Comparative Study of the Effect of Sophora alopecuroides Plant Extract on Trichomonas vaginalis Parasite In Vitro

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Background
Trichomoniasis is the most common non-viral sexually transmitted disease in the world, especially in developing countries.1 According to the latest statistics, the prevalence of trichomoniasis was 6.3% in women and 0.6% in men.2 Approximately 170 million people in the world are infected with this parasite. Trichomonas vaginalis is a flagellated protozoan parasite that is transmitted through sexual contact and is limited to the reproductive system.3 Trichomoniasis is related to reproductive years in women in the age range of 35-40 years old, while other non-viral sexually transmitted diseases usually occur in the ages of 15-25 years old.4 The symptomatic cases of trichomycosis are generally associated with itching, painful vaginal intercourse, vaginal discharge, and the like.5 Further, clinical symptoms include foamy and foul-smelling yellow-green discharge, redness and inflammation in the vaginal area, burning urine, and pain in the abdomen area6 that usually increases during menstruation. In addition, the parasite causes premature birth and birth of babies with low weight.6 Men with T. vaginalis are typically asymptomatic, but symptomatic infection is associated with urethritis (burning urine and discharge). Trichomonas in men resolve without treatment.7 Metronidazole is one of the drugs of choice for trichomoniasis treatment, which has many side effects (e.g., renal failure, neurotoxicity, and the like) due to long-term use. Recently, reports of resistance to metronidazole are increasing.4 Therefore, many attempts have been made to replace metronidazole or to introduce anti-Trichomonas vaginalis drugs and continue until now. The present study sought to investigate the effect of the extracts of S. alopecuroides plant against T. vaginalis and compare it with metronidazole in vitro.

Materials and Methods
The archived cryopreserved T. vaginalis parasite was...
null cultured under sterile conditions in the Parasitology Department of Tarbiat Modares University. After three passages and making sure of the presence of active and motility parasites that are in the stage after the logarithmic phase of their growth (duration 48 to 72 hours), the number of 2 million parasites per milliliter was counted and the amount of 400 µL of this medium containing the parasite was added to 500 µL of fetal bovine serum in special screw-door cryotubes. In the last step, 100 µL of dimethyl sulfoxide (DMSO) was added and immediately transferred to a -80-degree freezer.

Then, 500 g of the leaves and roots of S. alopecuroides was mixed with about 5 times distilled water and 70% alcohol. Next, it was covered and put on a shaker with a magnet at room temperature for 72 hours and checked every 24 hours. All the solutions were filtered with filter paper. The obtained solutions were poured into 50 mL Falcon tubes and centrifuged for 30 minutes with a refrigerated centrifuge at 5000 rpm. The aqueous extract was turned into powder by a lyophilizer. The alcoholic extract was transferred to a rotary device for drying to completely remove the alcohol, and then the residue was poured on large plates for a week and placed in the laboratory environment to evaporate the excess alcohol and turn it into powder. Subsequently, 0.02 g (20 mL) of the dry extract of each part was completely dissolved in 4 mL of sterile buffer. After passing through the 0.2 µ filter, the initial concentrations were prepared at 0.5, 1, 2, and 4 mg/mL and were kept in the refrigerator until encountering the parasite.

About 200 µL of the culture medium containing 10⁶ trophozoites was transferred under the biologic hood and next to the flame in completely sterile conditions to a 48-well plate, and then an amount of 200 µL of each of the test dilutions mentioned was added to each of the wells. Next, the microplates were transferred to a 37-degree incubator with 5% CO₂. For each dilution, 3 wells were replicated three times to ensure the work results. The effects of the extracts on the parasite were counted at both 24 and 48 hours, and for all tests, a negative control including the medium containing the parasite were counted at both 24 and 48 hours, and for all tests, a negative control including the medium containing the parasite and PBS with 1% DMSO solution and a positive control including the medium containing the parasite and metronidazole were taken into consideration.

To count the cells in completely sterile conditions, 200 µL of the medium containing the parasite was transferred to a 200 mL microtube after appropriate pipetting, and 20 µL of trypan blue dye was added to it so that the parasite was properly placed in the vicinity of the dye and entered the dead cells. It was well pipetted, and then 10 µL of it was taken and injected under the neobar slide for counting. To check the cytotoxicity level, the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-H-tetrazolium bromide technique was used on mouse peritoneal macrophage cells, and the percent survival of macrophages after exposure to plant dilutions and metronidazole was calculated finally.

### Statistics

Half-maximal inhibitory concentration (IC₅₀) and 50% cytotoxic concentration (CC₅₀) values were evaluated by PRISM GraphPad software, and the analysis of variance and T-test were used for statistical comparisons by SPSS software, version 23. The P value for statistical analysis was considered >0.05.

