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Short Article

Anti-Trichomonas Vaginalis Activity of Ethanolic Extracts of *Medicago Sativa* and *Satureja Hortensis*, *In Vitro* Study

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ABSTRACT

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Key words
Medicago sativa
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Background and Aims: *Trichomonas vaginalis* is a flagellated protozoa that is associated with vaginitis, cervicitis, urethritis and other vaginal disorders. Current study aimed to evaluate the anti-Trichomonas activity of *Medicago sativa* and *Satureja hortensis*, *in vitro*.

Materials and Methods: Ethanolic extract of *Medicago sativa* and *Satureja hortensis* were obtained by rotary evaporator. anti-Trichomonas vaginalis activities of the extracts in different concentrations were evaluated after 24, 48 and 72 hr of incubation of the cultured media.

Results and Conclusions: The data showed a significant difference between concentration and time regarding the *Satureja hortensis* and *Medicago sativa* extracts compared to the negative control ($p<0.05$). According to the results, the anti- trichomonas activity of the *Medicago sativa* and *Satureja hortensis* extracts may make it possible to use them in the treatment of trichomoniasis.

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Introduction

Natural products provide a large foundation of medications and are the basis of the drug compounds development. The therapeutic preparations from these plants, being available around, are inexpensive and easily affordable and are used more than chemical remedies in the treatment of different diseases [1]. Today, the number of proper herbal drugs used in the treatment of diseases in comparison to the total number of approved drug in the world is increasing. It is estimated that 250,000 to 500,000 species of plants exist on the Earth. Only 1 to 10% of these are used as foods by both humans and other animals. Therefore, it is conceivable that even more are used for therapeutic purposes [2]. Potential effects of plants as source of new antiprotozoal medications is proven by examples such as emetine, quinine and artemisinin obtained from *Cephaelisi pecacuhana*, Cinchona species and *Artemisia annua*, respectively [3]. *Satureja hortensis (S. hortensis)* and *Medicago sativa (M. sativa)* have found popularity as important herbal remedies against infectious diseases and for other medical purposes. *S. hortensis* has been traditionally used as stomachic, stimulant, expectorant, carminative, antidiarrheal, antioxidant, anti-inflammatory, sedative and aphrodisiac for the treatment of different types of infectious diseases [4]. *M. sativa* which is one of the most reputed medicinal plants is traditionally used to cure kidney pain, cough, antidiabetic, antioxidant, anti-inflammatory, antimicrobial and as well as central nervous system disorders. Phytochemical studies have shown flavonoids, alkaloids, phytoestrogens, coumarins, digestive enzymes, triterpenes, saponins and phytosterols on *M. sativa* plant [5]. *Trichomonas vaginalis (T. vaginalis)* is the agent of Trichomoniasis that is related with vaginitis, urethritis, cervicitis, prostatitis, epididymitis, cervical cancer, infertility, pelvic inflammatory, foul-smelling discharge, vaginal orurethral expulsion, pruritus and dysuria. This infection is prevalent in 5 to 74% of women and 5 to 29% of men with the estimate of 250 million patients worldwide. Current available drugs for parasitic diseases are effective in many cases. But they also have some limitations. Metronidazole and tinidazole are recommended drugs for the treatment of human trichomoniasis. But, many side-effects such as potential carcinogenic, mutagenicity, embryogenic and drug-resistant of *T. vaginalis* have been described. Of course, common bad effects comprise headache, glossitis, urticaria, pruritus, vertigo, nausea, dry mouth, bitter metallic taste and vomiting [6]. Given the importance of the disease and the problems of controlling and combating due to sexual transmission, the drug resistance and the absence of appropriate and effective vaccines, seem to be the acceptable, safe, cheap and available medical approaches. Therefore, the present study aimed to examine the anti-*trichomonas vaginalis* effect of *S. hortensis* and *M. sativa in vitro*.

Materials and Methods

Preparation of crude extracts

Leaves of *M. sativa* and *S. hortensis* were dried under shade, and were powdered mechanically

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using a commercial electrical blender. To achieve the ethanolic extract, 50 gr of dry powder was added to 500 ml of ethanol 80% and mixed steadily for 1 hr using amagnetic stirrer. The gained solutions were left at room temperature for 72 hr. After filtration, the solvent was then removed by rotary evaporator. The gained filtrate (5.5 g) was put into a sterile glass and stored at -20°C for further use [7].

