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Original Article

In Vitro Evaluation of the Effects of *Pelargonium quercetorum* Agnew Extracts on *Trichomonas vaginalis*

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Abstract

Background: *Trichomonas* infection is a common sexually transmitted infection, and concerns about drug resistance to this infection are increasing. The aim of this study was to evaluate the in vitro activity of *Pelargonium quercetorum* extracts on *Trichomonas* protozoans.

Methods: One isolate of *Trichomonas vaginalis* was subjected to susceptibility testing against ethyl acetate, n-hexane, and aqueous extracts of *P. quercetorum* using the microtiter plate method. The minimum lethal concentration (MLC) of the extracts was measured in comparison with metronidazole under aerobic conditions.

Results: All extracts had antiprotozoal activities against *Trichomonas*. After 48-hour exposure, the most antitrichomonal activity of the extracts belonged to the ethyl acetate, with an MLC of 250 µg/mL, followed by the n-hexane (500 µg/mL) and aqueous (25 mg/mL) extracts in comparison with metronidazole, with an MLC of 3.1 µg/mL.

Conclusion: The results of this study indicated that *P. quercetorum* has potential properties against *Trichomonas* protozoans, although further studies are needed to evaluate the antiprotozoal activity of its components.

Keywords: *Trichomonas vaginalis*, *Trichomonas* infections, *Pelargonium quercetorum*, Plant extracts

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Introduction

Trichomonas vaginalis is a flagellated protozoan parasite that causes trichomoniasis, one of the most common non-viral sexually transmitted infections in the world. According to the estimate of the World Health Organization (WHO) in 2016, out of 376.4 million cases of sexual infection in the world, 156 million were related to trichomoniasis, and its prevalence in women (5.3%) was higher than that in men (0.6%) (1). In Iran, the prevalence of this infection is on average 8%, and it has been reported to be more than 38% in some special social groups (2). Symptomatic trichomoniasis is often observed in women and is accompanied by vaginitis and symptoms such as foamy and foul-smelling secretions, itching, burning, and dyspareunia. Trichomoniasis in pregnancy may be associated with adverse outcomes, particularly early rupture of the uterine membrane, preterm delivery, and low birth weight. Male trichomoniasis is often asymptomatic and rarely leads to symptomatic infection, but urethritis may be observed in male patients (3,4).

Metronidazole has been the drug of choice for the treatment of trichomoniasis since 1961, and in addition to having side effects, cases of treatment failure with this drug have been reported as well. According to the Centers for Disease Control and Prevention, 2%–5% of clinically isolated *Trichomonas* have some levels of resistance to metronidazole in the United States (5,6). In addition, the use of metronidazole in some patients is associated with intolerance and side effects, and its administration is prohibited in the first three months of pregnancy (7). Herbs have long been widely used for the treatment of many diseases, and today, they are considered primary sources of many synthetic drugs. The genus *Pelargonium* belongs to the Geraniaceae family, with more than 220 species in the world. *P. quercetorum* Agnew, with the local name “gala revaci”, is native to the Kurdistan region of western Iran. Traditionally, the aerial parts of this herb

in crude or baked forms are used as antiparasitic agents to deworm intestinal parasites in indigenous people. Other biological effects of this plant include antioxidant, antimicrobial, and anticancer activities. Further, some bioactive compounds have been recognized in the essential oil of *P. quercetorum*, including α-pinene, α-fenchyl acetate, limonene, and trans-β-caryophyllene (8,9).

Therefore, according to the evidence related to the antiparasitic properties of *P. quercetorum*, this study aimed to investigate the effectiveness of the *P. quercetorum* extract on the growth of the *T. vaginalis* parasite in vitro.

