




# Anti-*Trichomonas vaginalis* Effect of Methanolic Extracts of *Sambucus nigra* in Comparison with Metronidazole

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## Abstract

**Background:** Trichomoniasis is the most common non-viral sexually transmitted disease caused by a flagellated protozoan living in the genitourinary tract, which infects both men and women. Metronidazole is the treatment of choice for trichomoniasis. Researchers are seeking an alternative to metronidazole because of its inevitable side effects and toxicity.

**Objectives:** This study aimed to evaluate the effect of the methanolic extract of *Sambucus nigra* against *Trichomonas vaginalis* in vitro.

**Methods:** Plants were collected from different areas of Mazandaran Province, northern Iran. Fruits were separated, shade-dried, milled, and their methanolic extract was prepared in concentrations of 100, 200, 400, and 800  $\mu\text{g}/\text{mL}$ . Parasites were obtained from patients referring to different health centers of Mazandaran province, cultured in Dorset medium, and incubated at 37°C. The effects were evaluated and compared to a control group. The data were analyzed by SPSS 18 using the ANOVA test.

**Results:** The exposure time and concentration of the extracts had a direct effect on anti-parasitic activity so that increasing extract concentration and incubation time heightened the anti-trichomoniasis effects. The concentrations of 400 and 800  $\mu\text{g}/\text{mL}$  of the plant had 100% efficacy after 72 and 48 hours, respectively.

**Conclusions:** It can be concluded from our results that the methanolic extract of *S. nigra* has a remarkable ability to destroy *T. vaginalis* and it can be considered an effective drug against *T. vaginalis* with further studies in human and animal models.

**Keywords:** *Trichomonas vaginalis*, Methanolic Extract, *Sambucus nigra*, Metronidazole

## 1. Background

Trichomoniasis is one of the most common non-viral sexually transmitted diseases (STDs), whose causative agent is a flagellated protozoan living in the genitourinary system called *Trichomonas vaginalis* (*T. vaginalis*). It can cause trichomoniasis in both men and women (1, 2). Trichomoniasis infection is more common in women and is associated with several clinical problems and symptoms, including awful smell vaginal discharge, painful urination, genital excitability, disorders after sexual intercourse, premature rupture of membranes, preterm birth, low birth weight, and increased risk of infection with HIV (1, 3-5). The worldwide studies and statistics indicate that in developed countries, a significant percentage (more than 50%) of patients referring to STD clinics suffer from trichomoniasis, and the rate of infection is increasing (6, 7). Also, studies in our country have shown that Iran is the endemic focus of this infection (5, 8, 9).

Metronidazole has been known as the gold standard

treatment for trichomoniasis, which has a significant effect on the treatment of this disease (10). However, side effects such as nausea, vomiting, bad taste, gastrointestinal disorders, exanthem, urticaria, angioedema, vertigo, peripheral neuropathy, and transient neutropenia are observed with the use of this drug, which reduces the willingness to use it by the patients (10-12). Additionally, in recent years, the evidence of drug resistance to metronidazole has been reported in some studies, which could be another reason for research on alternative trichomoniasis treatment (13).

The use of medicinal herbs has been common in many countries around the world for many years. Today, the tendency to use them is increasing due to the benefits including fewer side effects, better access, cost-effectiveness, and adaptability of the physiological function of the body to their therapeutic effects (14, 15). Studies on the anti-parasitic effects of plants, particularly against trichomoniasis, have been increasing in recent years, often accompa-

nied by satisfactory results (16-18). For example, Yousefi et al. (19) conducted a study to investigate the effect of the hydroalcoholic extract of Peppermint and *Salvia officinalis* on *T. vaginalis* parasites, and found that the parasite did not grow at a concentration of 2 mg/mL of *S. officinalis* extract and 4 mg/mL of Peppermint extract (19). Also, Arefkhah et al. (20) conducted another study to investigate the effect of the hydroalcoholic extract of *Scrophularia striata* Boiss on *T. vaginalis* and concluded that this plant extract could completely inhibit the growth at a concentration of 4 mg/mL, and the inhibition rate of parasite growth was 99% at a concentration of 2 mg/mL (20). Besides, *Sambucus* spp., belonging to the Caprifoliaceae family, is known and used as an antiseptic, antiviral, antioxidant, anti-inflammatory, analgesic, and anti-proliferating agent due to the presence of euosialic acid in its extract (21-23). Rahimi Esboei et al. (24) investigated the anti-parasitic effect of this plant against hydatid cyst protoscolex and *Giardia lamblia* cyst and observed an acceptable ability against this infection (24, 25).

