

Effect of *Echinophora platyloba*, *Stachys lavandulifolia*, and *Eucalyptus camaldulensis* plants on *Trichomonas vaginalis* growth *in vitro*

Hossein Ali Youse¹, Afsaneh Kazemian, Manijeh Sereshti, Elham Rahmanikhoh, Elham Ahmadiania, Mahmood Rafeian, Reza Maghsoodi², Hossein Y. Darani¹

¹Department of Parasitology and Mycology, Faculty of Medicine, Isfahan University of Medical Sciences, Isfahan, Department of Microbiology, Plant Research Center, ²Faculty of Medicine, Shahrekord University of Medical Sciences, Shahrekord, Iran

Abstract

Background: *Trichomonas vaginalis* is a protozoan parasite which causes vaginitis in women worldwide. Metronidazole with vast side effects is drug of choice for this infection. In search for an alternative drug, in this study the effect of three plants on *Trichomonas vaginalis* has been investigated *in vitro*.

Materials and Methods: Alcoholic and watery extracts of *Echinophora platyloba*, *Stachys lavandulifolia*, and *Eucalyptus camaldulensis* were prepared. In TYIS culture medium containing alive *Trichomonas vaginalis* different concentrations of extracts of three plants were added. Following, 24, 48, and 72 h incubation the number of parasite in each test tube was counted.

Results: *Eucalyptus camaldulensis* showed a strong effect on *Trichomonas vaginalis* growth. However, no significant effect was observed with *Echinophora platyloba* or *Stachys lavandulifolia* extracts.

Conclusion: *Eucalyptus camaldulensis* can be considered as an alternative drug for treatment of infective vaginitis which is caused by bacteria, fungi and parasites.

Key Words: *Echinophora platyloba*, *Eucalyptus camaldulensis*, plants, *Stachys lavandulifolia*, *Trichomonas vaginalis*

Address for correspondence:

Prof. Hossein Yousofi Darani, Department of Parasitology and Mycology, Faculty of Medicine, Isfahan University of Medical Sciences, Isfahan, Iran.

E-mail: H_yousofi@yahoo.com, Yousofi@med.mui.ac.ir

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INTRODUCTION

Trichomonas vaginalis, a protozoan parasite, is a common sexually transmitted infection in women and men.^[1,2] It is usually transmitted by sexual

relationship, by sharing contaminated underwear clothes or by nonhygienic vaginal examination.^[3] In women, it causes vaginitis and cystitis and in men it causes urethritis and prostatitis.^[4] *Trichomonas vaginalis* is considered as a common cause of vaginitis and as a causative factor for preterm birth and low birth weight.^[5] It has also been associated with increased human immunodeficiency virus transmission.^[6]

Metronidazole with vast side effects is now considered as a drug of choice for this infection with a cure rate of approximately 95%.^[7] Clinical resistance to this drug has been reported since 1962.^[8] Therefore, it would be very important to search for an alternative drug.

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Echinophora plants have four species in Iran and distributed in the west and western north of Iran.^[9] The genus *Stachys* is widespread throughout the world. In Iran, many species of this genus are present, from which, 13 are endemic. *Stachys lavandulifolia* has been used as an anxiolytic and sedative in Iranian folk medicine.^[10] Analysis of the crude extracts of the *Eucalyptus* spp. revealed the presence of saponin, saponin glycosides, steroid, cardiac glycoside, tannins, volatile oils, phenols and balsam.^[11]

Antibacterial, antifungal or antiparasitic effects of *Echinophora platyloba*,^[12-17] *Stachys lavandulifolia*,^[18,19] and *Eucalyptus camaldulensis*^[9,20] plants have been reported. In some local area of Iran these plants may be used for vaginal infection treatment. In this work, the effect of *Echinophora platyloba*, *Stachys lavandulifolia*, and *Eucalyptus camaldulensis* with antiparasitic activity^[15] on *Trichomonas vaginalis* has been investigated *in vitro*.

MATERIALS AND METHODS

Echinophora platyloba, *Stachys lavandulifolia* were collected from Chahar Mahal va Bakhtiari mountains and *Eucalyptus camaldulensis* was collected from Khostan trees. All plants were collected in spring of 2010 and confirmed by experts in medical plant research center of Shahrekord university of medical sciences. Leaves of those three plants were washed with water, dried, and then ground finely in a spice small electric mill. The resulting powders were mixed with ethanol or water to make extractions.

In order to prepare alcoholic extracts, 40 g of each dried powder plant was added to 1 L of 96% ethanol and left for 12 days. The mixture clarified by filtration through a sterile filter paper. The product was placed in the vacuum rotary evaporation device to remove solvent alcohol and it was dried in the 40°C oven.