Materials and Methods

Plant, Parasite Isolates, and Chemicals

The flowers, leaves, and stems of *P. quercetorum* were collected from Kurdistan province, west of Iran. The plant was confirmed by a botanist and dried in the shade. One isolate of *T. vaginalis* (TVH13 strain) was obtained from the Parasitology Research Laboratory of Hamedan University of Medical Sciences. Metronidazole (M3761) and dimethyl sulfoxide (D2650, BioReagent) were purchased from Sigma-Aldrich Chemical Company (St. Louis, MO, USA). Ethyl acetate (100863), n-hexane (100795), and other chemicals were purchased from Merck (Darmstadt, Germany).

Preparation of Extracts

The dried aerial parts of *P. quercetorum* were powdered and subjected to extraction by the maceration method. Briefly, the powder (100 g) was macerated in distilled water, ethyl acetate, and n-hexane (3 × 1 L, RT for 72 hours). The mixtures were filtered by Whatman filter paper (No. 40) and evaporated by a rotary evaporator under a vacuum at temperatures below 40 ˚C. Then, the extracts were preserved in a dark, airtight container at 4 ˚C until use (10).

Trichomonas vaginalis Culture and Susceptibility Assay

Trichomonas vaginalis trophozoites were grown axenically in the TYI-S-33 medium and used for susceptibility testing in the log/exponential growth phase (11). Metronidazole and the aqueous extract were dissolved in phosphate-buffered saline, and the other extracts were dissolved in dimethyl sulfoxide. The sensitivity was measured based on the 96-well microtiter plate method. Metronidazole and the extracts were diluted in the culture medium by successive dilutions. Next, 100 µL of the *Trichomonas* culture (1×10^5 cells/mL) was added to the wells until obtaining the desired concentrations. Then, the plates were aerobically incubated at 35.5 ˚C for 12, 24, and 48 hours. The plates were examined microscopically to determine the minimum lethal concentration (MLC) and growth inhibitory percentage (GI%). Experiments were performed in sterile conditions and against control groups in duplicate and repeated three times.

Statistical Analysis

Data were reported as MLC, GI%, and half-maximal inhibitory concentration (IC_{50}) values. The data were analyzed by SPSS statistical software (version 16) using a one-way ANOVA test, and a P value of < 0.05 was used to determine the level of significance.

Results

The susceptibility assay showed that the extracts of *P. quercetorum* had potent activity against *Trichomonas* cells. The activities of the extracts were dependent on time and concentration. The highest activity was observed after 48 hours of exposure, with MLCs of 250 µg/mL, 500 µg/mL, and 25 mg/mL for the ethyl acetate, n-hexane, and aqueous extract, respectively ($P < 0.001$). At sublethal concentrations (sub-MLCs), the extracts were able to inhibit the growth of the *Trichomonas* cells (Table 1). The IC_{50} values were calculated by non-linear regression analysis processed on dose–response curves using GraphPad Prism (version 8) software. The IC_{50} of the ethyl acetate extract ($IC_{50} = 89$ µg/mL) was lower than that of the n-hexane ($IC_{50} = 130$ µg/mL) and aqueous ($IC_{50} = 2.3$ mg/mL) extracts (Figure 1). According to drug susceptibility testing, the *Trichomonas* strain was sensitive to metronidazole with an MLC of 3.1 µg/mL after an incubation time of 48 hours (Table 2).

Discussion

Increasing antimicrobial resistance as an important and worrying health problem has continuously occupied the minds of researchers. Although the issue of drug resistance in parasitic infections is less important than that in bacterial infections, it is of great health importance in protozoal infections such as malaria and leishmaniasis (12). Since 1962, cases of metronidazole-resistant trichomoniasis have been reported, which are constantly increasing, and some reports have estimated the rate of metronidazole-resistant trichomoniasis in the United States to be up to 10% (13). Other nitroimidazole derivatives are also effective against *Trichomonas vaginalis*, and tinidazole has been licensed for the treatment of trichomoniasis in the United States, but cross-resistance has been reported due to the similar mechanism of this group (14).

