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RESEARCH ARTICLE

ANTILEISHMANIAL EFFICACY OF *HERACLEUM PERSICUM* DESF. EX. FISCH. EXTRACTS AGAINST *LEISHMANIA TROPICA* PROMASTIGOTES

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Abstract

Background: *Heracleum persicum* known as "suhotu" is widely distributed in Adana, Hakkari Van and used in the production of famous Van herb cheese. The antioxidant, antiinflammatory, antidiabetic, antiepileptic, hepatoprotective, and antifungal properties were previously investigated on *H. persicum* extracts.
Objectives: The purpose of the present study was to comparatively evaluate the *in vitro* antileishmanial potential of *H. persicum* extracts prepared by solvents having different polarities.
Methods: The extracts of aerial parts of *H. persicum* were obtained by maceration, and *in vitro* antileishmanial efficacy was assayed on *L. tropica* promastigotes using CellTiter-Glo® Cell Viability assay.
Results: The diethylether extract of *H. persicum* was found to be the most active extract on *L. tropica* promastigotes with IC_{50} value at 151.6 µg/ml. The n-hexane extract with 160.2 µg/ml IC_{50} value exhibited higher antileishmanial activity than methanol, ethylacetate and etanol extracts which had IC_{50} values at 229, 343.5, and 4708 µg/ml, respectively
Conclusions: The n-hexane and the diethylether extracts of *H. persicum* which showed remarkable *in vitro* antileishmanial activities could constitute a valuable source for leishmanicidal compounds.
Keywords: Antileishmanial, *Heracleum persicum*, *Leishmania tropica*, promastigotes.

INTRODUCTION

The genus *Heracleum* was named by Carl Linnaeus, the father of plant taxonomy. He was inspired by Heracles, Greek mythological character, considering the size of plants in this genus. The Greek philosopher Theophrastus also described this genus as "Hercules' panacea"[1-3]. In recent years *Heracleum* species have great potential for food, cosmetic, perfumery and pharmaceutical industries due to their ethnobotanical and pharmacological properties[4].

Heracleum persicum (*H. persicum*) locally known as golpar or hogweed had driven attention with its traditional use in Iran. In Albroz region, the stems of this plant are prickled and the fruits are used as spice in folk medicine to treat indigestion, epilepsy and infections[5,6]. In Turkey, *H. persicum* named as "suhotu" is widely distributed in Adana, Hakkari and Van and used in the production of famous Van herb cheese[7].

The records of *Heracleum* species in Europe is thought to have spread from Iran by England and Denmark colonies. In Norway, this ornamental plant is identified as invasive species. Toromso is the city where *Heracleum* species are most common and even the symbol of the city is characterised by "Toromso palm" whereas Toromsapalme international film festival derives its name from *Heracleus* genus[8].

H. persicum (*Apiaceae*) drives attention with its characteristic anise odor. It reaches up to 75 cm to 200 cm height with stalked base. The plant reaches up to 42 cm in with. The lower leaves with 5-7 leaflets are ovate and the tips are glabrous and lanceolate. The stem of the lower part is greenish, sparsely pubescent and can grow up to 100 cm in height. The middle stem leaves with 3-5 leaflets have lanceolate tips and sparsely pubescent. The scabboard reddish-green in color can grow up to 10 cm and sparsely covered with hairs. The color of flowers are white to pale green. They have 5 petals and 5 stamens having 2 pistils with fused carpels

and small sepals and arranged convexly, similar to an umbrella. Umbrellas on lateral branch are smaller than those on the main branch[9].

H. persicum is rich in phenolic compounds and furocoumarins. Cinnamic acid, *p*-coumaric acid, ferulic acid and rosmarinic acid are the main phenolic acids isolated from this plant. *H. persicum* has a pungent odor and yellow colored essential oil consisting β-ocimene, limonene and terpinolene as major constituents[6,10-11]. In addition to phytochemical analysis, the antioxidant, antiinflammatory, anti-diabetic, antiepileptic, hepatoprotective, antifungal and antileishmanial properties of *H. persicum* were previously investigated[12-19].

