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Original Scientific Paper

**ANTHELMINTIC POTENTIAL OF CORIANDER ESSENTIAL OIL
(*CORIANDRUM SATIVUM L.*) IN SHEEP****Filip ŠTRBAC^{1*}, Antonio BOSCO², Kosta PETROVIĆ¹, Dragica STOJANOVIĆ¹, Radomir RATAJAC³, Nataša SIMIN⁴, Dejan ORČIĆ⁴, Giuseppe CRINGOLI², Laura RINALDI²**¹ University in Novi Sad, Agricultural Faculty, Department for Veterinary Medicine, Novi Sad, Serbia² University of Naples "Federico II", Department of Veterinary Medicine and Animal Production, Naples, Italy³ Scientific Veterinary Institute "Novi Sad", Novi Sad, Serbia⁴ University in Novi Sad, Faculty of Sciences, Department of Chemistry, Biochemistry and Environmental Protection, Novi Sad, Serbia

*Corresponding author: Filip Štrbac, strbac.filip@gmail.com

Summary

The development of anthelmintic resistance in sheep gastrointestinal nematodes to commercial anthelmintics has led to enormous economic losses in many parts of the world. For this reason, new sustainable strategies for the control of these parasites are being actively searched, which include the use of different alternatives such as plant essential oils. The aim of this study was to determine the chemical composition of the essential oil of coriander (*Coriandrum sativum L.*) and to test its anthelmintic potential against gastrointestinal nematodes of sheep. Qualitative and semi-quantitative chemical characterization of the essential oil was performed using gas chromatography coupled with mass spectrometric detection (GC-MS analysis). The anthelmintic potential of the essential oil was tested using the *in vitro* egg hatching test (EHT), which was carried out with eight different concentrations of the tested oil (50; 12.5; 3.125; 0.781; 0.195; 0.049; 0.025 and 0.0125 mg/mL). Thiabendazole in concentrations of 0.025 and 0.0125 mg/mL was used as the positive control, and the emulsifier 3% Tween 80 v/v and distilled water was used as the negative control. GC-MS analyzes revealed a rich chemical composition of the tested oil, where a total of 17 different ingredients from different chemical groups were found, of which 15 were identified. The dominant ingredient was linalool (84.5%) along with γ -terpinene (5.04%), camphor (3.26%) and α -pinene (2.88%). The EHT results showed a high

anthelmintic potential of coriander oil with inhibition of larval hatching, i.e. an ovicidal effect of 29.0-88.7% depending on the concentration, with an IC₅₀ concentration of 0.04 mg/mL and a clear dose-dependent effect ($R^2 = 0.90$). This study confirmed the possible importance of botanical anthelmintics in the sustainable control of nematodes in animals and the fight against anthelmintic resistance. The obtained results indicate a possible role of coriander in future therapeutic approaches to infections caused by gastrointestinal nematodes of sheep, after further *in vivo* tests and toxicity studies.

Keywords: gastrointestinal nematodes, anthelmintic resistance, essential oil, coriander, gas chromatography, larval hatching test.

INTRODUCTION

Gastrointestinal nematodes lead to negative effects in sheep in various ways, from subclinical weight loss and decreased production of milk, meat and wool, to the appearance of anemia, diarrhea, anorexia, protein losses, reduced fertility, and in severe cases, even death (Giovanelli et al., 2018; Bosco et al., 2020; Beleckè et al., 2021). Control of infections caused by these parasites still mostly relies almost exclusively on the use of commercial anthelmintic drugs such as benzimidazoles, macrocyclic lactones and imidazothiazoles (Dyary, 2018). Although these drugs have been successfully used for decades in the therapy of the mentioned infections (Kaplan, 2020), their irrational use in terms of frequent and intensive treatments, high or low doses or consecutive use of anthelmintics from only one class led to the development of resistance in gastrointestinal nematodes (Bosco and et al., 2018; Pinto et al., 2019; Beleckè et al., 2021). The development of resistance has led to a decline in the effectiveness of the mentioned drugs and consequent large economic losses due to reduced production in animals and deaths, as well as treatment costs (Szewc et al., 2021). Due to all of the above, gastrointestinal nematodes represent one of the biggest obstacles facing modern sheep farming (Giovanelli et al., 2018).

