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# In vivo evaluation of antiparasitic effects of Artemisia abrotanum and Salvia officinalis extracts on Syphacia obvelata, Aspiculoris tetrapetra and Hymenolepis nana parasites

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#### PEER REVIEW

#### Peer reviewer

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#### Comments

This is a good research in which the authors determined effects of two local plant extracts on three various parasites in mice. The results are interesting that *S. officinalis* and *A. abrotanum* extracts had good antiparasitic effects on three various parasite.

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#### ABSTRACT

**Objective:** To evaluate the effects of *Salvia officinalis* and *Artemisia abrotanum* extracts against digestive system parasites of mice.

**Methods:** The ethanol extract was prepared and dissolved in distilled water. The mebendazole was used as positive control and distilled water as negative control. After counting eggs per gram feces, infected mice with 16 eggs per gram feces contained two to three parasites of *Syphacia obvelata*, *Aspicoloris terepetra* and *Hymenolipis nana* designated in 4 groups. The first group was given extracts of *Artemisia* (150 mg/kg), the second group was given *Salvia* extract (150 mg/kg), the third group was given mebendazole (10 mg/kg) and finally the fourth group was given distilled water (2 mL/kg).

**Results:** The ethanol extracts of *Artemisia* and *Salvia* plants reduced the number of parasite eggs per gram of feces. Results showed significant reduction (*P*–value<0.001) in the number of eggs excreted by *Hymenolepis nana*, *Aspiculuris tetraptera*, *Syphacia obvelata* in mice.

**Conclusions:** These results revealed that antiparasitic effects of *Artemisia* and *Salvia* are reasonable and these two plants might be used as antiparasitic natural products.

### KEYWORDS

Salvia officinalis, Artemisia abrotanum, Mice, Parasite, Syphacia obvelata, Aspicoloris terepetra, Hymenolipis nana

#### 1. Introduction

Parasitic infections cause major public health problems leading to morbidity and sometimes mortality of the victims. Chemical drug against parasites is beneficial, but this method has some problems such as drug resistance, residue and unwanted side effects associated. Therefore, study on alternative therapies are important<sup>[1]</sup>. For

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centuries, medicinal plants have been used to eliminate parasites in many parts of the world, and humans used the plant to treat various diseases. Herbal medicines are used in recent years due to the high reliability and effectiveness, and cost is also taken into consideration<sup>[2,3]</sup>. In ethnoveterinary medicine, which draws inspiration from traditional practice, there seems to be various plants suitable for treating almost every parasitic disorder of livestock. Although the majority of the evidence on antiparasitic activity of plants has been customarily based on anecdotal observations, there are currently an increasing number of controlled experimental studies aimed to verify, validate and quantify such plant activity in a scientific manner. Currently, there are 119 drugs composed of plants, and only 90 of the 250 000 known plant species, has been obtained[2,3]. In Chaharmahal va Bakhtiari and Kerman provinces in Iran, Artemisia abrotanum (A. abrotanum) and Salvia officinalis (S. officinalis) are used as medicinal plants against parasites.

Cultivars of S. officinalis are quite variable in size, leaf and flower color and foliage pattern with many variegated leaf types. The Old World type grows approximately 0.61 m tall and wide with lavender flowers, the most common color. And the flower can also be white, pink, or purple. The plant flowers bloom in late spring or summer. The leaves are oblong, ranging in size up to 6.4 cm long by 2.5 cm wide. Leaves are grey-green, rugose on the upper side, and nearly white underneath due to many short soft hairs. Modern cultivars include leaves with purple, rose, cream, and vellow in many variegated combinations. Sage leaf contains tannic acid, oleic acid, ursonic acid, ursolic acid, cornsole, cornsolic acid, fumaric acid, chlorogenic acid, caffeic acid, niacin, nicotinamide, flavones, flavonoid glycosides, and estrogenic substance[3]. Its leaves contain essential oils and tannins that can help to facilitate digestion with anticonvulsant, anti-fever, antiseptic and anti-diabetic effect. Modern evidence shows possible uses as an antisweating, antibiotic, antifungal, astringent, antispasmodic, estrogenic, hypoglycemic and tonic agent. This plant is also used for the treatment of gout, chronic rheumatism, dizziness and headache. Investigations have be performed to use sage as a medication for Alzheimer's disease.

Wormwood is used to treat liver, spleen and stomach disorders. It is seldom used for medicinal purpose today, except in Germany, where poultices are placed on wounds, splinters and skin conditions and it is employed occasionally to treat frostbite[4].

In the present study, the antiparasitic effects of *Artemisia* and *S. officinalis* plants were investigated on the most common gastrointestinal tract parasites such as *Hymenolepis nana* (*H. nana*), *Aspiculuris tetraptera* (*A. tetraptera*) and *Syphacia obvelata* (*S. obvelata*).

# 2. Materials and methods

The methanolic extracts of *A. abrotanum* and *S. officinalis* were prepared by adding a 1:3 ratio of garlic and methanol, respectively, and this was subjected to Soxhlet extraction for 72 h according to the prescribed method of Eidi *et al*<sup>[5]</sup>. After extraction, the solvent was filtered<sup>[5]</sup>. The information about the studied plants is presented in Table 1.

