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The Effect of Hydroalcoholic Extract of *Quercus brantii* and *Artemisia aucheri* Boiss Against *Trichomonas vaginalis In vitro*

Quercus ve Artemisia'nın Trichomonas vaginalis'e Etkisi ve Artemisia aucheri Boiss'in Hidro-Alkolik Ekstraktının Trichomonas vaginalis'e Karşı Terapötik Etkileri

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ABSTRACT

Objective: Trichomoniasis is the most common sexually transmitted protozoan infection worldwide. Metronidazole is widely considered as the drug of choice for treating of trichomoniasis but considering its potential side effects, we aimed to assess the therapeutic influences of hydro-alcoholic extracts of *Quercus brantii* and *Artemisia aucheri* Boiss as alternative medications against *Trichomonas vaginalis* (*T. vaginalis*).

Methods: The trophozoites were cultured in TYI-S-33 medium at a density of $5x10^5$ trophozoites/mL. Subsequently, they were incubated with varying concentrations of the plant extracts (32, 64, 125, 250, 500, and 1,000 µg/mL) and metronidazole (16, 32, 64, 125, 250, and 500 µg/mL), as the positive control. The number of trophozoites in each well plate was quantified after 2, 4, 6, 24, 48, and 72 hours using trypan blue staining. Finally, the viability of the parasite was assessed by vital methylene blue staining.

Results: The hydro-alcoholic extracts of *Q. brantii* and *A. aucheri* Boiss at concentrations of 125, 250, 500, and 1,000 µg/mL demonstrated significant efficacy against the parasite. Our findings indicated that the minimum effective concentrations were 125 µg/mL and hydro-alcoholic extracts of *Q. brantii* and *A. aucheri* Boiss have the ability to effectively eliminate *T. vaginalis* after 48 and 72 hours of treatment.

Conclusion: The findings of the present study showed that hydro-alcoholic extract of *Q. brantii* and *A. aucheri* Boiss can induce death in *T. vaginalis*. However, further complementary *in vivo* studies are needed to assess the components of these plants in the treatment of *T. vaginalis*.

Keywords: Hydro-alcoholic extract, *Quercus brantii*, *Artemisia aucheri* Boiss, *Trichomonas vaginalis*

ÖZ

Amaç: Trichomoniasis, dünya çapında en yaygın cinsel yolla bulaşan protozoa enfeksiyonudur. Metronidazol yaygın olarak trichomoniasis tedavisi için tercih edilen ilaç olarak kabul edilir, ancak potansiyel yan etkileri göz önüne alındığında, *Trichomonas vaginalis*'e karşı alternatif ilaçlar olarak *Quercus brantii* ve *Artemisia aucheri* Boiss'in hidro-alkolik ekstraktlarının terapötik etkilerini değerlendirmeyi amaçladık.

Yöntemler: Trofozoitler, $5x10^5$ trofozoit/mL yoğunlukta TYI-S-33 ortamında kültürlendi. Daha sonra, farklı konsantrasyonlarda bitki ekstraktları (32, 64, 125, 250, 500 ve 1.000 µg/mL) ve metronidazol (16, 32, 64, 125, 250 ve 500 µg/mL) ile inkübe edildiler. pozitif kontrol. Her gözenekli plakadaki trofozoitlerin sayısı, tripan mavisi boyama kullanılarak 2, 4, 6, 24, 48 ve 72 saat sonra ölçüldü. Son olarak, parazitin canlılığı hayati metilen mavisi boyaması ile değerlendirildi.

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Türkiye PARAZİTOLOJİ Dergisi

Bulgular: *Q. brantii* ve *A. aucheri* Boiss'in 125, 250, 500 ve 1.000 ug/mL konsantrasyonlardaki hidro-alkolik özleri, parazite karşı önemli etkinlik gösterdi. Bulgularımız, minimum etkili konsantrasyonların 125 µg/mL olduğunu ve *Q. brantii* ve *A. aucheri* Boiss'in hidro-alkolik ekstraktlarının, 48 ve 72 saatlik tedaviden sonra *T. vaginalis*'i etkili bir şekilde yok etme yeteneğine sahip olduğunu gösterdi.
Sonuç: Bu çalışmanın bulguları, *Q. brantii* ve *A. aucheri* Boiss'in hidro-alkolik ekstraktının *T. vaginalis*'te ölüme neden olabileceğini göstermiştir. Bununla birlikte, *T. vaginalis*'in tedavisinde bu bitkilerin bileşenlerini değerlendirmek için daha fazla tamamlayıcı *in vivo* çalışmalara ihtiyaç vardır.
Anahtar Kelimeler: Hidro-alkolik özü, *Quercus brantii*, *Artemisia aucheri* Boiss, *Trichomonas vaginalis*

