



## REVIEW: A Review on Herbal Treatments for Trichomonas Vaginalis

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### ARTICLE INFO

**Submitted:** 01 May 2021

**Accepted:** 30 May 2021

**Published:** 29 Jun 2021

### Keywords:

**Herbal Treatment;  
Trichomonas vaginalis;  
Trichomoniasis**

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### Citation:

Rahmani F, Ehteshaminia Y, Mohammadi H, Mahdavi SA. A Review on Herbal Treatments for Trichomonas vaginalis. Tabari Biomed Stu Res J. 2021;3(2):48-57.

10.18502/tbsrj.v3i2.6673

### ABSTRACT

**Introduction:** Trichomonas vaginalis is a protozoan parasite that infects the urogenital tract of men and women and causes trichomoniasis, a common sexually transmitted disease in both men and women. The infection is often asymptomatic, but it can be accompanied by symptoms such as severe inflammation, itching and burning, foamy discharge and foul-smelling mucus. In one year, 250 million cases of Trichomonas vaginalis were reported worldwide.

**Material and Methods:** In this study, the websites of PubMed, Google Scholar, SID, and Margiran were searched and related articles were reviewed.

**Results:** Today, the most common treatment for this disease is the use of metronidazole. However, its side effects, which include hematological and neurotoxic effects, cannot be ignored. Because of these side effects, researchers are looking for a suitable replacement for metronidazole in the treatment of trichomoniasis. Researchers' desire to use herbs can be due to various reasons such as fewer side effects, better patient acceptance, recommendation of traditional medicine, lower prices of herbs and also compatibility with the normal physiological function of the human body.

**Conclusion:** Considering the inhibitory effects of medicinal plants on the growth and proliferation of Trichomonas vaginalis in vitro, it can be concluded that the use of these plants can have many applications in the treatment of trichomoniasis. As a result, by studying more about their advantages and disadvantages, it is possible to make a drug that has higher therapeutic effects with fewer side effects.

## Introduction

Trichomoniasis is a common sexually transmitted disease in both men and women that is often asymptomatic but can be accompanied by symptoms such as severe inflammation, itching and irritation, foamy discharge, and foul-smelling mucus (1). There are differences in the prevalence of

trichomoniasis in different societies because cultural, social and health factors affect the prevalence of sexually transmitted diseases such as trichomoniasis (2). 250 million cases of Trichomonas vaginalis were reported worldwide in a year (3). Clinical manifestations in women, include vaginitis,

itching and irritation, as well as increased discharge can cause side effects such as premature birth, miscarriage, birth with low weight, premature rupture of the bladder, ectopic pregnancy, etc. Clinical manifestations in men include urethral itching, clear or mucous discharge, severe irritation and itching after urination (4). The prevalence of this disease in Iran has been reported between 2.15-1.7% (5). The prevalence of this disease in women is 5-74% and in men 5-29% and having high-risk sexual intercourse as well as having multiple sexual partners increases the risk of infection. Women aged 16-53 are also at the highest risk of developing trichomoniasis (3). Recent studies have also shown that HIV-positive people have a higher risk of contracting the infection than other people (6). One of the most important ways to prevent trichomoniasis is to use a condom when having sex (7).

Diagnostic methods for *Trichomonas vaginalis* infection, are wet slide, PCR and culture methods, among which, wet slide method is one of the most common due to its low duration and accuracy (8). Metronidazole is the drug of choice in the treatment of *trichomoniasis vaginitis* (9). However, it should be noted that the use of metronidazole is also associated with limitations, and in certain cases such as allergies to metronidazole, pregnancy, other systemic treatments in the individual and the presence of severe side effects, it can no longer be used in treatment (10). Other side effects of metronidazole include headache, inflammation of the tongue, itching and irritation, dizziness, drowsiness, hallucinations and delusions, mania, nausea and vomiting, dry mouth, and a metallic taste in the mouth (11). In addition to the aforementioned side effects, it should be noted that the use of metronidazole in the first trimester of pregnancy is contraindicated. As a result, in pregnant women with trichomoniasis, the treatment process is controversial (12). Evidence from ancient times shows that the ancients used plants as medicine to treat diseases. However, with the passage of time and the expansion of various branches of science, researchers tended to use

