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Origanum vulgare Essential Oil Demonstrates *In Vivo* and *In Silico* Activity Against a Mouse Nematode *Syphacia obvelata* (Rudolphi, 1802)

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ABSTRACT Oxyurid nematode infections are common in animals and often recurrent, necessitating the development of new anthelmintic agents. *Origanum vulgare* L. subsp. *hirtum* essential oil (OEO) was evaluated for its nematocidal effects against the *Syphacia obvelata* nematode in naturally infected mice. Mice received oral OEO at 300 and 600 mg/kg doses, with egg counts monitored via the perianal tape method. Gas chromatography identified carvacrol (84.0%) as the dominant compound, while in silico docking revealed β-caryophyllene exhibited strong binding affinities to β-tubulin and carnitine palmitoyltransferase 2, key nematode drug targets. OEO treatment significantly reduced parasite egg counts by up to 98% at 7 days post-treatment, with sustained effects at 14 days. These findings suggest that OEO, particularly β-caryophyllene, possesses promising anthelmintic activity against oxyurid nematodes, as a preliminary study. Further studies are warranted to explore its therapeutic potential and mechanisms of action.

Keywords: *Anthelmintic properties, Essential oils, Molecular docking, Nematocidal activity, Origanum vulgare.*

ÖZ

Origanum vulgare Esansiyel Yağının Fare Nematodu *Syphacia obvelata*'ya (Rudolphi, 1802) Karşı *İn-Vivo* ve *İn-Siliko* Etkisi

Oksyurid nematod enfeksiyonları hayvanlarda yaygındır ve sıklıkla tekrarlar. Bu nedenle yeni anthelmintik ajanların geliştirilmesi gerekmektedir. *Origanum vulgare* L. subsp. hirtum uçucu yağı (OEO), *Syphacia obvelata* nematodu ile doğal enfekte farelerde, nematosidal etkiler açısından değerlendirilmiştir. Farelere 300 ve 600 mg/kg dozlarında oral OEO verilmiş ve yumurta sayıları perianal bant yöntemi ile izlenmiştir. Gaz kromatografisi, karvakrolü (%84.0) baskın bileşik olarak tanımlarken, in-siliko yerleştirme, β-karyofilenin β-tubulin ve karnitin palmitoil transferaz 2'ye, yani nematod ilaçlarının temel hedeflerine güçlü bağlanma eğilimi gösterdiğini ortaya koymuştur. OEO tedavisi, tedaviden sonraki 7. günde parazit yumurta sayısını %98'e kadar önemli ölçüde azaltmış ve etkilerin 14. günde devam etmesini sağlamıştır. Bu bulgular, OEO'nun, özellikle β-karyofilenin, oksyurid nematodlara karşı umut verici bir anthelmintik aktiviteye sahip olduğunu bir ön çalışma olarak göstermektedir. Terapötik potansiyelini ve etki mekanizmalarını araştırmak için daha fazla çalışmaya ihtiyaç vardır.

Anahtar Kelimeler: *Anthelmintik özellik, Esansiyel yağ, Moleküler yerleştirme, Nematosidal aktivite, Origanum vulgare.*

INTRODUCTION

Oxyurid infections in domestic animals are significant due to their impact on animal health and welfare, as well as potential implications for zoonotic transmission. Oxyurid nematodes primarily affect various domestic animals, including rodents, reptiles, and some mammals, leading to a spectrum of health issues ranging from asymptomatic infections to severe clinical manifestations (Taylor et al. 2016). Despite numerous studies (Santoro et al. 2007; Castro et al. 2013; Pensel et al. 2014) investigating the anthelmintic properties of plant extracts, the number of in silico studies examining the action mechanism of active compounds remains insufficient. However, *in silico* studies provide fast and reliable results for this type of investigation when compared with classical application methods.

Benzimidazoles inhibit microtubule formation by binding β-tubulin in nematodes (Lacey 1990; Martin et al. 2014). Anthelmintic drugs have been used for an extended period due to their broad spectrum, good tolerability, and low price (Lanusse et al. 2014). Excessive and unnecessary use of this type of drug leads to the emergence of resistant

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nematode populations (dos Santos et al. 2014). Albendazole's common adverse effects are abdominal pain, nausea, vomiting, dizziness, temporary hair loss, headache, and fever (Jung and Gonzales-Esquivel 2002). Therefore, further research is required to determine whether plant extracts and essential oils will be applied to infected animals in order to discover new drugs that can be used safely.

