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RESEARCH ARTICLE

IN VITRO INHIBITORY ACTIVITY OF *BERBERIS VULGARIS* L. AGAINST *LEISHMANIA TROPICA* PROMASTIGOTES

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Abstract

Objective: In the present study it was aimed to determine the *in vitro* antileishmanial activity of *Berberis vulgaris* L. against *Leishmania tropica* promastigotes. The aerial parts of *Berberis vulgaris* were collected from Spil Mountain, Manisa.
Method: The ethanolic extract of the plant material was prepared. The consecutive concentrations of the plant extract (25-100 µg/ml) were set for *in vitro* antileishmanial assays. In addition to *in vitro* inhibitory activities against *Leishmania tropica* promastigotes, the cytotoxicity of the plant extract was also measured by WST-1 Cell proliferation assay. The percentages of parasite inhibition in the presence of *B. vulgaris* ethanol extract in comparison with glucantime reference group at time interval of 12-72 hours were observed between 88.0 and 100.0%. The plant extract was found to have cytotoxic activity with 444, 81±2, 12 µg/ml IC_{50} value.
Conclusion: This is the first study that involves the *in vitro* antileishmanial activity of *B. vulgaris* which is wildly growing in Manisa, Turkey. Initial results demonstrated that the ethanolic extract of *B. vulgaris* gave promising results and it could be used as an antileishmanial agent in future.
Keywords: Antileishmanial activity, inhibitory activities, *Leishmania tropica* promastigotes, plant extract.

INTRODUCTION

Berberis vulgaris L. (Barberry, family Berberidaceae) is native to central and southern Europe, western Asia and northwest Africa. The root, bark, leaves; and fruits of barberry are used in traditional medicine. The plant is a shrub, 1–3 m tall, spiny, with yellow wood and small, oval leaves, bearing yellow flowers and red oval fruits (barberry)[1-3]. Medicinal properties for all parts of the plant have been reported, including tonic, antioxidant, antimicrobial, antiemetic, antipyretic, anti-pruritic, anti-inflammatory, antinociceptive, hypoten-sive, antiarrhythmic, anticholinergic, sedative, and cholagogue actions. It has been used in some cases like cholecystitis, cholelithiasis, dysentery, leishma-niasis and malaria[4]. The main bioactive components of this plant are reported to be the alkaloids such as berbamine, palmatine and particularly berberine[1,5]. Leishmaniasis is a protozoan parasitic disease found in 16 developed and 72 developing countries with 12 million case[6]. The cutaneous leishmaniasis (CL), most common type of leishmaniasis was reported to be and affecting 1.5 million people annually, worldwide. Over 90% of cases are reported from countries such as Afghanistan, Iraq, Pakistan, Iran[7]. Visceral leishmaniasis (VL) is known to be the most severe form of leishmaniasis in the world[8]. Plant derived compounds and extracts are known to be valuable sources for the treatment of various diseases. The extract prepared from the roots and fruits of *B. vulgaris* were previously reported to possess *in vitro* leishmanicidal activity against *L. tropica* and *L. infantum*[9,10].

The aim of the present study was to determine the *in vitro* antileishmanial efficacy of ethanol extract prepared from the aerial parts of *Berberis vulgaris* collected from Spil Mountain, Manisa, Turkey. In addition to *in vitro* antileishmanial activity against *L. tropica* promastigotes, cytotoxic activity of the plant extract was also measured using a WST-1 cell proliferation assay[11,12].

MATERIALS AND METHODS

Plant material

B. vulgaris aerial parts are collected from Spil Mountain, Manisa, Turkey. The plant species were identified by Dr. Cenk Durmuskahya (Izmir Katip Celebi University, Faculty of Forestry, Department of Forest Engineering, Balatcik, İzmir Turkey)

Preparation of plant extract

The air dried and ground aerial parts of *B. vulgaris* were extracted in ethanol with stirring at room temperature. The extraction yield was determined as 3.6%.

Phytochemical analysis of plant extract

Phytochemical screening tests for plant secondary metabolites such as tannins, terpenoids, flavonoids and alkaloids were conducted on plant extract[13].

***In vitro* antileishmanial assay**

A range of concentrations of the plant extract (25-500 µg/mL) were prepared for *in vitro* antileishmanial assays. The haemocytometer counting of living *L. tropica* promastigotes in RPMI 1640 medium was preferred for *in vitro* assessments. All the experiments were run in triplicate and results were expressed as mean percentage inhibition of parasites. Glucantime was used as a reference drug[11].