chemicals in the production of drugs, but after a while they came to the conclusion that chemical drugs, in addition to their therapeutic effects, have side effects. As a result, researchers are once again interested in using plants to produce medicine (4). Regarding the use of chemical drugs, in addition to the side effects, it should be noted that the excessive increase of antibiotics and mutation of metronidazole-resistant gene in *Trichomonas vaginalis*, leads to increased drug resistance against these drugs have been used and this issue has become one of the reasons why researchers turn to medicinal plants (5, 13). The tendency to use medicinal plants can be due to various reasons such as fewer side effects, better patient acceptance due to the recommendation of traditional medicine, use of previous generations, lower price of medicinal plants and also adaptation to the normal physiological function of the human body. Iran has 7500 plant species, 10-15% of which are herbal medicines. However, the use of herbals in Iran is only 3-5% (14). Medicinal plants also contain compounds that have a low toxicity on the body's cells (15). In the meantime, increasing awareness about the use of herbs and potential drug interactions among physicians may help reduce unwanted complications and adverse outcomes of the disease (16).

## Methods

In this study, herbal remedies related to trichomoniasis infection and the effects of these therapies on *Trichomonas vaginalis* were investigated. To collect this information, databases in PubMed, Google scholar, SID and Iran Medex by the keywords *Trichomonas vaginalis*, Trichomoniasis, herbal remedies and their English equivalent have been searched. During this study, original research articles in two languages, Persian or English, as well as *in-vivo* and *in-vitro* studies from 2011 to 2020 were investigated.

## Results

### Rhubarb

Rhubarb, scientifically known as *Rheum ribes*, is a perennial plant that has laxative, appetite-stimulating and pain-relieving properties, and its stem and root are used to treat diseases (17). In 2015, Niyati et al. studied the trichomoniasis effect of rhubarb. For this purpose; aqueous extract of rhubarb was prepared at concentrations of 100, 200, 250 and 300 mg/ml. Then, *Trichomonas vaginalis* trophozoite parasite was prepared from the vagina of women with trichomoniasis and cultured in tubes containing TYIS33 medium that had already reached room temperature. These media were then incubated at 37 °C. When the trophozoites had grown sufficiently, they placed 1000 microliters of the medium in sterile borosilicate tubes next to the flame and then added 1000 microliters of each concentration of rhubarb extract to the same tubes. They also placed two tubes as positive and negative controls. Positive control contained parasite, culture medium and metronidazole and negative control contained parasite and culture medium. Finally, 1000 µl of each of the prepared tubes was removed and transferred to a sterile microtube and stored in a 37 °C incubator. Finally, the data showed that with increasing concentration, the inhibitory effect of rhubarb also increased and at a concentration of 300 mg/ml after 24 hours, inhibited the growth of parasitic trophozoites up to 100% (4).

### Fennel

Fennel with the scientific name of *Foeniculum vulgare* is a medicinal plant belongs to the Umbelliferae family that is used in the pharmaceutical and food industries (18). In 2015, Niyati et al. studied the anti-trichomonal effect of fennel. In this study, concentrations of 75, 150, 300, 600 and 800 µg/ml were prepared from the aqueous extract of fennel. *Trichomonas vaginalis* trophozoites, previously cultured in culture medium, were placed in sterile borosilicate tubes, and each of these concentrations was added to specific tubes, with two tubes under positive and negative control. The positive

control contained metronidazole and the negative tube did not. Finally, it was found that the highest inhibitory percentage of fennel extract at a concentration of 800 µg/ml after 48 hours, which has a 79% inhibitory effect on *Trichomonas vaginalis* (4).

