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

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

Short Title:
Biological activities of *Tanacetum falconeri*

***Tanacetum falconeri*'nin biyolojik aktiviteleri**

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ABSTRACT
Objectives: Secondary metabolites from plants can offer realistic alternatives to conventional synthetic chemicals when used as part of active principals. The aim of present research was to test the *T. falconeri* Hook. f. against 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 solvents fractions were further subjected to different bioassays. Antibacterial activity and cytotoxicity were determined by broth micro dilution method and MTT assay, respectively. Whereas nematocidal activity was assayed by using second-stage juvenile (J2) of *M. incognita,* while insecticidal, and phytotoxicity were measured with the help of *Rhyzopertha dominica* and *Tribolium castaneum,* and fronds of *Lemna minor* L.,

respectively. Compounds **1** was isolated by using liquid chromatography and its structure was deduced by spectroscopic data as *cis*-dehydromatricaria ester.
Results: The excellent nematocidal activity of 100% motility of compound **1** against root-knot nematode was obtained at 1% concentration after 72h incubation time whereas, it was 95 and 75% at the conc. of 0.5 and 0.125%. Similarly, the mortality was 90 and 82% at 1 and 0.5% conc. (w/v), respectively after 24 h of the treatment. Compound **1** also exhibited the excellent insecticide activity against *S. oryzae* at 1% concentration (100% mortality) with EC_{50} of 0.08 mg/L in comparison to phosphine used as standard (0.07 mg/L). Two fractions of *T. falconeri* showed moderate cytotoxic activity against 3T3 cells lines at the concentration of 30 mg/mL with IC_{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** with potent mortality (100%) against root-knot nematodes and *S. oryzae* can be use as prototype to produce a marketable product in the end.
Keywords: Pesticides, *Tanacetum falconeri*, secondary metabolites, root-knot nematodes, toxicity
ÖZET
Amaç: Bitkilerden elde edilen biyoaktif sekonder metabolitler, sentetik kimyasallara alternatif olarak kullanılabilirler. Bu araştırmada, *T. falconeri* Hook. f. türünün nematocidal, insektisidal, antibakteriyel, sitotoksik ve fitotoksik ajan olarak kullanılabilirliğinin ortaya konulması için biyoaktivite testlerinin yapılması amaçlandı. Ayrıca, *T. falconeri* türünün kimyasal bileşenleri de çalışıldı.
Gereç ve Yöntemler: *T. falconeri'nin* metanol ekstresi farklı polaritedeki çözücüler kullanılarak fraksiyonlandırıldı ve fraksiyonların da biyoaktiviteleri incelendi. Antibakteriyel aktivite broth mikro dilüsyon yöntemiyle ve sitotoksik aktivite ise MTT metodu kullanılarak test edildi. Buna ilaveten, nematocidal aktivite, *M. incognita'*nın ikinci aşama juvenil'i (J2) kullanılarak, insektisidal ve fitotoksisite aktiviteleri ise sırasıyla *Rhyzopertha dominica* ve *Tribolium castaneum* ve *Lemna minor* L. yaprakları yardımıyla ölçüldü. Bileşik **1** sıvı kromatografiyle izole edildi ve kimyasal yapısı spektroskopi verileri kullanılarak *cis*-dehidromatricaria ester olarak aydınlatıldı.
Bulgular: Bileşik **1**'in kök-düğüm nematoduna karşı %100 mortalite (ölüm oranı) gösteren en yüksek nematocidal aktivitesi, %1 konsantrasyonda 72 saatlik inkübasyondan sonra elde edildi. Bileşik **1** %0,5 ve %0,125 derişimlerde ise %95 ve %75 mortalitede nemotocidal aktivite gösterdi. Benzer şekilde, mortalite, 24 saatlik inkübasyondan sonra sırasıyla %1 ve %0,5 konsantrasyonda sırasıyla %90 ve %82 oranlarında izlendi. Ayrıca standart olarak kullanılan fosfine (EC_{50}: 0,07 mg/mL) ile karşılaştırıldığında, bileşik **1** (EC_{50}: 0,08 mg/L) %1 konsantrasyonda *S. oryzae*'yı %100 öldürerek mükemmel bir insektisit aktivitesi gösterdi. *T. falconeri* türünün TfP (IC_{50}: 22,4 mg/L) ve TfM (IC_{50}: 25,8 mg/L) kodlu fraksiyonları 30 mg/mL'lik konsantrasyonda 3T3 hücre hatlarına karşı orta düzeyde sitotoksik aktivite gösterdi.
Sonuç: Bu çalışma, kök-düğüm nematodlarına ve *S. oryzae'ye* karşı güçlü mortaliteye (%100) sahip *cis*-dehydromatricaria ester'in (**1**) bileşiğinin etkili bir ürün üretmek için prototip olarak kullanılabileceğini göstermektedir.
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 subfamilies and 1620 genera[1]. The family is quite diverse group of vascular plants containing shrubs, trees and vines widely grown in sub-tropical and lower temperature latitudes regions[2]. Worldwide this family is widely used in medicines, cosmetic and pesticidal products[2,3]. The