Origanum vulgare L. subsp. hirtumis a shrubby species in the Lamiaceae family, found in Mediterranean and Asian regions (Ietswaart 1982; Celep and Dirmenci 2017). It has 12 pairs of twigs per stem, with leaves that are petiolate or subsessile, measuring 6-40×5-30 mm. The spicules measure 3-30×2-8 mm, and the corolla is purple, pink, or white, measuring 3-10 mm (Ietswaart 1982). The essential oil has antiparasitic activity due to its thymol and carvacrol compounds (Santoro et al. 2007). In a comparative study, *Origanum vulgare* L. essential oil demonstrated potential anticancer activity against hepatocellular carcinoma (Hep3B), human breast adenocarcinoma (MCF-7), and human lung carcinoma (A549) cell lines. Antiproliferative activity of essential oil was found to be significant on these cell lines as compared to standards (cisplatin and 5-fluorouracil) (Erenler et al. 2023).

Origanum essential oil contains several monoterpenes and sesquiterpenes, including β-caryophyllene. Although β-caryophyllene is a sesquiterpene found widely in nature, its anthelmintic properties have never been investigated before. The investigation of β-caryophyllene, a compound found in plants such as *Cannabis sativa*, *Cinnamomum verum*, and *Syzygium aromaticum* (Ludwiczuk et al. 2017), which can be cultivated in Asian and African countries where helminthic infections are prevalent, may contribute to the discovery of promising natural anthelmintic components needed in these regions.

Enzymes in the carnitine palmitoyl transferase (CPT) family (EC 2.3.1.21) transport long-chain fatty acids from the cytoplasm to the mitochondria, which can be oxidised to release energy (Taylor et al. 2013). A drug called Perhexiline has been reported to target CPT homolog enzymes in humans. Inhibition of CPT 2 is effective against *Caenorhabditis elegans* larvae, a harmful parasitic nematode *Haemonchus contortus*, and the filarial nematode *Onchocerca linealis* (Ceccarelli et al. 2011). Therefore, CPT 2 has been considered a significant antinematodal drug target (Tyagi et al. 2018).

In domestic rodents, oxyurid infections, especially by species like *Syphacia* and *Aspicularis*, have been noted to cause a range of health problems despite often being regarded as relatively benign under low infestation levels. *Syphacia obvelata* (Rudolphi 1802) is a common oxyurid nematode of mice and is characterized by its distinct morphology, including a tapered tail and a prominent cephalic structure. This nematode is often found in the intestines of its hosts, where it can cause various gastrointestinal issues (Pisanu et al. 2003).

In this study, we aimed to assess the anthelmintic efficacy of *Origanum vulgare* subsp. hirtum essential oil (OEO) on mice naturally infected with the oxyurid nematode *Syphacia obvelata* (Rudolphi 1802). We analyzed the bioactive components in OEO and evaluated how these components bind to antinematodal drug target proteins. This research is necessary to identify natural alternatives that can effectively combat drug resistance in animal parasites, thereby improving treatment options and reducing reliance on synthetic medications.

MATERIAL AND METHODS

Ethics Committee Approval

This study was conducted with the permission of Bursa Uludağ University, Animal Experiments Local Ethics Committee on 11.07.2017 with the number 2017-10/07.

Preparation of Origanum Essential Oil

Origanum shoots were cultivated in Eskişehir obtained from the Atatürk Horticultural Central Research Institute, Yalova, Türkiye. Aerial parts of *Origanum vulgare* subsp. *hirtum* were collected on 26 July 2017 from Eskişehir.

A hydrodistillation method was used to extract fresh oregano essential oil from the aerial parts of *Origanum vulgare* subsp. *hirtum*, following the procedure outlined in the European Pharmacopoeia (2025). The oil was then stored at 4 °C in a refrigerator until use.

Gas Chromatography (GC) and Gas Chromatography - Mass Spectrometry (GC/MS) Analysis

An Agilent 6890N GC system was used for the GC analysis. The system was equipped with an FID detector set at a temperature of 300 °C. A duplicate column was used for simultaneous auto-injection under the same conditions to ensure the same elution order with GC-MS. The FID chromatogram displayed the relative percentage amounts of the separated compounds.