INTRODUCTION

Trichomoniasis is caused by *Trichomonas vaginalis* (*T. vaginalis*) and is considered the most common sexually transmitted protozoan infection worldwide (1,2). The prevalence of trichomoniasis varies among different countries and is influenced by factors such as cultural and socioeconomic status, as well as access to healthcare (2,3). It is estimated that 5% to 10% of women, equivalent to approximately 276 million cases annually, are affected in the overall female population worldwide (4). The prevalence of trichomonas infection has been reported more in sexual transmitted disease clinics to range from 25% to 36% (5). In Iran, the prevalence rate of this disease has been observed to range from 2% to 8%, with the possibility of reaching up to 42.9% percent in high-risk areas (2,6). These findings highlight the varying levels of trichomoniasis occurrence within the country. The infection can manifest with a wide range of signs, varying from asymptomatic states to severe inflammation. In women, clinical symptoms may include urinary discomfort, redness, itching, vaginal discharge, abdominal pain, and genital soreness. Among men, trichomoniasis typically affects the urethra and may cause symptoms such as burning sensation after ejaculation and urination, as well as a discharge from the penis. It is important to note that trichomoniasis can persist for an extended period, even years, if left untreated (7-9). The nitroimidazoles, including tinidazole and metronidazole, are the sole class of drugs known to possess efficacy against this parasitic infection as confirmed by the Food and Drug Administration (10). Despite being considered the first-line treatment for this disease (11), long-term use of metronidazole can result in treatment failure due to the emergence of drug-resistant strains of the protozoan. Furthermore, metronidazole is associated with several adverse effects, including nausea, dizziness, hypersensitivity reactions, and dermatological disorder manifestations (12). Therefore, it is necessary to explore alternative pharmaceutical options for the treatment of this infection. to fulfill this objective some plants with pharmacological potential were examined (13). *Artemisia aucheri* Boiss, a native plant in Iran, is commonly utilized in traditional medicine as an antiparasitic agent. Notably, this plant possesses artemisinin, a compound known for its antiparasitic properties (14).

*Quercus brantii***,** also known as west oak or zagros oak grows in western Iran. Decocted extract of this plant is used to treat diarrhea, inflammation and burns and it also has anibacterial properties (15-18). The aim of this study was to evaluate the therapeutic effects of hydro-alcoholic extract of *Q. brantii* and *A. aucheri* Boiss against *T. vaginalis*.

METHODS

Ethics Approval

The protocol of the present study was reviewed and approved by the Ethics Committee of the Jundishapur University of Medical Sciences (approval no: IR.AJUMS.REC.1400.536). It should be noted that since this study was conducted *in vitro*, it does not involve any patients or human subjects.

Preparation of hydro-alcoholic extracts

The fruits of *Q. brantii* and leaves of *A. aucheri* Boiss were obtained from a reputable herbal shop located in Khuzestan province, Ahvaz, Iran. The botanical identification of both plants was confirmed by Prof. Amir Siahpoosh at the Medicinal Plant Research Center, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran, and verified as *Quercus brantii* and *Artemisia aucheri* Boiss species. The *Quercus brantii* voucher specimen has been assigned code number A2116401021 and the *Artemisia aucheri* Boiss voucher specimen has been assigned code number A2111101012. Following the drying process, the plants were pulverized using an electric mill and subsequently stored in refrigerator until extraction time. The hydro-alcoholic extract was prepared by dissolving 100 g of the plant powder in 1,000 mL of 50% ethanol solution. In the subsequent step, the solution was mixed for 72 hours at a temperature of 25 °C using an electrical sieve shaker. Subsequently, the solution was filtered through Whatman's filter paper and placed in an incubator at 37 °C to allow for solvent evaporation. The hydro-alcoholic extracts were then stored at -20 °C until use. Finally, various concentrations of the extracts (32, 64, 125, 250, 500, and 1,000 µg/mL) were prepared in a dimethyl sulfoxide (DMSO) solution (19). *T. vaginalis* isolates were obtained from Isfahan University of Medical Sciences, Isfahan, Iran. In the following step, the *T. vaginalis* isolates were cultured in a TYI-S-33 medium consisting of trypticase-yeast extract-maltose (pH=6,8), 10% Fetal Calf Serum, 100 U/mL streptomycin and penicillin, and a vitamin mixture, at a temperature of 37 °C. Typically, this culture medium maintained a concentration of approximately $5x10^5$ cells/mL within 48 hours (11).

