

# Anti-helminthic Activity of *Myrtus communis* L. Fruit Ethanol Extract on Nematodes of *Caenorhabditis elegans* and The Determination of Possible Active Ingredients

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## ABSTRACT

*Myrtus communis* L. (Myrtaceae) is a plant widely found in the Middle East and Mediterranean region, both naturally grown and cultivated. In our study, *C. elegans* nematodes were used as an anti-helminthic model and by determining the anti-helminthic effect of *Myrtus communis* L. fruit, it was aimed to find the active ingredients with possible anti-helminthic effects by GC-MS analysis. In our study, the anthelmintic effect of the ethanol extract of *Myrtus communis* L. fruit on *C. elegans* was investigated. Ethanol fruit extracts were added to NGM separately at final volume of 10 mg/mL, 5 mg/mL, 2.5 mg/mL, 1.25 mg/mL and experimental groups were formed. When the data were evaluated statistically, the difference between all experimental groups, negative control group and positive control group (pyrantel pamoate), was significant ( $p < 0.05$ ). According to the results of GC-MS analysis, the highest detected ingredients were 1,8-Cineole (3.46%), Linalyl acetate (3.06%), and Palmitic acid (2.90%). It was observed that *Myrtus communis* L. fruit extract was more effective on *C. elegans* nematodes than pyrantel pamoate at the same concentration (5 mg/mL), and 20 different active ingredients were determined according to our GC-MS analysis results. By studying the forms of these active ingredients separately or in different combinations, the ingredients and combinations with the strongest anti-helminthic activity can be determined, and it is thought that our findings may lead to further studies.

## Keywords:

*Myrtus communis* L.; *Caenorhabditis elegans*; anti-helminthic effect; 1,8-Cineole; Linalyl acetate; Palmitic acid

## INTRODUCTION

*Myrtus communis* L. (Myrtaceae) is one of the characteristic representatives of the Mediterranean flora. This species widely found in the Middle East and Mediterranean Region is both naturally grown and cultivated [1]. *Myrtus communis* L. is an evergreen shrub that can grow to 1-5 meters approximately. The oppositely arranged leaves are ovate-lanceolate, 2-5 cm long, coriaceous, glabrous, punctate-glandular, and entire. The flowering period lasts from June to September. Subglobose to ellipsoid fruits are blue-black (or rarely yellowish-white) when ripe [2]. Different parts of this plant such as fruits, branches, leaves, and seeds have been extensively used in traditional medicine. Traditionally used as an antiseptic, disinfectant, and hypoglycemic agent, its leaves and fruits have been used as an antiseptic drug in villages of Turkey [3]. Similarly, in Italian traditional medicine, the fruit of this plant is used in the treatment of many types of infectious diseases, including diarrhea

and dysentery [4]. The leaves are used as an antiseptic and anti-inflammatory agent and as a mouthwash in the treatment of candidiasis [4, 5]. In addition, antibacterial [6], antifungal [7], and antioxidant [8] effects are frequently mentioned in the literature.

Helminthiasis is one of the important parasitic diseases, many of which are zoonotic, especially in developing countries and especially in countries with hot and humid climates. Although not as much as in tropical countries, helminthiasis is quite common in some settlements of Turkey due to insufficient infrastructure and lack of education. Intestinal parasitosis can cause significant manifestations at all levels of the gastrointestinal tract, as well as anemia and growth retardation. Cholecystitis, cholangitis, liver abscess, pancreatitis, ileus, and acute appendicitis are significant complications [9]. In addition, helminth infections are one of the most important causes of growth deficiency and intellectual

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development retardation in developmental ages [10]. Parasitic diseases cause many problems, especially in children of developmental age, such as malnutrition, developmental retardation, anemia, attention deficit, learning difficulties and consequently decreased success levels. Such health problems observed in primary and secondary education children cause children to lag behind in both physical and psychological development compared to their peers. Studies have mentioned the effects of helminth infections on the school performance of infected children and the future of the country [11]. In addition, it has been reported that these infections may have a detrimental effect on cognition and educational success in terms of intellectual development [11-13]. As a result, people's turn to anthelmintic drugs has also been one of the reasons for the development of resistance [14, 15]. As with many disease agents, the development of resistance to the drug of parasites causes parasitic diseases to be a public health problem and difficulties in their treatment [14, 15]. In recent years, the use of herbal medicines has been increasing exponentially. Many plants are used as a means of treatment for various diseases, and the effects that can replace synthetic drugs are seen. Products obtained from plants have been used since ancient times as a traditional treatment method, especially in the eradication of pathogens that develop drug resistance [16].