The growing and more pronounced problem of resistance requires the design of new strategies for the control of the mentioned parasites, which today are mainly based on an integrated approach to therapy (Keeton, 2016). This approach implies, on the one hand, the rational use of anthelmintics, i.e. targeted treatments (treatment of only certain herds) and targeted selective treatments (treatment of only certain animals that require treatment within the herd), with a combination of several anthelmintics or their rotation (Calvete et al., 2020). On the other hand, along with the rational use of commercial preparations, an integrated approach implies the introduction of various alternatives such as the selection of genetically

resistant animals, pasture management, nutritional manipulation, biological control (use of nematophagous fungi or bacteria), development of vaccines as well as the use of botanical anthelmintics (Zeineldin et al., 2018; Pinto et al., 2019). At the same time, among the listed alternatives, phytotherapy is considered particularly promising in view of the obtained results (Borges and Borges, 2016). It implies the use of whole plants or their parts in animal feed, but also different plant products such as extracts and essential oils.

Essential oils represent aromatic, concentrated and complex mixtures of volatile and non-polar compounds isolated from plant material (Štrbac et al., 2022a). They are present in specialized cells or glands of certain plants and play a role in protecting them from predators and vermin, as well as in attracting pollinators (Butnariu and Sarac, 2018). In veterinary medicine, essential oils are increasingly used in the treatment and prevention of various diseases, although primarily in monogastric animals such as pigs and poultry (Mucha and Witkovska, 2021), but also in dogs and cats (Štrbac et al., 2021). However, there is more and more evidence about the anthelmintic effect of essential oils against gastrointestinal nematodes of sheep (Štrbac et al., 2022b). Their biological effect originates from their rich chemical composition and compounds belonging to different groups such as terpenes, terpenoids or phenylpropanoid compounds (Dhifi et al., 2016, Fokou et al., 2020).

Coriander (*Coriandrum sativum L.*) is an aromatic, herbaceous annual plant from the *Apiaceae* family (Chahal et al., 2017). It has a long history of use in cooking as a source of aromatic compounds with antibacterial, antifungal and antioxidant activity, so it is useful in food preparation as an aroma and auxiliary agent (Mandal and Mandal, 2015). It is also known for its wide range of medicinal properties and is used for various gastrointestinal disorders such as anorexia, dyspepsia, flatulence, diarrhea and vomiting. Coriander fruit is also known as a cooling agent, tonic, diuretic and aphrodisiac, while its essential oil is considered useful in flatulence, rheumatism and neuralgia, with known antimicrobial, antioxidant and pesticidal properties (Chahal et al., 2017).

The aim of this study was to examine the anthelmintic activity of coriander essential oil against gastrointestinal nematodes of sheep, with the determination of its chemical composition and the identification of compounds significant for anthelmintic activity.

MATERIALS AND METHODS

Analysis of chemical composition

Coriander essential oil was purchased from the Institute of Field and Vegetable Crops in Novi Sad. Qualitative and semi-quantitative chemical characterization of the essential oil was performed at the Faculty of Science in Novi Sad using gas chromatography coupled with mass spectrometric detection (GC-MS). In the gas chromatograph (Agilent Technologies series 6890), 1 μ L of the solution of the tested oil (10 μ L/mL in hexane) was injected in split mode, with a split ratio of 1:10, at a temperature of 250°C. The components were separated on a non-polar poly (tetramethyl-1,4-silphenylenesiloxane) column HP-5 ms (Agilent Technologies) with dimensions 30m \times 0.25mm, layer thickness 0.25 μ m. The column was eluted in a temperature-programmed regime, with a starting temperature of 50°C, ramp 8°C/min to 120°C, 15°C/min to 230°C, 20°C/min to 270°C/min, and retention at the final temperature of 16.92 min (total analysis time 35 min). High-purity helium (5.0) was used as carrier gas in a constant flow regime of 1.0 mL/min. The effluent was sent via a transfer line maintained at 280°C to a mass spectrometer with electron ionization (Agilent Technologies series 5975). The mass spectrometer parameters were: electron energy 70eV, ion source temperature 230°C, quadrupole temperature 150°C. Scan acquisition mode was applied, in the m/z range 35-400, and with a solvent delay of 2.30 min. In order to achieve a better agreement between the experimental and library spectra, the standard spectra tune was used. Data were processed using Agilent Technologies MSD ChemStation software (revision E01.01.335) in combination with AMDIS (ver. 2.64) and NIST MS Search software (ver. 2.0d). AMDIS was used for deconvolution of mass spectra of coeluting compounds, and NIST MS Search provided a library search algorithm complementary to ChemStation's PBM algorithm. Wiley Registry of Mass Spectral Data 7th Edition and NIST/EPA/NIH Mass spectral Spectral Library 05 libraries were used to identify mass spectra. The identity of the compound was confirmed by comparing the linear retention indices with literature data. The relative proportions of the compounds were determined by the normalization method, based on the area of the peaks in TIC.