Treatment and cytotoxicity assay

A density of $5x10^5$ trophozoites/mL was incubated in triplicate in the 24-well plate at 37 °C with different concentrations of the plant extracts (32, 64, 125, 250, 500, and 1,000 µg/mL). Trichomonas trophozoites were also incubated in the presence of metronidazole (16, 32, 64, 125, 250, and 500 µg/mL) as the positive control, and culture media without any treatment served as the negative control. In addition, the viability of trophozoites was assessed at different time intervals ranging from 2 to 72 hours using by vital trypan blue staining. The IC50 values for the plant extracts were also determined.

Statistical Analysis

Data analysis was carried out using GraphPad Prism and SPSS software. Group comparisons were performed using the one-way ANOVA test and $P<0,05$ was considered for significant difference.

RESULTS

The effect of the various concentrations of hydro-alcoholic extract of *Q. brantii* and *A. aucheri* Boiss against *T. vaginalis* was assessed after 2, 4, 6, 24, 48, and 72 hours of treatment, respectively (Table 1 and 2). The hydro-alcoholic extract of *Q. brantii* and *A. aucheri* Boiss at concentrations of 125, 250, 500, and 1,000 µg/mL exhibited significant efficacy against the parasite. The most promising results were observed at concentrations of 500 and 1,000 µg/mL after 48 and 72 hours of treatment. However, all concentrations (16,32,64,125,250, and 500 µg/mL) of metronidazole demonstrated complete cytotoxicity against *T. vaginalis* after 24 hours of treatment (Table 3). In addition, the effect of hydro-alcoholic extract of *Q. brantii* and *A. aucheri* Boiss (1,000 µg/mL) against *T. vaginalis* at different time points (2, 4, 6, 24, 48, and 72 h) was compared to that of metronidazole (500 µg/mL), which served as the positive control. The comparison of their effects is illustrated in Figure 1. It is notable to mention that the concentrations of 500 and 1,000 µg/mL of both plants exhibited comparable efficacy to metronidazole after 48 and 72 h of treatment. Figure 2 and 3 present a comparison of the effects of hydro-alcoholic extract of *Q. brantii* and *A. aucheri* Boiss at concentrations of 32, 64, 125, 250, 500, and 1,000 µg/mL against *T. vaginalis* over the course of 2, 4, 6, 24, 48, and 72 hours. Notably, the most favorable outcome was observed at 48 and 72 hours with a concentration of 500 and 1,000 µg/mL. Based on the data presented in Table 4, the IC50 values of *A. aucheri* Boiss were determined to be 315.6, 144.5, and 22.19 µg/mL after 24, 48, and 72 hours, respectively. In comparison, the IC50 values of *Q. brantii* were found to be 321.7, 255.1, and 37,6 µg/mL after the same time intervals. Notably, no significant difference was observed between the hydro-alcoholic extracts of the two plants ($p>0.05$). However, statistical analysis indicated a significant difference in the IC50 values of the extracts at different time points.

DISCUSSION

Since the 1960s, metronidazole has been widely used as the main treatment for trichomoniasis (21). However, this drug has been associated with certain drawbacks. It has been found to exhibit mutagenic effects in bacteria and has shown carcinogenic potential in mice and rats. Additionally, prolonged

Table 1. Mortality rates of *T. vaginalis* exposed to different concentrations of the *Q. brantii* at different times

Concentrarion (µg/mL)	2 hrs	4 hrs	6 hrs	24 hrs	48 hrs	72 hrs
1,000	22.2	45	58.1	100	100	100
500	10	19.4	28.3	77.7	98	100
250	0.0	9.0	14.5	33.2	44.6	91.2
125	0.0	0.0	10.0	12.4	28.5	89.3
62	0.0	0.0	0.0	4.5	12.6	82.4
32	0.0	0.0	0.0	1.3	9.5	61.5