Studies on the anthelmintic activities of medicinal plants are very few. One of the reasons is that most of the helminths cannot be cultured in the laboratory environment. Therefore, laboratory animals such as rats or mice are generally preferred as models in such studies. However, animal studies bring many problems such as requiring a lot of labor, shortage of workspace, and being expensive [16]. *Caenorhabditis elegans* (*C. elegans*), Class: Chromadorea, Order: Rhabditida, Family: Rhabditidae, Genus: *Caenorhabditis*, Species: *Caenorhabditis elegans* is a nematode also called threadworm. *C. elegans* can be a good anti-helminthic model, studies have reported [16-18].

In our study, *C. elegans* nematodes were used as an anti-helminthic model and the anti-helminthic effect of *Myrtus communis* L. fruit was determined, and it was aimed to determine the active ingredients with possible anti-helminthic effects by GC-MS analysis.

## MATERIAL AND METHODS

### Plant Material

The *Myrtus communis* L. fruit used in our study was purchased in equal amounts from 10 different vendors sold commercially in Hatay province.

### Preparation of Plant Extracts

*Myrtus communis* L. fruit plant extracts were used in our study. The samples were first washed with tap water and then pure water, then dried on drying paper. Fruits that purchased from different vendors were grounded in equal proportions and homogenized. 100 g was taken from the powdered plant materials. Added 300 mL ethanol on top. It was kept at room temperature in the shaker at 150 rpm for 24 h. At the end of that time, it was filtered twice by the No:1whatman filter paper. Ethanol in the resulting liquid part was completely evaporated in the evaporator (Buchi R-100 equipped with Vacuum Pump V-300 and Control unit I-300).

### GC-MS Analysis

For GC-MS, the HP-5 MS IU capillar column (30 m X 250  $\mu$ m X 0.25  $\mu$ m) and the 7890A (Agilent) model GC-MS device with 5975C (Agilent) inert MSD mass detector were used. In GC-MS, an electron ionization system with 70 eV ionization energy and He was used as carrier gas with 99.999% purity. The carrier gas entered the He column with a flow rate of 1.5 mL/min and a starting pressure of 17,897 psi. After keeping the oven temperature at the starting temperature of 50°C for 2 minutes, the rate of temperature rise in all stages was 5°C/min and was increased to 80°C (waiting 2 minutes at this temperature), 100°C (waiting 1 minute at this temperature), 150°C (waiting 1 minute at this temperature), 240°C (waiting 1 minute at this temperature) and 270°C (waiting 7 minutes at this temperature), respectively.

The GC-MS analysis was performed by the Giresun University Central Research Laboratory Application and Research Centre, Giresun, Turkey.

### Anti-helminthic Activity

#### Obtaining *C. elegans* Strains

The strain of wild type *C. elegans* N2 was purchased from the *Caenorhabditis* Genetic Center (University of Minnesota, Minneapolis, USA).

#### Synchronization of *C. elegans* nematodes

About 20 adults *C. elegans* were transferred to the Nematot Growth Media (NGM) petri dish containing *E. coli* OP50. After laying for 4-6 hours, adults *C. elegans* were removed from the petri dish. These eggs formed synchronized offspring. These were used in the study when it came to adult form at the end of the 3rd day.

This procedure was carried out with 5 petri at the same time to provide sufficient nematodes for the study.

### Preparation of Nematode Growth Media

A 2.5 g peptone, 3 g NaCl, and 20 g agar were dissolved in 1 L of distilled water. After autoclaving at 125°C for 15 min, the mixture was cooled to 55°C. Homogenization was obtained by adding 1 mL MgSO<sub>4</sub> (1M), 1 mL cholesterol (5 mg/mL), 1 mL CaCl<sub>2</sub> (1M), 25 mL KH<sub>2</sub>PO<sub>4</sub> buffer (pH 6), which had been previously prepared and filtered through a 0.2 µm mesh, to the medium. *Myrtus communis* L fruit extracts were added to NGM separately, with final concentrations of 10 mg/mL, 5 mg/mL, 2,5 mg/mL, 1,25 mg/mL and experiment groups were formed. In addition, 10 mL was taken from Kontil (250 mg/5mL), which is used as an anthelmintic drug and whose active ingredient is pyrantel pamoate and dissolved within 100 mL NGM and prepared in a concentration of 5 mg/mL. It formed this positive control group. The negative control group was created only from NGM. NGM was transferred 10 mL each to petri dish and, the prepared *E. coli* OP50 strain was added to NGM.