### Eucalyptus

Eucalyptus with the scientific name of *Eucalyptus camaldulensis* is a medicinal plant belongs to the myrtaceae family that grows in harsh environmental conditions (19). In 2011, Kazemian et al. studied the effect of eucalyptus against *Trichomonas vaginalis*. During this study, the plant extract was prepared by percolation method and concentrations of 15, 60 and 90 µl were placed in tubes containing culture medium. Then, 100 live parasites were added to all environments and kept in a 37 °C incubator for 24 to 72 hours. The two tubes were also assigned positive and negative control. Finally, it was found that eucalyptus, in different concentrations, has different inhibitory effect on *Trichomonas vaginalis* and in concentrations of 60 and 90 mg, it has the most inhibitory effect. However, at lower concentrations, the growth of the parasite was lower than the control group, which could indicate the inhibitory effect of this plant (20).

### Geranium

In 2014 Fakhrieh Kashan et al. studied the anti-trichomonal effect of aqueous and alcoholic extracts of geranium flower with the scientific name of *Pelargonium roseum*. In this study, aqueous and alcoholic extracts of geranium were prepared by percolation method. They then prepared a 48-cell plate and placed 100 micrograms per milliliter of TYI-S-33 culture medium in each well. Then, concentrations of 25, 50, 100, 200, 400, 800 and 1000 µg/ml of the prepared extract were added to the wells. Two wells were assigned to positive and negative control. Finally, concentrations of 400 and above of geranium extract were found to completely inhibit the growth of the parasite after 24 hours and in fact have a similar

effect to metronidazole (15).

### **Turmeric**

Turmeric, scientifically known as *Curcuma longa*, has long been used for food and medicine in Asian countries. In addition to food and medicinal uses, the rhizome of this plant also has pharmacological properties (21). Curcumin in the rhizome of turmeric plant, through various cellular and molecular mechanisms, causes multiple pharmacological and therapeutic effects, the most important of which is antioxidant, anti-inflammatory, and anti-cancer effects (22). In 1397, Fooladvand et al. studied the effect of curcumin in turmeric on *Trichomonas vaginalis*. During this study, different concentrations of curcumin were first prepared from 70 and 90% pure curcumin. Then, a certain amount of parasite cultured in the medium was placed in plate wells and each of the concentrations prepared from curcumin was added to one well. They also allocated two wells for positive and negative control and incubated the plate at 37 °C. Finally, it is found that 90% curcumin is a better alternative to metronidazole because of its lower manufacturing cost and greater inhibitory effect than metallic curcumin. In addition to the above, it should be noted that 70% curcumin cannot be used as a substitute for metronidazole, because this compound, despite having a similar inhibitory effect to 90% curcumin, has a toxic effect that can cause side effects in people. Therefore, it is better not to use this combination in the treatment of disease (23).

### **Peppermint, pennyroyal, and savory**

Peppermint, scientifically known as *Mentha piperita L*, is a perennial herbaceous plant that is cultivated for its essential oil and medicinal and spice purposes. Essential oils are also extracted from the dried plant body (24). Pennyroyal, scientifically known as *Menthe longifolia L*, is a valuable medicinal and aromatic plant belonging to the mint family (25). Savory with the scientific name of *Menthe hortensis L*, is an aromatic plant belonging to the mint family and its oil, which

has antioxidant, anti-inflammatory and analgesic properties, is used in the pharmaceutical and food industries (26). In 1397, Eghbal et al. investigated the effect of different concentrations of hydroalcoholic extracts of peppermint, pennyroyal, savory and their double and triple mixtures against *Trichomonas vaginalis*. For this purpose, the plants used in this study were collected and dried at room temperature and finally the powder obtained from them was used to prepare a hydroalcoholic extract of these plants. Then, different concentrations of plant extracts were added to the tubes containing the culture medium and the parasite trophozoite, and then the tubes were placed in an incubator at 37 °C. Two tubes were considered as positive and negative controls, the positive control tube contained metronidazole and the negative control tube contained DMSO. Finally, it was found that the anti-trichomonal potency of these three plants at concentrations of 5, 8 and 10 mg/ml is similar to metronidazole. However, the extracts of these three plants, in concentrations of 2 mg and less, do not show much inhibitory effect (5).