Table 2. Mortality rates of *T. vaginalis*. Exposed to different concentrations of the *A. aucheri* Boiss at different times

Concentration (µg/mL)	2 hrs	4 hrs	6 hrs	24 hrs	48 hrs	72 hrs
1,000	26.1	48.2	65.0	95.7	100	100
500	12.3	22.4	35.5	79.2	100	100
250	0.0	11.3	15.3	40.0	86.5	98.2
125	0.0	0.0	11.2	15.3	28.5	98.2
62	0.0	0.0	0.0	8.7	24.2	83.4
32	0.0	0.0	0.0	4.16	21.5	72.1

Table 3. Mortality rates of *T. vaginalis* exposed to different concentrations of the metronidazole at different times.

Concentration (µg/mL)	2 hrs	4 hrs	6 hrs	24 hrs
500	61	79	90	100
250	37	54	71,5	100
125	15	38	60	100
64	4	24	53	100
32	0,0	15	44	100
16	0,0	8	38	100

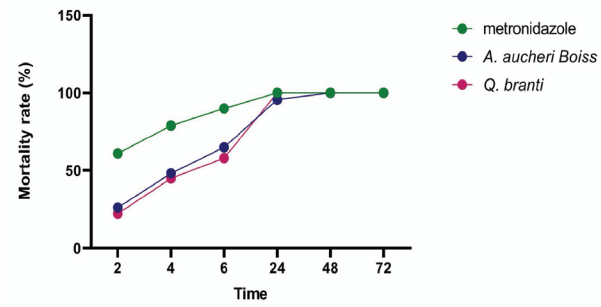

Figure 1. Mortality rate (%) of *T. vaginalis* vs. comparison of the logarithm of two extracts plants *A. aucheri* Boiss and *Q. brantii* against of concentration 1,000 µg/mL and metronidazole of concentration 500 µg/mL

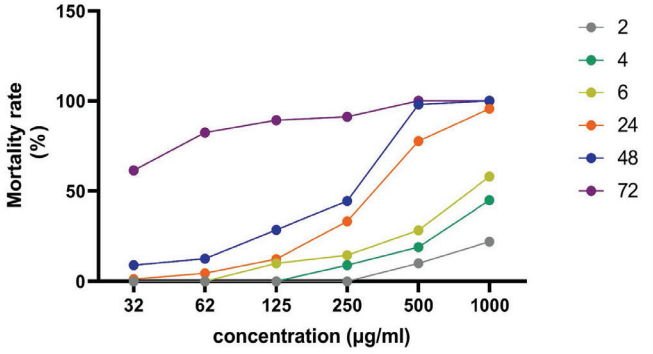

Figure 2. Mortality rate (%) of *T. vaginalis* vs. logarithm (Log) of concentration of *A. aucheri* Boiss in six times, 2, 4, 6, 24, 48 and, 72 h

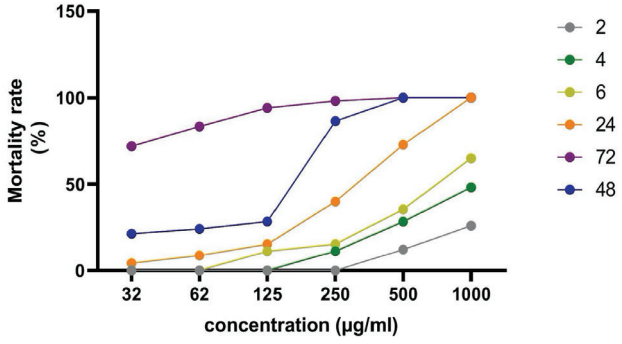

Figure 3. Mortality rate (%) of *T. vaginalis* vs. logarithm (Log) of concentration of *Q. brantii* in six times, 2, 4, 6, 24, 48 and, 72 h

Table 4. The IC50 values determined for extracts of *A. aucheri* Boiss and *Q. brantii* against *T. vaginalis*

