1.0c	65 \pm 1.1b	68 \pm 0.5a	0.01 \pm 0.1
TfP	24	10 \pm 0.5b	10 \pm 0.5b	15 \pm 0.2a	4.5 \pm 0.2
	48	30 \pm 2.0b	30 \pm 2.0b	32 \pm 1.0a	4.5 \pm 0.6
	72	50 \pm 0.5a	50 \pm 2.0a	50 \pm 1.0a	4.5 \pm 0.6
Carbofuran (furadan)	24	40 \pm 1.5b	100 \pm 0.0a	100 \pm 0.0a	0.13 \pm 0.02
	48	80 \pm 2.5b	100 \pm 0.0a	100 \pm 0.0a	0.07 \pm 0.3
	72	100 \pm 0.0a	100 \pm 0.0a	100 \pm 0.0a	0.07 \pm 0.3

*Values are in mg/L. The concentrations of 1%, 0.5%, and 0.125% were prepared by dissolving extract in 5% DMSO (w/v). Means followed by the same letter are not significantly different according to Tukey's test ($p \leq 0.05$). TfA: *T. falconeri* aqueous, TfB: *T. falconeri* butanolic, TfE: *T. falconeri* ethyl acetate, TfH: *T. falconeri* hexane, TfM: *T. falconeri* methanolic, TfP: *T. falconeri* whole plant extract, EC_{50} : Effective dose 50, SE: Standard error, DMSO: Dimethyl sulfoxide

TfP was found inactive against *B. subtilis*, *P. aeruginosa*, and *Salmonella typhi*.

Phytotoxicity

The phytotoxic and insecticidal constituents are vital for the development of green herbicides and insecticides that are more eco-friendly than synthetic ones. The phytotoxic effect of the studied samples on *L. minor* was analyzed to have dose-dependent activity because low activity was noticed in TfP, TfM, TfE, TfB, and TfA fractions, with 12.5%, 0%, 20%, 26.56%, and 0% inhibition at 100 µg/mL, respectively, and 0% inhibition at 10 µg/mL for all, in comparison to the Paraquat (0.015 µg/mL) as a standard drug and control. Among the six fractions, *n*-hexane fraction had moderate and good phytotoxic activity (31.25%, 77.08% inhibition) at concentration at 10 and 100 µg/mL, respectively. A very significant phytotoxic effect (100.0% inhibition) was observed at concentration of 1000 µg/mL for all fractions except TfB (68.75%) and TfA (25%) fractions of *T. falconeri*.

DISCUSSION

Meloidogyne incognita belongs to the family of nematodes and commonly called as root-knot nematode. The root-knot nematode found worldwide damages the roots of plants and ultimately decreases both quality and quantity of the plant. The affected plants show relatively slow growth rate and poor performance. To control the population of nematodes usually synthetic nematocides are used but these conventional nematocides are more vulnerable to the non-targeted organisms and ecologies. Hence more risk for environmental pollution problems arises. In these circumstances the green nematicodes (botanical nematicodes) are the best substituent of conventional chemicals to control the nematodes. The present study discussed certain botanical nematocides against *M. incognita*. In the present study, antibacterial, nematocidal, insecticidal, cytotoxic, and phytotoxic activities of *T. falconeri* were evaluated with the help of standard assay protocols. Extraction method is a key factor in obtaining the maximum quantity of active formulations from

Table 2. Insecticidal activity compound-1 isolated from *T. falconeri* against rice weevils

Sample	Time (h)	Concentration in % (w/v)			*EC ₅₀ (± SE)
		0.125*	0.5*	1*	
Compound-1	24	30±1.5c	52±1.0b	62±1.0a	0.44±0.17
	48	45±1.0c	70±1.2b	80±2.5a	0.163±0.08
	72	62±1.5c	88±1.2b	100±0.0a	0.08±0.03
Phosphine	24	80±0.5c	90±1.0b	100±0.0a	0.05±0.3
	48	90±2.0b	100±0.0a	100±0.0a	0.07±0.3
	72	100±0.0a	100±0.0a	100±0.0a	0.07±0.3

*Values are in mg/L. The concentrations of 1%, 0.5%, and 0.125% were prepared by dissolving extract in 5% DMSO (w/v). Means followed by the same letter are not significantly different according to Tukey's test ($p \leq 0.05$). EC₅₀: Effective dose 50, SE: Standard error, DMSO: Dimethyl sulfoxide

Table 3. Cytotoxic activity of two fractions of *T. falconeri* against 3T3 cell lines

Extract	Concentration (mg/mL)	% Inhibition	*IC ₅₀ ± SD
TfP	30	55	22.4±2.8
TfM	30	67	25.8±2.5
Std: Cyclohexamide	30	70	8±0.2

*Values are in mg/L. TfM: *T. falconeri* methanolic, TfP: *T. falconeri* whole plant extract, IC₅₀: Inhibitory concentration 50, SD: Standard deviation, Std: Standard

Table 4. Antibacterial susceptibility of *T. falconeri* against standard bacteria

