Original Article

Effects of different extracts of *Eucalyptus camaldulensis* on *Trichomonas vaginalis* parasite in culture medium

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Abstract

Background: *Trichomonas vaginalis* is considered one of the main causes of vulvovaginitis in women. Metronidazole with vast side effects is now the drug of choice for treatment of this infection. In an attempt to find an alternative drug, the effect of *Eucalyptus camaldulensis* on this parasite was shown in previous studies. In this investigation, the effect of different extracts of this plant on *T. vaginalis* in culture medium has been investigated.

Materials and Methods: Five different extracts including total extract, diethyl ether, chloroform, ethyl acetate, and water fractions were prepared. The extracts were dried using vacuum rotary evaporator and then they were used for *in vitro* anti-trichomonas experiments.

Results: Crude extract of *E. camaldulensis* showed 80% growth inhibition (GI) in a concentration of 12.5 mg/ml during 24 h. Diethyl ether extract in a concentration of 25 mg/ml showed 100% GI during 24 h. With ethyl acetate extract, 100% GI was detected with the minimum concentration of 12.5 mg/ml in the first 24 h. Finally, water extract in a concentration of 50 mg/ml showed 80% and 100% GI after 48 and 72 h, respectively.

Conclusion: Ethyl acetate fraction is the extract which showed the highest percentage of GI (100%) with the least concentration (12.5 mg/ml) after 24 and 48 h.

Key Words: *Eucalyptus camaldulensis*, fractions, *trichomonas vaginalis*

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INTRODUCTION

Reported as a common medical problem in women, vulvovaginitis can lead to considerable discomfort and repeated medical visits. Infection, allergy, irritation, and systemic diseases can happen after vulvovaginitis. The most widespread causes of vaginal discharge before menopause are bacterial vaginitis, vulvovaginal candidiasis, and *Trichomonas vaginalis*.[1]

Trichomoniasis, the most prevalent nonviral sexual infection, with roughly 170 million new infected people worldwide per year, has been related to various discomforts such as pre-term delivery, high infant mortality, or low birth weight, and makes patients more susceptible to HIV infection.[2-5]

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The mainstay medication for trichomoniasis is metronidazole, but some resistant strains to this treatment have been detected;[6,7] thus, efforts need to be taken for finding new alternative drugs in order to control trichomoniasis.

For a long time, there is widespread acceptance of using medical herbs in both developing and non-developing societies because of their significant benefits like fewer side effects, better patient tolerance, lesser cost, and treating the main cause of disease.[8]

Eucalyptus, a large genus of Myrtaceae, represented by 900 species and subspecies, can be found the world over. Its leaves were used by Australians for its benefits like wound healing and for treating fungal infection.[9,10] In addition, the leaf extract of *Eucalyptus* has been confirmed to be used as a food additive. Also, in some cosmetics' formulation, *Eucalyptus* extracts can be found. Based on researches, various biological effects of *Eucalyptus*, including antimicrobial, antihyperglycemic, and antioxidant activities, have been shown,[11] and it seems that the essential oils of *Eucalyptus*, containing different compounds such as terpenoid and phenolic ones, play an important role in its biological activities.[12] Moreover, it has been reported that the extracts of *Eucalyptus camaldulensis* have anti-trichomonas activity.[13,14]

The aim of this study was to prepare phenolic and non-phenolic extracts of *E. camaldulensis* leaves and evaluate which extract fractions are more effective against *T. vaginalis*.

MATERIALS AND METHODS

Preparation of extract

The crude extracts were prepared by maceration of 500 mg of dried powder of leaves of *E. camaldulensis*, which were collected from Shooushtar area, a city in Khouzestan, Iran, in July 2011, in 50% methanol and 1% HCl 6 N for 3 days continuously at room temperature.

The solvents of extract were evaporated using rotary evaporator. Then, the crude extract was partitioned into distilled water and diethyl ether phases. The pH of aqueous phase was adjusted to 8-9 with 20% NaOH, which converted phenolic compound to its sodium salt. Then chloroform was used for extraction in order to remove the impurities. Then, the pH of residual aqueous phase was decreased to 3-4 using HCl 6 N and extracted with ethyl acetate to provide phenolic fraction. Five extracts were obtained including total extract, diethyl ether, chloroform, ethyl acetate, and water fractions. The extracts were dried using vacuum rotary evaporator. The dried extracts were used for *in vitro* anti-trichomonas experiments.[15]

Preparation of test microorganism

Test microorganism was obtained from parasitological lab of Isfahan University of Medical Science, which was isolated from vaginal discharge of female patients attending Obstetric and Gynecology Clinic in Shahrekord, Iran. In all experiments, a pool of four isolates was used. The parasite, *T. vaginalis*, was cultured *in vitro* at 37°C in TYIS33. Log phase culture of *T. vaginalis* was diluted with TYIS33 medium for obtaining 2×10^5 cells/ml.

