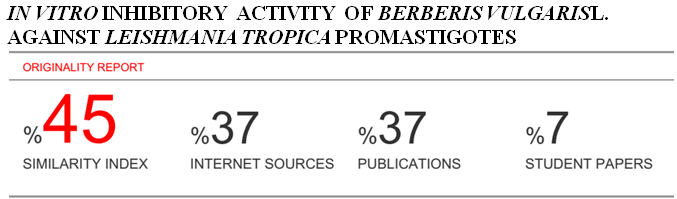
**Reviewer’s Comments**

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

**ABSTRACT**

In the present study it was aimeddetermine the *in vitro*antileishmanialactivity of *Berberis vulgaris* L. against *Leishmaniatropica* promastigotes. The aerial parts of *Berberisvulgaris*were collected from Spil Mountain, Manisa.Theethanolic 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*inhibitoryactivities against *Leishmaniatropica* 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 IC50value.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**

**INTRODUCTION**

*Berberis vulgaris* L. (Barberry, family Berberidaceae) is native to central and southern Europe, western Asia and northwest Africa. The root, bark, leaf, fruits of barberry are used in traditional medicine. The plant is a shrub, 1–3 m tall, spiny, with yellow wood and small, ovalleaves, 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, antipruritic, 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 berberine1,5.

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.Theextract prepared from the roots and fruits of *Berberis vulgaris* were previously reported to possess *in vitro*leishmanicidalactivity against *Leishmaniatropica*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 *Leishmaniatropica* promastigotes, cytotoxic activity of the plant extract was also measured using a WST-1 cell proliferation assay11,12.

**MATERIAL AND METHODS**

**Plant material**

*Berberisvulgaris*aerial parts are collected from Spil Mountain, Manisa, Turkey. The plant species were identified by Dr. CenkDurmuskahya (Izmir KatipCelebi 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 extract13.

***In vitro*antileishmanial assay**

A range of concentrations of the plant extract (25-500µg/mL) were prepared for *in vitro* antileishmanialassays.Thehaemocytometer counting of living *Leishmaniatropica* 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 drug11.

**Determination of Cytotoxic Activities (IC50) of Plant Extract**

The consecutive concentrations of plant extracts within 1 nM-100 μM were prepared and IC50 levels were determined by using “xCELLigence Real-Time Cell Analyzer” in 96 hours. A total of 2x106/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. IC50 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 % CO2, 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 reader12.

**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 linesby real-time analyser. The plant extract was found to have cytotoxic activity with 444,81±2,12 µg/ml IC50value.Thenumber 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 IC50 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*spaecies, 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 parasites8. The ethanolicextractprepared from fruits of *B. vulgaris* were found to be active against *L.tropica*promastiogesand amastigotes with IC50 4.8 and 24.03 µg/ml respectively10. The previous studies support our findings and further studies should be conducted.

**CONCLUSION**

This is the first study that involves the assessment *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 antileishmanialactivity.Theresults demonstrated that the ethanolic extract of *B. vulgaris* is promising and it could be used as a source forantileishmanialagents infuture.

#### ACKNOWLEDGEMENT

#### This study was support by TUBITAK (The Scientific and Technological Research Council of Turkey) with 110S289 number.

#### CONFLICT OF INTEREST

There is no conflict of interest associated with this work.

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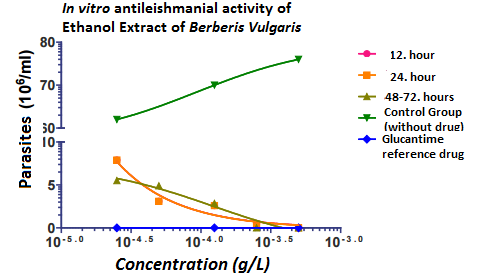
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**Figure 1. The parasite counts at different concentrations and time intervals**

**Table 1. The parasite inhibition percentages of *Berberisvulgaris*ethanolic extracts**

