

## Comparison anti-giardia activity of *Satureja hortensis* alcoholic extract and metronidazole *in vitro*

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### ABSTRACT

**Background and aims:** Giardiasis is known as intestinal infection and created by the flagellate protozoan *Giardia lamblia*. Some studies showed that phenolic components, present in plant extracts and essential oils, have anti-Giardia activity. The current study was performed to compare anti-Giardia activity of *Satureja hortensis* (SH) alcoholic extract and metronidazole *in vitro*.

**Methods:** In this Laboratory-experimental study, it was separated cysts from the feces by Bingham procedure with minor modification. The numbers of cysts were calculated with Hemusytometr and the purified cysts of *Giardia lamblia* (500 µl) were exposed with 500 µl of extract at concentrations of 10, 100 and 200 mg/ml and 125 mg/kg of metronidazole for 30, 60 and 120 min. The numbers of dead and live cysts was enumerated with a microscope.

**Results:** Findings in the currnt study showed an increase in anti-Giardia activity of extract at high concentrations with increasing time. SH, at 100 mg/ml killed 53% in 30 min, 68% in 60 min and 78% in 120 min. Also, the extract killed by 62% in 30 min, about 88% in 60 minutes and 92% in 120 minutes. The drug had similar effects in 60 and 120 minutes, but it killed 83% in 30 minutes.

**Conclusion:** SH alcoholic extract showed anti-Giardia activity at high rates and more time. Thus, SH extract at 200 mg may be suitable alternative for Metronidazole, without side effects. However, anti-Giardia activity of SH needs to more attentions, especially clinical investigations.

**Keywords:** Cyst, *Satureja hortensis*, *Giardia lamblia*, Metronidazole.

### INTRODUCTION

The *Giardia* species, from branching eukaryotes present in intestine, can stick to mammal's intestinal epithelium.<sup>1,2</sup> The most common symptom for involvement with *Giardia* species is diarrhea. Since *Giardia* involved in intestine system, it may decrease absorption of macronutrients and some

vitamins (A or B12) and subsequently developed retardation and delay in physical growth especially in younger peoples.<sup>3-5</sup> Treatment of giardiasis was done by 5-nitroimidazole, derivatives of metronidazole, nitroimidazoles, benzimidazoles and benzimidazole derivatives. These drugs

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have usual side effects and carcinogenic impacts.<sup>6-9</sup> The researchers tried to find efficient or guard and safe substances for the therapy of giardiasis infection.<sup>10-12</sup> The herbal medicines and their derivatives are important sources of new bioactive components, because they have wide variety of metabolites with potential therapeutic value.<sup>13-15</sup> The genus *Satureja* L. was presented in Mediterranean as origin and some areas of southern and south eastern Europe, Asia Minor, and northern Africa. The phenolic components especially carvacrol are most enough components in genus *Satureja* L.<sup>16</sup> The components of *Satureja hortensis* (SH) have anti-diarrheal activity, antioxidant properties and antibacterial action.<sup>17-20</sup> There are reports showing effects of plant extracts in prevention of proliferation, and adherence *G. lamblia* trophozoites in intestine.<sup>21-25</sup> Thus, the present experiment was done to compare anti-Giardia activity of SH with metronidazole under *in vitro* condition.

## METHODS

The fresh leaves of *Satureja hortensis* were collected from Sahand mountain slopes (Maragheh, Iran) in spring 2015 and immediately transferred to herbarium for detection by botanist in Maragheh University (Voucher No 2015-153). The leaves of *S. hortensis* were dried by oven at a temperature of 41°C, and then fined to moderate powders (290-650 µm) by mill. The powder of *S. hortensis* (100 g), were extracted using maceration and percolation by 75% ethanol for three times consecutive in both extractive processes. We concentrated extract solutions by a decrease in pressure using a rotary evaporator. The residues water was re-suspended in pH 7.1 phosphate buffered solution (PBS). These solutions were sterilized using filtration by a 0.24-µm membrane, and kept at sterile bottles in 5°C until analysis. Also

Metronidazole was prepared of Alborz-daru Company (Tehran-Iran).