Larval hatching test

The anthelmintic potential of coriander essential oil against gastrointestinal nematodes of sheep was tested using the egg hatch test (EHT), which measures the effect of the application of a substance on the inhibition of hatching of larvae from parasite eggs, i.e. the ovicidal effect. The study was conducted in the regional center for monitoring parasitosis KREMOPAR (Eboli, Salerno, southern

Italy). For the isolation of nematode eggs, samples of sheep feces taken directly from the rectal ampoule, from sheep from two separate farms in this region, were used, and the nematode eggs were isolated using the so-called recovery method (Bosco et al., 2018).

Initially, feces samples were homogenized and filtered under flowing water through sieves with openings of different sizes (1 mm, 250 μ m, 212 μ m and 38 μ m), in order to separate eggs from feces. The eggs retained on the sieve of the smallest size were then washed with distilled water into test tubes and centrifuged at 1500 RPM, after which the precipitate was removed. After that, centrifugation was performed so that the eggs that floated to the surface were isolated in new test tubes to which distilled water was added. Finally, the obtained solutions were centrifuged several more times to remove debris and obtain an aqueous solution with eggs.

The larval hatching test was conducted according to the instructions from the literature (Ferreira et al., 2018) with certain modifications. Eight different concentrations (50; 12.5; 3.125; 0.781; 0.195; 0.049; 0.025 and 0.0125 mg/mL) of coriander oil were tested using well plates. In the wells, solutions of the mentioned decreasing concentrations of oils emulsified in 3% Tween-uu 80, v/v, were made, and then supplemented with the obtained aqueous solution with eggs (about 40 ml, i.e. 100-150 eggs per well) and distilled water to a total amount of 0.5 mL per well.

The required amount of oil for the highest concentration was determined by calculation, and then the other concentrations were obtained using the dilution method.

Thiabendazole in concentrations of 0.025 mg/mL and 0.0125 mg/mL was used as a positive control, and 3% Tween 80, v/v and distilled water were used as negative controls. All oil concentrations and controls were tested in a total of three replicates. After making the mentioned solutions, the plate with wells was incubated at 27°C for 48 hours, and after that 1-2 drops of Lugol's solution were added to each well, in order to stop the hatching of nematode larvae from the eggs. The prepared solutions were then observed under a microscope with the aim of counting eggs and hatched larvae at each concentration of oil and controls.

Statistical processing

The percentage of inhibition of egg hatching into the larval form was calculated using the following formula (Coles et al., 1992; Pinto et al., 2019):

$$\text{IH} = \text{number of eggs} / (\text{number of eggs} + \text{number of larvae}) \times 100\%$$

After that, the final value for each concentration and control was calculated as the arithmetic mean between three repetitions. One-factor analysis of variance with

post hoc Tukey test ($p < 0.05$) was used to compare the obtained values between different concentrations of the tested oil, as well as to compare them with the controls. On the other hand, nonlinear regression and logarithmic distribution were used to determine the concentration that inhibits 50% of eggs from developing into larval form (Ferreira et al., 2018; Štrbac et al., 2022a). Statistical analyzes were performed in the GraphPad Prism 8.4.3 program (GraphPad Holdings, LLC, San Antonio, CA, USA).

RESULTS

Analysis of chemical composition

The results of the chemical analysis of the composition obtained by gas chromatography and mass spectrometry showed a rich chemical composition of coriander essential oil. The total number of compounds was 17, of which 15 were identified (Table 1), from different chemical groups, and the dominant compounds were linalool (84.53%), γ -terpinene (5.08%), camphor (3.26%)) and α -pinene (2.88%).