## 2. Objectives

As there are no published documents on the anti-trichomoniasis effects of *S. nigra*, the present study was conducted to evaluate the in-vitro anti-trichomoniasis effects of *S. nigra* in laboratory conditions.

## 3. Methods

### 3.1. Plant Collection and Extraction

The plant was collected from different regions of Mazandaran Province and was registered at the Faculty of Health of Mazandaran University of Medical Sciences after confirmation of the scientific name and plant herbarium with registration no. 137-95. The fruits of the plant were shade-dried and crushed by electric grinding, and the powders were stored in dark containers. Then, 500 mL of methanol (Merck, Germany) was added to the plant powder and placed in the laboratory for 48 h. In the next step, the plant waste was separated and the solvent in the solution was evaporated under vacuum conditions by an evaporator. The dried powder was prepared at concentrations of 100, 200, 400, and 800  $\mu\text{g/mL}$ , and used for anti-parasitic evaluation (24).

### 3.2. Preparation and Culture of *Trichomonas vaginalis* Parasite in Culture Medium

Vaginal samples were gathered by using sterile cotton swabs. Direct examinations were undertaken microscopically and then cultures were done in Dorset culture media. All test tubes were incubated at 37°C for eight days and

microscopic observations were done every two days. The genus and species of the parasite were confirmed by morphological and morphometric methods. In this study,  $5 \times 10^5$  parasites were used for each reaction. Metronidazole as positive control and dimethyl sulfoxide (DMSO) as negative control were also used in this study (26).

### 3.3. Investigation of the Anti-Parasitic Effect of *Sambucus nigra*

This study was carried out by a 24-well cell culture plate. In this test,  $5 \times 10^5$  parasites of *T. vaginalis* were added to each well and 100  $\mu\text{L}$  of different concentrations of herbal extracts were added, as well. The plate was placed in an incubator at 37°C, and the viability percentage of the parasite was evaluated at 12, 24, 48, and 72 h intervals using the microscopic method. Besides, 1% trypan blue was used for each concentration, as well as positive and negative control groups, and the growth inhibition was calculated using the following formula (27):

