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Effect of *Chaerophyllum macropodum* extracts on *Trichomonas vaginalis in vitro*

Maryam Jabari[1], Gholamreza Asghari[1], Mostafa Ghanadian[1], Azizullah Jafari[1], Hosseiail Yousefi[2], Rasool Jafari[2], Seyedeh Marayam Sharafi[3], Hossein Yousofi Darani[2*]

[1]Department of Phamacagnozi, Isfahan University of Medical Sciences, Isfahan, Iran
[2]Department of Parasitology, Isfahan University of Medical Sciences, Isfahan, Iran
[3]Infectious Diseases and Tropical Medicine Research Center, Isfahan University of Medical Sciences, Isfahan, Iran

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ABSTRACT

Introduction: *Trichomonas vaginalis (T. vaginalis)* is a protozoan parasite causing trichomoniasis 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.

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.

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Introduction

Trichomonas vaginalis is a flagellate protozoan parasite causing trichomoniasis, one of the sexually transmitted diseases (STD). Trichomoniasis is the most prevalent non-viral sexually transmitted disease worldwide that only in the United States 3.7 million people are affected with 173 million of new cases worldwide (1-3).

Trichomoniasis can result in pelvic inflammatory disease in women with HIV. Also it increases the risk of tubal infertility, HIV transmission and cervical neoplasia. During pregnancy, trichomoniasis may cause premature rupture of membranes, preterm delivery and low birth weight. *T. vaginalis* infected men are almost asymptomatic, but may present some symptoms such as dysuria or clear mucopurulent discharge. Also prostatitis, epididymitis, infertility and prostate cancer are complications of the infection in men (1,4-10).

Metronidazole and tinidazole are two effective agents against trichomoniasis, but these drugs have their own side effects. One of the important side effects of metronidazole is its' carcinogenic effect, but due to inadequate evidence, it is not regarded as a risk factor for cancer in man. Also antimicrobial resistance against metronidazole and tinidazole has been reported frequently (2,11). Therefore it is necessary to provide a substitute treatment for human trichomoniasis.

During recent years the use of medicinal plants have been attracted the attention of researchers, so that at 1996 herbal plants were stand for nearly half of medications in the United States (12). There are eight species of

***Corresponding author**: Prof. Hossein Yousofi Darani, Department of Parasitology, Isfahan University of Medical Sciences, Isfahan, Iran. Email: Yousofidarani@gmail.com

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 grinned 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 1×10^4/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 10^4 *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/a100×. 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.*

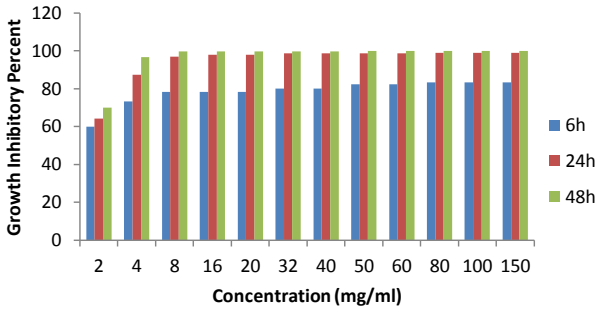

Figure 1. The inhibitory effect of crude extract of the *C. macropodum* on *T. vaginalis* growth in different concentrations and incubation times.

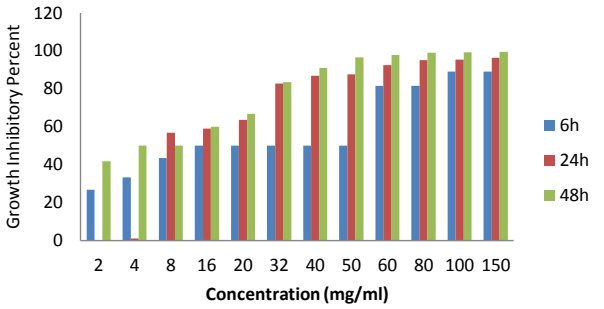

Figure 2. The inhibitory effect of the water extract of the *C. macropodum* on *T. vaginalis* growth in different concentrations and incubation times.

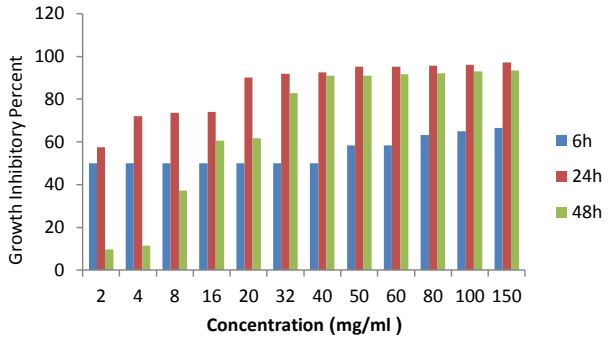

Figure 3. The inhibitory effect of the ethanol-water extract (20-80) of the *C. macropodum* on *T. vaginalis* growth in different concentrations and incubation times.

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 buafulia, Neurolarea 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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