### Determination of Anti-helminthic Activity

In order to determine the anti-helminthic effect of plant extracts on *C. elegans*, 20 synchronized adult *C. elegans* were transferred to NGMs containing plant extracts and *E. coli* OP50. The number of live and dead nematodes was recorded under a stereo microscope every day for 21 days. Each concentration was studied with 5 petri dishes, and the work was repeated 2 times. Nematodes who had completely lost their pharynx pumping movements were considered dead.

### Statistical analysis

The data obtained in our study were evaluated in the SPSS (Ver: 22.0) program and the One- Way Anova and Tukey test was used, the level of error was taken as 0.05.

## RESULTS

According to our study results, no adult nematodes were found in the sample of *Myrtus communis* L. fruit extract on the 6th day at all concentrations included in the study. It was found that there are no living nematodes at the end of the second day at the first concentration of the study, 10 mg/mL, at the end of the third day at the concentration of 5 mg/mL, at the end of the fifth day at the concentration of 2.5 mg/mL, and at the end of the 6th day at the concentration of 1.25 mg/mL. According to the data of pyrantel pamoate (5 mg/mL) used as a positive control

were examined, the presence of live nematodes was observed until the 10th day. In the negative control group, which was not exposed to any active substance, live nematodes were observed until the 21st day (Fig. 1, Fig. 3, Fig. 4, Table 2). When the data were evaluated statistically, the difference between all experimental groups, negative control group and positive control group (pyrantel pamoate), was significant ( $p < 0.05$ ). According to our findings, *Myrtus communis* L. fruit extract was found to be more effective on *C. elegans* nematodes than pyrantel pamoate at the same concentration (5 mg/mL).

The main components determined as a result of GC-MS analysis are shown in Table 1, Figure 2. According to the analysis, the highest detected ingredients were 1,8-Cineole (3.46%), Linalyl acetate (3.06%), and Palmitic acid (2.90%).

## DISCUSSION

In our study, the anti-helminthic activity of *Myrtus communis* L. fruit extract on *C. elegans* nematodes was investigated. Many medicinal effects of *Myrtus communis* L. have been mentioned in the literature [3-7]. In addition, there are studies showing the antiparasitic effect of *Myrtus communis* L. In a study, potent prophylactic effects of essential oils of *Myrtus communis* L. were shown in mice infected with *Toxoplasma gondii*, especially at doses of 200 and 300 mg/kg [19]. Another study was carried out on promastigote and amastigote forms of *Leishmania tropica* and it was shown that the essential oils of *Myrtus communis* L. significantly inhibited their growth

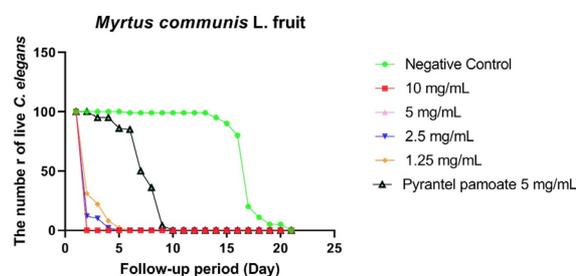


Figure 1. Anti-helminthic activity of *Myrtus communis* L. fruit extract on *C. elegans* nematodes