$$\%GI = \left( 1 - \frac{GR_{extract}}{GR_{control}} \right) 100 \quad (1)$$

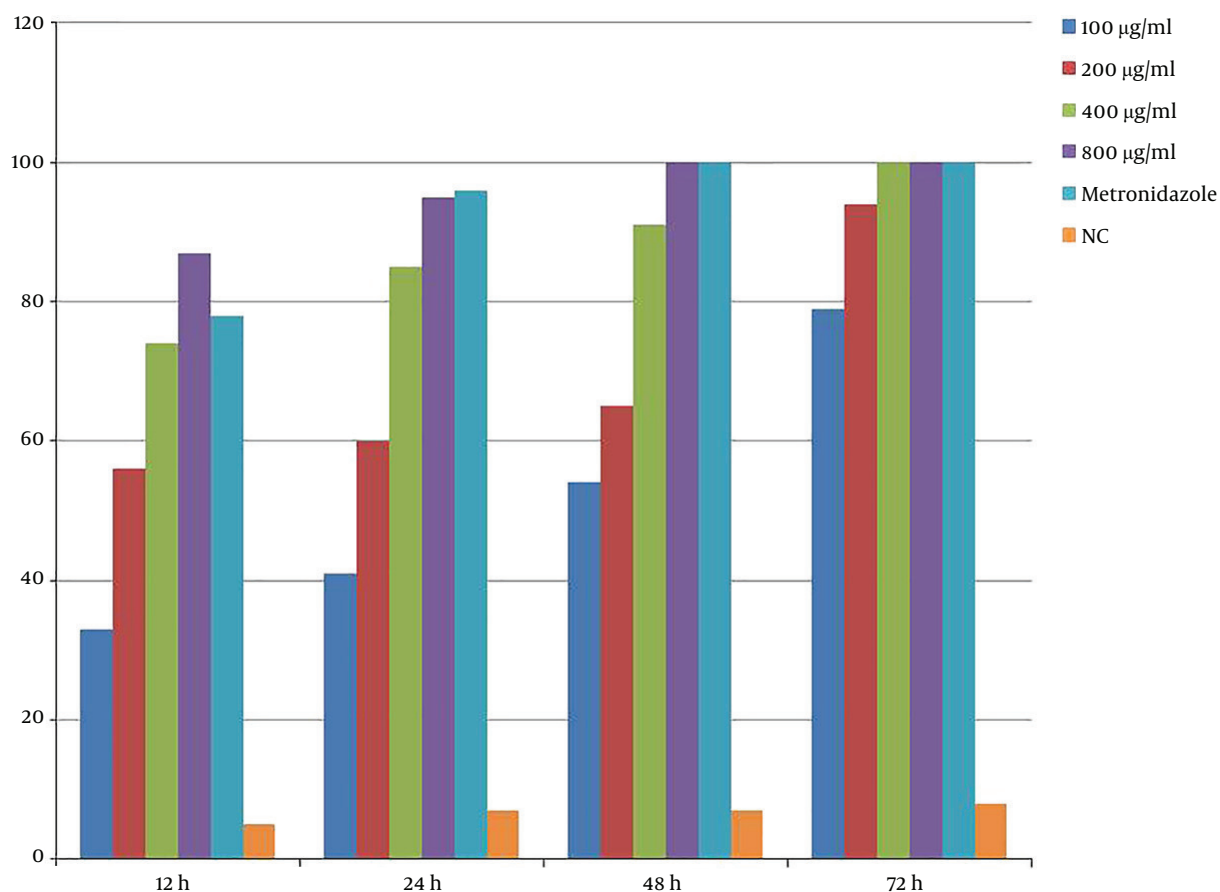
Finally, the number of parasites was counted at certain times using Neubauer slides and the minimum inhibitory concentration (IC<sub>50</sub>) was calculated.

### 3.4. Statistical Analysis

The experiments were conducted in triplicate. Finally, the obtained data were analyzed using SPSS software with the chi-square test.

## 4. Results

This study was carried out using the methanolic extract of the plant to evaluate its anti-trichomoniasis effects at concentrations of 100, 200, 400, and 800  $\mu\text{g/mL}$  at exposure times of 12, 24, 48, and 72 h. The results are shown in Table 1 and Figure 1. As observed, the acceptable efficacy of plant extract against *T. vaginalis* was observed at concentrations of 400 and 800  $\mu\text{g/mL}$ . It is also noticeable that the effect of the extract had a direct relationship with exposure time, implying that the inhibition of parasite growth enhanced with increasing exposure time. In addition, the best inhibitory effect on parasite growth was observed at an extract concentration of 800  $\mu\text{g/mL}$ . At this concentration, the anti-trichomoniasis effect of the extract was equal to the anti-trichomoniasis effect of metronidazole (as an anti-trichomoniasis standard drug). The inhibition rate of parasite growth was not significant in a negative control environment (Table 2).



**Figure 1.** Growth inhibitory effect of the methanolic extract of *Sambucus nigra* at concentrations of 100, 200, 400, and 800 µg/ml against *Trichomonas vaginalis* after 12, 24, 48, and 72 h compared to the positive control (metronidazole) and negative control (medium with DMSO).

**Table 1.** Growth Inhibitory Effect of the Methanolic Extract of *Sambucus nigra* at Concentrations of 100, 200, 400, and 800 µg/ml Against *Trichomonas vaginalis* After 12, 24, 48, and 72 h Compared to the Positive Control (Metronidazole) and Negative Control (Medium with DMSO).

Concentration	100 µg/ml	200 µg/ml	400 µg/ml	800 µg/ml	NC	Metronidazole	P value
12 hours	33	56	74	87	5	78	0.031
24 hours	41	60	85	95	7	96	0.011
48 hours	54	65	91	100	7	100	0.000
72 hours	79	94	100	100	8	100	0.000

Abbreviation: NC, negative control (DMSO).