### Results

Based on the results of Table 1, after 24 hours at a concentration of 2.5%, the root aqueous extract had the greatest effect with 69.4%, while the leaf aqueous extract had the least effect with 34.68% of growth inhibition. However, none of the extracts had a greater inhibitory effect than metronidazole, but after 48 hours, the effect of the extract caused more inhibition due to the effect of time so that a significant difference was observed between all the extracts at 24 and 48 hours after performing a statistical test (P<0.01). Furthermore, the percentage of growth inhibition in the aqueous and alcoholic extract of the root on the parasite increased with increasing time and concentration, and this increase was significant compared to the metronidazole group.

Regarding the effect of the extracts on parasite T. vaginalis, the percentage of life decreased with increasing the concentration in 24 and 48 hours. The decrease in the percentage of life in all concentrations was significant compared to the control group, and there was also a significant difference in comparing different concentrations with each other between 24 and 48 hours (P<0.01), indicating that time is effective in reducing

### Table 1. Parasite Growth Inhibition (Percentage) Under the Influence of Extracts and Metronidazole in 24 and 48 Hours

<table>
<thead>
<tr>
<th>Extract</th>
<th>Concentrations</th>
<th>0.25</th>
<th>0.5</th>
<th>1</th>
<th>2</th>
<th>2.5</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>24 h</td>
<td>48 h</td>
<td>24 h</td>
<td>48 h</td>
<td>24 h</td>
<td>48 h</td>
</tr>
<tr>
<td>Root (Alcoholic)</td>
<td>26.78</td>
<td>66.47</td>
<td>27.16</td>
<td>67.24</td>
<td>36.41</td>
<td>70.32</td>
</tr>
<tr>
<td>Root (Aqueous)</td>
<td>5</td>
<td>60.86</td>
<td>14.45</td>
<td>64.84</td>
<td>41.04</td>
<td>69.82</td>
</tr>
<tr>
<td>Leaf (Alcoholic)</td>
<td>4.04</td>
<td>62.53</td>
<td>6.35</td>
<td>72.80</td>
<td>9.82</td>
<td>75.29</td>
</tr>
<tr>
<td>Leaf (Aqueous)</td>
<td>5.97</td>
<td>69.03</td>
<td>6.35</td>
<td>70.98</td>
<td>9.07</td>
<td>74.62</td>
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<td>Metronidazole</td>
<td>78.6</td>
<td>90.71</td>
<td>89.21</td>
<td>93.20</td>
<td>89.98</td>
<td>99.96</td>
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</tbody>
</table>

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the percentage of life. The average number of parasites after exposure to different concentrations of aqueous root extract in both 24 and 48 hours with the increase in concentration, was showed decrease in survival percentage (significant) compared to the control group. Moreover, comparing the percentage of life between different concentrations in 24 and 48 hours, except for the concentration of 2.5, the decrease in the percentage of life was significant, but there was no significant difference in the concentration of 2.5. Considering the aqueous and alcoholic extracts of leaves at 24- and 48-hour time points with the increase in concentration, the percentage of life decreased significantly compared to the control group. Further, the comparison of the percentage of life between different concentrations at 24- and 48-hour time points was significant; therefore time was effective in decreasing viability ($P < 0.01$). Furthermore, the decrease in viability between different concentrations of metronidazole in all concentrations and both 24 and 48 hours was significant.

Examining the Toxicity of Drugs Using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium Bromide Assay and Half-maximal Inhibitory Concentration

Mouse macrophage cells were used to determine the toxicity of drugs, and the percentage of cell viability was checked at different concentrations of drugs and metronidazole, the results of which are provided in Table 2. The cytotoxicity effect of leaf and root extracts of Sophora alopecorides plant after exposing mouse macrophages to the amount of CC$_{50}$ of the concentrations prepared from each of the extracts was calculated using Graphpad prism5 software which is given in the Table 2.

The IC$_{50}$ was determined for the tested extracts, and then the results were compared with each other. They were almost the same, but after 48 hours, the results demonstrated that the IC$_{50}$ of the alcoholic and aqueous leaf extracts was reduced in the alcoholic extract of the root after 24 hours (Table 3).

The effectiveness of any drug combination depends on its therapeutic selectivity index (SI), implying that the drug must inhibit the parasite and simultaneously have the least toxicity for the host cell. The value of the SI (which is obtained by dividing the amount of CC$_{50}$ by the amount of IC$_{50}$) was calculated, and numbers related to metronidazole and the extracts used in the research after 24 and 48 hours are presented in Table 3. The results revealed that after 48 hours, root and alcoholic leaf extracts were more effective than metronidazole, and the aquatic leaf extract had a good effect with the least toxicity.

Discussion

Trichomonas vaginalis is considered the most important non-viral sexually transmitted infection (Trichomoniasis) agent, affecting about 60-80 million people annually. Since 1960, metronidazole and other 5-nitroimidazole compounds such as tinidazole have been employed to treat trichomoniiasis effectively; however, resistance to metronidazole is increasing, and allergic reactions and side effects of this drug are a major concern. Moreover, 5-nitroimidazole compounds are destructive to the host's DNA. The need for wider research to replace medicinal plants as new therapeutic agents has been proven. Natural products, especially medicinal plants, are considered a rich source of bioactive molecules, which have been used for the treatment of various diseases previously.