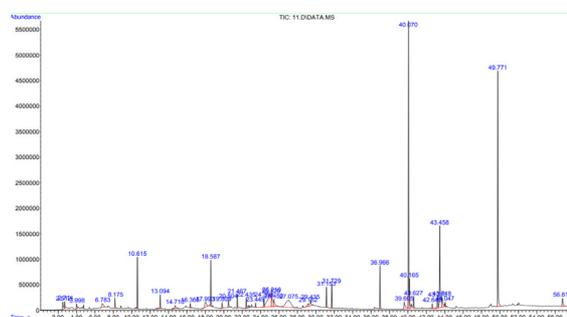
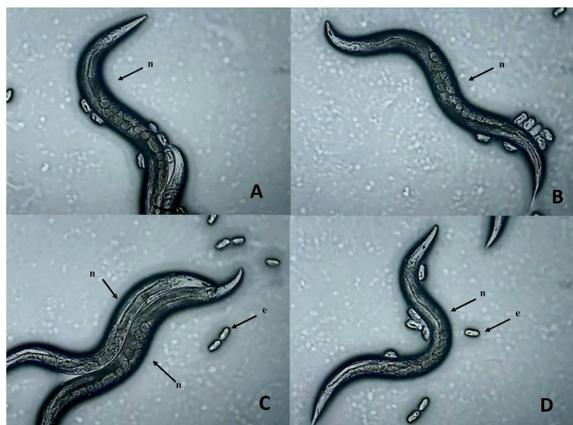


Figure 2. GC-MS chromatogram of *Myrtus communis* L. fruit ethanol extract



**Figure 3.** *C. elegans* live nematodes (n: *C. elegans* L4 form, e: egg)



**Figure 4.** A, B, C Dead nematodes

**Table 1.** GC-MS results of *Myrtus communis* L. fruit extract

Bileşen Adı	RT	Ethanol(%)
Acetic acid	2.513	0.81
9-Octadecenamide	2.513	0.81
2-Propenoic acid	3.995	0.45
2-Heptenal	8.178	0.82
1,8-Cineole (Eucalyptol)	10.616	3.46
Linalool	13.093	1.37
4H-Pyran-4-one	14.713	0.78
β-Fenchyl Alcohol	16.366	0.38
5-Hydroxymethylfurfural	17.991	1.29
Linalyl acetate	18.586	3.06
2,4-Decadienal	20.503	0.72
Camphene	21.465	0.90
(+)-4-Carene	21.465	0.90
Geranyl acetate	22.437	0.69
trans-Caryophyllene	23.450	0.32
beta-Selinene	24.360	0.77
Palmitic acid	36.965	2.90
9,12-Octadecadienoic acid	39.603	1.58
Stearic acid	40.627	0.98
D-alpha-Tocopherol	56.809	1.25

in a dose-dependent manner. In a study, The IC50 values of essential oil and methanolic extract were reported as 8.4 and 28.9 µg/mL against promastigotes, and 11.6

**Table 2.** Number of live *C. elegans* exposed to different doses of *Myrtus communis* L. fruit ethanol extract

Days	Control	Pyrantel pamoate 5 mg/mL	10 mg/mL	5 mg/mL	2.5 mg/mL	1.25 mg/mL
1	100	100	100	100	100	100
2	100	100	0	1	12	31
3	100	95	0	0	10	22
4	100	95	0	0	2	8
5	100	86	0	0	0	2
6	99	85	0	0	0	0
7	99	50	0	0	0	0
8	99	36	0	0	0	0
9	99	4	0	0	0	0
10	99	0	0	0	0	0
11	99	0	0	0	0	0
12	99	0	0	0	0	0
13	99	0	0	0	0	0
14	95	0	0	0	0	0
15	90	0	0	0	0	0
16	80	0	0	0	0	0
17	20	0	0	0	0	0
18	11	0	0	0	0	0
19	5	0	0	0	0	0
20	5	0	0	0	0	0
21	0	0	0	0	0	0

and 40.8 µg/mL against amastigote forms, respectively. In addition, it was emphasized in the study that *Myrtus communis* L. was not cytotoxic in the J774 cell line at the working concentrations [20]. In addition to these studies, the anti-parasitic effects of *Myrtus communis* L. on *Giardia lamblia* [21] and *Trichomonas vaginalis* [22,23] protozoa were also mentioned. In the literature review, there were not enough studies on the anti-helminthic activity of *Myrtus communis* L. fruits. In a study, water and ethanol extracts of the leaves of *Myrtus communis* L. investigated for the anti-helminthic activity on cattle strongyles nematodes, and this effect was evaluated by egg screening test and larval mortality test. It was determined that *Myrtus communis* L. leaf water and ethanol extracts have a potential anthelmintic activity on cattle strongyles parasite larvae [24].

As a result of GC-MS analysis, 1,8-Cineole (Eucalyptol), the oxygenated monoterpenes that we detected with the highest rate, were also detected at high rates in the essential oils of *Artemisia lancea* (34.56%), *Piper aduncum* (55.8%),

and *Rosmarinus officinalis* (42.11%). It was observed that essential oils of these species show high ovicidal and larvicidal activity on *H. contortus* [25-27].