Parasites culture

The organisms used in this study were isolated from vaginal discharge of female patients attending to the health care centers of Shahrekord city, Iran. The isolates were cultured in modified Diamonds media and kept in Parasitology Research Laboratory in Yazd University of Medical Sciences for examination. The protozoa were cultured axenically in modified Diamonds medium (pH 6.2) supplemented with 10% heat-inactivated bovine serum and incubated at 37°C with 5% of CO_2. Log phase culture of *T. vaginalis* was diluted with modified Diamonds medium for obtaining 10^5 cells/ml. The parasites were used, showing normal motility and morphology during the logarithmic phase of growth.

***In vitro* susceptibility assays**

To explore anti-trichomonas effects of *S. hortensis* and *M. sativa*, the extracts were diluted with dimethyl sulfoxide with a final concentration of 0.1% in test solution and transferred to microtubes for providing final concentrations of 50, 100, 200, 400, 600, 800 and 1000 µg/ml. *T. vaginalis* trophozoites were incubated for 24, 48 and 72 hr at 37°C in the presence of different concentrations (50- 1000 µg/ml) of the ethanolic extracts and the total numbers of viable protozoa were assessed using a hemocytometer. The eosin stain with the concentration of 0.1% was used to check the viability of the trophozoites. The DMSO and metronidazole were used as negative and positive controls, respectively. The experiments were carried out in triplicate. All microscopic examinations were performed blindly by two investigators. Results of parasite counting have been described as percentage of growth inhibition (%GI). The numbers of parasites were compared with the positive and negative controls and the percentage of %GI was calculated for each experiment with respect to the growth control as follows:

$$\%\ \mathrm{GI} = \left(1 - \frac{\mathrm{GR}_{\mathrm{extract}}}{\mathrm{GR}_{\mathrm{control}}}\right)100$$

The 50% inhibitory concentration was (IC50) calculated and figure was plotted using SigmaPlot™13 (Systat Software Inc, USA) [7]. This study was approved by the Ethics Committee of Shahid Sadoughi University of Medical Sciences, Yazd, Iran.

Statistical analysis

The normal distribution of data was evaluated by Kolmogorov–Smirnov (K-S) test. Afterwards, the interaction between time and concentration was calculated by repeated-measures analysis of variance for each extract. Additionally, two-tailed t test analysis based on different concentrations was used to reveal the statistical difference between each of the extracts and the negative control group at a given hour.

Results and Discussion

The results of Anti-trichomonas activity of various concentrations of *M. sativa* and S.

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hortensis were shown in figures 1 and 2, respectively. The IC50 values are shown in table 1 as well. For the positive control, metronidazole killed all the cells after 24 hr of incubation time. Ethanolic extract of *M. sativain* in concentration of 600 µg/ml showed 80% GI during 72 hr and the concentrations of 800 µg/ml revealed more than 90% GI after 48 hr. Complete inhibition of growth (100% GI) of the trophozoites was seen in concentrations of 1000 µg/ml after 48 hr. 100% GI was shown in concentrations of 1000 µg/ml after 72 hr using ethanolic extract of S. hortensis. Also, more than 80% GI has been observed in concentration of 600 µg/ml after 72 hr. Regarding the effect of two evaluated extracts on *T. vaginalis*, there were significant differences between *S. hortensis* as well as *M. sativa* and their negative controls in various times (Fig. 2). Furthermore, for *M. sativa* compared to the negative control at 24, 48, and 72 hr were 0.02, 0.03 and 0.05 respectively. Then for the *M. sativa* and *S. hortensis* extracts, these were significant statistically. ($p<0.05$).

Table1. Anti-trichomonal activity of ethanolic extracts of *Medicago sativain* and *Satureja hortensis*

Time	IC50 (ug/ml) with 95% confidence	
	Medicago sativain	*Satureja hortensis*
After 24 hr	474.38	539.96
After 48 hr	361.95	441.34
After 72 hr	197.43	396.99

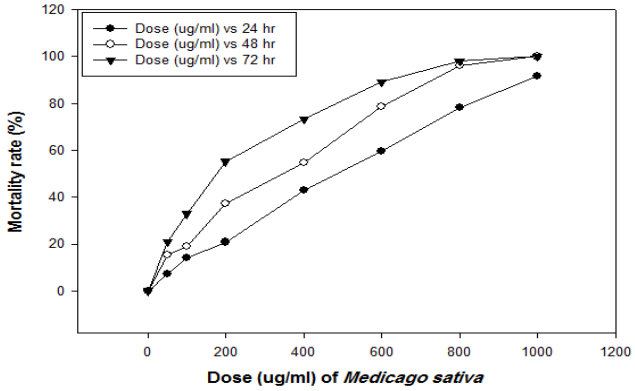

Fig. 1. The effect of *Medicago sativa*in concentrations of 50, 100, 200, 400, 600, 800 and 1000 µg/ml on *T. vaginalis* after 24, 48 and 72 hour of incubation times, *in vitro*.