The results of the present study, which was conducted on the antiprotozoal properties of *P. quercetorum*, demonstrated that this medicinal plant has antitrichomonal activity and is able to inhibit the growth of *T. vaginalis* trophozoites. The effects of this plant depended on the concentration and exposure time. The different extracts of *P. quercetorum* were able to kill all exposed parasites from a concentration of 250 µg/mL (the ethyl acetate extract) to 25 mg/mL (the aqueous extract). Moreover, at lower concentrations, they could inhibit the growth of the parasite. The most antitrichomonal activity was found in the ethyl acetate and n-hexane extracts. This is probably due to the higher solubility of the antiprotozoal bioactive compounds of *P. quercetorum* in non-polar

Table 1. Efficacy of Different Extracts of *Pelargonium quercetorum* Agnew on *Trichomonas vaginalis* Cells

Incubation Time	Growth Inhibition Percentage (Mean ± SD) at Different Concentrations						
Ethyl Acetate Extract							
	15.6 (µg/mL)	**31.2 (µg/mL)**	**62.5 (µg/mL)**	**125 (µg/ml)**	**250 (µg/mL)**	**500 (µg/mL)**	**1000 (µg/mL)**
12 hours	2.4 ± 7.6	6.3 ± 5.5	13.2 ± 6.3	35.9 ± 4.1	75.5 ± 3.3	100 ± 0.0 [a]	100 ± 0.0
24 hours	5.5 ± 5.8	19.2 ± 6.3	30.3 ± 7.5	56.5 ± 8.1	81.9 ± 6.4	100 ± 0.0 [a]	100 ± 0.0
48 hours	3.2 ± 6.5	17.3 ± 4.7	25.3 ± 5.2	68.1 ± 4.3	100 ± 0.0 [a]	100 ± 0.0	100 ± 0.0
n-Hexane Extract							
	15.6 (µg/mL)	**31.2 (µg/mL)**	**62.5 (µg/mL)**	**125 (µg/ml)**	**250 (µg/mL)**	**500 (µg/mL)**	**1000 (µg/mL)**
12 hours	1.2 ± 5.2	5.3 ± 6.5	10.4 ± 8.3	32.9 ± 5.2	58.8 ± 6.7	100 ± 0.0 [a]	100 ± 0.0
24 hours	4.5 ± 4.9	8.1 ± 5.1	20.4 ± 3.1	38.8 ± 4.8	65.9 ± 3.2	100 ± 0.0 [a]	100 ± 0.0
48 hours	7.1 ± 6.2	13.4 ± 3.2	23.5 ± 6.8	44.9 ± 5.1	71.6 ± 4.4	100 ± 0.0 [a]	100 ± 0.0
Aqueous Extract							
	0.8 (mg/mL)	**1.6 (mg/mL)**	**3.1 (mg/mL)**	**6.2 (mg/ml)**	**12.5 (mg/mL)**	**25 (mg/mL)**	**50 (mg/mL)**
12 hours	1.5 ± 8.2	7.5 ± 1.2	21.4 ± 2.2	31.2 ± 7.4	85.6 ± 4.4	100 ± 0.0 [a]	100 ± 0.0
24 hours	5.6 ± 4.7	21.3 ± 5.1	31.9 ± 4.5	77.6 ± 4.9	91.6 ± 4.8	100 ± 0.0 [a]	100 ± 0.0
48 hours	9.6 ± 3.7	31.6 ± 3.4	65.6 ± 3.6	91.7 ± 3.3	98.8 ± 5.1	100 ± 0.0 [a]	100 ± 0.0

Note. SD: Standard deviation. [a] Minimum lethal concentration is associated with the lowest concentration of the extract that kills all trichomonas cells.