Compounds such as beta-seline, palmitic acid, 9,12-Octadecadienoic acid, and camphene, which we determined as a result of GC-MS analysis of *Myrtus communis* L. fruit ethanol extract, were found in many plants have anthelmintic effects [28-34].

In a study by Taur et al. [35], the anti-helmintic activity of essential oil isolated from *Eucalyptus globulus* on the adult Indian worms *Pheretima posthuma*, which is anatomically and physiologically similar to human intestinal worm parasites, was investigated. As a result of this study, it was concluded that *E. globulus* oil has anthelmintic potential due to the presence of borneol, linalool, cineol, geranyl acetate, anethole, and saffron. The presence of these herbal components, linalool and geranyl acetate, in the fruit extract of *Myrtus communis* L., was determined as a result of our GC-MS analysis.

The active ingredients in herbal extracts sometimes act alone or sometimes in combination with several active ingredients. There are studies using some of these active ingredient combinations to prove anti-helmintic activity. For example, in a study with fatty acids such as palmitic acid and stearic acid as a result of GC-MS analysis, it was determined that some combinations of palmitic acid and stearic acid prevented the hatch rate of *H. contortus* species by 100%. Likewise, as a result of GC-MS analysis, the highest rate of linalyl acetate (3.06%), which we determined highest than the 1,8-Cineole (3.46%) in the fruit extract of *Myrtus communis* L., was different combinations of  $\alpha$ -pinene, p-cymene, thymol octanoate, and thymol acetate compounds were tested on pigs infected with *Ascaris suum* eggs, and the combination of  $\alpha$ -pinene, linalyl acetate, p-cymene, and thymol octanoate was found to be quite effective [36].

In a study conducted by Matos-Rocha et al. [37], the schistosomicidal activity of sesquiterpenes such as alpha-humulene and trans-caryophyllene in adult individuals of *Schistosoma mansoni* was evaluated in vitro and it was found that sesquiterpenes at non-lethal concentrations reduce the egg production and motor activity of the worms and cause death in some way depending on the concentration (100 and 200  $\mu$ g/mL). Trans-caryophyllene is one of the ingredient that we detected as a result of GC-MS analysis in our study.

## CONCLUSSIONS

In our study, the anti-helmintic activity of *Myrtus communis* L. fruit extract on *C. elegans* nematodes was inves-

tigated, and it was found that *Myrtus communis* L. fruit extract was more effective on *C. elegans* nematodes than pyrantel pamoate at the same concentration (5 mg/mL). According to our GC-MS results of *Myrtus communis* L. fruit extract, 20 different active ingredients were found. In the literature reviews, it has been shown in previous studies that some compounds such as 1,8-Cineole (Eucalyptol), linalool, geranyl acetate, linalyl acetate, and trans-caryophyllene have shown anti-helmintic activity. The presence of these active ingredients supports our experimental results. Via studying the forms of these active ingredients separately or in different combinations, the ingredients and combinations with the strongest anti-helmintic activity can be found, and our findings may pave the way for further studies.

## CONFLICT OF INTEREST

The author declares no financial, or otherwise, conflicts of interest.

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## References

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1. Traveset A, Riera N, Mas RE. Ecology of fruit-colour polymorphism in *Myrtus communis* and differential effects of birds and mammals on seed germination and seedling growth. *Journal of Ecology* (2001) 749-760.
2. Morel J-P, Mercuri AM. Plants and Culture: seeds of the cultural heritage of Europe: Centro Europeo per. I Beni Culturali Ravello Edipuglia (2009) 1-284.
3. Baytop T. Therapy with medicinal plants in Turkey (Past and Present). Publication of the Istanbul University, Istanbul, 1999.
4. Aleksic V, Knezevic P. Antimicrobial and antioxidative activity of extracts and essential oils of *Myrtus communis* L. *Microbiological Research* 169 (2014) 240-254.
5. Gortzi O, Lalas S, Chinou I, Tsaknis J. Reevaluation of bioactivity and antioxidant activity of *Myrtus communis* extract before and after encapsulation in liposomes. *European Food Research And Technology* 226 (2008) 583-590.
6. El Hartiti H, El Mostaphi A, Barrahi M, Ben Ali A, Chahboun N. Chemical composition and antibacterial activity of the essential oil of *Myrtus communis* leaves. *Karbala International Journal of Modern Science* 6 (2020) 249-258.
7. Sadeghi Nejad B, Erfani Nejad M, Yusef Naanaie S, Zarrin M. Antifungal efficacy of *Myrtus communis* Linn. *Jentashapir Journal of Health Research* 5 (2014) e21879.
8. Tuberoso CIG., Rosa, A., Bifulco E, Melis MP, Atzeri A, Pirisi FM, & Dessi MA. Chemical composition and antioxidant activities of *Myrtus communis* L. berries extracts. *Food Chemistry* 123 (2010) 1242-1251.
9. Gul C, Nazligul Y. A Current Approach to Helminthiasis. *Dirim Medical Journal* 83 (2008) 40-48.
10. Bethony J, Brooker S, Albonico M, Geiger, SM, Loukas A, Diemert D, & Hotez PJ. Soil-transmitted helminth infections: ascariasis,