Table 1. Chemical composition of coriander essential oil determined by gas chromatography and mass spectrometry

Arithmetic retention index	Compound	% Total peak area
925	α -Thujone	0.02
932	α -Pinene	2.88
946	Camphene	0.30
971	Sabinene	0.02
976	β -Pinene	0.21
989	Myrecene	0.37
1016	α -Terpinene	0.05
1023	p-Cymene	1.08
1027	Limonene	1.12
1029	1,8-Cineol	0.14
1056	γ -Terpinene	5.08
1087	Terpinolene	0.30
1100	Linalool	84.53
1143	Camphor	3.26
1165	Unknown	0.12
1176	Terpinen-4-ol	0.27
1191	Unknown	0.16

Larval hatching test

Coriander essential oil showed high anthelmintic potential against gastrointestinal nematodes of sheep with inhibition of larval hatching (ovicidal activity), which varied from 29.0 to 88.7% depending on the applied concentration (Table 2). The activity was high (70%) even at a relatively low concentration of 0.049 mg/mL, which resulted in a very low obtained IC₅₀ value of 0.04 mg/mL. At all tested concentrations, the effect was significantly greater ($p < 0.05$) than both negative controls, while at the highest concentration of 50 mg/mL it was similar to the positive control ($p > 0.05$). Anthelmintic activity was largely dose-dependent, with an R² value of 0.90.

Table 2. Percentage efficiency (arithmetic mean \pm standard deviation) of coriander essential oil against the hatching of sheep gastrointestinal nematode larvae at different concentrations

Concentration [mg/mL]	Inhibition of larval hatching (%)
50	88.7 \pm 1.53 ^{AB}
12.5	85.7 \pm 1.16 ^B
3.125	81.0 \pm 1 ^{BC}
0.781	77.3 \pm 2.08 ^{CD}
0.195	76.0 \pm 4.36 ^{CD}
0.049	70.0 \pm 1 ^D
0.025	36.3 \pm 4.16 ^E
0.0125	29.0 \pm 2 ^E
Thiabendazole, 0.025	96.3 \pm 1.53 ^A
Thiabendazole, 0.0125	95.0 \pm 1 ^A
3% Tween, v/v	14.2 \pm 3.34 ^F
Distilled H ₂ O	6.6 \pm 1.92 ^F

* Values with different capital letters indicate a statistically significant difference ($p < 0.05$)

DISCUSSION

Due to the development and spread of anthelmintic resistance and the increasing consequent economic losses, there is a growing interest in alternative strategies for the treatment of nematode infections in animals. In this context, many researchers agree that bioactive ingredients produced from medicinal plants represent a promising alternative to conventional anthelmintic preparations, or an important addition to the range of preparations and methods in an integrated approach to the control of these parasites (Macedo et al., 2010; Borges and Borges, 2016; Zeineldin et al., 2018). However, before the introduction of individual herbal preparations for wider use in practice, it is necessary to prove

their effectiveness with the assistance of reliable tests. At the same time, *in vitro* tests are very important for the initial assessment of the anthelmintic potential of a substance and the basis for their selection for further *in vivo* tests and toxicity studies (Fonseca et al., 2013). The larval hatching test is considered a reliable and precise test that is recommended and widely used both for the detection of anthelmintic resistance in commercial preparations and for the discovery of new anthelmintic substances such as essential oils (Coles et al., 1992; André et al., 2018; Štrbac et al., 2022b).

The results of this study proved the anthelmintic activity of coriander essential oil against gastrointestinal nematodes of sheep. Although maximum efficiency was not reached at the tested concentrations, ovicidal activity was high at even six tested concentrations, i.e. from 0.049 to 50 mg/mL (70.0-88.7%). Due to that, the IC₅₀ value (a parameter that is often used in such tests as an indicator of efficiency and for comparison with other results) was very low (0.04 mg/mL), which indicates a very high ovicidal activity of coriander oil. If this result is compared with other oils tested so far (André et al., 2018; Štrbac et al., 2022b), it can be observed that it is one of the most effective, which indicate the high anthelmintic potential of coriander and its convenience for further tests.

