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

ABSTRACT
In the present study it was aimed 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.

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, oval leaves, bearing yellow flowers and red oval fruits (barberry). 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 malaria. The main bioactive components of this plant are reported to be the alkaloids such as berbamine, palmatine and particularly berberine.

Leishmaniasis is a protozoan parasitic disease found in 16 developed and 72 developing countries with 12 million case. 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. Visceral leishmaniasis (VL) is known to be the most severe form of leishmaniasis in the world.
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 Leishmaniatropica and L. infantum. 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 assay.

MATERIAL AND METHODS

Plant material

Berberis 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, Izmir 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.

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 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 drug.

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 reader.

RESULTS AND DISCUSSION

The preliminary phytochemical analysis results for the ethanolic extract of aerial parts of B. vulgaris was 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
The plant extract was found to have cytotoxic activity with $444.81 \pm 2.12 \mu g/ml$ $IC_{50}$ 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 $88.0 \pm 0.04$ and $100 \pm 0.00$ % 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 $IC_{50}$ value of $444.81 \pm 2.12 \mu g/ml$ 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 leishmanial parasites. 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. 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 antileishmanial activity. The results demonstrated that the ethanolic extract of $B. vulgaris$ is promising and it could be used as a source for antileishmanial agents in future.

**ACKNOWLEDGEMENT**

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

**CONFLICT OF INTEREST**

There is no conflict of interest associated with this work.

**REFERENCES**

1. Imanshahidi M, Hosseinzadeh H. Pharmacological and therapeutic effects of *Berberis vulgaris* and its active constituent, berberine. Phytother Res2008; 22:999–1012.

2. Sahin K, Orhan C, Tuzcu M, Borawska MH, Jablonski J, Guler O, Sahin N, Hayirli A. *Berberis vulgaris* root extract alleviates the adverse effects of heat stress via modulating hepatic nuclear transcription factors in quails. Brit Jour of Nutri2013:110:609–616.

3. Abd El-Wahab AE, Ghareeb DA, Sarhan EEM, Abu-Serie MM, El Demellawy MA. *In vitro* biological assessment of *Berberis vulgaris* and its active constituent, berberine: antioxidants, anti-acetylcholinesterase, anti-diabetic and anticancer effects. BMC Complem and AlternMedi2013; 13:218.
4. Khosrokhavare, R., Ahmadiani, A., Shamsa, F. Antihistaminic and Anticholinergic Activity of Methanolic Extract of Barberry Fruit (*Berberis vulgaris*) in the Guinea-Pig Ileum. J Med Plants 2010; 9(35):99-105.

5. Kupeli, E., Kosar, M., Yesilada, E., Baser, KHC. A comparative study on the anti-inflammatory, antinociceptive and antipyretic effects of isoquinoline alkaloids from the roots of Turkish Berberis species. Life Sci. 2002; 72:645-57.

6. World Health Organization, Control of the Leishmaniasis. Geneva: WHO Technical Report Series 949, 2010, 5-12.

7. Desjeux, P. Leishmaniasis: current situation and new perspectives. Comp ImmunolMicrobiol Infect Dis. 2004; 27:305-318.

8. Mahmoudvand, H., Sharififar, F., Sharifi, I., Ezatpour, B., FasihiHarandi, M., Makki, MS, Zia-Alin, Jahanbakhsh S. *In vitro* Inhibitory Effect of *Berberis vulgaris* (Berberidaceae) and Its Main Component, Berberine against Different *Leishmania* Species. Iranian J Parasitol 2014, 9(1):28-36.

9. Mishra, BB, Kale, RR, Prasad, V, Tiwari, VK, Singh, RK. Scope of natural products in fighting against leishmaniasis. Bioact Nat Prod, World Sci Pub Comp 2011, 1: 121-154.

10. Mahmoudvand, H., Sharififar, F., Rahmat, MS, Tavakoli, R., Dezaki, E., Jahanbaksh, S., Sharifi, I. Evaluation of antileishmanial activity and cytotoxicity of the extracts of *Berberis vulgaris* and *Nigella sativa* against *Leishmaniatropica*. J Vector Borne Dis 2014; 294:299.

11. Ozbilgin, A., Durmuskahya, C., Kayalar, H., Erhabaklar, H., Gunduz, C., Ural, IO, Zeyrek, F., Kurt, O., Cavus, I., Toz, SO, Ozbel, Y. Antileishmanial Activity of Selected Turkish Medicinal Plants. Trop J Pharm Res 2014; 13:2047-2055.

12. Urcan, E., Haertel, U., Styilou, M., Hickel, R., Scherthan, H., Reichi, FX. Real-time xCELLigence impedance analysis of the cytotoxicity of dental composite components on human gingival fibroblasts. Dent Mater 2010; 6:51-58.

13. Evans WC., Trease and Evans Pharmacognosy, 16th ed., Elsevier, 2009.
Figure 1. The parasite counts at different concentrations and time intervals

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

| B. vulgaris Ethanol Extract (µg/ml) | Parasite inhibition % |
|-----------------------------------|------------------------|
|                                   | 12hrs | 24hrs | 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    |