- trichuriasis, and hookworm. *The Lancet* 367 (2006) 1521-1532.
11. Drake L, Bundy D. Multiple helminth infections in children: impact and control. *Parasitology* 122 (2001) 73-81.
  12. Gatti S, Lopes R, Cevini C, Ijaoba, B, Bruno A, Bernuzzi AM, Scaglia M. Intestinal parasitic infections in an institution for the mentally retarded. *Annals of Tropical Medicine & Parasitology* 94 (2000) 453-460.
  13. Tappeh KH, Mohammadzadeh H, Rahim RN, Barazesh A, Khashaveh S, Taherkhani H. Prevalence of intestinal parasitic infections among mentally disabled children and adults of Urmia, Iran. *Iranian Journal of Parasitology* 5 (2010) 60-64.
  14. Kaminsky R, Ducray P, Jung M, Clover R, Rufener L, Bouvier J & Mäser P. A new class of anthelmintics effective against drug-resistant nematodes. *Nature* 452 (2008) 176-181.
  15. Smout MJ, Kotze AC, McCarthy JS, Loukas A. A novel high throughput assay for anthelmintic drug screening and resistance diagnosis by real-time monitoring of parasite motility. *Plos Neglected Tropical Diseases* 4 (2010) e885.
  16. Ozpinar N. A model Organism for Antihelminthic Activity: *Caenorhabditis elegans* and *Nigella sativa*. *Turkish Journal of Parasitology* 44 (2020) 31.
  17. Hernando G, Turani O, Bouzat C. *Caenorhabditis elegans* muscle Cys-loop receptors as novel targets of terpenoids with potential anthelmintic activity. *Plos Neglected Tropical Diseases* 13 (2019) e0007895.
  18. Kaewintajak K, Cho PY, Kim SY, Lee ES, Lee HK, Choi EB, & Park, H. Anthelmintic activity of KSI-4088 against *Caenorhabditis elegans*. *Parasitology Research* 107 (2010) 27-30.
  19. Shaapan RM, Al-Abodi HR, Alanazi AD, Abdel-Shafy S, Rashidipour M, Shater AF, & Mahmoudvand H. *Myrtus communis* Essential Oil; Anti-Parasitic Effects and Induction of the Innate Immune System in Mice with *Toxoplasma gondii* Infection. *Molecules* 26 (2021) 819.
  20. Mahmoudvand H, Ezzatkah F, Sharififar F, Sharifi I, Dezaki ES. Antileishmanial and cytotoxic effects of essential oil and methanolic extract of *Myrtus communis* L. *The Korean Journal of Parasitology* 53 (2015) 21-27.
  21. Abdalla SF, Ramadan NI, Mohamed AA, El-Deeb HK, Al-Khadrawy FM, Badawy AF. A study on the effect of *Myrtus communis* and *Olibanum* on *Giardia lamblia* infection in Egypt. *Parasitologists United Journal* 4 (2011) 89-100.
  22. Azadbakht M, Ziaiye H, Abdollahi F, Shabankhani B. Effect of Methanolic essence and extract of *Myrtus communis* on *Trichomonas vaginalis*. *Journal of Guilan University of Medical Sciences* 12 (2004) 8-13.
  23. Abdollahy F, Ziaei H, Shabankhani B, Azadbakht M. Effect of essential oils of *Artemisia aucheri* Boiss. *Zataria multiflora* Boiss, and *Myrtus communis* L. on *Trichomonas vaginalis*. *Iranian Journal of Pharmaceutical Research* (2010) 35.
  24. Moussouni L, Besseboua O, Abdelhanine A. Anthelmintic activity of aqueous and ethanol extracts of *Urtica dioica* L. and *Myrtus communis* L. leaves on bovine digestive strongyles: in-vitro study. *Ataturk University Journal of Veterinary Sciences* 14 (2019) 273-283.