Table 1: Composition of the essential oil of *Origanum vulgare* subsp. *Hirtum.*

RRI	Compounds	%	Identification Method
1032	a-Pinene	0.5	t_R, MS
1035	a -Thujene	0.6	MS
1076	Camphene	0.2	t_R, MS
1118	*b*-Pinene	0.1	t_R, MS
1132	Sabinene	0.1	t_R, MS
1174	Myrcene	0.9	t_R, MS
1176	a -Phellandrene	0.1	t_R, MS
1188	a-Terpinene	0.8	t_R, MS
1203	Limonene	0.1	t_R, MS
1213	b -Phellandrene	0.1	t_R, MS
1255	**g-Terpinene**	**3.5**	t_R, MS
1280	***p*-Cymene**	**4.6**	t_R, MS
1290	Terpinolene	0.1	t_R, MS
1452	1-Octen-3-ol	0.1	t_R, MS
1474	*trans*-Sabinene hydrate	0.3	t_R, MS
1553	Linalool	tr	t_R, MS
1556	*cis*-Sabinene hydrate	0.1	t_R, MS
1611	Terpinen-4-ol	0.6	t_R, MS
1612	b-Caryophyllene	1.4	t_R, MS
1687	a-Humulene	0.1	t_R, MS
1706	a-Terpineol	0.2	t_R, MS
1719	Borneol	0.5	t_R, MS
1737	b-Bisabolene	0.4	t_R, MS
1757	Carvone	0.1	t_R, MS
2008	Caryophyllene oxide	0.2	t_R, MS
2181	Isothymol	tr	MS
2198	Thymol	0.3	t_R, MS
2228	Isocarvacrol	tr	t_R, MS
2239	**Carvacrol**	**84.0**	t_R, MS

RRI: Relative retention indices experimentally calculated against *n*-alkanes; % calculated from FID data; **tr**: Trace (<0.1 %); **t_R**: Identification based on comparison with co-injected with standards on an HP Innowax column; **MS**: identified based on computer matching of the mass spectra with those of the in-house Baser Library of Essential Oil Constituents, Adams, MassFinder and Wiley libraries.

The oregano essential oil was analysed using an Agilent GC-MSD system from Agilent Technologies Inc. (Santa Clara, CA) with capillary GC/MS. Compounds were separated using an HP-Innowax FSC column from Hewlett-Packard (U.S.A.). The column had a length of 60 meters, an inner diameter of 0.25 millimetres, and a film thickness of 0.25 micrometres. Helium was used as the carrier gas at a flow rate of 0.8 millilitres per minute. The GC oven temperature was initially set to 60 °C and maintained for 10 minutes, then increased to 220 °C at a rate of 4 °C per minute and held there for 10 minutes. Finally, the temperature was raised to 240 °C at 1°C per minute. The split flow was adjusted to 40 millilitres per minute with a split ratio of 40:1, and the injector temperature was set at 250 °C. Mass spectra were taken at 70 eV with a mass range of m/z 35-450.

To identify the oregano essential oil compounds, their mass spectra were compared with those in the Baser Library of Essential Oil Constituents, Wiley GC/MS Library (McLafferty and Stauffer 1989), Adams Library (Adams 2007), and Mass Finder Library (Hochmut 2008). The analysis involved comparing the retention indices of the samples. To calculate the relative retention indices (RRI), a homologous series of n-alkanes was used as reference points. The FID chromatograms were used to calculate the relative percentages of the compounds that were separated. The GC/MS analysis results were presented as the mean percentage in Table 1.

In vivo Procedures

Balb/c mice of both sexes, weighing between 28 and 41 grams, of different ages,were obtained from the Experimental Animals Breeding Centre of Bursa Uludag University in Bursa, Türkiye. These mice were imported from the Netherlands and were not transgenic. They were housed in plastic cages at room temperature (20-24 °C) with a 12-hour light/dark cycle and 55% relative humidity. The animals were fed *ad libitum* and had continuous access to water. Fresh air was provided daily through the ventilation system.

Experimental animals naturally infected with *Syphacia obvelata* were selected using perianal cellophane tape and Fülleborn fecal flotation methods. Each group consisted of six animals, except for the ABZ drug control group, which included eight animals. In total, 26 mice were involved in this study.