genus *Tanacetum* commonly known as tansy belongs to the Asteraceae and contain 200 species across the globe and abundantly found in Europe and Western Asia[4,5]. These species contain a number of 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 different species of *Tanacetum*. Biologically, plants of genus *Tanacetum* have shown a variety of activities including insect antifeedant and antimicrobial properties[7]. In addition to this, different species of genus *Tanacetum* also have biological activities like antimicrobial, cytotoxicity, growth regulating, phytotoxic, antiulcer, anthelmintic, antifungal 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 *Tanacetum chiliophyllum* have been reported in 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 reported *cis*-dehydrometricaria ester (compound **1**) for the first time from *T. falconeri* whereas, it has been further tested for it 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 trustworthy chemical companies e.g., E. Merck, Fisher Scientific. For soaking and extraction commercial grade of different solvents (methanol, *n*-hexane, ethyl acetate, dichloromethane and butanol) 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 determination of samples purity pre-coated silica gel plates (GF 254, TLC) were used. Bruker AM-400 and AMX-500 MHz instrument was utilized for ^{1}H-and ^{13}C NMR (1D and 2D spectra). By using reference solvent proton signal of $CDCl_3$, 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. as a whole (5.2 Kg*)* was carried out in July 2017 from Astore (Daosai). The identification of plant was done in Karakoram International University Gilgit Baltistan, through 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 x 2 L) with 95% methanol at 20 °C by soaking for 3 days each time. Following filtration, the combined methanol extracts were evaporated by using rotary evaporator at 40 °C to dryness to obtain whole plant extract (105g, TfP). The combined and concentrated whole plant extract was further dissolved in water (500 mL) and extracted with *n*-hexane (3 x 500 mL) first and water was evaporated by using rotary evaporator to get the concentrated MeOH extract (91g,

TfM). The methanolic extract was further fractionated through successive solvent-solvent extractions with ethyl acetate (3 x 300 mL), and *n*-butanol saturated with H_2O (3 x 250 mL) in a separatory funnel. Each extract, as well as its remaining aqueous phase (R-H_2O) after solvent extractions were evaporated to dryness under reduced pressure to yield an *n*-hexane fraction (14g, TfH), EtOAc fraction (37g, TfE), *n*-BuOH fraction (13g, TfB), and R-H_2O fraction (25g, TfA), respectively.

*Isolation of compound **1***

The EtOAc extract (37g) 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. E1-MS m/z: 172.1. Molecular formula: $C_{11}H_8O_2$. ^{1}H-NMR: ($CDCL_3$, 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). ^{13}C NMR: ($CDCL_3$, 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 6 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 cavity block with water. The cavity block was placed under conditions that promote the development of egg hatching at ambient temperature for 72h. In 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% 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 observed percentage death rate (mortality) after an interval of 24, 48, and 72h. Nematodes were considered dead when no movement was detected after mechanical nudge. Then the nematodes bodies were transferred into distilled water for conformation of irreversible mobility.