Extract	Ic50 (µg/mL)		
	24 h	48 h	72 h
Aucheri Boiss	315.6	144.5	22.19
Quercus brantii	321.7	255.1	37.62

use of metronidazole has been linked to the development of metronidazole-resistant *T. vaginalis* infections (22) and can result in adverse effects such as nausea, dizziness, hypersensitivity reactions, and dermatological symptoms (12). It should also be avoided during pregnancy, particularly in the first trimester, due to potential risks and safety concerns (22). Therefore, Alternative drugs, such as herbal medications, are deemed necessary for the treatment of the infection (22). Certain plants have the potential to serve as sources of new antiparasitic and antiprotozoal drugs, offering advantages such as lower cost, reduced toxicity, and potent activity (23). *Q. brantii* is a native plant found in western Iran, and has a long-standing use in traditional medicine for treatment of diarrhea, inflammation, burns, and possess antibacterial properties (17,18). Similarly, *A. aucheri* Boiss, another native plant in Iran, is commonly employed in traditional medicine as an antiparasitic agent. Notably, this plant contains artemisinin, a compound known for its antiparasitic properties (14). Based on the mentioned considerations, the primary objective of this study was to assess the therapeutic effects of hydro-alcoholic extracts derived from *Q. brantii* and *A. aucheri* Boiss against *T. vaginalis* infection in an *in vitro* assay. The results of the present study showed that the concentrations of 500 and 1,000 µg/mL of hydro-alcoholic extract of *Q. brantii* and *A. aucheri* Boiss have comparable efficacy to metronidazole after 48 and 72 hours of treatment. Both extracts of *Q. brantii* and *A. aucheri* Boiss demonstrated time-dependent dose-dependent inhibition of *T. vaginalis* growth after 24, 48, and 72 hours. Notably, *A. aucheri* Boiss extract displayed higher efficacy against *T. vaginalis* trophozoites. The current study represents the first investigation conducted on the hydro-alcoholic extract of *Q. brantii* and *A. aucheri* Boiss. In alignment with our investigations, several previous studies have examined the efficacy of herbal medicines in treating this particular parasite. In Iran, *Lavandulifolia Stachys* (24), *Eucalyptus* (25), *Myrtus, Zataria multiflora*, and *Artemisia* (26) have been the subject of studies investigating their efficacy in treating trichomoniasis. Similarly, in different parts of the world, researches have focused on the use of other plants such as *Harungana madagascariensis*, *Polygala decumbens*, *Haplophyllum myrtifolium*, and *Rheum rhabarbarum* for the treatment of this infection (27-30). In previous investigations, *A. aucheri* Boiss has been shown to possess antiparasitic (14), antimalarial (31), and *leishmanicidal* (32) properties. The essential oil of *A. aucheri* Boiss is composed of compounds such as trans-verbenol (8.1%), 1.8-cineole (8.3%), camphor (21.0%), and verbenone (21.5%) (14), its major constituents include mesitylene (7.41%), α-pinene (8.33%), chrysanthenone (18.16%), and 1.8-cineole (22.8%) (14). Furthermore, the plant contains artemisinin, a component known for its antiparasitic properties (14). On the other hand, *Q. brantii* is known for its antibacterial properties (17,18) and contains various constituents such as sugars, oils, pentosan, amydone, quercetin, and tannin (33). The beneficial effects of this plant are attributed to its secondary metabolites, including antioxidants, phenols, flavonolic compounds, and flavonoids (34).

Study Limitations

Primary strength of our research was the evaluation of novel plants, however it is important to mention the limitations of this study such as the absence of a detailed analysis on the components of these plants and the signaling pathways related to their antiparasitic effects Due to the above-mentioned points, it is recommended that future researchers isolate the components of these plants and separately evaluate their effectiveness in the treatment of *T. vaginalis* infection in an *in vitro* assay.

CONCLUSION

Based on our results, the concentrations of 500 and 1,000 µg/mL of hydro-alcoholic extract of *Q. brantii* and *A. aucheri* Boiss had ability to kill *T. vaginalis* after 48 and 72 h treatment. Therefore, the use of medicine plants can be considered as an alternative treatment for *Trichomoniasis*. However, more complementary investigations are needed to evaluate the specific components of these plants that are effective in the treatment of *T. vaginalis*.

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* Ethics

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* Authorship Contributions

Concept: M.R., Design: M.R., Data Collection or Processing: S.T.R.,H.A.Y., Analysis or Interpretation: S.T.R., H.A.Y., Literature Search: M.R., A.H., Writing: M.F.K., A.H., A.Z.

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