Annals of Medical Research

DOI: 10.5455/annalsmedres.2019.07.409 2020;27(1):252-8

Original Article

Anthelmintic effect of essential oil and extract produced from *Salvia Sclarea* L., (*Lamiacea*) on nematodes living in gastrointestinal system of sheep

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Abstract

Aim: This study aims to investigate anthelmintic effect of the essential oil and crude extract of ***Salvia sclarea*** L.,Lamiaceae (clary sage), which are widespread in the region of Julfa and Kangarlı districts in Nakhchivan Autonomous Republic. The essential oil was extracted from top flowers and fresh leaves of the plant by ether distillation. Crude extracts were prepared by boiling the dry parts of the plant. Than the extracts were administered separately, in vivo and in vitro targeting on the nematodes localized in the digestive tract of sheep. The results revealed that both extracts have strong anthelmintic effect on nematodes.

Material and Methods: The essential oil output of the plant was found to be 0.3-0.4%. The composition of the essential oil was further analyzed by gas chromatography. Total 26 different substances were identified 26 in essential oil extract. Linalylacetate (41.2%) and linalool (18.9%) were found to be the major components of essential oil, extracted from the clary sage.

Results: The success in the treatment of nematodes (intensive efficacy) in vivo was 84.3% for essential oil extract, and 69.4% for the crude extract of clary sage species. The extensive efficacies of both products were found to be 60% and 40% respectively.

Conclusion: The results confirm that clary sage has high anthelmintic effect in vivo and in vitro on gastrointestinal nematodes in sheep.

Keywords: S.sclarea L.; clary sage; essential oil extract; nematodes; anthelmintic effect

INTRODUCTION

Parasitic diseases can cause major morbidity and mortality in small ruminants worldwide. Helminths occupy the first place among the gastrointestinal parasites of livestock. Nematodes are the main helminths causing substantial loses to the livestock industry worldwide. Nematodes could also have considerable negative impact on animals' welfare and the productivity of livestock animals leading to production losses and death in severely affected animals (1-4).

Control of nematodes relies largely on the use of anthelmintic drugs. The excessive use of such drugs can lead to widespread resistance in these nematodes to most classes of anthelmintics, seriously compromising the control of parasites in many countries (5-8).

Nematodes can damage animal wellbeing and the development of animal breeding by reduced appetite and weakening of the intestinal function in the body. The weakening of the animal's development and immune system, reduction of carbohydrates, energy exhaustion in the tissues, and the reduction of microelements causes various diseases including iron impairment in protein and hemoglobin synthesis and anemia (9). Therefore, animal protein, carbohydrate and some micronutrient deficiencies occur.

There has been a resurgence of interest in traditional health practices in both the industrialized and developing countries. In animal health, this interest encompasses ethnobotany and the use of herbal remedies (10-13).

Received: 16.07.2019 **Accepted:** 29.11.2019 **Available online:** 18.02.2020
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Natural compounds from plants provide a unique opportunity in the search for new, effective and safe anthelmintic. Despite the fact that the control of these nematodes has relied mainly on the use of chemical medicinal products, some bioactive plants have been demonstrated to have negative effects to the parasites. Many herbal medicines have been reported that they could destroy parasites, eliminate many deficiencies in body organisms and dramatically increase defence function (14-17)

Nakhchivan is characterized by moderate climate conditions, rich biodiversity, complex geological feature and soil types. Plants in Lamiceae Family are common species in this region. Soil coverage by residue protects soil and land resources from erosion, conserves soil water, and maintains soil quality. No-till and chemical weed control is management practices that increase soil coverage by residue. This has further enriched the essential oil composition of plants (18-20).

Plant flora of Nakhchivan Autonomous Republic is rich of traditional herbal remedies that can be used for the treatment of variety of diseases (21,22). One of the best known plant in this region is clary sage, which is a medicinal herb with antispasmodic and calming features in human use. An essential oil obtained from the flowering stems of this plant is called 'Muscatel oil', which is used in cosmetics. There are essential oils, sugars, pectin substances, vitamins C and E, microelements in the structure of clary sage grown in this area in Nakhchivan. However, information on antiparasitic usage in livestock animals is limited (23-26).

MATERIAL and METHODS

Study design

The essential oil content of S sclarea L. was separated by using ether distillation and the crude extract was prepared as crude mixture in boiling water, to be tested for anthelmintic effect.

For in vivo studies, 24 sheep with suspected infection of nematodes were divided into 3 groups, 8 animals for each group. Animals in groups 1 and 2 were allocated as experimental group, and group 3 as control group. The essential oil extract were given to animals in group 1, and the mixture extract was given to animals in group 2. No plant extracts were given to control animals in group 3.