Infected mice were divided into four groups consisting of three treatment groups and one negative control group. These groups were generated according to the observed parasite egg numbers inmice by considering the similar average egg numbers in all groups. All cages were labelled according to the mouse number. Egg numbers were obtained from Fülleborn faecal flotation and perianal tape methods performed on two consecutive days. Group 1 (n=6) and Group 2 (n=6) received 300 mg/kg and 600 mg/kg of *Origanum* Essential Oil (OEO), respectively. Group 3 (n=6) received soybean oil (4 ml) as solvent control, and Group 4 (n=8) received Albendazole 20 mg/kg as drug control by completing the 4 ml total volume with soybean oil. Each mouse was given pellets containing the solutions and kept in separate cages. We observed whether the mice fed on the pellets. Groups 1, 2, and 3 were treated for seven consecutive days, but Group 4 was treated for three days at one-day intervals to see the results of a different drug application. The steps of the *in vivo* experiment are shown in Figure 1.

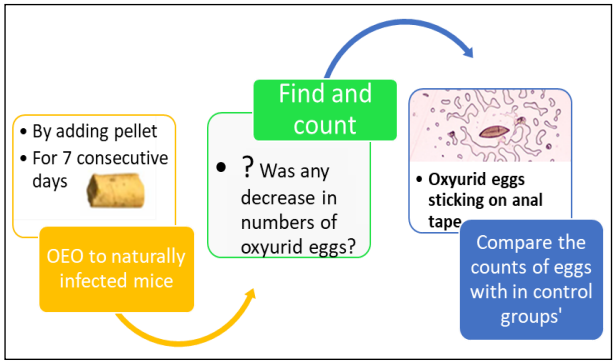

Figure 1: Steps of the *in vivo* experiment.

After the acclimatisation of animals, the perianal tape (cellophane) method was selected to detect the nematode infections. To evaluate the efficacy of the plant extract on egg numbers, the parasite egg count on each mouse's rectum was calculated by counting eggs on anal tape collected from each mouse before the 7th day of treatment and on the 0th, 7th, and 14th days after treatment. To apply this method, approximately 9 × 1 cm cellophane tape was pressed onto the rectum of each mouse 10-15 times. After sticking it to a microscopy slide, *S. obvelata* eggs were counted.

In the perianal band, the reduction percentage of oxyurid eggs was counted as follows (Pagano and Gauvreau 2018):

$$reduction\ \% = 100 \times (\frac{I - L}{I})$$

I: Initial number of parasite eggs in each group according to the arithmetic mean.

L: Last number of parasite eggs in each group according to the arithmetic mean.

The reduction in the number of parasite eggs across all application groups was compared. The findings were analyzed using the IBM-SPSS statistical software, employing Kruskal-Wallis post hoc analysis (IBM Corporation 2020). Due to the variability in variance caused by the uneven distribution of parasite eggs among the groups, the nonparametric Kruskal-Wallis test was chosen. A p-value of less than 0.05 was considered statistically significant.

In silico Molecular Docking

In our study, we selected the most abundant active compounds found in the essential oil of *Origanum vulgare* subsp. *hirtum*. We conducted a docking study with antinematodal drug target proteins to further investigate their potential antinematodal effects. The ten compounds examined in this study include α-thujene, β-caryophyllene, γ-terpinene, borneol, terpinene-4-ol, p-cymene, carvacrol, myrcene, α-pinene, and α-terpinene, all of which were obtained from the PubChem database (Kim et al. 2021). *Haemonchus contortus* β-tubulin (pdb ID: 1OJ0) (Robinson et al. 2004) and *Rattus norvegicus* Carnitine Palmitoyltransferase 2 (CPT 2, pdb ID: 2H4T, resolution: 1.90 Å) (Hsiao et al. 2006) were obtained from the Protein Data Bank (2025). Docking files were created using the ADT program (Huey et al. 2007; Morris et al. 2009). The results were analyzed with the Biovia Discovery Studio Client 2020 software, developed by Dassault Systèmes, Biovia Inc. (2020). The root mean square deviation (RMSD) was calculated using the DockRMSD tool from the Zanglag web server (Bell and Zhang 2019). For the docking simulations involving the β-tubulin protein and the herbal ligands, the coordinates of the original ligand bound to the

protein in the co-crystallized structure were chosen as the center point. The previous docking experiments were useful for predicting the center coordinates of a protein, so we utilized the docking coordinates from the grid box provided by Taylor et al. (2013) for the CPT2 enzyme. In our approach, all protein atoms were considered rigid, while only the hydrogen atoms were allowed to move freely within the protein's active site.

