Original Article

Phytotoxic, Cytotoxic and Insecticidal Activities of *Chrysophthalmum dichotomum* Boiss. and Heldr.

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ABSTRACT

Background: *Chrysophthalmum dichotomum* Boiss. and Heldr. (Asteraceae) is an endemic herbaceous plant to Southern part of Turkey. **Aim:** To investigate *in vitro* phytotoxic, cytotoxic and insecticidal activities of *C. dichotomum*. **Methods:** The MeOH extract of *C. dichotomum* was fractionated through subsequent solvent extractions in increasing polarity with *n*-hexane, chloroform and *n*-butanol. The MeOH extract and its fractions were evaluated for their biological acitivities using *in vitro* screening bioassays such as cytotoxicity on brine shrimps, phytotoxicity against *Lemna minor* and insecticidal activity against *Rhyzopertha dominica* and *Tribolium castaneum*. **Results:** The *n*-hexane and chloroform fractions showed significant phytotoxic activity (100 % growth inhibition) at 1000 µg/ml against *L. minor*. The brine shrimp lethality test revealed that the chloroform and remaining water fractions of *C. dichotomum* have moderate and positive lethality with LD_{50} values of 169.48 and 46.26 µg/mL, respectively. In addition, the chloroform and *n*-butanol fractions had low and moderate insecticidal activity with 20 and 40 % of mortality against *Tribolium castaneum*, respectively. **Conclusion:** This study demonstrates that *C. dichotomum* consists of bioactive constituents responsible for phytotoxicity, cytotoxicity on brine shrimps and insecticidal activity.

Key words: *Chrysophthalmum dichotomum*, Asteraceae, Phytotoxicity against *Lemna minor*, Cytotoxicity on brine shrimps, Insecticidal activity.

INTRODUCTION

The genus *Chrysophthalmum* Schultz Bip. (Asteraceae, tribus: Inulaeae) and consists of four species all over the world. In Turkey, the genus is represented by three species, namely *C. montanum* (DC.) Boiss., *C. dichotomum* Boiss. and Heldr. and *C. gueneri* Aytac and Anderb. *C. dichotomum* is an endemic herbaceous plant that grows in wooded or shrubby valley beds in the district of Antalya, Turkey.[1] Up to date, no phytochemical and biological data has been reported on the plant. In our previous study, we firstly evaluated the cytotoxicity of *C. dichotomum* against selected cancer cell lines by Sulforhodamine B (SRB) assay.[2]

As a part of our continuing researches on the genus *Chrysophthalmum*, we recently evaluated the crude methanol extract and its fractions of *C. montanum* for their *in vitro* phytotoxic, cytotoxic and insecticidal activities. In that study, the crude extract, *n*-hexane and chloroform fractions have positive lethality on brine shrimp as well as *n*-hexane and chloroform fractions had significant phytotoxicity (100% of growth inhibition) at 1000 µg/mL.[3] In other our previous study, we found that chloroform extract of *C. montanum* and its isolated sesquiterpene lactones have significant cytotoxic activities on different cancer cell lines using SRB assay.[4] Family Asteraceae is characterized by structurally diverse sesquiterpenes and, therefore it has been extensively studied in point of cytotoxic and anticancer activities. Sesquiterpenes have also been reported to use as toxic or insect-feeding deterrent.[5-6]

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Our ongoing researches on the genus *Chrysophthalmum*, we now aimed to investigate *in vitro* phytotoxic, cytotoxic and insecticidal activities of *C. dichotomum*.

MATERIALS AND METHODS

Plant material

Chrysophthalmum. dichotomum were collected from damp places in opening of *Pinus nigra* forest around Tınaztepe cave, in Konya 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. dichotomum* (150 g) were extracted four times (4×1500 mL) with 80% methanol at 25°C by stirring for 2 days. Following filtration, the combined methanol extracts were evaporated *in vacuo* at 40°C to dryness. The concentrated MeOH extract (25.8 g for CD) were further fractionated by successive solvent extractions with *n*-hexane (11×200 mL), chloroform (8×200 mL) and *n*-butanol saturated with H_2O (7×200 mL) in a separatory funnel. Each extract and remaining water phase (R-H_2O) after solvent extractions were evaporated to dryness under reduced pressure to yield "*n*-Hexane fraction" (1.1 g CDH), "$CHCl_3$ fraction" (2.6 g for CDC), "*n*-BuOH fraction" (7.9 g for CDB) and "R-H_2O fraction" (11.6 g CDR), respectively.