Preparations of plant extracts for in vivo and in vitro use

Plant extracts were prepared as follows. Essential oil content was extracted from upper leaves and flowers of the plant by using ether distillation. This compound was used both in vivo and in vitro conditions. The essential oil separated by distillation was diluted in 2% (v/v) ethyl alcohol at the proportion of 1:5 for in vitro use. For in vivo use it was diluted in olive oil at 1:3 (v/v) ((27-29).

The mixture extracted from the dried parts of the plant in boiling water was further studied in vivo and in vitro conditions.

Analysis of chemical composition of essential oil separated from the plant

The chemical composition of essential oil of S.sclarea L. was analyzed using gas-liquid chromatography. The results were depicted in Table 2, identifying 26 different substances in this essential oil.

Analysis of essential oil content of S sclarea L. plant

S.sclarea L. (Alpine sage) plant from the 2600-2800 meter altitude of Julfa and Kangarli districts of Nakhchivan Autonomous Republic has been collected in the mass flowering phase. The upper parts and leaves of the plant's flower group were dried in shade, and the essential oil has been separated by ether distillation and the content of essential oil was analysed by gas chromatography (30-32). The results were given at the Tables 1, 2.

In vitro anthelmintic effect of the plant extracts

For in vitro examination, 15-20 helminths taken from slaughtered sheep were kept in essential oil extract and in the mixture separately for 15 minutes. After that the helminths were transferred into physiologic saline. This procedure was repeated until all helminthes were death, and the time required were recorded. Meanwhile, control parasites which were kept in physiological saline solution were monitored further for survival.

In vivo anthelmintic effect of the plant extracts

Animals with suspected nematode infection in each group were treated each morning with essential oil/olive oil preparation as follows; the animals, in group 1 received 5ml, animals in group 2 received 100ml and the control animals in group 3 were kept under similar conditions without any extract administration.

The trial has lasted for 5 days. At the end of the 5th day of the trial, the fecal samples were collected from each animal. Eventually, the Strongyloides eggs were counted in each sample. The findings were compared with the results obtained before the experiment. Finally, the anthelmintic efficacy of the preparations was evaluated.

Helminthoscopy

After the treatment period, circa 3 gram of fecal samples were taken separately from each animal in 3 groups and were examined in the laboratory. The Strongyloides eggs were counted under microscopy. The statistical means were calculated (Table 3).

The infected gastrointestinal tissue of slaughtered sheep were further examined for helminths by helminthoscopy and identified according to their morphological features. Each type of helminths were kept separately as control in physiologic saline solution (33-36).

The efficacy of plant S.sclarea L. was further studied on helminths Strongyloides infected sheep in two groups. Each group has 10 animals that each animal was around 20 years of age. Before starting the experiment 1g feces was collected from each animal. Each sample was examined under microscope, the strongyloid eggs were counted. The statistical means were calculated for each

group. Animals in the group 1, were given essential oil extract and animals in group 2, were given the extract mixture for 5 days as explained before.

At the end of 5th day, the samples of feces from each animal were examined for live Strongyloides eggs. At the same time, possible toxic effects of this plant at experimental dose on sheep's body temperature, heart beats, respiratory functions, and oropharengeal structures were investigated. No such toxic effect was observed.

RESULTS

The essential oil content of the plant was separated by distillation in ether from the plant collected in an area of 2600-2800 m altitude in Julfa and Kangarli districts of Nakhchivan. The results were given in Table 1.

Table 1. The amount of essential oil *S.sclarea* L. (by weight % in dry weight)

The name of the plant	Place of and altitude (m)	Amount of essential oil (ml)	Moisture content of dry plant (%)	Amount of essential oil (%)
S.sclarea L.	2600 m in Julfa	0.36	9.1	0.4
	2800m in Kangarlı	0.27	10.4	0.3

Among the substances identified in Table 2; linalyl acetate is 41.2%, linalool is 18.9%, germakren D (9.4%), α-terpineol (7.3%) and geranyl acetate (5.3%) were the majority chemicals found in the essential oil (Table 2).

Table 2. Substances found in the essential oil extracted from the S.sclarea L.