We set the dielectric constant to 10 and established the ionic strength at 0.145. The dimensions of the grid box were defined as 60×60×60, with a grid point spacing of 0.375 Å. The maximum number of generations was set to 27,000, and the maximum number of evaluations was adjusted to 2,500,000, given that the number of rotational bonds was fewer than 10.

For the docking, we chose the AutoDock 4.2 program, employing a Lamarckian genetic algorithm for the procedure (Morris et al. 1998). The docking simulations were conducted with a total of 20 runs. An overview of the process is summarized in Figure 2.

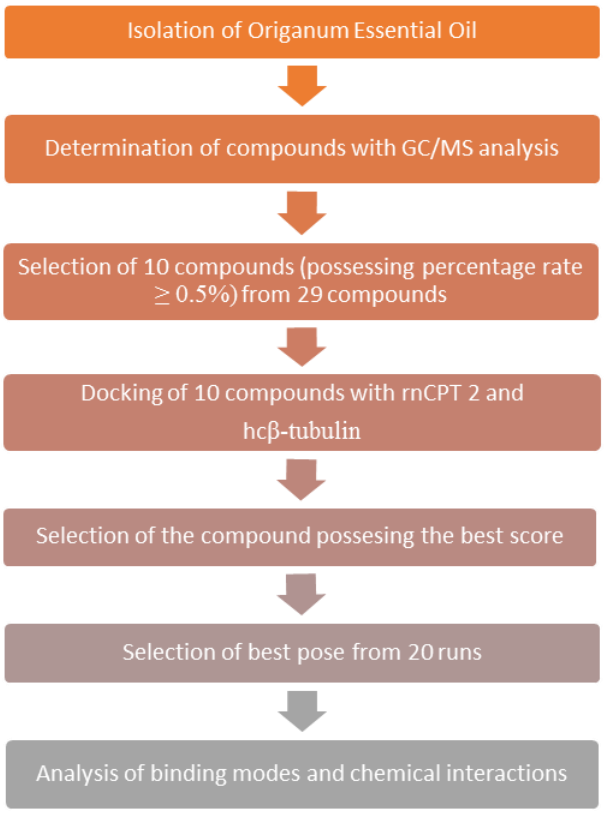

Figure 2: Steps of compound selection and docking.

According to docking simulations of the herbal ligands, the best molecules were estimated via the SwissADME web server (Daina et al. 2017) for ADME (Absorption, Distribution, Metabolism, Excretion) properties. SwissADME web server makes a series of predictions based on chemical formulations and calculations to show the molecule's ability in terms of oral bioavailability, penetration of the blood-brain barrier, and gastrointestinal absorption.

RESULTS

The study demonstrated that *Origanum* essential oil has antinematodal effects against oxyurid nematodes. Additionally, the study searched for interactions between target proteins and bioactive molecules found in the essential oil to discover new drug scaffolds. This was done using *in silico* methods.

In the chemical analysis of *Origanum* essential oil, carvacrol was identified as the dominant compound, comprising 84.0% of the total content. A total of 29 compounds were identified (Table 1). According to the data presented in Table 1, oxygenated sesquiterpenes account for a larger proportion, while monoterpene hydrocarbons exhibit greater diversity in composition. The high concentration of carvacrol indicates the excellent medicinal quality of *O. vulgare* subsp. *hirtum*, which is grown in Türkiye (Baser 2008).

For the docking test, ten compounds with a presence greater than 0.5% were selected. Among these, four compounds exceeded the 1% threshold: γ-terpinene (3.5%), p-cymene (4.6%), β-caryophyllene (1.4%), and carvacrol (84.0%). In contrast, linalool, isothymol, and isocarvacrol were found in much smaller amounts, each below 0.1%.

The mean number of *S. obvelata* eggs counted by the perianal tape method after applying OEOfor seven days is shown in Figure 3. From the 7th day, a significant difference emerged between the group in which soybean oil was given as solvent control and the group in which Albendazole was given as drug control at 20 mg/kg for three days at one-day intervals in the number of parasite eggs.

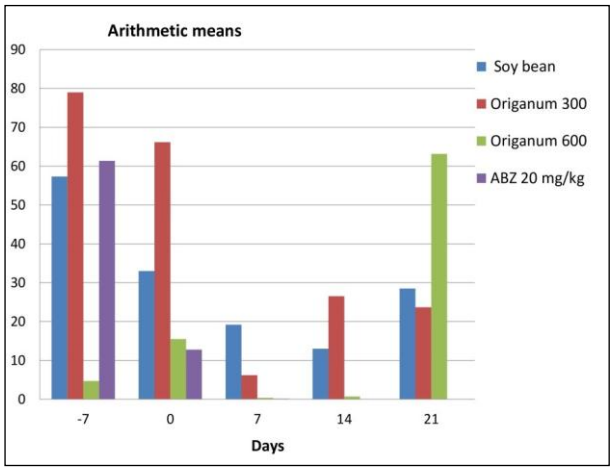

Figure 3: Averages of *S. Obvelata* parasite eggs counted on cellophane in each mouse group.