Bacteria	% Inhibition of different extracts (conc. 3000 µg/mL)						% Inhibition of drug
	TfP	TfM	TfH	TfE	TfB	TfA	Ofloxacin
<i>E. coli</i>	11.32	18.01	10.84	22.55	27.37	6.80	95.52
<i>B. subtilis</i>	0.00	1.23	16.37	17.65	22.42	29.57	95.19
<i>Staphylococcus aureus</i>	2.04	33.64	19.17	6.74	0.19	4.82	90.93
<i>P. aeruginosa</i>	0.00	2.08	5.95	15.29	5.05	2.35	90.99
<i>Salmonella typhi</i>	0.00	0.24	14.67	20.23	1.69	1.73	92.15

Means followed by the same letter are not significantly different according to Tukey's test ($p \leq 0.05$). TfA: *T. falconeri* aqueous, TfB: *T. falconeri* butanolic, TfE: *T. falconeri* ethyl acetate, TfH: *T. falconeri* hexane, TfM: *T. falconeri* methanolic, TfP: *T. falconeri* whole plant extract

target plant species. Methanol was used initially to ensure the maximum extraction of secondary metabolites from *T. falconeri* whereas, four different solvents with the variable degrees of polarity along with water were used in this study to divide the main extract into separate fractions, based on ingredients polarity. The methanolic and ethyl acetate fractions were more efficient than solvents with both lower polarity (hexane) and higher polarity (water). These results suggest that nematocidal compounds probably possess an intermediate degree of polarity or that *T. falconeri* contains several nematocidal compounds with different degrees of polarity.

Although the sensitivity of plant parasitic nematodes to nematocides varies among different extractives in general, in present study, the results are closely related to each other except the results for the methanolic extract. The results strongly support the profound ethnobotanical application of *Tanacetum* species and *cis*-dehydrometracaria ester (1). These results also demonstrate its potential for use in botanical pest control strategies.²⁰ Control treatment fails to kill nematode while standard control carbofuran gave 100% mortality after 72 h of incubation with EC_{50} value 0.07 ± 0.3 mg/L, but it is toxic and extremely lethal to the mammals and the wildlife. Whereas in human beings, it causes reproductive disorders, genotoxic abnormalities, and endocrine disrupting activity.²¹ Carbofuran (Furadan[®]) is used to control broad spectrum of insect is highly soluble white crystalline solid chemical and possess low adsorption properties in soil.²² The results of extractives and compound-1 showed an excellent activity and may be used as safe alternatives to replace the hazardous chemical products in the market. We recommend *in vivo* testing of active extracts, which have never been reported yet to promote the green practices for sustainable agriculture and the protection of the environment. In line with nematocidal activities, the insecticidal activity of the compound-1 was found to be exceptional, which is consistent with the literature data. It has been reported that *cis*-dehydrometracaria ester isolated from *Artemisia ordosica* was tested against *Tribolium castaneum* at different concentrations (62.91, 12.58, 2.52, and 0.5 nL/cm²). The pest repellent value has been reported higher than 90% at both 2 and 4 h incubation time.²⁰ Phosphine pesticide has led to the selection of strong resistance against the major stored grain pest, including *Sitophilus oryzae*, *Cryptolestes ferrugineus*, *Rhyzopertha dominica*, *Tribolium castaneum*, and *Liposcelis bostrychophila*. It causes lethargy in humans which has been referred to as narcosis or anesthesia in animals.²³ The promotion and development of green practices is in utmost need for the sustainable agriculture and the protection of the environment. The methanolic extract and different fractions of *T. falconeri* were found inactive against cytotoxicity, antibacterial, and phytotoxicity assays. This indicates the safe and effective nature of formulations from *T. falconeri* to the plants and animals for its sustainable application in agriculture, as well as health and well-being.

In summary, the results of the present study indicate that medium polar extracts in general and compound-1 of *T. falconeri* potentially be developed into a commercial nematocide.

Although plant-based materials and extractives can be used in sustainable and organic farming, but isolation and identification of the nematocidal compounds is essential for further development of commercial products. Similarly, synthesis of active compounds or their derivatives with a higher nematocidal activity are likely to be a more promising means of developing a nematocide based on *T. falconeri* or related plant species.

Study limitations

Compound-1 could not have been tested for antibacterial, cytotoxic, and phytotoxic activities due to the limited quantity in hand. The nematocidal activity have been carried out on *Meloidogyne incognita* only whereas insecticidal activity against *Rhyzopertha dominica* and *Tribolium castaneum*. The results of *T. falconeri* extracts and compound-1 are limited to the microorganisms stated in this study.

CONCLUSION

The investigation on green pesticides from plant origin is essentially vital for the progress of new botanical pesticides, especially in view of the vast worldwide flora. In summary, the current study revealed the ability of the different fractions of *T. falconeri* for their potential as cytotoxic, antibacterial, phytotoxic, nematocidal, and insecticidal agent. The results indicated that in development of plant-based green pesticides these plant species are a new potential source. These plants species may provide best alternative paths for controlling the different pests than synthetic pesticides with no worse impacts on ecology. Hence, detailed studies are required to find and investigate the functioning compounds, and their path of mechanism of action of these plant formulations, to introduce more safe products to the market, and substitute some of the present toxic chemicals already available and in practice. Opportunities exist to reduce chemical inputs to the environment provided by exploring the potential of pesticides based on plant products.

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