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

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

Fatma Ayaz[1], Nurgün Küçükboyacı[1], Barış Bani[2], Bilge Şener[1], Muhammad Iqbal Choudhary[3]

[1]Department of Pharmacognosy, Faculty of Pharmacy, Gazi University, 06330 Ankara, Turkey
[2]Department of Biology, Faculty of Arts And Science, Kastamonu University, 37200 Kastamonu, Turkey
[3]International Center for Chemical and Biological Sciences, University of Karachi, 75270 Karachi, Pakistan

ABSTRACT

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

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 biological acitivities 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 research 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 have moderate phytotoxic activities at 1000 µg/ml. Otherwise, all samples had no toxicity on the brine shrimps. In addition, the remaining water fraction had low insecticidal activity with 20 % of mortality against Tribolium castaneum.

DISCUSSION AND CONCLUSION: Our results exerted that the n-hexane and chloroform fractions of C. gueneri had potential phytotoxic effects.

Keywords: Chrysophthalmum gueneri, Asteraceae, brine shrimp lethality, phytotoxicity, insecticidal activity

INTRODUCTION

Medicinal plants which contain various constituents are the most important sources for developing the candidates of new drugs and therapeutic agents. Our country has a rich still unexplored medicinal flora. Traditional medicines have the economic resource for the treatment of the diseases. 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 brine shrimp lethality test, phytotoxicity and insecticidal effect can be used as major prescreening assays.[1-4]

The genus *Chrysophthalmum* Schultz Bip. belonging to family Asteraceae, tribe Inulaeae, is represented by four species all over the world.[5] In Turkey, the genus *Chrysophthalmum* has three species, namely *C. montanum* (DC.) Boiss., *C. dichotomum* Boiss. & Heldr. and *C. 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] Up 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 against some cancer cell lines by Sulforhodamine B assay for the first time.[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*, have been 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, toxicity on brine shrimps and insecticidal effect of the plant.

EXPERIMENTAL

Plant material

The whole plants of *Chrysophthalmum gueneri* Aytac and Anderb. were collected from wet places among pine forest around of 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. Ayaz 46) was deposited at Herbarium of Gazi University (GAZI), Ankara, Turkey.

Preparation of extracts

The air-dried whole plants of *C. gueneri* (780 g) were extracted four times (4×4000 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 (15×250 mL), chloroform (10×250 mL) and *n*-butanol saturated with H_2O (9×250 mL) in a separatory 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 toxicity of test samples on *Artemia saline* (Leach) shrimp larvae. Brine shrimp eggs (50 mg) were sprinkled in a rectangular dish (22x32 cm, a hatching tank) 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 by keeping over night. 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 ºC 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 lighted background in each vial and LD_{50} values with 95% confidence intervals were determined with Finney computer software.[11,12]

Pytotoxicity assay

The phytotoxicity assay was carried out 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 get 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 growth cabinet for seven days at 30 ºC. At the end of incubation period, the number of fronds per flasks were counted and recorded. The growth regulation (GR) in percentage (%) was determined by calculated with the formula given below:

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

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

Insecticidal activity

The methanol (80%) extract and subsequent solvent fractions of *C. gueneri* were tested against *Rhyzopertha dominica* and *Tribolium castaneum* by impregnated filter paper method.[14] To prepare 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 plates 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 positive control; methanol was used as negative control. The plates were incubated at 27 ºC 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:

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

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).

Table 1. Toxicity of the extract and fractions of *C. 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)

The phytotoxicity assay is a useful primary screen for weedicides search. It is also observed that natural antitumor compounds can inhibit the lemna growth.[13] *C. gueneri* showed variable effects in terms of phytotoxicity against *L. minor.* It was found that the tested samples have 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 is no phytotoxicity on all tested samples at 10 µg/mL (Table 2).

Table 2. Phytotoxic activity of the extract and fractions of *C. 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)

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

Table 3. Insecticidal activity of the extract and fractions of *C. 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 data presented in the present paper firstly depicted that *C. gueneri* exhibited a variety of biological activities, such as phytotoxic and insecticidal. According to our results, especially *n*-hexane and chloroform fractions of the plant were found as promising samples due to having 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 presented results also showed that the *n*-hexane and chloroform fractions of *C. gueneri* contained bioactive constituents and these fractions can lead to the discovery of important agents.

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 fewer risks to human health and the enviroment. Therefore, further investigations in order to identify the responsible bioactive compound(s) are going on *C. gueneri*.

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