Table 2. Efficacy of Metronidazole on *Trichomonas vaginalis* Cells

Incubation Time	Growth Inhibition Percentage (Mean ± SD) at Different Concentrations of Metronidazole									
	0.2 (µg/mL)	**0.4 (µg/mL)**	**0.8 (µg/mL)**	**1.6 (µg/mL)**	**3.1 (µg/mL)**	**6.2 (µg/mL)**	**12.5 (µg/mL)**	**25 (µg/mL)**	**50 (µg/mL)**	**100 (µg/mL)**
12 hours	5.8 ± 3.7	18.3 ± 4.5	37.9 ± 5.1	58.2 ± 8.1	63.6 ± 5.3	76.5 ± 6.3	89.2 ± 4.5	100 ± 0.0 [a]	100.0 ± 0.0	100.0 ± 0.0
24 hours	11.3 ± 5.7	31.8 ± 2.6	54.0 ± 4.3	71.6 ± 2.3	84.6 ± 2.5	92.4 ± 1.6	100 ± 0.0 [a]	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0
48 hours	23.9 ± 4.8	45.0 ± 2.6	74.4 ± 2.5	91.4 ± 3.3	100 ± 0.0 [a]	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0

Note. SD: Standard deviation. [a] Minimum lethal concentration is related to the lowest concentration of metronidazole that kills all trichomonas cells.

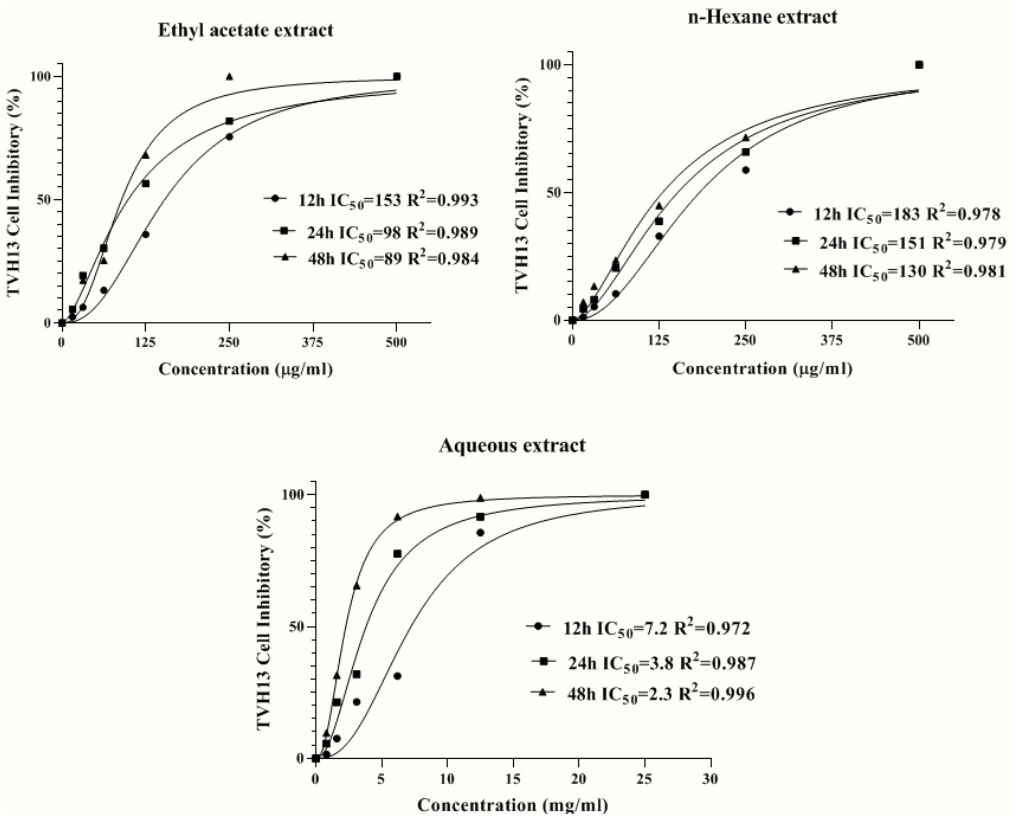

Figure 1. Percentage of Growth Inhibition of *Trichomonas* Cells (TVH13 Strain) at Different Concentrations of the *Pelargonium quercetorum* Extracts and 12, 24, and 48 Hours of Exposure

extraction solvents. Nonetheless, phenolic and flavonoid compounds are the most abundant phytochemicals that have been extracted by polar extraction solvents from *P. quercetorum* (15).