The positive and fresh samples of human feces were collected by parasitology laboratory of Abhar town (Zanjan-Iran). In this Laboratory-experimental study, the cysts were separated as previously explained by Bingham et al.<sup>26</sup> It was selected the samples *Giardia lamblia*-contained. Briefly, 5 gr of feces samples mixed with 10 ml physiological serum for 30 min and subsequently were percolated by 4-layer filter. Then, the samples were centrifuged for 3 min at 1500 rpm. The upper solution removed and added 10 ml sucrose solution (2M) to residue and centrifuged for 10 min at 1800 rpm. The upper solution (having cyst) separated and added 10 ml normal saline (0.9%) to above solution and centrifuged for 5 min at 1000 rpm. Finally, 2 ml of tube end (having cyst) was kept for analysis as described by Farsangi et al.<sup>27</sup>

To this purpose, it was prepared the extracts at different concentrations (10, 100 and 200 mg/ml) by DMSO (dimethyl-sulfoxide) solvent. 500 µl of extracts at different concentrations and 125 mg/kg of metronidazole as well as mixed with 500 µl of suspension cyst-contained for 30, 60 and 120 min. Then, it was added five hundred µl from eosin dye as explained by Farsangi et al.<sup>27</sup> After 3 min, it was calculated 100 cysts by light microscope and compared percentage of active and dead cysts with control sample (having cyst and physiological serum). On basis of Bingham et al. procedure, dead and active cysts were seen in colors red and non-color, respectively<sup>26</sup>.

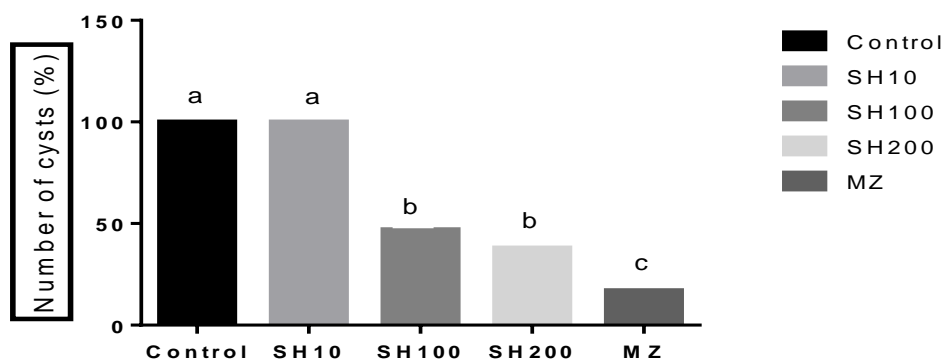
The data were analyzed by ANOVA procedure of SPSS software and subsequently the means compared by Tukey-test.

## RESULTS

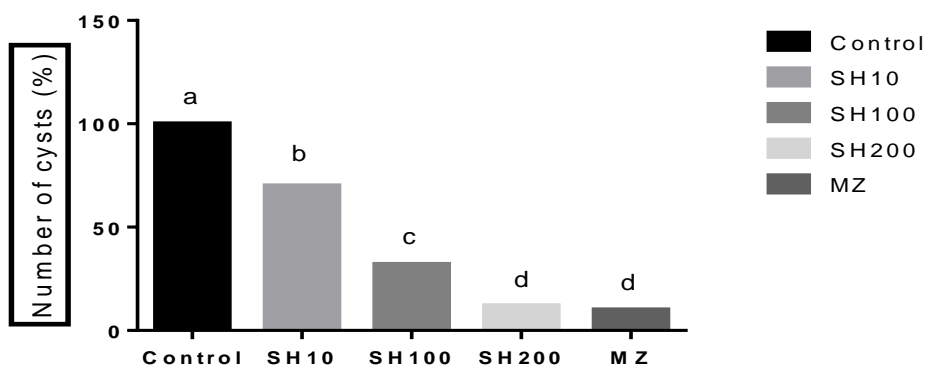
Figures 1-3 compare anti-Giardia activity of SH and metronidazole on Giardia

cysts at different times. Figure 1 presents a mean of anti-Giardia activity of extract at different concentrations and metronidazole for 30 min. As results indicate, the extract at concentrations of 100 and 200 mg significantly showed higher activity against Giardia cysts compared with control group and 10 mg ( $P<0.05$ ). Although SH at high concentration (100 and 200 mg) showed higher anti-Giardia activity but metronidazole had better activity compared with SH (100 or 200 mg). An increase in anti-Giardia activity was seen with increasing extracts concentration ( $P<0.001$ ). On the basis our findings in Figures 2-3, the extract at different levels showed anti-Giardia activity when compared with

