

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

Hulya Ozpinar 

Sivas Cumhuriyet University, Department of Pharmaceutical Botany, Sivas, Turkey

## 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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**Correspondence to:** Hulya Ozpinar, Sivas Cumhuriyet University, Faculty of Pharmacy, Department of Pharmaceutical Botany, 58140 Sivas/Turkey  
E-Mail: hulya1177@yahoo.com.tr



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

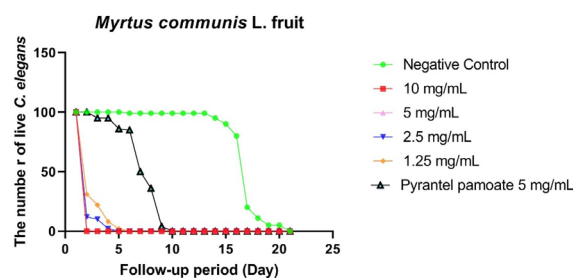


Figure 1. Anti-helminthic activity of *Myrtus communis* L. fruit extract on *C. elegans* nematodes

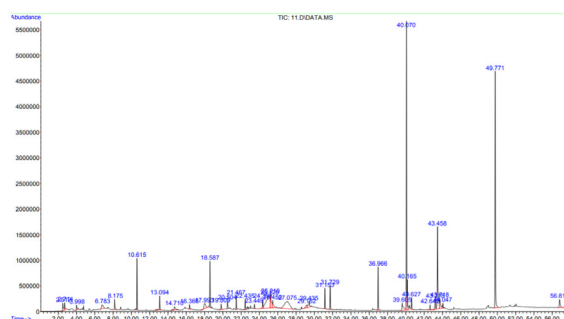


Figure 2. GC-MS chromatogram of *Myrtus communis* L. fruit ethanol extract



**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%),



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