### **Feverfew**

Feverfew plant with the scientific name of *Tanacetum parthenium* is a long-day plant that grows in rocky and sometimes humid areas and due to its medicinal and antioxidant properties, it is one of the valuable plants in Iran, Iraq and Turkey (27). In 2013, Arefkhah et al. studied the effect of hydroalcoholic extract of Feverfew plant on *Trichomonas vaginalis*. In this study, the hydroalcoholic extract of the plant was prepared first and then concentrations of 1, 2, 4, 5, 8 and 10 mg/ml were prepared. Then, each of these prepared concentrations was added to the microtubes containing the parasite and the culture medium, and two microtubes were assigned to the positive and negative controls. The positive control containing metronidazole and the negative control without any additives. Finally, it was observed that concentrations of 4 and above, show a 100% inhibitory effect on *Trichomonas vaginalis*

after 24 hours (12).

### **Periploca aphylla and Pergularia tomentosa**

In 2017, Fooladvand et al. conducted a study on the lethality of two plants on *Trichomonas vaginalis*. During this study, different concentrations of aqueous, methanolic, chloromethane and N-hexane extracts of both plants were prepared and 100 µl of each of these concentrations was added to wells with culture medium and parasite. It was incubated for 48 hours. Finally, it was found that among the four extracts prepared from these two plants, dichloromethane extract at a concentration of 800 µg/ml has the highest inhibitory effect, which can be considered as a candidate for replacement with metronidazole. However, the aqueous extract of these plants does not show a good percentage of inhibition and therefore cannot be a good alternative to metronidazole (10).

### **White goosefoot**

White goosefoot, scientifically named *Chenopodium album*, belongs to the spinach genus and is one of the most important natural antioxidants that reduces oxidative stress in cells; It is also useful in the treatment of many cardiovascular and inflammatory diseases (28). In 2017, Ziaei Hezar Jaribi et al. investigated the inhibitory effect of hydroalcoholic extract of White goosefoot on *Trichomonas vaginalis*. In this study, the hydroalcoholic extract of the plant was prepared at concentrations of 600 and 1200 µg/ml and 100 µl of it was added to wells containing culture medium and parasite and incubated at 37 °C for 48 hours. Finally, it was found that concentrations of 600 and 1200 micrograms of this extract, 96 and 100%, respectively, inhibited the growth of parasite trophozoites, so the extract of this plant at a concentration of 1200 micrograms, can be a good alternative to metronidazole. However, lower concentrations of this extract showed less inhibitory effect, so concentrations below 600 may not be a suitable alternative to metronidazole (29).

### **Sweet violet**

Sweet violet plant with the scientific name of *Viola odorata* belongs to the violet genus, which has medicinal properties due to the presence of compounds such as carbohydrates and mucilage in its leaves and flowers (30). In 2013 Salehi et al. investigated the effect of different fractions of sweet violet on *Trichomonas vaginalis*. To perform this study, first the whole plant extract was obtained from the leaves and roots of the flower and finally different concentrations were prepared from the 3 prepared fractions. Then, for each fraction, seven tubes with culture medium and parasite were considered. To the first 5 tubes, different concentrations prepared from each fraction were added and the other two tubes were assigned to positive and negative control. Finally, it was found that the total extracts of leaves, flowers and roots of this plant for 24 hours, at concentrations of 1, 2 and 4 mg/ml, respectively, show a 100% inhibitory effect (31).