In vitro anti-trichomonas assay

To explore anti-trichomonas effects of *E. camaldulensis*, the extracts were diluted with dimethyl sulfoxide (DMSO) or phosphate buffer (depending on their solvents) and transferred to Eppendorf tubes for providing final concentration of 50, 25, 12.5, 6.25, 3.125 mg/ml. The DMSO and phosphate buffer were used as negative control accordingly, and metronidazole at a concentration of 100 μg/ml was used as positive control. All tubes were incubated at 37°C. After 24, 48, and 72 h, the samples were taken from each tube and viable parasites were counted with hemocytometer. Complete active and flagella active parasites were considered as viable ones.

Results of parasite counting have been reported as percentage of growth inhibition (GI %) using the following equation in which "a" stands for mean number of viable parasites in negative control tube and "b" stands for mean number of viable parasites in test tube.[16,17] For comparing GI in case and control tubes, statistical values and tests such as mean, standard deviation, and analysis of variance (ANOVA) test were used.

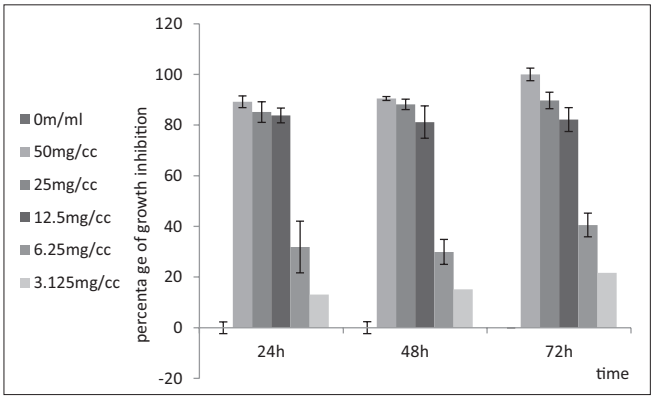

Figure 1: Effect of crude extract of *Eucalyptus camaldulensis* on *Trichomonas vaginalis* in culture medium following 24, 48, or 72 h. Results are presented as mean ± SD, and ANOVA followed by *post hoc* test was used for statistical analysis

$$GI\% = \frac{a-b}{a} \times 100$$

RESULTS

Crude extract of *E. camaldulensis* showed 80% GI in a concentration of 12.5 mg/ml during 24 h. Most of the growth inhibition was detected in a concentration of 50 mg/ml after 72 h. This is shown in detail in Figure 1.

Diethyl ether extract of *E. camaldulensis* in a concentration of 25 mg/ml showed 100% growth inhibition during 24 h, and this extract in concentrations of 6.25 and 12.5 mg/ml resulted in more than 80% GI after 48 h. Following 24 h, the minimum concentration which showed 100% GI was 6.25 mg/ml. This is shown in detail in Figure 2.

When ethyl acetate extract of *E. camaldulensis* was used, 100% GI was shown with the minimum concentration of 12.5 mg/ml, at the first 24 h. Also, more than 80% GI was detected with a concentration of 6.25 mg/ml after 72 h. This is shown in detail in Figure 3.

Water extract of *E. camaldulensis* in a concentration of 50 mg/ml showed 80% and 100% GI after 48 and 72 h, respectively, while at concentrations of 6.25 and 12.5 mg/ml it improved the survival of the parasite. This is shown in detail in Figure 4.

DISCUSSION

Considering the percentage of GI results showed in Figures 1 and 4, at concentrations of 50 and 25 mg/ml, diethyl ether and ethyl acetate extracts showed 100% of GI after 24 h. In these concentrations, the least GI % was shown by water fraction. When the concentration of 12.5 mg/ml was used, 100% of GI was observed in ethyl acetate fraction after 24 h. However, the same results were recorded for diethyl ether extract after 48 h. The interesting point to note is that water fraction improved the growth of *T. vaginalis*. Diethyl ether fraction, at a concentration of 6.25 mg/ml, was the only extract that caused 100% of GI after 72 h. Diethyl ether fraction in a concentration of 3.12 mg/ml revealed 50% and 68% of GI after 48 and 72 h, respectively.

It can be concluded that ethyl acetate fraction showed the highest percentage of GI (100%) at the least concentration (12.5 mg/ml) after 24 and 48 h ($P > 0.05$ in comparison to positive control. It seems the difference between the activities of the extracts can be due to the presence of different compounds related to the solvent polarity used to form the extract.