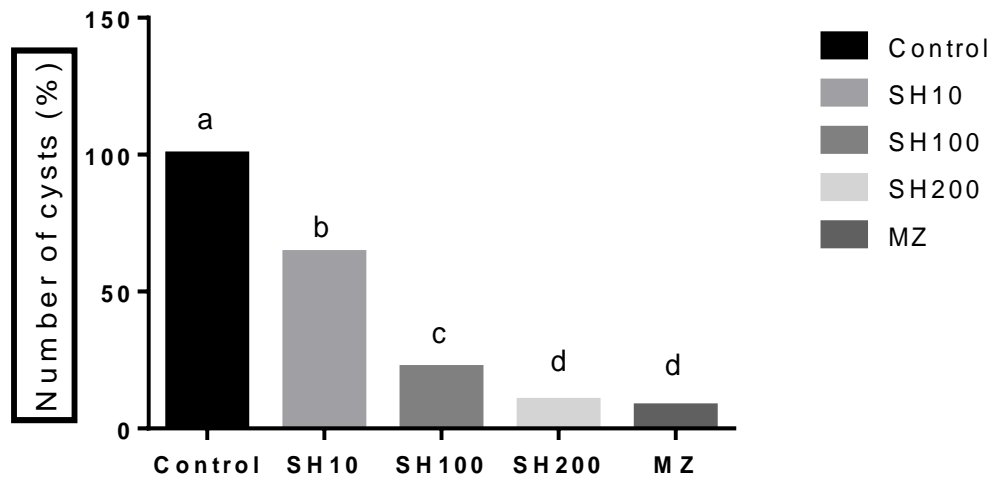
control group for 1 and 2 hours ( $P<0.05$ ). There were no statistical significant differences between SH (200 mg) and metronidazole ( $P<0.05$ ) for 60 and 120 min. Overall, SH at 100 mg/ml killed 53% in 30 min, 68% in 60 min and 78% in 120 min. Also, the extract killed by 62% in 30 min, about 88% in 60 min and 92% in 120 min. The drug had similar effects in 60 and 120 min, but it killed 83% in 30 min. Figure 4 presents effects of SH at rates (10, 100 and 200 mg) and metronidazole in different times. There were significant differences between treatments at different times, so that samples treated with SH at 60 and 120 min showed higher anti-Giardia activity compared with 30 min.



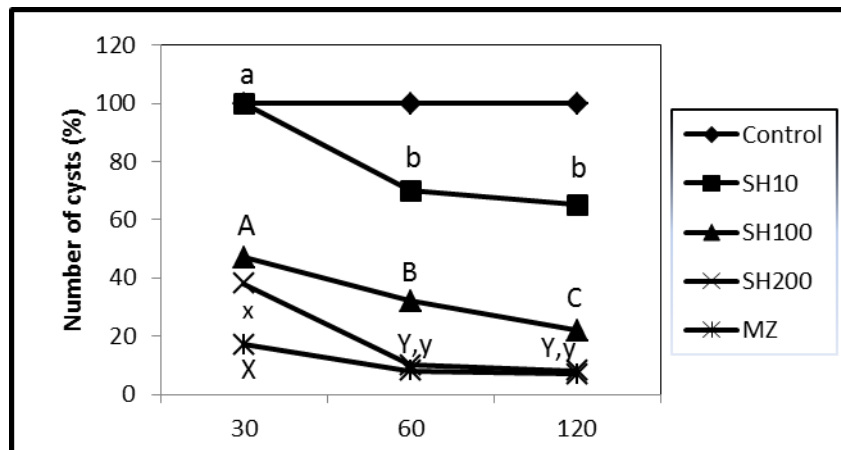
**Figure 1:** Comparison the effect of *Satureja hortensis* (SH) alcoholic extracts at rates (10, 20 and/or 200) and metronidazole (MZ) on Giardia cysts to 30 min  
<sup>a-c</sup> shows significant differences at ( $P<0.001$ ) between treatments.