## 5. Discussion

Based on the results, the methanolic extract of *S. nigra* significantly decreased the number of *Trichomonas* parasites at different times. The antiparasitic effect of the plant extract increased with its concentrations from 100 to 800 mg/L, and the best results were observed at 800 µg/ml, which was equal to the efficacy of metronidazole. Also, the time was effective on the effect of the plant extract on the

parasite so that the highest efficacy was observed at the highest exposure time for each concentration.

Although there is an effective drug against this disease, consumers have complaints and are not satisfied, and drug resistance has led to conducting various studies for finding new drugs (15). Medicinal herbs have become one of the most attractive options in recent years to achieve effective drugs with lower side effects against

**Table 2.** Minimum Inhibitory Concentration (IC<sub>50</sub>) of Methanolic Extract of *Sambucus nigra* at Concentrations of 100, 200, 400, and 800 µg/mL Against *Trichomonas vaginalis* After 12, 24, 48 and 72 h

Time	Minimum Inhibitory Concentration (IC <sub>50</sub> ), µg/mL
12 hours	151.15
24 hours	121.95
48 hours	92.59
72 hours	63.29

all types of pathogens (17). Many studies are annually carried out in Iran and other parts of the world to investigate the ability of these types of plants against pathogenic agents (28). The use of herbal medicines and studies on the anti-parasitic properties of herbs against trichomoniasis have been also taken into consideration (17). *Sambucus nigra* is known as an antiseptic, antiviral, antioxidant, anti-inflammatory, analgesic, and antiproliferative agent due to the presence of eurosialic acid in its extract. Anthocyanins, especially cyanidin 3-sambubioside and cyanidin 3-glucoside, are polyphenolic compounds responsible for anti-oxidative effects (29), with antibacterial, antiviral, and anti-allergic effects, as well as immune-stimulating properties in humans (30-32).

Uncini Manganelli et al. showed the antiviral activity of *S. nigra* in the contradiction of the feline immunodeficiency virus using feline kidney Crandell cells. In another study, Razi Jalali et al. (33) used this plant to evaluate its anti-parasitic effect against hydatid cyst in an in vivo study and observed the acceptable ability of the plant against this infection. Their results indicated that in all infected groups, the number, size, and volume of cysts were significantly lower in the test groups than in the control group ( $P < 0.05$ ) (33). Daryani et al. (23) reported that the fruit extract of *S. nigra* at concentrations of 5, 10, 25, and 50 mg/mL was effective against *Toxoplasma gondii* in an in vitro study. The concentrations used in this study were significantly lower than those in other work. In the current work, 100% efficacy was observed at a concentration of 400 µg/ml after 48 hours of incubation. Given the research done, as well as the presence of effective compounds such as anthocyanins and eurosialic acid, it is expected that more research is done on this plant (22).

In the current work, the methanolic extract of *S. nigra* was used to evaluate its anti-trichomonas activity and our results indicated that this plant was fully effective. Up to now, several plants have been used; but, there has not been any success in the production of medication. *Ferula szowitiana* (34), *Artemisia*, *Zataria*, *Myrtus* (35), *Freula assafoetida*, *Allium sativum* (36), *Taxus baccata* (37), *Saponaria officinalis* (16), and many others plants have shown a variety of ef-

fects. Although many studies have been carried out and many plants have been studied, research on finding an effective plant remains ongoing. The comparison of our results with the results of the above-mentioned studies illustrates that the methanolic extract of *S. nigra* was very effective in removing *T. vaginalis*.

### 5.1. Conclusions

In our study, the exposure time for complete parasite removal was detected to be lower than in previous studies. Moreover, it was found that the methanolic extract of *S. nigra* was equal in effectiveness to metronidazole against *T. vaginalis*, which indicates the high potential of this plant to be examined as a drug candidate for the treatment of trichomoniasis. The results confirmed the potential of *S. nigra* against *T. vaginalis* and showed that, by supplementary studies on the effects of this plant, it can be considered a good alternative to metronidazole. It is suggested that animal and human models be used to study the effect of this plant on the growth and amplification of *T. vaginalis*.

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### Footnotes

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