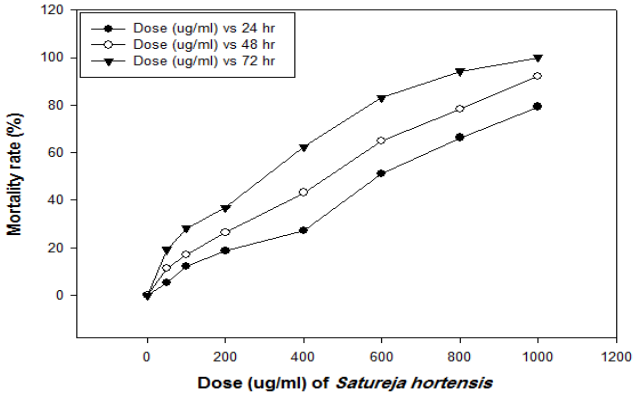

Fig. 2. The effect of *Satureja hortensis* in concentrations of 50, 100, 200, 400, 600, 800 and 1000 µg/ml on *T. vaginalis* after 24, 48 and 72 hour of incubation times, *in vitro*

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Metronidazole is the choice treatment for trichomoniasis and most of intestinal protozoan diseases. Many studies indicated the resistance of this drug and its carcinogenic and teratogenic complications (especially in the first trimester). Also safety in pregnancy is unreliable. These defects had the scientists search for alternative drugs with fewer side effects [8]. There is a great need for available and inexpensive therapeutic agents particularly in the underdeveloped and developing countries. Natural products are the talented basis of active molecules that can be widely used for their minimal side effects, better acceptance of patient, as well as availability and non-toxicity profile [9]. This study was designed to survey the anti-Trichomonas vaginalis efficacy of *S. hortensis and M. sativa in vitro*. According to the results, both extracts tested in the current investigation indicated remarkable anti-Trichomonas activity against trophozoites of *T. vaginalis*. However, the *M. sativa* ethanolic extract was more effective than *S. hortensis* as it demonstrated a lower IC50 values against trophozoite of *T. vaginalis* with 100% GI at hour 48 and 72. The difference in the effect of two extracts on the parasite can be explained by the presence of different chemical compounds in the plants. To date, many studies have been conducted around the activity of some medicinal plants against *T. vaginalis* [10]. *Allium hirtifolium*, *Mikania cordifolia* from Asteraceae family, *Scutia buxifulia* from Asteraceae family and *Lobalia neurolarea* from Rhamnaceae family, *Carcia papaya*, *Cocos nucifera* and *Praneem polyherbal* tablets are shown to have anti-Trichomonas activity *in vivo* and *in vitro*. Calzada et al. in their *in vitro* study examined the anti-Trichomonas activity of Mexican medicinal plants and showed that thyme with a minimum inhibitory concentration of 126.4 µg/ml is ineffective for *Trichomonas* trophozoite [11]. In a similar study, Mirzaei et al. indicated the cytotoxicity of *S. hortensis* extract and demonstrated that *S. hortensis* has anti- leishmanial effect with IC50=298/42 ug/ml after 72 hr of exposure time [7]. Diba et al. revealed that the alcoholic extract of *S. hortensis* can inhibit the growth of some species of *Candida* and *Aspergillus in vitro* and also can kill them in higher concentrations. Species of Satureja (*S. khuzestanica*, leaf) comprise effective compounds which can serve as an alternative agent in the control of leishmaniasis [12].

Conclusion

Based on the results, it is concluded that ethanolic extracts of *S. hortensis* and *M. sativa* are potent inhibitors of the growth of *T. vaginalis*. The current study provided valuable data as for new active products against *T.vaginalis*. It may act as a favorable anti-trichomonacidal remedy in the future. Additional investigations are recommended for isolation of active fractions and components.

Conflict of Interest

No conflict of interest is associated with this work.

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