**Table 1**Scientific name, English name, part used, extract type, and traditional treatment of *A. abrotanum* and *S. officinalis*.

Scientific name	English name	Part used	Extract	Traditional use[6]
A. abrotanum	Wormwood	Leaf	Ethanolic extract	Antiparasite
S. officinalis	Garden sage	Leaf	Ethanolic extract	Antiparasite

S. officinalis (garden sage, common sage) is a small, perennial, evergreen subshrub, with woody stems, grayish leaves, and blue to purplish flowers. It is a family member of Lamiaceae and is native to the Mediterranean region, and it has been naturalized in many places throughout the world. It has a long history of medicinal and culinary use, and in modern times as an ornamental garden plant.

Wormwood or southernwood (A. abrotanum) is a flowering plant. Southernwood is antiseptic and kills intestinal worms.

First, a few infected mice were randomly divided into four groups. The mice were treated with doses of 20, 50, 100 and 150 mg kg body weight of ethanol extracts or normal saline (2 mL/kg), orally. All animals were tested during 48 h for symptoms of poisoning and death. After that, 16 mice with equal weight, nutritional conditions, infected with *H. nana*, *A. tetraptera* and *S. obvelata* were tested for four groups that each group consisted of four mice. The Lynn Clayton test was employed for detecting parasite eggs per gram feces. These groups were treated as following:

Group 1: treated with 150 mg/kg Salvia extract for 5 d.

Group 2: treated with 150 mg/kg Artemisia extract for 5 d.

Group 3: treated with  $10\ mg$  /kg mebendazole for  $5\ d$  as positive control .

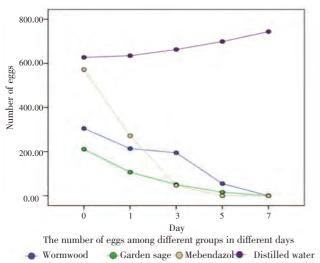
Group 4: treated with 2 mL/kg normal saline as negative

The parasite eggs were counted on the first, second, third and fifth day. On the seventh day, one mouse from each group was killed and the intestine was checked regarding the presence of parasites. The differences between the control and treated groups were analyzed using M-stat program.

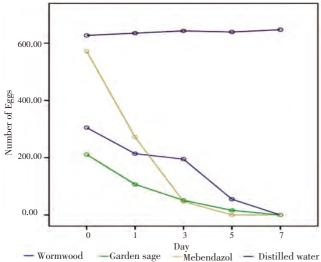
### 3. Results

In the present study, the ethanol extracts of wormwood, and garden sage, and mebendazole was examined for the effect on digestive tract parasites of mice. The results showed that in all groups which received drug or herbal extracts, but not in control group, the number of eggs excreted in per gram of feces was reduced one day after treatment. Results showed significant differences (*P*-value<0.001) in the number of eggs excreted by the *H. nana*, *A. tetraptera* and *S. obvelata* in mice, among the groups which had used the wormwood or garden sage and control group.

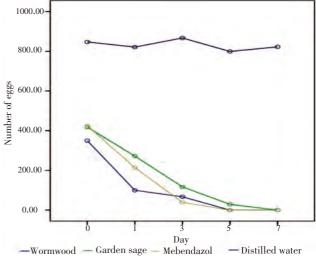
This study showed a noticeable effect for wormwood and garden sage over time against parasites. So that on the seventh day, the number of parasite eggs in feces was at the lowest amount (P<0.001). The garden sage and mebendazole revealed better effects on H. nana and A. tetraptera in comparison to Wormwood. Wormwood, however, had better effect on S. obvelata in comparison to garden sage. Average number of eggs in per gram of feces excreted by S. obvelata, A. tetrapetra and H. nana on Day 0, 1, 3, 5 and 7 after treatment in four groups is specified in Figure 1, 2 and 3. The mean and standard deviation of the different treatment groups is shown in Figure 4.



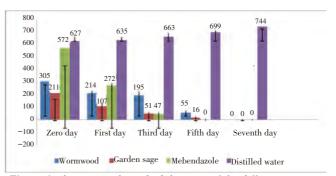
**Figure 1.** Average number of eggs in per gram of feces excreted by *H. nana* on Day 0, 1, 3, 5 and 7 after treatment in four groups.



**Figure 2.** Average number of eggs in per gram of feces excreted by *A. tetraptera* on Day 0, 1, 3, 5 and 7 after treatment in four groups.



**Figure 3.** Average number of eggs in per gram of feces excreted by *S. obvelata* on Day 0, 1, 3, 5 and 7 after treatment in four groups.