Insecticidal activity

By using 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 sample (200 mg) in methanol (3 mL). On petri plates with the help of micropipette samples (1019.10 µg/cm^2) were applied to filter paper of size 9 cm or 90 mm. The solvent was evaporated after 24h break. From each species 10 insects were set in each plate for test and control. For positive and negative control 239.5 µg/cm^2 of Permethrin and methanol were used respectively. After maintaining ambient temperature and 50% humidity in growth chamber the test plates were incubated in the chamber for 24h. The next day, from each species the number of survivals were calculated, and percentage mortality *(%M*) was determined by applying the formula:

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

*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 compounds and concentration separately. The Petri dishes were sealed by parafilm (PM-996) and kept at 28 ± 2 °C. Pesticide phosphine was chosen as standard drug. Mortality was recorded after 24, 48 and 72h 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 ($P \leq 0.05$). Probit analysis was done under survival analysis for EC_{50} values by SAS, 2000 software.

Antibacterial activity

With the help of broth microdilution method minimum inhibition concentration (MIC) was recoded. 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 to 25 ppm) were used. A concentration of 10 µL of indicator solution and 10 µL of Mueller Hinton Broth were mixed to each well. 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 3 values. The sample plates were then set-in incubator for 24h at 37 °C. The lowest concentration was obtained by observing the color change with necked eye. The growth of microorganism was indicated by turbidity[15,16].

Cytotoxicity (MTT)

The cytotoxicity of different extracts of *T. falconeri* was studied by applying the standard MTT (3-(4,5-dimethylthiazole-2-yl)-2,5-diphenyl-tetrazolium bromide) 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 (FBS), 100 IU/mL of penicillin and 100 µg/mL of streptomycin in 75 cm^2 flasks. The flasks were placed in incubator at 37 °C having 5% CO_2. Hemocytometer was used to calculate the growing cells and the dilution of cells with medium was also done. Cell suspension ($5x10^4$cells/mL) was prepared and added (100 µL/well) into 96-well plates. Afterwards 12h incubation, medium was removed and 200 µL of fresh medium containing different concentrations of samples (1-30 mg/L) was added. After 48h medium was removed and 200 µL MTT (0.5 mg/mL) was added to each well after 48h and was kept for further 4h incubation. Later, 100µL of DMSO was mixed to each well. The amount of MTT decrease to formazan within cells was obtained with the help micro plate reader by determining the absorbance at 540 nm. The cytotoxic activity was noted as IC_{50} for 3T3 cell. At last, % inhibition of cells was determined by applying the formula:

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

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 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 prepared stock solution. Three type of concentrations (10, 100, and 1000 µg/mL) were obtained after dilution of stock solution of the extract. A total of nine flasks were obtained, among them, three flasks 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 medium used as a positive control and Paraquate (reference plant growth inhibitor) as negative control. The sample flasks were placed in growth cabinet of the incubator for one week at 30 °C. After incubation the number of fronds in each sample flasks were measured and by using formula growth regulation (GR) as a percent regulation was calculated.

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

RESULTS

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

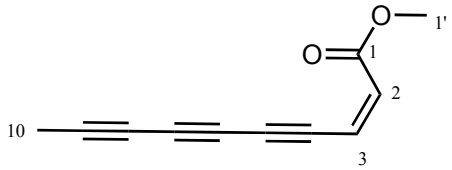

Figure: Structure of *cis*-dehydrometicaria ester (**1**) isolated from *T. falconeri*.

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 aqueous (TfA), butanolic *(*TfB), ethyl acetate (TfE), hexane (TfH), methanolic (TfM), and whole plant (TfP) of *T.* falconeri were more consistent at the conc. of 1, 0.5 and 0.125% with total larval mortality rate of 50-68% after 72h 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 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 72h of treatment, respectively. Whereas the activity at 1, 0.5 and 0.125% after 48h of treatment was recorded as 90, 82 and 37% mortality of root-knot nematodes (Table 1).

Insecticidal activity

The insecticidal effect of *T. falconeri* whole plant extract and all solvent fractions were studied against *R. dominica* and *S. oryzae*. The insecticidal activity of all 6 fractions of the extract of *T. falconeri* was carried out by using 1019.10 µg/cm^2 with reference to standard drug permethrin (239.5 µg/cm^2). The results reveal that activity of all fractions remain insignificant at all concentration. Therefore, the data results have been not 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, mortality rate was

increased with increasing the concentration of compounds and exposure time. Compounds **1** showed 100% mortality at 1% conc. comparable to standard pesticide (phosphine). The Ec_{50} values of compound **1** against *S. oryzae* was 0.0897 mg/L at 1% conc. after 72h of incubation (Table 2).

