Effect of Chaerophyllum macropodum extracts on Trichomonas vaginalis in vitro

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Introduction

Trichomonas vaginalis (T. vaginalis) is a protozoan parasite causing trichomonia or trichomonal vaginitis. The infection is considered as non-viral sexually transmitted disease (STD). Metronidazole and Tinidazole are now the drugs of choice for the treatment of this infection. However, resistant to these drugs has also been reported. So it is necessary to search for effective alternative drugs with fewer side effects. Chaerophyllum macropodum (C. macropodum) plant have been used against some parasites. Therefore, in this study the effects of different extracts of this plant on T. vaginalis in culture media have been investigated.

Methods: In this experimental study hydro-ethanol extracts of C. macropodum leaves were prepared. Anti-T. vaginalis activities of the extracts were tested in concentrations of 2, 4, 8, 16, 32, 40, 50, 60, 80, 100 and 150 mg/ml following 24, 48 and 72 hours of incubation of cultured media.

Results: All extract concentrations showed some degrees of growth inhibition activity on T. vaginalis. However crude extract was more efficient.

Conclusion: C. macropodum showed an anti-T. vaginalis activity. More investigations are recommended to use this plant as an antiparasitic drug.

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A B S T R A C T

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Implication for health policy/practice/research/medical education:
The results of this study showed that the different extracts of C. macropodum with different concentrations have anti-trichomonal activity, and might be used in human.

Chaerophyllum plant and Iran is home for two species of them. It is reported that the essence of *C. macropodum* possesses notable antibiotic potentials. Furthermore, the anti-oxidant activity of the plant has also been documented (13). Given that the anti-bacterial effects of *C. macropodum* has been documented on the plenty of bacterial isolates, the present study aimed to evaluate the anti-microbial effect of the extract of the plant on *T. vaginalis*.

### Material and Methods

#### Plant collection
In this experimental study, shoots of *C. macropodum* were collected from Kohgiluyeh and Boyer-ahmad province, south-west of Iran. Species identification and authentication were done in the botany section of Yasuj University, Yasuj, Iran and a plat specimen was deposited there.

#### Preparation of water extract of the plant
The shoots of the collected plant were dried in shade and grinded in powder form. The water extract of the plant was performed by maceration of 50 g of dried plant's powder in 500 ml deionized sterile water in room temperature for 24 hours. Then the macerated material cleared through Buchner funnel.

#### Preparation of alcohol extract of the plant
The alcohol extract of the plant was prepared by maceration of 370 g of dried powder of plant's shoots in absolute methanol four a week at room temperature. The materials were filtered by Buchner funnel. Rotary evaporator was used for evaporation of the solvent. Then, fractions prepared using invert phase chromatography. The crud water and ethanol extracts, with different ratios were added to the invert phase column and 20%, 60% and 95% ethanol-water fractions were yielded. In this study 20% ethanol-water fraction was used for experimentation of parasite growth inhibition.

#### Microorganism preparation
*T. vaginalis* strains isolated from vaginal discharge of women with trichomonal vaginitis referred to the health care centers of Shahrekord city. The isolates were cultured in TYIS33 media and kept in Parasitology Research Laboratory in Isfahan University of Medical Sciences until the examination. *T. vaginalis* cells were collected from logarithmic growth phase and their count number were estimated using hemocytometer slide. Finally, a count number of 1×10⁵/ml cells were used for antimicrobial effect of *C. macropodum*.

#### Evaluation of the anti-trichomonal effect of the extract in vitro
For evaluation of the anti-trichomonal effect of the *C. macropodum* extract concentrations of 2, 4, 8, 16, 32, 40, 50, 60, 80, 100 and 150 mg/ml were disposed in distilled sterile water and added to the microtubes. Metronidazole (100 mg/ml) and distilled sterile water were used as positive and negative controls, respectively. Approximately 10⁶ *T. vaginalis* added to each TYIS33 media, which were containing the prepared concentrations of the extract. The media were incubated at 37 °C for 72 hours. For evaluation of the count of live parasites in different times, samples were taken from the incubated media at 6, 24, 48 and 72 hours after incubation initiated. In every sample in each time *T. vaginalis* live cells were counted using hemocytometer slide. The active parasites and those with moving flagellum were considered as live cells. The Growth Inhibitory Percent (GI%) were calculated and reported using the following formula; GI% = a–b/a×100. In the formula, a; stands for average of live parasites in positive control tube and b; stands for the average live parasite count in test tube.

