

# *In vitro* Activity of *Arbutus unedo* Against *Leishmania tropica* Promastigotes

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**SUMMARY:** Pentavalent antimonials are the first choice for the treatment of anthroponotic cutaneous leishmaniasis (ACL) in health centers in Turkey, however in rural areas, traditional plants may be preferred for the treatment of lesions. In recent years a number of papers are published related to the natural products especially plant derivatives. Our aim is to investigate the antileishmanial effect of *Arbutus unedo* which is a wild plant mainly grown in maquis and rocky places of the seabord in South Europe. In the present study, the ethanolic, water and n-hexane extracts from the leaves of *Arbutus unedo* were originally tested *in vitro* against *Leishmania tropica* promastigotes. The ethanol extract of *Arbutus unedo* leaves at the concentrations of 100, 250, 500 µg/ml were found to be more effective than the other extracts (p:0.000). Our study showed that the ethanolic extract of *Arbutus unedo* leaves can be a promising antileishmanial agent and further experiments are needed.

**Key Words:** *Arbutus unedo*, *Leishmania tropica*, Plant extract.

## *Arbutus unedo* Yaprak Ekstrelerinin *Leishmania tropica* Promastigotları Üzerine *In vitro* Etkisinin Araştırılması

**ÖZET:** Antropoonotik kutanöz leishmaniasis (ACL)'in tedavisi sağlık merkezlerinde antimon bileşikleriyle yapılmakta fakat özellikle kırsal bölgelerde hastaların değişik bitkilerden hazırlanan karışımları tedavi amacı ile kullandıkları bilinmektedir. Son yıllarda doğal ürünlerle, özellikle bitki ekstreleri ile ilgili çok sayıda çalışma yayınlanmaktadır. Bu çalışmada özellikle Güney Avrupa'da kayalık ve makilik alanda yetişen doğal bir bitki olan *Arbutus unedo* yapraklarından hazırlanan etanol, su, heksan ekstrelerinin *Leishmania tropica* promastigotları üzerine *in vitro* etkileri ilk kez araştırılmıştır. Etanol ekstresinin 100, 250, 500 µg/ml konsantrasyonlarda etkili olduğu ve diğerleri ile karşılaştırıldığında istatistiksel olarak daha etkin olduğu saptanmıştır (p: 0.000). Bu ekstrelerin umut verici anti-leishmaniasidal bir ajan olabileceği ve ileri araştırmaların yapılması gerektiği düşünülmüştür.

**Anahtar Sözcükler:** *Arbutus unedo*, *Leishmania tropica*, Bitki ekstresi

## INTRODUCTION

Anthroponotic cutaneous leishmaniasis (ACL) caused by *Leishmania tropica* (*L. tropica*) has long been known to exist in the southeastern Turkey. New localities with ACL patients were reported in western part of the country in recent years (7, 9).

Treatment for cutaneous leishmaniasis is unsatisfactory. Various forms of local treatment as heating, freezing, curettage, and infiltration with antimonial, have their own advantages and disadvantages, however none of them is suitable for all forms of the disease. Systemic treatment with

antimonial compounds is expensive and relatively ineffective besides unacceptable side effects. An effective topical preparation would be of great value as it would permit an unsupervised and safely treatment with a reasonable cost (3, 4).

*Arbutus unedo* (strawberry tree), was grown wild, mainly in maquis and rocky places of the Mediterranean seabord. The leaves contain flavonol glycosides (afzelin, juglanin, avicularin, quercitrin, hyperin), phenol glycosides (arbutin, methylarbutin), lipids, tannins and vitamin E (6). In traditional medicine, the herbal teas and preparations of *Arbutus unedo* leaves have been generally used to treat hypertension, anxiety, diarrhea and hemorrhoids (2, 10). n-Hexane and water extracts were found to be cytotoxic while ethanol extract was not shown cytotoxicity by brine shrimp lethality bioassay (8). In our study, we aimed to search the activity of these extracts against *L. tropica* promastigotes *in vitro*.

