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ORIGINAL ARTICLE

Phytotoxicity, Toxicity on Brine Shrimp and Insecticidal Effect of *Chrysophthalmum gueneri* Aytac & Anderb. Growing in Turkey

Türkiye'de Yetişen *Chrysophthalmum gueneri* Aytac & Anderb.'in Fitotoksisitesi, Tuzlu Su Karidesi Üzerine Toksisitesi ve İnsektisidal Etkisi

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ABSTRACT

Objectives: The aim of this study was to investigate the probable toxicity on brine shrimp, phytotoxicity, and insecticidal activity of *Chrysophthalmum gueneri* Aytac & Anderb.

Materials and Methods: The MeOH (80%) extract obtained from the whole plant of *C. gueneri* was fractionated through subsequent solvent extractions in increasing polarity with *n*-hexane, chloroform, and *n*-butanol. The MeOH (80%) extract and all fractions of *C. gueneri* were evaluated for their biologic activities using *in vitro* screening bioassays such as brine shrimp lethality test and phytotoxicity against *Lemna minor*, as well as insecticidal activity against *Rhyzopertha dominica* and *Tribolium castaneum*.

Results: The findings showed that the *n*-hexane and chloroform fractions of the plant had significant phytotoxic activities with 100% growth inhibition (GI) at 1000 µg/mL against *L. minor*. Moreover, the MeOH (80%) extract (53% GI) and *n*-butanol fraction (46.6% GI) of the plant had moderate phytotoxic activities at 1000 µg/mL. Otherwise, no samples had toxicity on the brine shrimps. In addition, the remaining water fraction had low insecticidal activity with 20% mortality against *T. castaneum*.

Conclusion: Our results show that the *n*-hexane and chloroform fractions of *C. gueneri* had potential phytotoxic effects.

Key words: *Chrysophthalmum gueneri*, Asteraceae, brine shrimp lethality, phytotoxicity, insecticidal activity

ÖZ

Amaç: Bu çalışmanın amacı, *Chrysophthalmum gueneri* Aytac & Anderb.'in tuzlu su karidesi üzerine olası toksisite, fitotoksisite ve insektisidal aktivitesinin incelenmesidir.

Gereç ve Yöntemler: *C. gueneri*'nin tüm bitki kısımlarından hazırlanan metanollü (%80) ekstresi artan polaritede *n*-hekzan, kloroform ve *n*-butanol ile art arda fraksiyonlanmıştır. *C. gueneri*'nin metanollü (%80) ekstresi ve tüm fraksiyonları tuzlu su karidesi letalite testi, *Lemna minor*'e karşı fitotoksisite ile *Rhyzopertha dominica* ve *Tribolium castaneum*'a karşı insektisidal aktiviteleri olmak üzere *in vitro* tarama testleri kullanılarak biyolojik aktiviteleri bakımından incelenmiştir.

Bulgular: Çalışmamızın bulguları bitkinin *n*-hekzan ve kloroform fraksiyonlarının 1000 µg/mL'de %100 büyüme inhibisyonu (Bİ) ile *L. minor*'e karşı önemli fitotoksik aktiviteye sahip olduğunu göstermiştir. Bunun yanı sıra, bitkinin metanol (%80) ekstresi (%53 Bİ) ve *n*-butanol fraksiyonu (%46.6 Bİ) 1000 µg/mL'de orta derecede fitotoksik aktiviteye sahip bulunmuştur. Diğer taraftan, tüm örnekler tuzlu su karidesi üzerinde toksisiteye sahip bulunmamıştır. Ayrıca kalan sulu fraksiyonu %20 mortalite ile *T. castaneum*'a karşı düşük insektisidal aktivite göstermiştir.

Sonuç: Sonuçlarımız *C. gueneri*'nin *n*-hekzan ve kloroform fraksiyonlarının potansiyel fitotoksik etkiye sahip olduğunu ortaya koymuştur.

Anahtar kelimeler: *Chrysophthalmum gueneri*, Asteraceae, tuzlu su karidesi letalitesi, fitotoksisite, insektisidal aktivite

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INTRODUCTION

Medicinal plants that contain various constituents are the most important sources for developing candidates of new drugs and therapeutic agents. Turkey has a rich, still unexplored medicinal flora. Traditional medicines have been used for the treatment of diseases to develope new biological agents from natural sources. Throughout the study of medicinal plants, finding bioactive components prior to structural elucidation from plant extracts, it is necessary to evaluate their biological activity. For this reason, several bench-top assays such as the brine shrimp lethality test, phytotoxicity, and insecticidal effect can be used as major prescreening assays.[1-4]

The genus *Chrysophthalmum* Schultz Bip., which belongs to the family Asteraceae, tribe Inulaeae, is represented by four species around the world.[5] In Turkey, the genus *Chrysophthalmum* has three species, namely *Chrysophthalmum montanum* (DC.) Boiss., *Chrysophthalmum dichotomum* Boiss. & Heldr. and *Chrysophthalmum gueneri* Aytac & Anderb. growing in Turkey.[6] Among them, *C. gueneri* is an endemic herbaceous plant with linear-lanceolate leaves and slender peduncles that grows around Cirlasun bridge, Alanya, Turkey.[7] To date, no phytochemical data has been reported on *C. gueneri*.