		S.sclarea L. Essential oil			
N	Components	%	N	Components	%
1	β-pinene	0.1	14	linalyl acetate	41.2
2	myrcene	0.4	15	thymol	-
3	α-terpinene	0.1	16	carvacrol	0.1
4	p-simen	0.3	17	neryl acetate	2.4
5	limonene	-	18	geranyl acetate	5.3
6	β-osimen (E)	-	19	caryophyllene	3.1
7	linalool	18.9	20	germakren D	9.4
8	α-tuyon	0.6	21	bitsiklogermakren	2.4
9	camphora	-	22	δ-kadinene	-
10	borneol	0.1	23	spathulenol	0.3
11	terpinen-4-ol	0.2	24	caryophyllene oxidemannol	0.6
12	α-terpineol	7.3	25	sclareol	0.1
13	neroli	1.5	26		1.8

Table 3. The helminths identified from the gastrointestinal system of farm sheep

Type of Helminths	Organs effected	Number of the sheep	Numbers of helminths
Marshallagia marshalli	Abomasum	3	243
Haemonchus contortus	Abomasum	4	168
Ostertagia osertagi	Small intestine	3	467
Bunostomum phlebotomum	Small intestine	3	313
Nematodirus spathiger	Small intestine	5	298
Bunostomum trigonocephalum	Small intestine	7	189
Strongyloides stercoralis	Small intestine	5	257
Chabertia ovina	Colon	4	358
Total number of nematodes found			**2293**

After slaughtering animals helminths, which were identified at in vitro studies, were presented on Table 3. The majority of helminths were found to be Marshallagia marshalli, Ostertagia ostertagi, Chabertia ovina.

Table 3, listed the number of sheep infected by helminthes in gastrointestinal system of 15 sheep. Eight different helminthes were identified in various part of the gastrointestinal system. Total number of helminth count was 2,293.

The study in vitro conditions, in which the anthelmintic properties of essential oils extract and the mixture extract of *Salvia sclarea* L. on nematodes has yielded the results given in Table 4.

Table 4. Anthelmintic effect of *S.sclarea* L. plant *in vitro* conditions

Type of helminths	The plant *S.sclarea* L.		Physiological saline solution (controls)
	Essential oil extracted through ether distillation	Mixture extracted in boiling water	
	Survival time before death of helminthes in two extracts		
Marshallagia marshalli	45 min.±12 min.	3h. 50 min.±1h.10 min	22h.±1 h. 30 min
Ostertagia osertagi	1h.15 min.± 18 min	3h. 40 min.±55 min	21h.± 1 h.
Bunostomum phlebotomum	1h.05min.±22 min	4h. 15min.±1h.	25h.±1 h.10 min
Chabertia ovina	2h 20 min.±20 min	6h.40 min.±1 h.15 min	29h.±1h.

Table 5. The extensive efficacy of anthelmintic effect of extracts from *S.sclarea* L. *in vivo* conditions (essential oils and the mixture preparations)

Group 1: (n=10 sheep) Treated with essential oil extract (in ethanol solution)			Group 2: (n=10 sheep) Treated with the crude extract (obtained by boiling water)		
	Number of eggs before the treatment, in 1g sheep feces	Number of eggs After the treatment, in 1g sheep feces		Number of eggs before the treatment, in 1g sheep feces	Number of eggs after the treatment, in 1g sheep feces
1	103,4	-	1	87,5	11,2
2	98,7	21,9	2	95,4	-
3	78,6	-	3	119,1	-
4	100,2	-	4	87,3	-
5	89,3	12,8	5	78,2	21,4
6	98,4	25,7	6	88,1	-
7	92,7	-	7	107,6	13,2
8	115,7	17,9	8	121,1	25,6
9	80,4	-	9	71,9	20,1
10	89,7	-	10	93,9	17,9

The findings showed that the nematodes were immobilized and eventually died in essential oil extract within 45 minutes to two hours twenty minutes (group-1), and 3-6 hours in the crude extract mixture (group-2). In contrary, the nematodes have survived significantly longer (up to 22 to 30 hours) in the saline solution (controls, group-3)

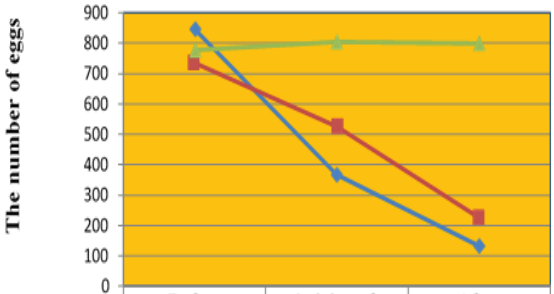
The eggs were counted in one gram feces of each sheep, which were allocated in 3 groups. The numbers of eggs were found as decreased significantly after in vivo treatments in animals of group-1 and group-2. No changes were observed in untreated animals (in controls, group-3). The results were given in Table 5 and in Figure 1.

In vivo treated six sheep out of ten in group-1 and four sheep out of ten in group 2, were cleansed completely from nematodes at the end of the five days trial period by using both extracts.

The results revealed the intensity of efficacy of *S.sclarea* L. as 84.3% for essential oils extracts and as 69.4% for the crude extract of the plant.