**Figure 2:** Comparison the effect of *Satureja hortensis* (SH) alcoholic extracts at rates (10, 20 and/or 200) and metronidazole (MZ) on Giardia cysts to 60 min  
<sup>a-d</sup> shows significant differences at ( $P<0.001$ ) between treatments.



**Figure 3:** Comparison the effect of *Satureja hortensis* (SH) alcoholic extracts at rates (10, 20 and/or 200) and metronidazole (MZ) on Giardia cysts to 120 min  
<sup>a-d</sup> shows significant differences at ( $P < 0.001$ ) between treatments.



**Figure 4:** Comparison the effect of *Satureja hortensis* (SH) alcoholic extracts at rates (10, 20 and/or 200) and metronidazole (MZ) at different times  
<sup>a-b</sup> shows significant differences for SH10 at different times. <sup>A-B</sup> presents significant differences for SH100 at different times. <sup>x-y</sup> indicates significant differences for SH200 at different times. <sup>X-Y</sup> indicates significant differences for SH200 at different times.

## DISCUSSION

The *G. lamblia*, an intestinal parasite that stick to intestinal epithelium, exhibits with different signs such as diarrhea and sickness in humans especially in infants.<sup>28,29</sup> Since anti-Giardia drugs have side effects, thus it is necessarily to find new, safe, and effective agents.<sup>28,29</sup> Plants and their derivates may be suitable alternatives for

drugs, because humans use them for treatment of diarrhea and other digestive disorders. Our findings showed that treatment with extracts at different times and concentration, except *S.hortensis* 10 mg/ml at 30 min, could show anti-Giardia activity when compared with control treatment. The extract at high concentration had powerful

anti-Giardia activity compared with low concentrations at different times, so that the most anti-Giardia activity was associated 200 mg treatment. It's interested that, an increase in time caused an increase in anti-Giardia activity of extracts at low concentrations (10 mg), so that the extracts (10 mg) at 120 min had more activity when compared with 30 min. *In vitro* and *in vivo* studies showed positive effects of some plant derivatives against *G. lamblia* cysts and trophozoites.<sup>23,30,31</sup> In partly similar studies, plant extracts prevented proliferation, and adherence *G. lamblia* trophozoites in intestine.<sup>21-25</sup> It is well-known that essential oils obtained from various species of *Satureja* have antidiarrheal activity.<sup>17,32</sup> Anti-Giardia activity of extracts may be associated to phenolic compounds (thymol and carvacrol) that prevent xanthine and xanthine oxidase activity.<sup>33</sup>

Seems SH extract show anti-Giardia activity through interference in cell structure and more contact (1 and 2 h) provides suitable opportunity for this association. The idea confirmed by other researchers Cristani et al. who showed that present compounds in plants inhibits membrane expansion and respiration, or elevate membrane fluidity and permeability, and change ion transport processes in parasites.<sup>34</sup> In this association, some researchers reported that treatment with essential oils increase Giardia cells, volume of the Giardia peripheral vesicles, and subsequently cell death.<sup>35,36</sup> Interestingly, SH at 200 mg had no significant differences with metronidazole for anti-Giardia activity for 1 and 2 h; suggesting use of natural compounds instead metronidazole.

## CONCLUSION

On basis our findings, SH at high concentrations has anti-Giardia activity of 30-120 min. Although with increasing time, SH at low concentrations (10 mg) indicated

anti-Giardia activity but it could compete with metronidazole. Extract at 200 mg could compete with Metronidazole at times 1 and 2 h. Thus, SH extract at 200 mg can be suitable alternative for Metronidazole, without side effects.

## CONFLICT OF INTEREST

The authors declare no conflict of interest.

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