Cytotoxicity

The cytotoxicity of *T. falconeri* (extract and all fractions) were examined at concentrations of 30 mg/mL, with reference to 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 bellow 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*, *Staphylococus aureus*, *P. aeruginosa,* and *Salamonella typhi*. The bacterial isolates were revived on nutrient agar (NA). 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 standard drug ofloxacine presented in Table 4. All extracts have low inhibitory activity against tested bacterial strains, whereas, TfP was found inactive against *B. subtilis*, *P. aeruginosa*, and *Salamonella 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 0 at 10 and 12.5, 0, 20, 26.56, 0% inhibition at 100 µg/mL, respectively in comparison to Paraquat (0.015 µg/mL) as a standard drug and control. Among the six fractions, *n*-hexane fraction has 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 family of nematodes and commonly called root-knot nematode. The root-knot nematode found worldwide, and it damage the roots of plants and ultimately decrease both quality and quantity of 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 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 target plant species. Methanol was used initially to ensure maximum extraction of secondary metabolites from *T. falconeri* whereas, four different solvents with variable degrees of polarity along with water were used in this study to divide the main extract into separate fractions on the bases of 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 *T. falconeri* contains several nematocidal compounds with different degrees of polarity.

The sensitivity of plant parasitic nematodes to nematocides although varies among different extractives in general, but 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 72h of incubation with Ec_{50} value 0.07±0.3 mg/L but it is one of the toxic and extremely lethal to the mammals and wildlife. Whereas in human it causes reproductive disorders, genotoxic abnormalities, endocrine disrupting activity[21]. Carbofuran (Furadan) used for controlling 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 excellent activities and can be used as safe alternatives and to replace the hazardous chemical products in the market. We recommend *In-Vivo* testing of active extracts, which have been never reported yet to promote the green practices for sustainable agriculture and the protection of the environment. In line with the nematocidal activities, the insecticidal activity of the compound **1** found to be exceptional where the results are 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/cm^2), the pest repellent value has been reported higher than 90% at both 2 and 4h 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 narcosis or anesthesia in animals[23]. The promotion and development of green practices is utmost need for the sustainable agriculture and the protection of 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 wellbeing.

In summary, the results of the present study indicate that medium polar extracts in general and compound **1** of *T. falconeri* in particular 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 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 considered to be a new potential source. These plants species may provide best alternative paths for controlling

different pests than synthetic pesticides with no worse impacts on ecology. Hence, a detail 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 in 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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REFERENCES