In order to prepare watery extracts, 40 g of each dried powder plant soaked in 1 L distilled water and left for 48 h. The mixture clarified by filtration through a sterile filter paper. Vacuum rotary evaporation device was used to remove the solvent water. Stock solutions of dried extracts were reconstituted in 50% DMSO.

Trichomonas vaginalis parasite was isolated from vaginal discharge of women referred to Hajar hospital clinic in Shahrekord city, Iran and transferred to TYIS culture medium. A pooled of five parasite isolates was used for experiments. In test tubes containing 10 ml of TYIS culture medium different concentrations of dried extracts of three plants were added. In one tube metronidazole (5 µg/ml) was added as positive control and one tube left intact as negative control. Then 50 µl of medium containing about 100 live *Trichomonas vaginalis* were added to each tube. All tubes incubated at 37°C and the number of parasites in each tube was counted following, 24, 48, and 72 h incubation. For parasite counting, each tube was first shaken and 10 µl of it was observed on a microscope slide. *Trichomonas vaginalis* parasites with active flagella were considered alive. Each experiment was performed in triplicate.

RESULTS

In tubes contained metronidazole (5 µg /ml), 60 or 90 µg Eucalyptus, no parasite was observed after 72 h, while in control test tubes parasite had a normal growth. Details of parasite counts in all test tubes have been presented in Table 1. No significant effect on *Trichomonas vaginalis* growth was observed when alcoholic or watery extract of aerial parts of *Stachys lavandulifolia* were used. Details of parasite counts of this experiment presented in Table 2. Also no significant effect was observed when *Echinophora platyloba* was used.

DISCUSSION

Results of this investigation revealed that *Eucalyptus*

Table 2: Number of *Trichomonas vaginalis* in 20 µl of TYIS-33 culture medium 72 h after treatment with different concentrations of *Stachys lavandulifolia* extracts or metronidazole

TYIS culture medium containing <i>Trichomonas vaginalis</i> treated with	Number of parasites in 20 ml of medium 72 h after treatment with
No treatment	1504
Metronidazole	0
Different watery extracts	1504
Different ethanolic extracts	1400
Distilled water	1504
Ethanol	577

Table 1: Results of *Trichomonas vaginalis* counts in test tubes treated with Eucalyptus extract in comparison with appropriate controls

Test tubes No.	1	2	3	4	5	6	7
Material added	Metronidazole (5 µg/ ml)	1.5 µg Eucalyptus extract	15 µg Eucalyptus extract	60 µg Eucalyptus extract	90 µg Eucalyptus extract	100 µl DMSO	–
Parasite counts following 48 or 72 hours.	0	18 After 48 h	0 After 48 h 15 After 72 h	0	0	65 After 48 h	65 After 48 h

camaldulensis but not *Echinophora platyloba* or *Stachys lavandulifolia* had a strong effects on *Trichomonas vaginalis* growth *in vitro*.

Antimicrobial, antifungal, and antiparasitic effects of *Eucalyptus* spp. have been shown in different investigations.^[12,13,15,16] Adeniyi demonstrated that *Eucalyptus camaldulensis* had a strong effect on *Helicobacter pylori* *in vitro*.^[12] Drug of choice for these bacteria is metronidazole, same drug for treatment of *Trichomonas vaginalis*. In another study Safaei *et al.* showed that essential oil of *Eucalyptus* spp. had a strong antimicrobial activities.^[13] Nathan *et al.* also showed that essential oil of *Eucalyptus* spp. possess anti trypanosomal activity *in vitro* and this effects was dose dependent.^[15] Ramazani *et al.* demonstrated antifungal activity of *Eucalyptus* spp.^[16] Satorelli *et al.* investigated antimicrobial and antifungal activities essential oil of *Eucalyptus* Spp. They demonstrated that this plant presented the highest growth inhibition against *Staphylococcus aureus*, *Escherichia coli*, and *Candida albicans*.^[17] Mahdi *et al.* studied effects of two plants, *Myrtus communcs* and *Eucalyptus comaldensis*, on *Trichomonas vaginalis* growth *in vitro*. They showed that *Eucalyptus* extract caused death of parasite at pH 5.3.^[14] Results of this investigation are in agreement with our findings.

CONCLUSION

According to results of present investigation *Eucalyptus camaldulensis* plant could be considered as an alternative drug for *Trichomonas vaginalis* treatment. So it will be very worthwhile to recognize the efficient components of this plant with anti *Trichomonas* activities in further investigation. Moreover, antifungal and antiparasitic activities have been reported for *Eucalyptus* spp.^[12-17] Therefore this plant has a potential to be considered as a unique drug for treatment of infective vaginitis which is caused by bacteria, fungi or parasites. Further investigations are recommended to test this hypothesis.

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