Determination of Cytotoxic Activities (IC_{50}) of plant extract

The consecutive concentrations of plant extracts within 1 nM-100 µM were prepared and IC_{50} levels were determined by using "xCELLigence Real-Time Cell Analyzer" in 96 hours. A total of $2x10^6$/ml cells were distributed for each cell line in the plates having 96 gold-coated wells, including the control group without plant extract. Each assessment was run in triplicate. IC_{50} levels of the plant extracts in each cell line were confirmed in a colorimetric fashion with WST1 (4-[3-(4-iodophenyl)-2-(4-nitrophenyl)-2H-5-tetrazolio]-1,3-benzene disulfonate) test; following the addition of WST1, all extracts were kept for 4 hours inside an incubator with 5% CO_2, and 95% humidity at 37°C. The colorimetric change was determined quantitatively at 450 nm and 600 nm reference intervals by using a Multiscan FC Thermo Scientific micro plate reader[12].

RESULTS AND DISCUSSION

The preliminary phytochemical analysis results for the ethanolic extract of aerial parts of *B. vulgaris* were positive for flavonoids, tannins, anthracenes, terpenoids and alkaloids. The cytotoxic activity of plant extract was determined against WI-38 foetal lung fibroblast cell lines by real-time analyser. The plant extract was found to have cytotoxic activity with 444, 81±2, 12 µg/ml IC_{50} value. The number of parasites at different concentrations of the extract and the reference drug glucantime was shown in Figure 1. Parasite inhibition was observed between 88.0±0.04 and 100% in the presence of *B. vulgaris* ethanol extract, when measured in comparison with a glucantime treated reference group at time intervals of 12-72 hours (Table 1).

Table 1: The parasite inhibition percentages of *B. vulgaris* ethanolic extracts.

B. vulgaris Ethanol Extract (µg/ml)	Parasite inhibition (%)		
	12 hrs	24 hrs	48-72 hrs
25	88.00	89.00	89.70
50	89.00	95.60	96.00
125	95.90	97.00	96.60
250	99.30	99.40	99.42
500	100.00	100.00	100.00

The plant extract with IC_{50} value of 444.81±2.12 µg/ml was not found to be significantly cytotoxic against lung fibroblast cell lines.

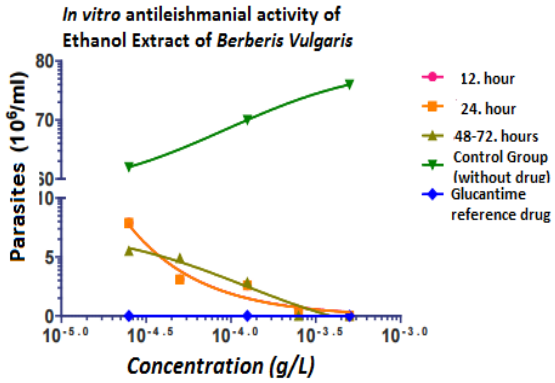

Figure 1: The parasite counts at different concentrations and time intervals.

In a previous work on investigation against different *Leishmania* species, the aqueous and methanolic extracts of aerial parts of *B. vulgaris* were reported to have inhibitory activities against *L. tropica* and *L. infantum.* Berberine, the biologically active component of *B. vulgaris* was also reported to have significant inhibitory effects on the promastigote and amastigote forms of the mentioned leishmanial parasites[8]. The ethanolic extract prepared from fruits of *B. vulgaris* were found to be active against *L. tropica* promastioges and amastigotes with IC_{50} 4.8 and 24.03 µg/ml respectively[10]. The previous studies support findings of current study and further studies should be conducted.

CONCLUSIONS

This is the first study that involves the assessment of *in vitro* antileishmanial activity of *B. vulgaris* growing wildly in Turkey. Further *in vivo* studies are required to elucidate the potential mechanism of action and identify the structures of compounds responsible for the observed antileishmanial activity. The results demonstrated that the ethanolic extract of *B. vulgaris* is promising and it could be used as a source for antileishmanial agent in future.

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AUTHOR'S CONTRIBUTION

Ozbilgin A: writing original draft, methodology. **Kayalar H:** investigation, formal analysis, conceptualization. **Cavus I:** writing, review and editing. **Durmuskahya C:** methodology, formal analysis, conceptualization. **Toktas U:** writing, review, and editing, methodology. **Gunduz C:** writing, review, and editing. Final manuscript was read and approved by all authors.

DATA AVAILABILITY

The datasets generated during this study are available from the corresponding author upon reasonable request.

CONFLICT OF INTEREST

There is no conflict of interest associated with this work.

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