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## MATERIAL AND METHODS

*Arbutus unedo* leaves were collected around Cicekliköy village located 40 km north of Izmir city, Turkey in November 1998. A voucher specimen was deposited in the herbarium of the Pharmacognosy Department of Pharmacy Faculty, Ege University (No: 1251).

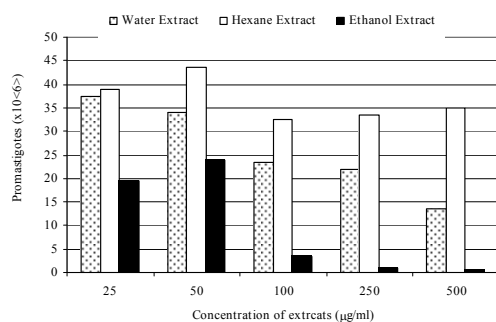
**Leaf extract Preparation:** Air-dried and powdered leaves of *Arbutus unedo* leaves were extracted with n-hexane and ethanol by percolation at room temperature. The water extract was prepared by infusion at room temperature. The extracts were evaporated to dryness in vacuum and weighed. All of the extracts were prepared at 100% concentration (g/ml).

**In vitro antileishmanial activity studies:** *L. tropica* MON53 promastigotes (MHOM/TR/99/EP40) were isolated from a patient from Şanlıurfa city located in southeastern part of Turkey using NNN medium. Promastigotes transferred to RPMI 1640 medium supplemented with 15% Fetal calf serum for mass cultivation. Test flasks were seeded with medium containing  $1 \times 10^6$  promastigotes per ml. Extracts diluted with DMSO transferred to the culture flasks to obtain final concentrations of 25, 50, 100, 250, 500 µg/ml. The highest concentration of DMSO and a normal culture flask were used as controls. Antileishmanicidal activity was studied after 48 and 72 hours incubation at 25 °C. Promastigotes were counted after adding 10% formaldehyde. Experiments were performed three times.

**Statistical Analysis:** The results of water and ethanol extracts of *Arbutus unedo* leaves were analysed by  $X^2$  test. The results of 48th and 72nd hours were correlated with using the T test.

## RESULTS

The extracts were diluted with DMSO and DMSO control was found to be inactive at the highest concentration used in this study. The n-hexane extract did not show activity while ethanol extract of *Arbutus unedo* leaves showed the strongest antileishmanial activity against *L. tropica* promastigotes (Figure 1).



**Figure 1.** Antileishmanial activity of the extracts from *Arbutus unedo* leaves.

$X^2$  analysis showed significantly difference between the antileishmanial activities of ethanol and water extracts of *Arbutus unedo* leaves as follows:

There was no difference in the efficacy of the water extract with the increasing of the dosage while there was a concordance in antileishmanial efficacy of the ethanol extract

with the increasing of the dosage. T test analyse did not show significantly difference in results after 48 and 72 hours.

## DISCUSSION

The perspectives for the cure of leishmaniasis are still uncertain. There is a strong need to easy synthesized and low cost therapeutic agents especially in undeveloped and developing countries as our country. Many natural products have already provided valuable clues for potentially antiparasitic compounds (1). However, *Leishmania* parasites are protected inside macrophages and the compounds that are toxic to the parasite are usually also toxic to the host (5).

Our study is providing useful data in order to find new active products against *L. tropica* parasites. Ethanol extract of *Arbutus unedo* leaves showed the strongest antileishmanial activity among three extracts. Interestingly, n-hexane and water extracts was found to be cytotoxic while ethanolic extract was found not to be cytotoxic by brine shrimp lethality bioassay previously (8).

As a conclusion, the ethanolic extract of *Arbutus unedo* leaves can be a promising antileishmanial agent in the future. Further experiments are needed for isolation of active fractions and identification of the active components of ethanol extract.

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