  25. Vickers NJ. Animal communication: when I'm calling you, will you answer too? *Current Biology* 27 (2017) 713-715.
  26. Oliveira GL, Vieira TM, Nunes VF, Ruas MDO, Duarte ER, Moreira DDL, & Martins ER. Chemical composition and efficacy in the egg-hatching inhibition of essential oil of *Piper aduncum* against *Haemonchus contortus* from sheep. *Revista Brasileira de Farmacognosia* 24 (2014) 288-292.
  27. Pinto, N. B., Castro, L. M. D., Azambuja, R. H. M., Capella, G. D. A., Moura, M. Q. D., Terto, W. D., ... & Leite, F. P. L. Ovicidal and larvicidal potential of *Rosmarinus officinalis* to control gastrointestinal nematodes of sheep. *Revista Brasileira de Parasitologia Veterinária* 28 (2019) 807-811.
  28. Pessoa L, Morais S, Bevilacqua C, Luciano J. Anthelmintic activity of essential oil of *Ocimum gratissimum* Linn. and eugenol against *Haemonchus contortus*. *Veterinary Parasitology* 109 (2002) 59-63.
  29. Malheiros DF, Sarquis IR, Ferreira IM, Mathews PD, Mertins O, Tavares-Dias M. Nanoemulsions with oleoresin of *Copaifera reticulata* (Leguminosae) improve anthelmintic efficacy in the control of monogenean parasites when compared to oleoresin without nanoformulation. *Journal of Fish Diseases* 43 (2020) 687-695.
  30. Lalthanpuii PB, Lalchhandama K. Phytochemical analysis and in vitro anthelmintic activity of *Imperata cylindrica* underground parts. *BMC Complementary Medicine and Therapies* 20 (2020) 1-9.
  31. Lalthanpuii P, Zokimi Z, Lalchhandama K. The toothache plant (*Acmella oleracea*) exhibits anthelmintic activity on both parasitic tapeworms and roundworms. *Pharmacognosy Magazine* 16 (2020) 193-198.
  32. Hernandez PM, Salem AZ, Elghandour MM, Cipriano-Salazar M, Cruz-Lagunas B, Camacho LM. Anthelmintic effects of *Salix babylonica* L. and *Leucaena leucocephala* Lam. extracts in growing lambs. *Tropical Animal Health and Production* 46 (2014) 173-178.
  33. Panda SK, Das R, Mai AH, De Borggraeve WM, Luyten W. Nematicidal Activity of *Hologarna caustica* (Dennst.) Oken Fruit Is Due to Linoleic Acid. *Biomolecules* 10 (2020) 1043-1054.
  34. Štrbac F, Bosco A, Amadesi A, Rinaldi L, Stojanović D, Simin N, Orčić D, Pušić I, Krnjajić S, Ratajac R. In vitro ovicidal activity of two chemotypes of the yarrow (*Achillea millefolium* L.) essential oil against sheep gastrointestinal nematodes. *Archives of Veterinary Medicine* 13 (2020) 59-76.
  35. Taur D, Kulkarni V, Patil R. Chromatographic evaluation and anthelmintic activity of *Eucalyptus globulus* oil. *Pharmacognosy Research* 2 (2010) 125-127.
  36. Kaplan RM, Storey BE, Vidyashankar AN, Bissinger BW, Mitchell SM, Howell SB, Mason ME, Lee MD, Pedrosa AA, Akashe A, Skrypec DJ. Antiparasitic efficacy of a novel plant-based functional food using an *Ascaris suum* model in pigs. *Acta Tropica* 139 (2014) 15-22.
  37. Matos-Rocha TJ, dos Santos Cavalcanti MG, Barbosa-Filho JM, et al. In vitro evaluation of schistosomicidal activity of essential oil of *Mentha x villosa* and some of its chemical constituents in adult worms of *Schistosoma mansoni*. *Planta Medica*. 79 (2013) 1307-1312.