Ethyl acetate with a polarity of 4.4 extracted the polar compounds of *E. camaldulensis* including

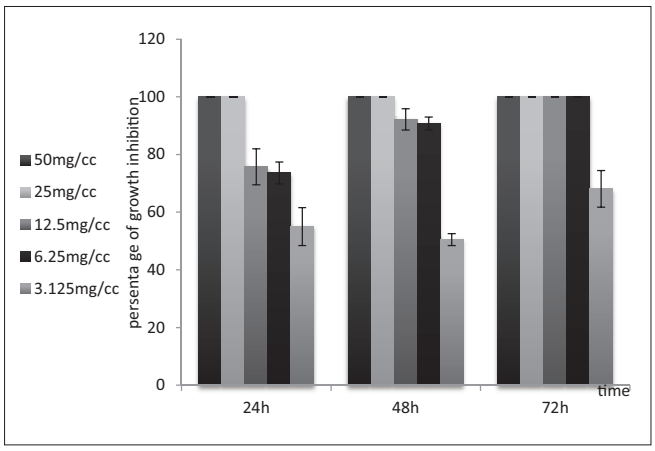

Figure 2: Effect of diethyl ether extract of *Eucalyptus camaldulensis* on *Trichomonas vaginalis* in culture medium following 24, 48, or 72 h. Results are presented as mean ± SD, and ANOVA followed by *post hoc* test was used for statistical analysis

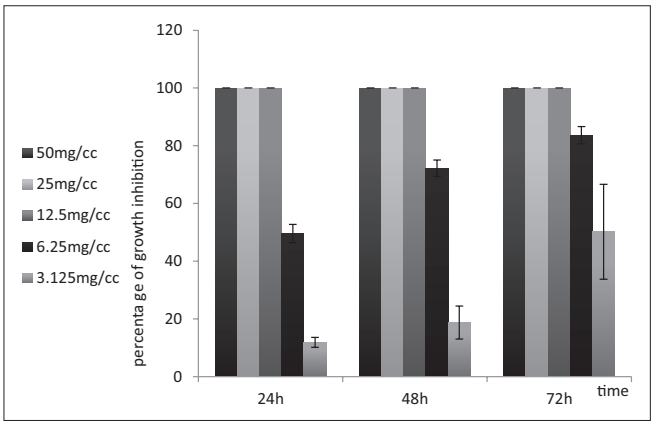

Figure 3: Effect of ethyl acetate extract of *Eucalyptus camaldulensis* on *Trichomonas vaginalis* in culture medium following 24, 48, or 72 h. Results are presented as mean ± SD, and ANOVA followed by *post hoc* test was used for statistical analysis

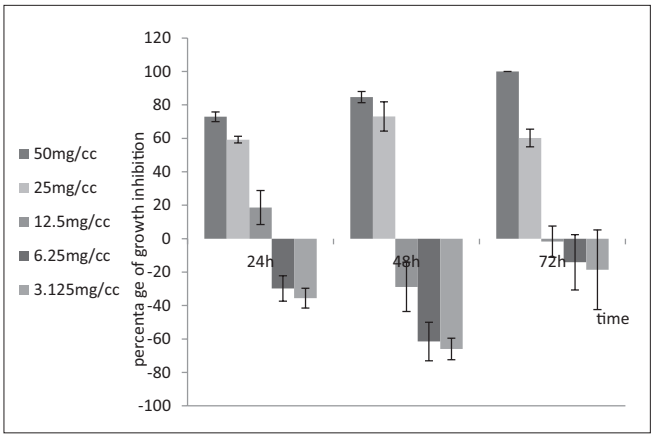

Figure 4: Effect of water extract of *Eucalyptus camaldulensis* on *Trichomonas vaginalis* in culture medium following 24, 48, or 72 h. Results are presented as mean ± SD, and ANOVA followed by *post hoc* test was used for statistical analysis

phenolic materials. It seems polar materials are more effective than semi-polar and non-polar compounds in *E. camaldulensis*. Also, other polar compounds like allicine and ajoene, which exist in *Allium hirtifolium*, could exhibit anti-trichomonas activity in comparison to metronidazole.[18] It has been reported in another study that berberin isolated from *Berberis arisata* had *in vitro* activity compared with metronidazole, on *T. vaginalis*.[19] Moreover, alcoholic extracts of *Calendula officinalis* and *Echinacea angustifolia* had *in vitro* efficacy against *T. vaginalis*.[20] Emodine present in the rhizome and root of *Rheum palmatum* manifested an inhibitory effect on *T. vaginalis*.[21]

Also, it is reported that ethyl acetate extract of *Arbutus anedo* leaves showed 100% GI at a concentration of 500 μg/ml.[22]

CONCLUSION

Growth stimulation was shown with lower concentration of water fraction in our study. It may be because of the presence of sugar and amino acids in this extract, which are water soluble, culture medium of the parasite contains also both sugar and amino acid. While 100% GI was reported after 24 h of *T. vaginalis* exposure to *Nigella sativa* aqueous extract in a concentration of 10 mg/ml,[17] the *N. sativa* water extract was prepared directly from the plant, but the water fraction used in our study was a fraction which was prepared after consequent extraction of the organic solvent.