**Figure 4.** The mean and standard deviation of the different treatment groups.

## 4. Discussion

In the present study, the ethanol extracts of wormwood, garden sage and mebendazole for treatment of the digestive tract parasites of mice was examined. The results showed significant differences (*P*-value<0.001) in the number of eggs excreted by the *H. nana*, *A. tetraptera* and *S. obvelata* in mice among the groups which had used the wormwood or garden sage and control group. This study found a noticeable effect of wormwood and garden sage over time, so that the number of parasite eggs in the feces was at the lowest amount on the seventh day.

In Iranian traditional medicine, one species of Artemisia, namely Artemisia aucheri Bioss, is known as antiparasitic plant. Ghasemi pirbalouti showed that Artemisia salina had antiparasite and anti-pesticide effect, Artemisia inculata had anti-parasite and laxative effects, and Saliva verbeneca L. had good effect for skin disorders[6]

The essence of *S. officinalis* can help to repair cell, stimulate, and strengthen the skin and hair, and decrease depression. Sharifi *et al.* revealed that the extraction of

Artemisia aucheri Bioss has an antiparasitic effect on the Leishmania major[7].

Mahrami Poor and Neghahban in 2007 showed that essential oils of *Artemisia scoparia* and *Artemisia sieberi* dependently reduced spawning, hatching eggs, and mortality of first instars larvae of the beetle (parasite of grains)[8]. Jalali *et al.* revealed the insecticide effect of the *Artemisia annua* L[9].

S. officinalis essential oil contains compounds such as thujine, cis humulen- $\alpha$ , 1,8-cineole, (E)-Caryophylene, borneole, monoterpenes, diterpenes and diterpenes. The artemisinin is one of the most important components of Artemisia<sup>[9]</sup>.

Results of study by Mirza showed that 32 compound has been identified on sage. Among the compounds identified, using gas chromatograph coupled with mass spectrometer,  $\beta$ -caryophyllene (16.3%), sclareol (13.3%), hexyl octanoate (12.2%) and bicyclogermacrene (10%) had the highest share, respectively<sup>[10]</sup>.

The results of Bahmani *et al.* showed that high doses of the plant extract on antiparasitic effect against leeches (*Limnatis nilotica*) was found[11]. Santonin is antiparasitic herb drug that is produced from the *Artemisia* plant and its active ingredient is artemisian (artemisinin)[6]. Perhaps the powerful antiparasitic effects of garden sage and wormwood plants are due to these compounds.

The antiparasitic effect of *Artemisia* on *Eimeria* has been previously shown and it has been introduced as an anticoccidian drug<sup>[12–14]</sup>. Bruce had demonstrated the effects of Artemisia extract on malarial protozoa<sup>[13]</sup>.

Esfandiari et al. has shown effect of different concentrations of Artemisia extract on Syphacia nematode<sup>[15]</sup>. Khosravierad and Allen et al. showed that the artemisinin, a component of Artemisia (different species) has appropriate antiparasite effect on Coccidia<sup>[16,17]</sup>.

Artemisia absinthium Linn. is used traditionally by people as a vermifuge in addition to its other livestock uses. The results of the study of Tariq et al. showed that Artemisia absinthium extracts are a promising alternative to the commercially available anthelmintics for the treatment of GI nematodes of sheep[18]. Study of Ezzatzadeh et al. showed that the plant Artemisia kulbadica had antimicrobials and antioxidants effects[19]. Studies of Arokiyaraj showed that Artemisia nilagirica showed good antibacterial effects[20]. In studies of Suresh it was found that phytochemical contents of Artemisia parviflora Roxb were alkaloids, sterols/terpenoids, flavonoids, tannins, phenols and coumarins[21]. Results of two studies showed

that traditional effects of antiparasitic on intestinal worms are introduced for *Artemisia herba–alba*[22,23].

This study and other studies revealed appropriate antiparasite effect of *Artemisia* and *S. officinalis* which indicate that they might be used instead of chemical drugs in parasites control programs.

# **Conflict of interest statement**

We declare that we have no conflict of interest.

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#### **Comments**

# Background

Parasitic infections in animals and humans are one of the medical problems. In recent decades the use of local medicinal herbs and non-chemical remedies instead of chemical drugs to treat diseases has been regarded. Herbal plants in different areas based on species and amount of active ingredient have different therapeutic effects.

#### Research frontiers

Studies are performed in order to determine effects of two local plant extracts on three various parasites in mice. Certain dose of two plant extracts were fed to ternary test groups of infected mice.

# Related reports

The data about the effects of two plant extracts on three parasites are different. This difference is probably due to extracts' main ingredients and drug resistance of parasites that cause various results during one week consuming of plant extracts.

#### Innovations & breakthroughs

Data regarding effects of different plant extracts on various parasites are scarce. This study showed that S. officinalis and A. abrotanum extracts can be use for progressive study on human and domestic animals.

# Applications

The results of this study showed that *S. officinalis* and *A. abrotanum* extracts had good antiparasitic effects on three various parasite, one of which is common in human. So we can test those extracts on other parasites in human and domestic animals.

# Peer review

This is a good research in which the authors determined effects of two local plant extracts on three various parasites in mice. The results are interesting, and *S. officinalis* and *A. abrotanum* extracts had good antiparasitic effects on three various parasite.

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