1. Pareek A, Suthar M, Rathore G, Bansal V. Feverfew (*Tanacetum parthenium* L.): A systematic review. Pharmacogn Rev. 2011;5:103-110.
2. Kumar V, Tyagi D. Chemical composition and biological activities of essential oils of Genus *Tanacetum* - a review. J Pharmacogn Phytochem. 2013;2:155-159.
3. Gao T, Yao H, Song J, Zhu Y, Liu C, Chen S. Evaluating the feasibility of using candidate DNA barcodes in discriminating species of the large Asteraceae family. BMC Evol Biol. 2010;10:324.
4. Ismail M, Hussain F, Ali S. Botanical pesticide and insects repellent prepared from *Tanacetum baltistanicum* endemic to Gilgit-Baltistan. J Biodivers Environ Sci. 2014;5:128-135.
5. [Orhan IE, Tosun F, Gülpinar AR, Kartal M, Duran A, Mihoglugil F, Akalgan D. LC-MS quantification of parthenolide and cholinesterase inhibitory potential of selected *Tanacetum* L. (Emend. Briq.) taxa. Phytochem Lett. 2015;11:347-352.
6. Salamci E, Kordali S, Kotan R, Cakir A, Kaya Y. Chemical compositions, antimicrobial and herbicidal effects of essential oils isolated from Turkish *Tanacetum aucheranum* and *Tanacetum chiliophyllum* var. *chiliophyllum*. Biochem Syst Ecol. 2007;35:569-581.
7. Bukhari IA, Khan RA, Gilani AUH, Shah AJ, Hussaun J, Ahmad VU. The analgesic, anti-inflammatory and calcium antagonist potential of *Tanacetum artemisioides*. Arch Pharm Res. 2007;30:303–312.
8. Polatoğlu K, Karakoç ÖC, Gökçe A, Goren N. Insecticidal activity of *Tanacetum chiliophyllum* (Fisch. & Mey.) var. monocephalum grierson extracts and a new sesquiterpene lactone. Phytochem Lett. 2011;4:432–435.
9. Polatoğlu K, Yücel YY, Nalbantsoy A, Yalcin HT, Goren N. Cytotoxic, antimicrobial activities, AChE and BChE inhibitory effects of compounds from *Tanacetum chiliophyllum* (Fisch. & Mey.) Schultz Bip. var. *oligocephalum* (D.C.) Sosn. and *T. chiliophyllum* (Fisch. & Mey.) Schultz Bip. var. *monocephalum* Grierson. Phytochem Lett. 2017;22:199-204.
10. Hegazy MEF, Hamed AR, Mohamed TA, Debbab A, Nakamura S, Mutsuda H, Pare PW. Anti-inflammatory sesquiterpenes from the medicinal herb *Tanacetum sinaicum*. RSC Adv. 2015;5:44895-44901.
11. Kumar A, Singh SP, Bhakuni RS. Secondary metabolites of Chrysanthemum genus and their biological activities. Curr. Sci. 2005;89:1489-1501.
12. Atta-ur-Rahman, Choudhary MI, Thomson WJ. Bioassay techniques for drug development. The Netherlands: Harwood Academic Publishers; 2001; p. 8-100.
13. Hagstrum DW, Phillips TW, Cuperus G. Stored Product Protection. USA: Kansas State University; 2012.
14. Green PWC, Belmain SR, Ndakidemi PA, Farrel IW, Stevenson PC. Insecticidal

activity of *Tithonia diversifolia* and *Vernonia amygdalina*. Ind Crops Prod. 2017;110:15-21.
15. Pettit RK, Weber CA, Kean MJ, Hoffmann H, Pettit GR, Tan R, Franks KS, Hotrton ML. Microplate alamar blue assay for *Staphylococcus epidermidis* biofilm susceptibility testing. Antimicrob Agents Chemother. 2005;49:2612-2617.
16. Sarker SD, Nahar L, Kumarasamy Y. Microtitre plate-based antibacterial assay incorporating resazurin as an indicator of cell growth, and its application in the in vitro antibacterial screening of phytochemicals. Methods. 2007;42:321-324.
17. Mosmann T. Rapid colorimetric assay for cellular growth and survival: Application to proliferation and cytotoxicity assays. J Immunol Methods. 1983;65:65-63.
18. Ayaz F, Küçükboyaci N, Duman H, Sener B, Choudhary MI. Cytotoxic, phytotoxic and insecticidal activities of *Chrysophthalmum montanum* (DC.) Boiss. Turkish J Pharm Sci. 2017;14:290–293.
19. Itokawa H, Oshida Y, Ikuta A, Inatomi H, Adachi T. Phenolic plant growth inhibitors from the flowers of *Cucurbita pepo*. Phytochemistry. 1982;21:1935-1937.
20. Zhang Z, Guo S-S, Zhang W-J, Geng Z-F, Lian J-Y, Du S-S, Wang CF, Deng Z-W. Essential oil and polyacetylenes from *Artemisia ordosica* and their bioactivities against *Tribolium castaneum* Herbst (Coleoptera: Tenebrionidae). Ind Crops Prod. 2017;100:132–137.
21. Mishra S, Zhang W, Lin Z, Pang S, Huang Y, Bhatt P, Chen S. Carbofuran toxicity and its microbial degradation in contaminated environments. Chemosphere. 2020;259:127419.
22. Rahman MA, Parvin A, Khan MSH, War AR, Lingaraju K, Prasad R, Das S, Hussain B, Bhattacharyya A. Efficacy of the green synthesized nickel-oxide nanoparticles against pulse beetle, *Callosobruchus maculatus* (F.) in black gram (*Vigna mungo* L.). Int J Pest Manag. 2020; https://doi.org/10.1080/09670874.2020.1773572
23. Alzahrani SM, Ebert PR. Oxygen and arsenite synergize phosphine toxicity by distinct mechanisms. Toxicol Sci. 2019;167:419-425.