Leishmania species were recorded in literature in the early nineteenth century, when pathologist William Boog Leishman and physiologist Charles Donovan found similar oval shaped bodies in the spleens of patients. Later, medical doctor Ronald Ross suggested that these oval bodies were a new protozoan and proposed the name "*Leishmania donovani*"[20]. In 1904, the new protozoan entered the literature and so far 53 *Leishmania* species have been identified of which 20 of them have been found to cause disease in human[21]. *Leishmania*sis is observed as visceral (VL), mucocutaneous (ML) and cutaneous forms (CL). VL is characterized by fever, weight loss, anemia and enlargement of the liver and spleen. CL is characterized by large scarring sores on the skin whereas ML involves the destruction of mucous mebranes of the nose, mouth and throat[22]. The oldest *Leishmania* species causing cutaneous *Leishmania*sis are *L. tropica*, *L. major*, *L. infantum*, *L. donovani* and *L. aethiopica*. The VL and CL forms were detected in Turkey caused by *L. infantum* and *L. tropica* respectively[23,24]. In a study conducted between 1990 and 2010, 46033 cases of CL were recorded and the cases were reduced to a great extend with *Leishmania*sis Strategic Plan implemented by the Turkish Ministry of Health in 2011[25]. Pentavalent antimony compounds are used in the treatment of *Leishmania*sis despite serious side effects, toxicity, resistance and difficulty in administration. In this context, World Health Organization recommended that herbal products which have been used in the treatment of infections since ancient times, should also be explored for their antileishmanial efficacies[23,26].

Antileishmanial effects of *H. persicum* extracts were previously reported against *L. major* and *L. infantum*[18,19,27]. The essential oil obtained from *H. persicum* was previously searched for its inhibitory effect against *L. tropica*[19]. To the best of our knowledge this is the first preliminary *in vitro* research on the comparative efficacy of *H. persicum* extracts against *L. tropica* promastigotes. Hence, the active extracts of *H. persicum* will constitute an important resource in the detection of potential antileishmanial compounds and herbal drugs to be prepared in the future.

MATERIALS AND METHODS

Plant material

H. persicum aerial parts were collected from Van province, Gürpınar district, Sarıçiçek neighborhood, Turkey in June, 2022. The plant was authenticated by Fatih Donmez, Van Yuzuncuyil University, Department of Biochemistry and a voucher specimen with 1262/4 herbarium number is deposited in the herbarium of Ege University Faculty of Pharmacy, Department of Pharmacognosy, Turkey.

Preparation of plant extracts

The aerial parts of the plant were dried at room temperature and powdered by electrical grinder. An amount of 5 g powdered aerial parts was accurately weighed and macerated with extraction solvents (ethanol, diethyleter, ethylacetate, methanol and n-hexane) individiually for 8 hours at room temperature with a plant: solvent ratio of 1:20. The filtrates were filtered through Whatman No.1 paper and solvent was evaporated at low pressure by using rotary evaporator. The extracts were then lyophilized and stored at -20ºC until analysis[24].

Antileishmanial activity assay

Production of *Leishmania tropica* isolate

L. tropica isolate with MHOM/TR/2012/CBCL-LT code was obtained from Manisa Celal Bayar University Faculty of Medicine Parasite Bank. The isolate was removed from the liquid nitrogen tank and thawed in 37ºC water bath for 2 minutes and then inoculated into Now-Mc Neal-Nicolle (NNN) media. NNN media was left for incubation in an oven at 26ºC. The NNN media was controlled under a microscobe for the growth of parasites on consecutive days. The parasites in 200-300 µl were inoculated into 3 ml RPMI-1640 culture media containing 10% fetal bovine serum (FBS) placed in a cell culture flask which was then incubated in an oven at 26ºC. On consecutive days, the medium was checked for the growth of parasites under an inverted microscope. The samples taken from flasks with sufficient growth density as 10^6 promastigotes/ml were stained with Trypan Blue on a Thoma chamber for counting, and the number of unstained cells (alive cells) was calculated. The average number of live parasites per square was multiplied by the amount of medium, the amount of dilution and 10^4. The flasks containing 10^6 promastigotes/ml were used for the analysis[28].

In vitro antileishmanial activity of plant extracts

The antileishmanial activity of plant extracts was evaluated using CellTiter®GloCell Viability kit with *L. tropica* promastigotes that reached the required reproductive density for the study. Amphotericin B was used as the control drug. The study was conducted in a sterile environment using 96-well flat bottom cell culture plates. Initially, 100 µl of RPMI-1640 medium containing 10% FBS was added to each well of the plate. The first three columns of the plate were grouped as parazite control, and the next three columns as blind control, the next three columns as drug control, and the subsequent columns were used for the various dilutions of extracts. 100 µl of medium containing the parasites was added to each well and the plates were incubated

at 26ºC. After 48 hours, the plates were processed according to manufacturer's instruction using CellTiter-Glo® Cell Viability kit to evaluate the antileishmanial activity of the extracts[24,28].

