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

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ABSTRACT

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. The ethanolic extract of the plant material was prepared. The consecutive concentrations of the plant extract (25-100µg/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 IC50 value. 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 northwestern 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)1,2,3. Medicinal properties for all parts of the plant have been reported, including tonic, antioxidant, antimicrobial, antiemetic, antipyretic, antitussive, anti-inflammatory, antinociceptive, hypotensive, antiarrhythmic, anticholinergic, sedative, and cholagogue actions. It has been used in some cases like cholecystitis, cholelithiasis, dysentery, leishmaniasis and malaria4. The main bioactive components of this plant are reported to be the alkaloids such as berbamine, palmatine and particularly berberine1,3.

Leishmaniasis is a protozoan parasitic disease found in 16 developed and 72 developing countries with 12 million case6. 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, Iran7. Visceral leishmaniasis (VL) is known to be the most severe form of leishmaniasis in the world8. 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 Berberis vulgaris were previously reported to possess in vitro leishmanicidal activity against Leishmania tropica and L. infantum9,10. 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 Leishmania tropica promastigotes, cytotoxic activity of the plant extract was also measured using a WST-1 cell proliferation assay\textsuperscript{11,12}.

**MATERIAL AND METHODS**

**Plant material**

*Berberis vulgaris* aerial parts are collected from Spil Mountain, Manisa, Turkey. The plant species were identified by Dr. Cenk Durmuşkahya (Izmir Katip Celebi University, Faculty of Forestry, Department of Forest Engineering, Balatçık, 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\textsuperscript{13}.

**In vitro antileishmanial assay**

A range of concentrations of the plant extract (25-5000 µg/mL) were prepared for in vitro antileishmanial assays. The haemocytometer counting of living *Leishmania 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\textsuperscript{11}.

**Determination of Cytotoxic Activities (IC\textsubscript{50}) of plant extract**

The consecutive concentrations of plant extracts within 1 nM-100 µM were prepared and IC\textsubscript{50} levels were determined by using “xCELLigence Real-Time Cell Analyzer” in 96 hours. A total of 2x10\textsuperscript{4} 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\textsubscript{50} levels of the plant extracts in each cell line were confirmed in a colorimetric fashion with WST1 (4-[3-(4-iодонил)-2-(4-нитрофенил)-2H-5-тетразолио]-1-бензил disulfonate) test; following the addition of WST1, all extracts were kept for 4 hours inside an incubator with 5 % CO\textsubscript{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 microplate reader\textsuperscript{15}.

**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\textsubscript{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±0.00 % 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). The plant extract with IC\textsubscript{50} value of 444.81±2.12 µg/mL was not found to be significantly cytotoxic against lung fibroblast cell lines. 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 antileishmanial parasites\textsuperscript{5}. The ethanolic extract prepared from fruits of *B. vulgaris* were found to be active against *L. tropica* promastigotes and amastigotes with IC\textsubscript{50} 4.8 and 24.03 µg/mL respectively\textsuperscript{10}. The previous studies support findings of current study and further studies should be conducted.

**CONCLUSION**

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.

**ACKNOWLEDGEMENT**

This study was supported by TUBITAK (The Scientific and Technological Research Council of Turkey) with 1105289 number.

**CONFLICT OF INTEREST**

There is no conflict of interest associated with this work.

**REFERENCES**


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

<table>
<thead>
<tr>
<th><em>B. vulgaris</em> Ethanol Extract (µg/ml)</th>
<th>Parasite inhibition %</th>
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<tr>
<td></td>
<td>12hrs</td>
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<tr>
<td>25</td>
<td>88.00</td>
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<td>50</td>
<td>89.00</td>
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<td>125</td>
<td>95.90</td>
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<td>250</td>
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<td>500</td>
<td>100.00</td>
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Figure 1: The parasite counts at different concentrations and time intervals