The extensive efficacy on nematodes was calculated in two groups treated with two different preparations.

Economic efficacy of both extract was calculated according to the economic analysis formula (5). The economic efficacy was found to be 60% for the essential oil extract and 40% for the mixture extract.



	Before experiment	3rd day of experiment	After experiment
Group 1. Treated with essential oils	845,9	367,5	132,4
Group 2. Treated with crude extract	734,6	524,7	224,9
Group 3. Controls	776,8	803,6	798,4

Figure 1. Anthelmintic effect of S.sclarea L. Species, in vivo, on gastrointestinal nematodes of sheep

DISCUSSION

Parasitic diseases are of supreme importance in many agro-ecological zones including Nakhchivan and still a serious threat to the livestock economy in our region. Helminthes infections remain one of the major constraints to sheep farming. Infection with gastrointestinal nematodes is regarded as one of the important factor causing production losses of livestock.

In Azerbaijan and Caucasians region, herbal remedies have been used for centuries to treat many disease conditions in animals and in humans including parasitic infections. Many recent studies have focused similarly on the topic of finding new herbal remedies for the sustainable treatment of helminthes in livestock animals in Nakhchivan. Resistance and increasing cost effects adversely on domestic livestock animal farming (20,22), which is rather prospective for sheep rearing due to its geo-climatic condition in Nakhchivan. Nevertheless, the sheep rearing is hindered by various problems of which parasitic diseases might be one of the major problems

Natural plants containing bioactive components comprise a structurally diverse class of natural products with diverse biological activity against different helminthes. Although the mode of action and the identity of their active component are, yet to be clarified (37).

An effective way to preventive and healing the helminth infections in livestock animals is to use natural bioactive plants. Clary sage is one of them and grows abundantly in this geography. There is no precise report available on usage of clary sage in parasitic disease in Nakhchivan. Therefore, the present study was designed to examine the clary sage for its anthelmintic characteristics in sheep.

The present study indicates that the extracts from S sclarea L. have considerable activity in vitro and in vivo against the parasitic larvae. The essential oil extracts was the most effective inhibitor of motility at the concentrations tested, but with no observable dose-dependent effect. These findings suggest that the effect of both extract appears to irreversibly inhibit motility and larval development. S Sclare L. has proven that it is a bioactive plant with strong anthelmintic properties on nematodes in sheep.

It was observed that the intensive efficacy for in vivo test was 84.3% for essential oil and 69.4 % for the extract mixture of clary sage and the extensive efficacy of the extract mixture was 60% for essential oil, and 40 % for the extract mixture. The present study revealed that clary sage (S.sclarea L.) has high anthelmintic effects on nematodes (Figure 1).

The extracts produced from this plant may be used for preventive and therapeutic purposes in the fight against Helminthiasis. Feeding animals with clary sage could reduce the parasitic burden of sheep farming. Use of clary sage in helminthes control in Nakhchivan would provide an opportunity for livestock farmers to reduce overheads and loses to food production.

Various drugs used for anthelmintic purposes have high activity (38), but they may have several disadvantages, such as accumulation in the body, undesirable effects on the functions of various systems such as digestion, respiration and excretion, as well as their high cost. Nevertheless, success in discovering new drugs without negative impact has been limited (39).

It is likely that many of these natural products may be acting on parasites using different therapeutic pathways rather than currently used anthelmintic drugs. However, for the majority of such natural products, there has been limited data on the systematic and scientific evaluation of the efficacy of these compounds. Despite the existence of large and diverse range of herbal medicinal product known, the scientific validation of the anthelmintic effects of many of these products is still lacking. It is, therefore, not many plant- based anthelmintic is yet commercially available for agro-farmers. This question has to be addressed by local researchers.

In agreement with literature, this study suggests new diet manipulation strategy that feeding animals with natural supplements containing clary sage could lead to an improved resilience against nematode infections. Alternative way could be exploring in local habitat for rotational grazing (40).

Integrated approaches are the only way ensuring an effective helminth control, which in turn, will help to secure the overall sustainability of the grazing livestock industries.

CONCLUSION

There is still a great need to evaluate further using clary sage for helminths control in combination when the means and the methods are relevant, affordable and available.

The results suggest that the extracts of clary sage may be a rich source of natural anthelmintic for the control of gastrointestinal nematodes. We recommend that future work should focus on attempting to fractionate the extract, in order to identify the most powerful constituent(s) that are active against nematodes, and then to explore which biological process are affected by these fractions.

Competing interests: The authors declare that they have no competing interest.

Financial Disclosure: From Nakhcivan State University.

Ethical approval: From Nachcivan State University Medical Faculty, Ethics Committe

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