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

Sample	Time (h)	Concentration in % (w/v)			*EC_{50} (± SE)
		0.125*	**0.5***	**1***	
Compound **1**	24	22±2.0c	35±2.5b	42±1.3a	3.4 ±06
	48	37±1.2c	82±1.0b	90±1.5a	0.18±0.04
	72	75±1.0c	95±1.4b	100±0.0a	0.04±0.1
TfA	24	10± 1.0c	15 ± 1.0b	25 ± 0.5a	2.1±0.1
	48	45 ± 1.5b	45 ± 1.5b	50 ± 2.0 a	1.09±0.5
	72	55 ± 1.1Cb	60 ± 1.5Ba	60 ± 0.5a	0.01±0.5
TfB	24	10 ± 2.0c	10 ± 1.0b	15 ± 1.0a	4.5±0.2
	48	35 ± 1.0a	35 ± 1.1a	35 ± 1.5a	4.5±0.2
	72	60 ± 1.0b	60 ± 1.1b	65 ± 1.0a	4.5±0.2
TfE	24	15 ± 1.5b	15 ± 1.0b	25 ± 1.1a	2.6±1.8
	48	40 ± 1.0b	40 ± 1.5b	45 ± 2.0a	1.92±0.5
	72	62 ± 1.0b	62 ± 1.0b	68 ± 1.5a	0.001±1.0
TfH	24	15 ± 0.5bc	18 ± 1.0b	20 ± 1.0a	4.8±0.3
	48	30 ± 1.0c	30 ± 2.0b	35 ± 1.5a	3.4±0.4
	72	60 ± 1.0bc	62 ± 0.2b	65 ± 1.5a	2.9±0.8
TfM	24	12 ± 1.0c	15± 2.0b	18 ± 1.0a	4.1±0.2
	48	32 ± 1.0b	32 ± 1.5b	35 ± 1.0a	5.0±0.8
	72	60 ± 1.0c	65 ± 1.1b	68 ±0.5a	0.01±0.1
TfP	24	10 ± 0.5b	10 ± 0.5b	15± 0.2a	4.5±0.2
	48	30 ± 2.0b	30 ± 2.0b	32 ± 1.0a	4.5±0.6
	72	50 ± 0.5a	50 ± 2.0a	50 ± 1.0a	4.5±0.6
Carbofuran (Furadan)	24	40±1.5b	100±0.0a	100±0.0a	0.13±0.02
	48	80±2.5b	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).

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

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 ($P \leq 0.05$).

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

Extract	Conc. (mg/mL)	% Inhibition	*IC_{50} ± SD
TfP	30	55	22.4 ± 2.8
TfM	30	67	25.8 ± 2.5
Std: cyclohexamide	30	70	0.8 ± 0.2

*Values are in mg/L
Abbreviations of extracts from *T. falconeri*: TfP: whole plant, TfM: methanolic.

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	Ofloxacine
E. coli	11.32	18.01	10.84	22.55	27.37	6.80	95.52
B. subtilis	0.00	1.23	16.37	17.65	22.42	29.57	95.19
Staphylococus	2.04	33.64	19.17	6.74	0.19	4.82	90.93
P. aeruginosa	0.00	2.08	5.95	15.29	5.05	2.35	90.99
Salamonella typhi	0.00	0.24	14.67	20.23	1.69	1.73	92.15

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

Figure:

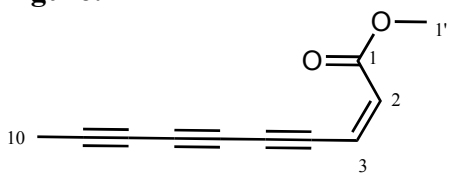

Figure Legend:

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