

# *In vitro* Activity of *Arbutus unedo* Leaf Extracts against *Trichomonas vaginalis* Trophozoites

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**SUMMARY:** *Trichomonas vaginalis* (*T. vaginalis*) is a flagellated protozoan commonly causing sexually transmitted disease. *T. vaginalis* infections are treated with a 5-nitroimidazole derivate. However, drug resistance has been known to occur for a long time and new alternatives are under investigation. *Arbutus unedo* is a wild plant mainly growing in maquis and rocky places of the seaboard in Southern Europe. In our study, ethanolic, water, hexane and ethyl acetate extracts of *Arbutus unedo* leaves were tested *in vitro* against *T. vaginalis* trophozoites and the ethyl acetate extract of *Arbutus unedo* leaves was found to be effective (Growth inhibition rate (GI): 100%, at the concentration of 500 µg/ml). It may be a promising anti-trichomonacidal agent in the future and further experiments are needed.

**Key Words:** *Trichomonas vaginalis*, *Arbutus unedo*, plant extract.

## *Arbutus unedo* Yaprak Ekstrelerinin *Trichomonas vaginalis* Trofozoitleri Üzerine *in vitro* Etkisinin Araştırılması

**ÖZET:** *Trichomonas vaginalis* (*T. vaginalis*) sık görülen, cinsel yolla bulaşan kamçılı bir protozoondur. *T. vaginalis* enfeksiyonu 5-nitroimidazol türevleri ile tedavi edilmektedir. Bununla birlikte ilaca karşı direnç uzun zamandır bilinmekte ve yeni alternatifler araştırılmaktadır. *Arbutus unedo* özellikle Güney Avrupa'da kayalık ve makili alanda yetişen doğal bir bitkidir. Bu çalışmada *Arbutus unedo* yapraklarında hazırlanan etanol, su, heksan ve etil asetat ekstrelerinin *T. vaginalis* trofozoitleri üzerine *in vitro* etkileri araştırılmış ve etil asetat ekstrelerinin etkili olduğu saptanmıştır (etil asetat ekstrelerinin 500 µg/ml'lik konsantrasyonda çoğalmayı engelleyici etkisinin %100 olduğu saptanmıştır). Bu ekstrelerin ileride umut verici anti-trichomonasidal bir ajan olabileceği ve ileri araştırmaların yapılması gerektiği düşünülmüştür.

**Anahtar Sözcükler:** *Trichomonas vaginalis*, *Arbutus unedo*, Bitki ekstresi

## INTRODUCTION

*Trichomonas vaginalis* is a flagellated protozoan which is the most common microorganism causing sexually transmitted disease. Worldwide *T. vaginalis* causes approximately 174 million new infections per year (12, 15, 19). This infections may be associated with an asymptomatic carrier state or symptoms ranging from acute vaginitis to perinatal complications such as premature rupture of membranes, preterm birth, and postpartum endometritis (6, 17). Recently some studies suggested that trichomoniasis may increase the risk of transmission of human immunodeficiency virus (HIV) (18).

Metronidazole is the major medication in trichomoniasis but some resistant strains to this drug have appeared (8, 10). Thus

efforts oriented to new alternative drugs need to be made in order to control of trichomoniasis.

*Arbutus unedo* (*A. unedo*), grows wild, mainly in maquis and rocky places of the seaboard in Southern Europe. The leaves contain flavonol glycosides (afzelin, juglanin, avicularin, quercitrin, hyperin), phenol glycosides (arbutin, methylarbutin), lipids, tannins and vitamin E (5). In traditional medicine, the herbal teas and preparations of *A. unedo* leaves have been generally used to treat hypertension, anxiety, diarrhoea and hemorrhoids (3). The ethanol and methanol extracts of *A. unedo* leaves were found to show antioxidant activity (14). We aimed to test the *in vitro* activity of the extracts of *A. unedo* leaves against *T. vaginalis* trophozoites in the present study.

## MATERIAL AND METHODS

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

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**Preparation of extract:** Air-dried and powdered leaves of *A. unedo* were extracted sequentially with n-hexane, ethanol and ethyl acetate by percolation at room temperature. The water extract was prepared by infusion at room temperature. The extracts were evaporated to dryness by vacuum and weighed.