### **Chinese lantern**

Chinese lantern, scientifically named *Physalis alkekengi*, is a plant from the Solanaceae family that is more adaptable than plants in the same family and has different species with high economic value (32). In 2016, Jalil Azar et al. conducted a study on the effect of aqueous and alcoholic extracts of physalis fruit on the growth and proliferation of *Trichomonas vaginalis*. In this study, first the aqueous and alcoholic extracts of the fruit of this plant were prepared by percolation method. Subsequently, different concentrations of aqueous and alcoholic extracts of the fruit were prepared in the range of 250 - 8000 µg/ml and added to wells containing culture medium and parasite, and the growth of the parasite was studied for 72 hours. Finally, it was found that 2 concentrations of 4000 and 8000 µg/ml of the extract of this plant, in all three time periods of 24, 48 and 72 hours are able to inhibit 100% of parasites. As a result, these two concentrations of this extract, which have a similar effect to metronidazole, can be considered as a suitable alternative in the treatment of trichomoniasis (**Table 1**) (33).

**Table 1. Characteristics of medicinal plants affecting *Trichomonas vaginalis* based on studies**

Plant name	Extract concentration	Percentage growth inhibition and lethality	Study time (h)	Result	reference
Rhubarb	250 $\frac{mg}{ml}$	97%	24	The higher the concentration of rhubarb extract, the greater the anti-trichomoniasis effect and the best concentration is 300 mg/ml.	(4, 17)
	250 $\frac{mg}{ml}$	100%	48		
	300 $\frac{mg}{ml}$	100%	24		
Fennel	300 $\frac{\mu g}{ml}$	78%	48	Fennel has a less inhibitory effect than metronidazole and cannot be a suitable alternative to this drug in the studied concentrations.	(4, 18)
	600 $\frac{\mu g}{ml}$	79%			
	800 $\frac{\mu g}{ml}$	79%			
Eucalyptus	15 $\frac{mg}{ml}$	76%	72	Eucalyptus has an acceptable inhibitory effect at higher concentrations and at concentrations of 60 and 90 mg/ml have a similar effect to metronidazole.	(19, 20)
	60 $\frac{mg}{ml}$	100%			
	90 $\frac{mg}{ml}$	100%			
Geranium	200 $\frac{\mu g}{ml}$	50%	24	Geranium extract at concentrations of 400 mg/ml and above has an acceptable inhibitory effect.	(15)
	400 $\frac{\mu g}{ml}$	80%			
	800 $\frac{\mu g}{ml}$	100%			
	1000 $\frac{\mu g}{ml}$	100%			
Turmeric (curcumin 90)	200 $\frac{mg}{ml}$	12%	24	Considering the IC50 among different curcumin derivatives, 90% curcumin seems to be a better candidate for drug manufacturing due to its lower manufacturing cost and higher impact than metallic curcumin.	(21, 23)
	600 $\frac{mg}{ml}$	18%			
	800 $\frac{mg}{ml}$	19%			
Mint Savory Pennyroyal	2 $\frac{mg}{ml}$	Low High High High	24	Extracts of these three types of plants in concentrations above 5 mg/ml, show a good inhibitory effect.	(5, 25, 26)
	5 $\frac{mg}{ml}$				
	8 $\frac{mg}{ml}$				
	10 $\frac{mg}{ml}$				
Feverfew	1 $\frac{mg}{ml}$	5%	24	Concentration of 4 mg/ml and more of this plant extract can be a good substitute for metronidazole	(12, 27)
	2.5 $\frac{mg}{ml}$	94%			
	4-10 $\frac{mg}{ml}$	100%			
Pergularia Tomentosa (dichloromethane)	100 $\frac{\mu g}{ml}$	90%	48	The greatest inhibitory effect of this plant is observed by its dichloromethane extract at a concentration of 800 $\mu g/ml$	(10)
	400 $\frac{\mu g}{ml}$	92.7%			
	800 $\frac{\mu g}{ml}$	95.2%			
Periploca aphylla (dichloromethane extract)	100 $\frac{\mu g}{ml}$	88.7%	48	The greatest inhibitory effect of this plant is observed by its dichloromethane extract at a concentration of 800 $\mu g/ml$	(10)
	400 $\frac{\mu g}{ml}$	89.8%			
	800 $\frac{\mu g}{ml}$	93.4%			