The anthelmintic effect of coriander essential oil against gastrointestinal nematodes of sheep has been proven in other studies. Its ovicidal effect against *Haemonchus contortus* of 14.8 - 99.0% at concentrations of 0.15 - 2.5 mg/mL (IC₅₀ 0.63 mg/mL) was proven in a study by Macedo et al. (2013). Although not so strong, in the same study, the larvicidal effect of coriander oil was also proven to be 10.1 - 99.5% at concentrations of 1.25-20.0 mg/mL (IC₅₀ 2.89 mg/mL), suggesting its activity against different stages of parasites. The inhibitory activity of coriander on the motility of larvae of different types of gastrointestinal nematodes was also proven, the strongest against *Teladorsagia circumcincta*, *Trichostrongylus vitrinus*, *T. axei* and *H. contortus* with IC₅₀ values of 0.11, 0.15, 0.25 and 0.26% (in the final concentration), respectively, while in mixed infection the IC₅₀ value was 0.22% (Helal et al., 2020). In our study, we also used mixed infection, and coproculture was examined in a previous study where the same farms were used, with the following percentage of individual genera: *Haemonchus* 53%, *Trichostrongylus* 29.5%, *Teladorsagia* 14.5% and *Chabertia* 3 % (Štrbac et al., 2022a). From these results, it can be concluded that coriander has anthelmintic efficacy against various types of gastrointestinal nematodes.

If the results of gas chromatography and mass spectrometry are observed, the presence of terpene and terpenoid compounds from different chemical groups is observed. By far the most abundant ingredient is linalool (84.53%), an acyclic monoterpenoid alcohol, and next to it, hydrocarbon terpenes (γ -terpinene, α -

pinene, limonene and p-cymene), as well as the terpenoid ketone camphor. Given that the biological properties of essential oils derive to the greatest extent from their main ingredient (Dhifi et al., 2016), it can be concluded that the effect of coriander originates from linalool, but the presence of other mentioned compounds is essential for the overall synergistic effect. High ovicidal activity of isolated linalool against *H. contortus* was demonstrated in the study done by Katiki et al. (2017) with an IC_{50} of 0.29 mg/mL, and linalool individually was the main constituent of *Arisaema franchetianum* (8.89%) and a significant constituent of *Arisaema lobatum* (6.67%), essential oils with a huge number of isolated compounds (over 50), which showed ovicidal and larvicidal activity against *H. contortus* (Zhu et al., 2013). Interestingly, isolated linalool alone showed a weaker activity in this study compared to whole oils, which indicates the importance of the presence of other compounds and the synergistic effect.

If the results of the analysis of the chemical composition of coriander essential oil are compared with other researches, a high level of agreement is observed. Thus, in the research done by Helal et al. (2020) the main ingredients were linalool (68.03%), camphor (11.76%), γ -terpinene (10.48%) and α -pinene (9.71%), while in the research done by Macedo et al. (2013) the main ingredients were β -linalool (73.21%), camphor (4.25%), α -pinene (4.20%) and terpinene (3.10%). In other studies, the chemical composition is also similar with the dominance of alcohol linalool, geraniol and terpinen-4-ol; hydrocarbons γ -terpinene, cymene, limonene and α -pinene; camphor ketone, but also esters of geraniol acetate and linalyl acetate (Mandal and Mandal, 2015). Although the chemical composition is similar in different studies, certain differences are still observed, most often in the percentage of individual compounds. These differences can be explained by various factors such as the place where the plant is grown (rainfall, light, soil - pH, structure, salinity), the part of the plant from which it is extracted, the age of the plant and the method of extraction itself (Fokou et al., 2020). Those factors can lead to certain differences in the biological activity of the oil itself.

The use of different plants and their products, such as essential oils, against gastrointestinal nematodes, but also parasites in general, has numerous advantages. Their rich chemical composition with compounds from different chemical groups and potentially different mechanisms of action can contribute to a high antiparasitic activity, but also to a lower susceptibility to the development of resistance. On the other hand, their natural origin is associated with a lower degree of toxicity for hosts, smaller amounts of residues in meat and milk, as well as better environmental acceptability compared to chemical preparations. Finally, the large number of available plant species favors the wide use of botanical anthelmintics, easy availability and favorable price in countries with developed

biodiversity (Ferreira et al., 2018; Štrbac et al., 2022b). However, further tests are necessary in order for plants and their products to be widely used in veterinary medicine.

CONCLUSION

The development and spread of anthelmintic resistance and the increasing consequent economic losses require the search for alternative tools, which include various botanical anthelmintics. In this study, coriander essential oil showed a high anthelmintic potential with regard to the ovicidal effect found in sheep gastrointestinal nematodes, which is why it can be considered suitable for further tests. Coriander's high ovicidal activity originates from its rich chemical composition, primarily linalool along with other compounds such as γ -terpinene, camphor and α -pinene. The results of this study indicate the possible role of phytotherapy in future sustainable approaches to the control of gastrointestinal nematodes in sheep, reducing the use of commercial preparations and anthelmintic resistance.

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