In our ongoing investigations on the genus *Chrysophthalmum*, the cytotoxic activity of *C. gueneri* was tested for the first time against some cancer cell lines using a sulforhodamine B assay.[8] In our previous studies on preliminary screening bioassays such as toxic effect on brine shrimp, phytotoxic and insecticidal activities, the *n*-hexane and chloroform fractions of two species, *C. montanum* and *C. dichotomum*, were found as promising plant sources due to having phytotoxicity and toxicity on brine shrimps.[9,10] Following our studies on *C. gueneri*, we now aimed to evaluate *in vitro* phytotoxicity and toxicity on brine shrimps and the insecticidal effect of the plant.

EXPERIMENTAL

Plant material

The whole plants of *C. gueneri* Aytac & Anderb. were collected from wet places among pine forest around Cirlasun bridge, Antalya, Turkey, at the flowering stage in August 2014. The plant was identified by one of our authors Barış Bani PhD, (Kastamonu University). Voucher specimen (F.A. 46) was deposited at the Herbarium of Gazi University (GAZİ), Ankara, Turkey.

Preparation of extracts

The air-dried whole plants of *C. gueneri* (780 g) were extracted four times (4x4000 mL) with 80% methanol at 25°C by stirring for 2 days. After filtration, the combined methanol extracts were evaporated *in vacuo* at 40°C to dryness. The concentrated MeOH extract (140.0 g, CG) were further fractionated by successive solvent extractions with *n*-hexane (15x250 mL), chloroform (10x250 mL), and *n*-butanol saturated with H_2O (9x250 mL) in a separator funnel. Each extract and remaining water phase (R-H_2O) were evaporated to dryness under reduced pressure to yield "*n*-hexane fraction" (5.6 g, CGH), "$CHCl_3$ fraction" (13.6 g, CGC), "*n*-BuOH fraction" (25.4 g, CGB) and "R-H_2O fraction" (67.0 g, CGR), respectively.

Brine shrimp lethality assay

In this assay, we investigated the toxicity of the test samples on *Artemia saline* (Leach) shrimp larvae. Brine shrimp eggs (50 mg) were sprinkled in a rectangular hatching tank (22x32 cm) half-filled with filtered brine solution. The methanol (80%) extract and subsequent solvent fractions of *C. gueneri* (20 mg) were dissolved in 2 mL of methanol. 10, 100, and 1000 µg/mL concentrations were prepared in three vials from stock solution. The solvent was evaporated overnight. After hatching (2 days), 30 shrimps were added in each vial with a volume adjusted to 5 mL using sea water. Under illumination, the vials were incubated at 25-27°C for 24 h. Other vials were supplemented with reference cytotoxic drug (etoposide: 7.46 µg/mL), and solvent, which served as positive and negative controls, respectively. The survived brine shrimps were counted macroscopically using a magnifying glass against a lit background in each vial and LD_{50} values with 95% confidence intervals were determined using Finney computer software.[11,12]

Pytotoxicity assay

The phytotoxicity assay was conducted for the methanol (80%) extract and subsequent solvent fractions of *C. gueneri* against *Lemna minor* L.[13] The medium was prepared by mixing various inorganic components in 1000 mL distilled water. KOH pellets were added for the adjustment of pH at 6.0-7.0. The extracts (30.0 mg) were dissolved in 1.5 mL of methanol (stock solution). The stock solutions of the extracts were diluted to obtain final concentrations as 10, 100 and 1000 µg/mL (nine flasks, three for each dilution). After evaporating the solvent overnight under sterile conditions, 20 mL medium and 10 plants were added to each flask, each one containing a rosette of two fronds of *L. minor*. Other flasks were supplemented with medium and reference plant growth inhibitor, Paraquate, as negative and positive controls, respectively. All flasks were incubated in a growth cabinet for seven days at 30°C. At the end of the incubation period, the number of fronds per flasks was counted and recorded. The growth regulation (GR) in percentage (%) was determined using the formula given below:

$$GR\ (\%) = \frac{100 - \text{Number of the fronds in the test samples}}{\text{Number of the fronds in the negative control}} \times 100$$

According to the criteria, the GR (%) means low activity in 0-39%, moderate activity in 40-59%, good activity in 60-69%, and significant activity in >70%.