Phytochemical analysis

The $CHCl_3$ extract of *C. gueneri* (1 mg/mL) was applied to silica gel plate. After developing with the mixture of chloroform: acetone (80:20) as mobile phases, TLC plate was evaluated under UV light at 254 and 366 nm for the determination of fluorescent compounds. Anisaldehyde reagent was sprayed on the plates to visualize the separated compounds and then plates were heated for 5 min at 100°C. Sesquiterpenes appeared with pink and purple coloration.

Phytotoxicity against *Lemna minor*

The *Lemna minor* L. phytotoxicity assay was performed for the extracts of *C. dichotomum* by adopting the protocol as described by Atta-ur-Rahman.[7] The medium was prepared by mixing various constituents in 1000 mL distilled water and pH was adjusted 6.0-7.0 by adding KOH pellets. 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. The solvent was allowed to evaporate overnight under sterile condition. To each flask was then added 20 mL medium and 10 plants, 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 kept in growth cabinet for 7 days. The number of fronds per flasks was counted and recorded at the end of the incubation period. The growth regulation as a percentage (%) was determined using the formula given below:

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

The activity criteria indicate that the growth regulation (%) of 0-39% for low activity, 40-59% for moderate activity, 60-69% for good activity and >70% for significant activity were detected.

Cytotoxicity on brine shrimps

Brine shrimp (*Artemia saline* Leach) eggs (50 mg) were sprinkled in a hatching tank (a rectangular dish 22x32 cm) half-filled with filtered brine solution. The extracts (20 mg) were dissolved in 2 mL of methanol (stock solution). The concentrations of 10, 100 and 1000 µg/mL of each extracts from stock solutions were prepared in three vials and the solvent was evaporated by keeping over night. 2 days of hatching later, 30 shrimps were added in each vial with the volume adjusted to 5 ml using sea water. The vials were incubated at 25-27°C for 24 h under illumination. Other vials were supplemented with solvent, and reference cytotoxic drug (Etoposide: 7.4625 µg/ml) which served as – ve and + ve controls, respectively. The number of brine shrimps that survived was counted in each vial and LD_{50} values with 95% confidence intervals were determined using Finney computer software.[8-9]

Insecticidal activity

The extracts of *C. dichotomum* were tested against *Rhyzopertha dominica* and *Tribolium castaneum* using impregnated filter paper method.[10] The extracts (200 mg) were dissolved in 3 mL of methanol (stock solution). The extracts 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 permethrin (239.5 µg/cm^2) was used as + ve control; methanol was used as – ve control] using a clean brush. 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 (%) was determined using the following formula:

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

RESULTS

In our study, we investigated the methanol extract and its fractions of *C. dichotomum* for their *in vitro* phytotoxic, cytotoxic and insecticidal activities. The phytotoxicity of the tested samples on *L. minor* was observed to have dose dependent activity, because low activity was detected in the chloroform and *n*-butanol fractions with 19.0 and 10.6% inhibition at 100 µg/mL, respectively, as well as in remaining water fraction with 37.5% inhibition at 1000 µg/mL. Moderate phytotoxic activity was found in the *n*-butanol fraction (42.6% inhibition) at 1000 µg/mL. Significant phytotoxic activity was shown in the *n*-hexane and chloroform fractions of the plant; 100.0% inhibition for each fraction at 1000 µg/mL (Table 1).

The cytotoxic properties of the extract and fractions of *C. dichotomum* were investigated at a concentration of 10, 100 and 1000 µg/mL, using etoposide as a standard. The chloroform and remaining water fractions had moderate and positive lethality with LD_{50} values of 169.48 and 46.26 µg/mL against the brine shrimps, respectively (Table 2).

The extract and fractions of *C. dichotomum* were also screened for their insecticidal effects against *Rhyzopertha dominica* and *Tribolium castaneum* using permethrin as a standard drug. The chloroform and *n*-butanol fractions had low and moderate insecticidal activities with 20 and 40% of mortality against *Tribolium castaneum*, respectively. There was no insecticidal effect on all tested samples against *Rhyzopertha dominica* (Table 3).

Table 1: Phytotoxic activities of the extract and fractions of *C. dichotomum*.