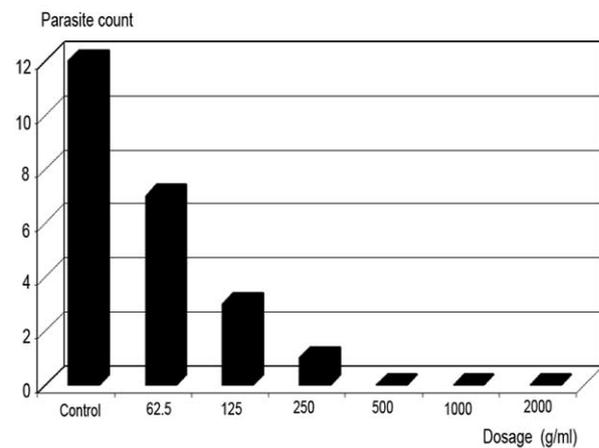
**Assays for trichomonacidal activity:** The antiproliferative activity of extracts versus strain of *T.vaginalis* (ADU-TV5) newly isolated from a woman attending a gynecology clinic in Aydin and maintained in continuous culture in Trypticase-yeast extract-maltose (TYM) medium supplemented with %10 heat inactivated foetal calf serum and penicillin and streptomycin. Test tubes (16X160mm, glass, screwcap tubes) were placed with 5ml medium containing  $1 \times 10^5$  *T.vaginalis* trophozoites per ml. Extracts were diluted with DMSO transferred to the culture tubes to obtain final concentrations of 62.5, 125, 250, 500, 1000, 2000 µg/ml. The highest concentration of DMSO and normal culture tubes were used as negative controls and metronidazole was used as a positive control. All tubes were incubated at 37 °C. After 24 and 48 hours viable *T.vaginalis* trophozoites were identified and counted microscopically with a hemocytometer on the basis of their aspect and motility. The numbers of parasites were compared with the positive and negative controls and percentage of inhibition was calculated for each experiment. The percentage of growth inhibition rate (GI %) was calculated with respect to the growth control as follows: %GI= (1- GR extract/GRcontrol)X100 (13). All tests were carried out in triplicate. All microscopic examinations were performed blindly by two investigators.

**RESULTS**

The DMSO control was found to be inactive at the highest concentration used in this study with similar parasite numbers as normal culture tube. The n-hexane, ethanolic and water extracts of *A. unedo* leaves did not show anti-trichomonacidal activity while the ethyl acetate extract possessed marked anti-trichomonacidal activity. There was a correlation of anti-trichomonacidal efficacy of the ethyl acetate extract with increasing dosage (Figure 1). Growth inhibition rates of the ethyl acetate extract are shown in Table 1. In our experiments no trophozoite was found in the reference tubes at a concentration of 10 µg/ml of metronidazole (Sigma, M-1547).

**Table 1.** Anti-trichomonacidal activity of the extracts from *Arbutus unedo* leaves

<b>Ethyl acetate extract (µg/ml)</b>	62.5	125	250	500	1000	2000
<b>Growth inhibition (%)</b>	50	75	95	100	100	100



**Figure 1.** Anti-trichomonacidal activity of the ethyl acetate extracts from *Arbutus unedo* leaves

**DISCUSSION**

The main drug, metronidazole used for the cure of trichomoniasis has toxic effects and safety in pregnancy is uncertain (19). There is a great need for easily synthesized and low cost therapeutic agents especially in undeveloped and developing countries. Many natural products have already provided valuable clues for potentially antiparasitic compounds (1).

Up to date, there have been many studies about in vitro activity of medicinal plants against *T. vaginalis* (4, 7, 9, 11). In a study which is held in Mexico, antitrichomonal activity is found in Mexican medicinal plants named *Carcia papaya* and *Cocos nucifera* (4). In another study, 29 extract of 18 medicinal plants are investigated and *Scaevola balansae* extract is found to show weak activity against *T. vaginalis* (7). In an Indian phase II clinical trial, all women with *T. vaginalis* infection can be cured by Praneem polyherbal tablets (PPT) (16).

Based on the results obtained in this study we can conclude that preparations made of *A. unedo* leaves are potent inhibitors of the growth of *T.vaginalis*. Additionally n-Hexane and water extracts were found to be toxic while ethanol extract and ethyl acetate extracts did not show toxicity using brine shrimp lethality bioassay (20). Our study provides useful data in order to find new active products against *T.vaginalis*. It may be a promising anti-trichomonacidal agent in the future. Further experiments are needed for isolation of active fractions and identification of the active components of ethyl acetate extract.

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