To the best of our knowledge, only one study has so far been conducted on the antiprotozoal activity of *P. quercetorum*. In their study, Maroufi et al investigated the antileishmanial activity of *P. quercetorum*. They found that the aqueous extract of *P. quercetorum* leaves could reduce the number of promastigotes and amastigotes in vitro, although this reduction was not dose-dependent. After 24 hours of exposure, the highest growth inhibition effect was observed at a concentration of 10 000 µg/mL. However, the growth inhibition effect at the concentration of 1000 µg/mL was greater than 5000 µg/mL. In addition, the IC_{50} of the aqueous extract of *P. quercetorum* was reported at 1508.99 µg/mL, indicating a greater effect on *Leishmania* than on *Trichomonas* (8).

So far, a significant number of studies have investigated the antitrichomonal effects of medicinal plants. Most plant species that have antitrichomonal activity belong to the three families of Asteraceae, Lamiaceae, and Myrtaceae (16). One of the most effective medicinal plants against *Trichomonas* is avocado (*Persea americana*). In the study by Jiménez-Arellanes, chloroform and ethanolic extracts of avocado seeds were effective against *Trichomonas*, with an IC_{50} of 0.524 and 0.533 µg/mL, respectively, compared to metronidazole at a concentration of 0.037 µg/mL after 72 hours of incubation (17). Another effective herb against the parasite is the Persian shallot (*Allium hirtifolium* Boiss). The MIC of Persian shallot after 48 hours of exposure was reported to be 10 µg/mL and 5 µg/mL for the hydroalcoholic and dichloromethane extracts, respectively. Probably, the anti-trichomonal properties of *A. hirtifolium* are related to organic sulfur compounds such as allicin, ajoene, diallyl sulfides, and alliin (18).

Satureja khuzestanica, *Marrubium vulgare*, and *Foeniculum vulgare* can be mentioned among other plants whose antimicrobial properties have been evaluated on *Trichomonas*. *S. khuzestanica*, with the Persian name "*Marzeh Khuzestani*", belongs to the Lamiaceae family. After 48 hours, the essential oil and n-hexane extract were the most potent natural products extracted from *Marzeh Khuzestani*, with an MLC of 200 µg/mL. Carvacrol, a phenolic monoterpenoid, is the main compound of *S. khuzestanica*, which has strong antitrichomonal activity (19). *M. vulgare*, another member of the Lamiaceae family, was investigated in another study. In this study, the average MIC of the essential oil, methanol, ethyl acetate, and n-hexane extract was 145 µg/mL, 500 µg/mL, 375 µg/mL, and 716 µg/mL, respectively (20). *F. vulgare* from the Apiaceae family, known as fennel and with the Persian name *Razianeh*, has a number of phytochemicals such as trans-anthole, coumarins, and sesquiterpenoids. According to the available data, the methanol and n-hexane extracts of *Razianeh* (MLC = 400 µg/mL) had more activity than the essential oil (MLC = 1600 µg/mL) on *Trichomonas* cells after 48 hours (21). These data confirm that the polar extracts of *S. khuzestanica*, *M. vulgare*, and *F. vulgare*, such as *P. quercetorum*, have an inhibitory effect on *Trichomonas* growth in laboratory conditions, although the effect of *S. khuzestanica* seems to be greater than others.

Conclusion

The results of the present study revealed that the extracted products from *P. quercetorum* have bioactive components with potential activities against *Trichomonas* protozoans, although further studies are required to analyze the antiprotozoal activity of its components.

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Authors' Contribution

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Visualization: Mohammad Matini, Dara Dastan, Mohammad Fallah.
Supervision: Mohammad Matini, Dara Dastan, Mohammad Fallah.
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Writing–review & editing: Mohammad Matini, Dara Dastan, Mohammad Fallah.

Competing Interests

The authors declare no conflict of interests.

Ethical Approval

This study was approved by the Research Ethics Committee of Hamadan University of Medical Sciences (Ethics code: IR.UMSHA.REC.1401.001).

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