Statistical analysis

The experiments were carried out as triplicate and results using Student's *t*-test was performed using SPSS 23.0 statistics. *p* value less than 0.05 was considered as statistically significant.

RESULTS AND DISCUSSION

The diethylether extract of *H. persicum* was found to be the most active extract on *L. tropica* promastigotes with IC_{50} value at 151.6 µg/ml. The n-hexane extract with 160.2 µg/ml IC_{50} value exhibited higher antileishmanial activity than methanol, ethylacetate and etanol extracts which had IC_{50} values at 229, 343.5, and 4708 µg/ml, respectively (Table 1). The viability percentages and comparision of the antileishmanial activity of plant extracts against *L. tropica* promastigotes is shown in Figure 1. In a previous work, the essential oil obtained by hydro-distillation of *H. persicum* leaves was found to be active on *L. tropica* and *L. major* promastigotes with IC_{50} values of 15.6 and 16 µg/ml respectively[19]. In another study, *H. persicum* exhibited antileishmanial activiy with IC_{50} at 29.3 mg/ml on *L majo*r promastigotes and IC_{50} at 14.7 mg/ml concentration on *L infantum* promastigotes[29].

Table 1: The *in vitro* IC_{50} values of *H. persicum* extracts against *L. tropica* .

Extracts	$IC_{50\pm}$SD (µg/ml)
Diethylether	151.6±2.51
N-hexane	160.2±2.00
Methanol	229.0±2.65
Ethylacetate	343.5±2.00
Ethanol	4708.0±2.08
Amphotericin B	0.500±0.00

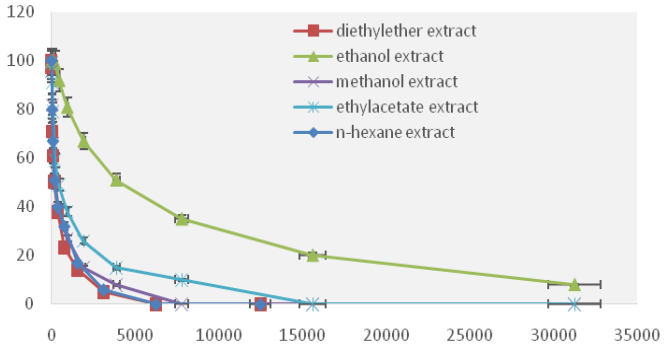

Figure 1: The viability percentages of *L. tropica* promastigotes obtained with various dilutions of *H. persicum* extracts (µg/ml).

The ethanol extract of *H. persicum* at 10 mg/ml concentration was shown to have 85% antimotility effect on *L. major*[27]. In the present study, the ethanol extract was found to be the least active extract with IC_{50} at 4.708 mg/ml. The hydroethanolic extract prepared from the fruits of *H. persicum* was reported to exhibit antileishmanial activity against *L. major* and *L. infantum* with IC_{50} values of 31.32 and 11.7 µg/ml, respectively[18]. When previous antileishmanial activity studies on *H. persicum* were examined, it was determined that ethanolic or hydroethanolic extracts were studied for their antileishmanial activities. In the present study, in addition to ethanolic extract, methanol, n-hexane, ethylacetate, diethylether extracts were comparatively analyzed against *L. tropica* whereas the n-hexane and the diethylether extract were found to posses remarkable antileishmanial activities on *L. tropica* promastigotes in microgram quantities in a dose-dependent manner. The aliphatic esters, coumarins and terpenoids which tend to be more soluble in nonpolar solvents such as n-hexane and dietylether might be the responsible constituents for the observed antileishmanial activity.

Limitation of the study

The study of plant material collected from a single region and the lack of *in vivo* study constitute the limitations of the study.

CONCLUSIONS

To the best of our knowledge the literature on the antileishmanial efficacy of *H. persicum* is scarce. Although various biological activity assays and phytochemical analysis were previously conducted on this plant, the information about the antileishmanial potential is insufficient. The n-hexane and the diethylether extracts of *H. persicum* which showed remarkable *in vitro* antileishmanial activities could constitute a valuable source for leishmanicidal compounds. Antileishmanial activity guided assay for the isolation of potential active constituents is planned for further studies.

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AUTHOR'S CONTRIBUTION

Ozgun G, **Cavus I**, **Alayli F**: investigation, data curation. **Donmez F**: plant material supply **Kayalar H** and **Ozbilgin A**: methodology and supervision. Final manuscript was read and approved by all authors.

DATA AVAILABILITY

The data will be available to anyone upon request from the corresponding author.

CONFLICT OF INTEREST

Authors declare that they have no conflict of interest.

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