The selection of the bitter plant with the scientific name Sophora and specifically the S. alopecuroides species is based on the research conducted by Wang et al, whose active ingredient is quinolizidine alkaloids, which have anti-depressant, sedative, analgesic, anti-pyretic, anti-inflammatory, anti-depressant, and anti-inflammatory properties. Additionally, it is against viral, bacterial, fungal, and protozoan infections. Several studies have been conducted in the field of the antiparasitic properties of Sophora flavescens and Torilis japonica plant extracts.

Different parts of Sophora plants, including the root, leaf, and stem of its seeds, each alone or simultaneously, are commonly applied in Chinese medicine for the treatment of eczema-vaginitis, acute respiratory tract infections. Many plant extracts and their metabolites are a rich source of biologically active substances that have antimicrobial and anti-parasitic effects. The presence of anti-parasitic compounds in plants allows for the identification of effective compounds against parasites for pharmaceutical purposes and bioactive additives in foods. In this way, a wide range of plants can be

<table>
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<th>0.25</th>
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<th>2</th>
<th>2.5</th>
<th>Control</th>
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<tr>
<td>Root (Alcoholic)</td>
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<td></td>
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<td>Root (Aqueous)</td>
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<td>Leaf (Alcoholic)</td>
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<td>Leaf (Aqueous)</td>
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<tr>
<td>Metronidazole</td>
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Table 2. Macrophage Survival Percentage After Exposure to Extracts and Metronidazole

<table>
<thead>
<tr>
<th>Extract</th>
<th>CC$_{50}$</th>
<th>SI</th>
<th>IC$_{50}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Root (Alcoholic)</td>
<td>2.858</td>
<td>1.66</td>
<td>1.72</td>
</tr>
<tr>
<td>Root (Aqueous)</td>
<td>5.788</td>
<td>3.40</td>
<td>1.70</td>
</tr>
<tr>
<td>Leaf (Alcoholic)</td>
<td>2.61</td>
<td>0.512</td>
<td>5.097</td>
</tr>
<tr>
<td>Leaf (Aqueous)</td>
<td>1.37</td>
<td>0.25</td>
<td>5.46</td>
</tr>
<tr>
<td>Metronidazole</td>
<td>3.107</td>
<td>4.438</td>
<td>0.5</td>
</tr>
</tbody>
</table>

Table 3. The Value of the SI, CC$_{50}$, and IC$_{50}$ Index Related to Metronidazole and the Extracts Used in the Research After 24 and 48 Hours

Note: SI: Selectivity index; CC$_{50}$: 50% cytotoxic concentration; IC$_{50}$: Half-maximal inhibitory concentration.
included in the diet and can replace common drugs for parasite control; this method can probably reduce the risk of developing drug resistance.\textsuperscript{16} \textit{S. alopecuroides} has alkaloid compounds and non-poisonous amino acids that have pharmacological effects. Considering that no research has been performed on the effect of different extracts of the Sophora plant on Trichomonas, this is the first investigation on the exposure of this extract to \textit{T. vaginalis}.

According to the obtained results, both aqueous and alcoholic extracts of both leaves and roots have an inhibitory effect on \textit{T. vaginalis}. According to the statistical test, it is determined that the effect of the extract is completely dependent on time and between 24 and 48 hours. The hour of growth inhibition has a significant difference in all concentrations, except for the concentration of 2.5, the aqueous root extract, in which time does not cause a significant difference.

Conclusion

Overall, the aqueous and alcoholic extracts of the leaves and roots of the \textit{S. alopecuroides} plant have good anti-trichomonas effects on the parasite. After 48 hours, the root extracts and the alcoholic leaf extract were more effective than metronidazole. Additionally, the aqueous leaf extract has a noble effect with the least toxicity. Considering that this research was conducted for the first time, a wider study is needed to generalize the results and to continue the work in \textit{in vivo} conditions.

Authors’ Contribution

Conceptualization: Javid Sadraei.
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Formal analysis: Majid Pirestani.
Funding acquisition: Javid Sadraei.
Investigation: Saeed Bahadory.
Methodology: Sedigheh Khoeeniha.
Project administration: Javid Sadraei.
Resources: Majid Pirestani.
Software: Saeed Bahadory.
Supervision: Javid Sadraei.
Validation: Majid Pirestani.
Visualization: Javid Sadraei.
Writing—original draft: Sedigheh Khoeeniha, Javid Sadraei, Majid Pirestani, Saeed Bahadory.
Writing—review & editing: Sedigheh Khoeeniha, Javid Sadraei, Majid Pirestani, Saeed Bahadory.

Competing Interests

The authors declare no conflict of interests.

References