**Table 1. Continued**

Pergularia Tomentosa (aqueous extract)	200 $\frac{\mu g}{ml}$	55%	48	The aqueous extract of this plant has little inhibitory effect, so it cannot be a good alternative to metronidazole.	(10)
	800 $\frac{\mu g}{ml}$	64%			
Periploca aphylla (aqueous extract)	200 $\frac{\mu g}{ml}$	59%	48	The aqueous extract of this plant has little inhibitory effect, so it cannot be a good alternative to metronidazole.	(10)
	800 $\frac{\mu g}{ml}$	70%			
White goosefoot	75 $\frac{\mu g}{ml}$	79%	48	Concentration of 1200 $\mu g/ml$ of the extract of this plant has a similar effect to metronidazole, so it can be a good alternative to metronidazole.	(28, 29)
	600 $\frac{\mu g}{ml}$	96%			
	1200 $\frac{\mu g}{ml}$	100%			
Sweet violet leaf extract	1 $\frac{mg}{ml}$	60%	24	The extract of sweet violet leaves at a concentration of 4 mg/ml has a similar inhibitory effect to metronidazole.	(30, 31)
	4 $\frac{mg}{ml}$	100%			
Sweet violet flower extract	1 $\frac{mg}{ml}$	65%	24	The inhibitory effect of the extract of sweet violet flowers is almost similar to that of the extract of violet leaves.	(30, 31)
	4 $\frac{mg}{ml}$	100%			
Sweet violet root extract	1 $\frac{mg}{ml}$	90%	24	The whole extract of violet root is more effective than the leaf and its flower, because at a lower concentration, it is 100% effective.	(30, 31)
	2 $\frac{mg}{ml}$	100%			
Chinese lantern	250 $\frac{\mu g}{ml}$		24	Concentrations of 4000 and 8000 $\mu g/ml$ of the extract of this plant show a similar effect to metronidazole.	(32, 33)
	500 $\frac{\mu g}{ml}$	42%			
	1000 $\frac{\mu g}{ml}$	61%			
	4000 $\frac{\mu g}{ml}$	90%			
	8000 $\frac{\mu g}{ml}$	100%			

## Discussion

*Trichomonas vaginitis* is one of the most common sexually transmitted infections that affects women between the ages of 16 and 53, especially pregnant women, and can affect the health of individuals. In this study, we investigated the effects of herbal remedies in eradicating or inhibiting the growth of *Trichomonas vaginalis*. Studies have shown that herbs can be effective in treatment and since natural remedies are less expensive, less risky and more accessible, they can be used as an alternative treatment, but it should be noted that most studies have been performed *in vitro* and the effectiveness of these

treatments is actually determined when they are tested in clinical trials and their effects on human health be proved. Because understanding the side effects of these plants can be important in deciding whether to use them as medicine or not.

## Conclusion

Considering the inhibitory effects of medicinal plants on the growth and proliferation of *Trichomonas vaginalis* *in vitro*, it can be concluded that the use of these plants can have many applications in the treatment of trichomoniasis. As a result, by studying more about their advantages and disadvantages, it is possible to make a drug

that has higher therapeutic effects with fewer side effects.

### Acknowledgments

The present study, has been approved by the Ethics Committee of the Vice-Chancellor for Research and Technology of Mazandaran University of Medical Sciences with the number IR.MAZUMS.REC.1399.948. The authors of the article express their gratitude to the Vice-Chancellor for Research and Technology and Student Research Committee of Mazandaran University of Medical Sciences for supporting this project.

### Conflicts of interest

Authors declare that there is no conflict of interests.

### Authors' contributions

All authors have intellectually committed to the study design and process. The final manuscript was revised and accepted by all authors.

### Funding

Mazandaran University of Medical Sciences.

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