Sample	% Growth inhibition		
	10 µg/mL	100 µg/mL	1000 µg/mL
CD	0	0	0
CDH	0	0	100.0
CDC	0	19.0	100.0
CDB	0	10.6	42.6
CDR	0	0	37.5

Standard drug: Paraquate (0.015 µg/mL)

Table 2: Cytotoxic activities of the extract and fractions of *C. dichotomum*.

Sample	No of survivals from 30 shrimps			LD_{50} (µg/mL)
	10 µg/mL	100 µg/mL	1000 µg/mL	
CD	23	22	17	5900.97
CDH	28	25	21	-
CDC	24	25	03	169.48
CDB	18	16	15	771.45
CDR	16	15	12	46.26

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

Table 3: Insecticidal activities of the extract and fractions of *C. dichotomum*.

Sample	% Mortality	
	Tribolium castaneum	*Rhyzopertha dominica*
CD	0	0
CDH	0	0
CDC	20	0
CDB	40	0
CDR	0	0

Reference insecticide: Permethrin (239.5 µg/cm²)

DISCUSSION

To date, a number of plant extracts prepared in different solvents was screened to determine the preliminary cytotoxic activity on brine shrimp larvae. Brine shrimp lethality assay is a preliminary method for the assessment of cytotoxic compounds which could show a significant correlation with their anticancer potentials.[11-13] In addition, to develop natural herbicides and insecticides that are safe and user friendly for the environment is important for screening in terms of phytotoxic and insecticidal activities of plants.[14]

According to our results, the chloroform fraction of *C. dichotomum* was found as a promising sample due to having cytotoxicity on brine shrimps. In our previous study, the chloroform fraction of *C. dichotomum* exhibited cytotoxicity on MCF-7, MDA-MB-231, LNCaP, PC-3 (lung) and HT-29 cancer cell lines using SRB assay with % viability values 22.99, 21.08, 24.27, 16.02 and 28.00, respectively.[2] Our preliminary phytochemical analysis of the bioactive chloroform fraction by TLC depicted that sesquiterpenes were detected as principal components.

CONCLUSION

The present study firstly showed that the potential of *C. dichotomum* on biological activities such as cytotoxicity on brine shrimps, phytotoxic and insecticidal effects *in vitro*. Further investigations are continuing on the isola-

tion and characterization for the responsible bioactive constituents from *C. dichotomum*.

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CONFLICT OF INTEREST

The authors declare no conflict of interest..

ABBREVIATIONS USED

SRB: Sulforhodamine B; **CD:** The Concentrated MeOH extract; **CDH:** n-Hexane fraction; **CDC:** CHCl3 Fraction; **CDB:** n-BuOH fraction; **CDR:** R-H2O Fraction.

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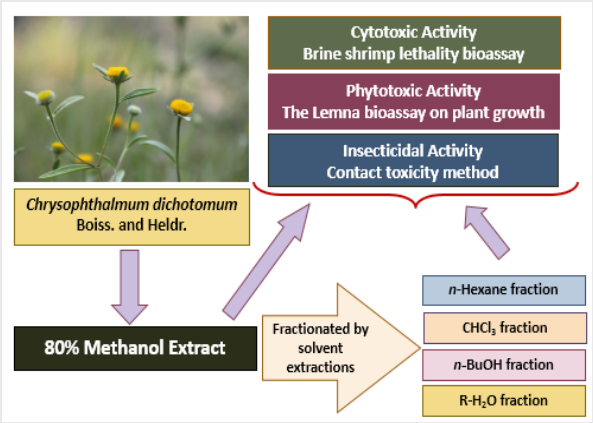
PICTORIAL ABSTRACT



SUMMARY

- In the present study was investigated on *C. dichotomum* on biological activities such as cytotoxicity
- brine shrimps, cytotoxicity and insecticidal effects *in vitro*.
- The chloroform fraction of *C. dichotomum* showed-moderate cytotoxicity on brine shrimps.
- Significant phytotoxic activity was shown in the *n*-hexane and chloroform fractions of the plant.

About Authors

Dr. Ayaz: Has been graduated from Hacettepe Univ., Fac. of Pharmacy in 2011 as the Second Degree. She has completed her Ph.D. (Pharmacognosy) with "Pharmacognosic Researches on Chrysophthalmum Schultz Bip. Species Growing in Turkey" in 2017. In 2018, she became an Assist. Professor in Selcuk Univ. Fac. of Pharmacy. She has been working in the Department of Pharmacognosy, Faculty of Pharmacy, Selcuk University, Konya, Turkey.

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