Data were analyzed by SPSS (version 16.2, SPSS Inc., Chicago, IL, USA) using descriptive tests.

### Results
The growth inhibitory effect of the crude extract of *C. macropodum* was 98% and 100% in the concentration of 20 mg/ml after 24 hours and 48 hours of incubation, respectively (Figure 1). The 80 mg/ml concentration of water extract of the plant had 95% and 99% of growth inhibitory effect in 24 hours and 48 hours of incubation, respectively, but concentration of 20 mg/ml showed only 63.5% of growth inhibition after 24 hours (Figure 2). The 20 and 150 mg/ml ethanol-water extract of the *C.
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Figure 3. The inhibitory effect of the ethanol-water extract (20-80) of the C. macropodum on T. vaginalis growth in different concentrations and incubation times.

macropodum showed 90% and 93.5% of growth inhibitory effect after 24 and 48 hours of incubation, respectively (Figure 3).

Discussion
In this study the effect of C. macropodum extract on T. vaginalis growth was evaluated. The result showed that after 24 hours of incubation of the parasite with the crude, water and ethanol-water extracts with 8, 60 and 20 mg/ml of concentrations or more, respectively had higher than 90% of growth inhibition. According to the results, the crude extract of the plant showed highest growth inhibitory effect on T. vaginalis with lowest concentration in the 24 and 48 hours of incubation comparing to the controls. So, it would be clear that the crude extract of the C. macropodum possesses the highest inhibitory effect on the growth on T. vaginalis.

During the history, mankind have used the plants for therapeutic and pain relief purposes. In the recent decades plenty of studies carried out in order to select and extract the herbal components for therapeutic ends. The therapeutic effects of some plants on T. vaginalis infection, which is a non-viral sexually transmitted disease, have been documented in different investigations (14-20). For example Ziaiye et al. evaluated the effect of methanolic extracts of Zataria multiflora Boiss, Artemisia aucheri Boiss and Myrtus communis on T. vaginalis growth. They reported that the effects of these plants on T. vaginalis were considerably similar to that of metronidazole (21). Anti-trichomonal effects of Scutia buafilia, Neuroloarea lobelia and Mikania cordifolia have been reported by Muelas-Serrano et al (22). In another study Ezatpour et al. reported that the Lavandula angustifolia essential oil in 0.1% of concentration killed all live T. vaginalis cells in 90 minutes (23). The notable inhibitory effect of Eucalyptus camaldulensis on growth of T. vaginalis has also been reported (15).

The inhibitory effect of Achillea millefolium, Juglans regia and Artemisia absinthium leaves extracts on Trichomonas vaginalis growth has been shown by Khalili et al (24). Eventually Sarkari et al. reported anti-Trichomonas activity of Ferula assafoetida and garlic extracts (25). Water with polarity of 10.2 dissolves polar components of C. macropodum. It can be concluded that the polar components have stronger anti-Trichomonas activity than semi- and non-polar components of the plant. Taran et al. concluded that the polar components of Allium hirtifolium such as allicin and ajoene have anti-Trichomonas activities (26). Considering the mentioned studies, there are plenty reports about the effect of medicinal plants on T. vaginalis. In the present study, the anti-trichomonal effect of C. macropodum was observed. Thus, it can be suggested that the pharmaceutical formulations of this plants be prepared and be tested in animal models and clinical trials.

Conclusion
The results of this study showed that the different extracts of C. macropodum with different concentrations have anti-trichomonal activity.

Authors’ contributions
All authors equally engaged in the study.

Ethical considerations
This work has been performed in culture medium; so ethical committee approval was not needed.

Conflict of interests
None of authors had conflict of interests.

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