Insecticidal activity

The methanol (80%) extract and subsequent solvent fractions of *C. gueneri* were tested against *Rhyzopertha dominica* and *Tribolium castaneum* using the impregnated filter paper method.[14] To prepare the stock solution, the samples (200 mg) were dissolved in 3 mL of methanol. The samples were applied to filter paper (1019.10 µg/cm^2) of appropriate size (9 cm or 90 mm) on Petri dishes using micropipette. The plates were left for 24 h to evaporate the solvent. The next day, 10 insects of each

species were placed in each plate (test and control) using a clean brush. Permethrin (239.5 µg/cm^2) was used as a positive control; methanol was used as a negative control. The plates were incubated at 27°C for 24 h with 50% relative humidity in the growth chamber.

For the calculation, the number of survivals of each species was counted and mortality (M) (%) was determined using the following formula:

$$M\ (\%) = \frac{100 - \text{Number of insects alive in the test samples}}{\text{Number of insects alive in the control}} \times 100$$

Ethics committee approval was not necessary for this study.

RESULTS AND DISCUSSION

In this study, we examined the methanol (80%) extract and the fractions of *C. gueneri* for their lethality, phytotoxicity, and insecticidal activity with primary screening bioassays. The brine shrimp lethality test on *C. gueneri* was investigated at concentrations of 10, 100 and 1000 µg/mL, using etoposide as a standard drug. All fractions and the methanol extract had no toxicity against the brine shrimps (Table 1).

The phytotoxicity assay is a useful primary screen for weedicide research. It is also observed that natural antitumor compounds can inhibit *Lemna* growth.[13] *C. gueneri* showed variable effects in terms of phytotoxicity against *L. minor.* It was found that the tested samples had dose dependent activity. The *n*-hexane and chloroform fractions of the plant showed significant phytotoxic activities with 100% growth inhibition (GI) at 1000 µg/mL. Moreover, the MeOH extract and *n*-butanol fraction had moderate phytotoxic activities with 53% and 46.6% of GI at 1000 µg/mL, respectively. In addition, low phytotoxicity was found in the remaining water fraction with 31.2% of GI at 1000 µg/mL, followed by the chloroform (21% GI), *n*-hexane (15.4% GI), *n*-butanol (13.3% GI), and methanol (80%) extract (6.2% GI) of the plant at 100 µg/mL. There was no phytotoxicity in any tested samples at 10 µg/mL (Table 2).

The methanol extract and fractions of *C. gueneri* were also screened for their insecticidal effects against *R. dominica* and *T. castaneum* using permethrin as a standard drug. The remaining water fraction had low insecticidal activity with 20% of mortality against *T. castaneum* (Table 3).

Table 1. Toxicity of the extract and fractions of *Chrysophthalmum gueneri*

Samples	No of survivors from 30 shrimps			LD_{50} (µg/mL)
	10 µg/mL	100 µg/mL	1000 µg/mL	
CG	27	27	26	-
CGH	19	15	15	464.2454
CGC	27	25	18	3695.8640
CGB	28	27	24	-
CGR	28	22	23	-

Standard drug: etoposide (LD_{50} =7.46 µg/mL),

Table 2. Phytotoxic activity of the extract and fractions of *Chrysophthalmum gueneri*

Samples	Growth regulation (%)		
	10 µg/mL	100 µg/mL	1000 µg/mL
CG	0	6.2	53.0
CGH	0	15.4	100.0
CGC	0	21.0	100.0
CGB	0	13.3	46.6
CGR	0	0	31.2

Standard drug: paraquate (0.015 µg/mL)

Table 3. Insecticidal activity of the extract and fractions of *Chrysophthalmum gueneri*

Samples (1019.10 µg/cm^2)	*Tribolium castaneum*		*Rhyzopertha dominica*	
	Mortality (%)	Insecticidal activity	Mortality (%)	Insecticidal activity
CG	0	No	0	No
CGH	0	No	0	No
CGC	0	No	0	No
CGB	0	No	0	No
CGR	20	Low	0	No

Reference insecticide: permethrin (239.5 µg/cm^2)

The present paper is the first to present data to show that *C. gueneri* exhibits a variety of phytotoxic and insecticidal biologic activities. According to our results, the *n*-hexane and chloroform fractions of the plant were found as promising samples because they had significant phytoxicity on *L. minor*. In our recent study, *n*-hexane and chloroform fractions of the plant also exhibited cytotoxicity on selected cancer cell lines.[8] Our results also showed that the *n*-hexane and chloroform fractions of *C. gueneri* contained bioactive constituents and these fractions could lead to the discovery of important agents.

CONCLUSIONS

In conclusion, according to conventional herbicides and pesticides, *C. gueneri* could be considered a potential source for developing natural constituents possessing weedicide and insecticide activities with less risk to human health and the environment. Therefore, further investigations are merited in order to identify the responsible bioactive compound(s) in *C. gueneri*.

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Conflict of Interest: No conflict of interest was declared by the authors.

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