In general, anti-trichomonas activity seems to depend on the solvent and plant used for extraction.

REFERENCES

1. Nyirjesy P, Peyton C, Weitz MV, Mathew L, Culhane JF. Causes of chronic vaginitis: Analysis of a prospective database of affected women. Obstet Gynecol 2006;108:1185-91.
2. Patel SR, Wiese W, Patel SC, Ohl C, Byrd JC, Estrada CA. Systematic review of diagnostic tests for vaginal trichomoniasis. Infect Dis Obstet Gynecol 2000;8:248-57.
3. Petrin D, Delgaty K, Bhatt R, Garber G. Clinical and microbiological aspects of Trichomonas vaginalis. Clin Microbiol Rev 1998;11:300-17.
4. Schwebke JR, Burgess D. Trichomoniasis. Clin Microbiol Rev 2004;17:794-803.
5. Upcroft P, Upcroft JA. Drug targets and mechanisms of resistance in the anaerobic protozoa. Clin Microbiol Rev 2001;14:150-64.
6. Edwards DI. Nitroimidazole drugs-action and resistance mechanisms II. Mechanisms of resistance. J Antimicrob Chemother 1993;31:9-20.
7. Grossman JH III, Galask RP. Persistent vaginitis caused by metronidazole-resistant trichomonas. Obstet Gynecol 1990;76 (3, Part 2):521-2.
8. Vermani K, Garg S. Herbal medicines for sexually transmitted diseases and AIDS. J Ethnopharmacol 2002;80:49-66.
9. Brooker MI, Kleinig DA. Field guide to Eucalyptus. Northen Australia. 2nd ed. Vol. 3. Melbourne: Blooming Books; 2004.
10. Chevalier A. Encyclopedia of medical plants. St. Leonards, NSW: Dorling Kindersely Pty Ltd.; 2001.
11. Takahashi T, Kokubo R, Sakaino M. Antimicrobial activities of Eucalyptus leaf extracts and flavonoids from Eucalyptus maculata. Lett Appl Microbiol 2004;39:60-4.
12. Conner D. Naturally occuring compounds. In: Davidson P, Branen AL, editors. New York: Marcel Dekker; 1993.
13. Mahdi N, Gany Z, Sharief M. Alternative drugs against Trichomonas vaginalis. East Mediterr Health J 2006;12:679-84.
14. Yousefi H, Kazemian A, Sereshti M, Rahmanikhoh E, Ahmadinia E, Rafiaian M, et al. Effect of echinophora platyloba, stachys lavandulifolia and eucalyptus camaldulensis plants on trichomonas vaginalis growth in vitro. Adv Biomed Res; 2012 in press.
15. Verma AR, Vijayakumar M, Mathela CS, Rao CV. In vitro and in vivo antioxidant properties of different fractions of Moringa oleifera leaves. Food Chem Toxicol 2009;47:2196-201.
16. Moon T, Wilkinson JM, Cavanagh HM. Antiparasitic activity of two Lavandula essential oils against Giardia duodenalis, Trichomonas vaginalis and Hexamita inflata. Parasitol Res 2006;99:722-8.
17. Tonkal A. In vitro antitrichomonal effect of Nigella sativa aqueous extract and wheat germ agglutinin. JKAU: Med. Sci, 2009;16:17-34.
18. Taran M, Rezaeian M, Izaddoost M. In vitro antitrichomonas activity of Allium hirtifloium (Persian Shallot) in comparison with metronidazole. Iran J Public Health 2006;35:92-4.
19. Soffar SA, Metwali DM, Abdel-Aziz SS, el-Wakil HS, Saad GA. Evaluation of the effect of a plant alkaloid (berberine derived from Berberisaristata) on Trichomonas vaginalis in vitro. J Egypt Soc Parasitol 2001;31:893-904.
20. Samochowiec E, Urbanska L, Manka W, Stolarska E. Assessment of the action of Calendula officinalis and Echinacea angustifolia extracts on Trichomonas vaginalis in vitro. Wiad Parazytol 1979;1:77-81.
21. Wang HH. Antitrichomonal action of emodin in mice. J Ethnopharmacol 1993;40:111-6.
22. Ertabaklar H, Kivcak B, Mert T, Toz So. In vitro activity of Arbutus unedo leaf extracts against Trichomonas vaginalis trophozoites. Turkiye Parazitol Derg 2009;33:263-5.

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