The study found that the reduction rates of *S. obvelata* eggs on cellophane tapes varied weekly depending on the doses of OEO applied. The most significant difference was observed on the seventh day after the application ended. In the OEO 300 group (Group 1), the reduction percentage was 90.63%, while in the OEO 600 group (Group 2), it reached 98.06% from days 0 to 7 post-application. Additionally, a decrease of 59.96% was noted in the mean number of parasite eggs in the OEO 300 group, and a 95.48% reduction was observed in the OEO 600 group by the 14th day when compared to the pre-treatment count on day 0.

In this study, for the first time, it was observed that administering Oregano Essential Oil (OEO) to laboratory mice infected with *S. obvelata* by adding it to their feed resulted in a reduction in the number of *S. obvelata* eggs. When Albendazole (ABZ) was administered three times at double the usual dose, it was noted that all eggs were eliminated, achieving a 100% cure of the helminthic infection, and there were no adverse effects on the overall health of the mice. However, since the OEO altered the

taste of the feed, the mice did not consume all of the food containing OEO. Consequently, the percentage reduction in the OEO 600 group was lower than anticipated. After treatment, all mice were provided with regular food, ensuring they did not remain hungry for long. This method was preferred because it was more suitable for animal welfare compared to the gavage method.

All groups experienced a significant decrease in the number of eggs by the seventh day of treatment. As expected, the ABZ group showed the most notable reduction, followed by Group 2. By the fourteenth day post-treatment, no eggs were counted in the ABZ group, while Groups 1 and 2 continued to show some infection, albeit with a considerable decrease compared to the initial counts (as shown in Table 2). This suggests that the effects of OEO lasted even after the seven days following treatment.

Table 2: Arithmetic mean of *S. obvelata* eggs counted on perianal tape.

Group	Application	-7. day	0. day	7. day	14. day
1	OEO 300 mg/kg	79	66.2	6.2	26.5
2	OEO 600 mg/kg	4.7	15.5	0.3	0.7
3	Soy oil (4 ml)	57.3	33	19.2	13
4	ABZ 20 mg/kg	61.4	12.8	0.1	0

The results of the Shapiro-Wilk test indicated that the groups did not follow a normal distribution. Additionally, Levene's test revealed that the variances were not homogeneous. Consequently, the Kruskal-Wallis test was conducted. The findings from the Kruskal-Wallis test showed no significant difference between the OEO groups and the solvent control (soy oil) group. However, significant differences were found between the ABZ 20 mg/kg group and both the OEO 300 group and the OEO 600 group. Additionally, a significant difference was noted between the OEO 600 and OEO 300 groups ($p < 0.05$).

Table 3: Docking results of the main bioactive substances in *Origanum vulgare* subsp. *hirtum* essential oil content.

	β Tubulin		CPT 2	
Compound	*Ki*	ΔG (kcal/mol)	*Ki*	ΔG (kcal/mol)
α-Thujene	13.96 uM	-6.62	34.46 uM	-6.09
β-Caryophyllene	873.99 nM	-8.27	1.19 uM	-8.08
γ-Terpinene	10.31 uM	-6.80	42.97 uM	-5.96
Borneol	15.06 uM	-6.58	9.78 uM	-6.83
Terpinen-4-ol	6.73 uM	-7.06	12.85 uM	-6.67
p-Cymene	37.39 uM	-6.04	134.63 uM	-5.28
Carvacrol	22.48 uM	-6.34	73.84 uM	-5.64
Myrcene	36.21 uM	-6.06	101.54 uM	-5.45
α-Pinene	22.50 uM	-6.34	32.89 uM	-6.12
α-Terpinene	10.30 uM	-6.80	41.19 uM	-5.98

No observable side effects were noted during the application period and two weeks afterward. All mice remained in good condition.

Before docking with the major components in the OEO, β-tubulin was re-docked with Albendazole. The calculated RMSD was found as 1.564 Å using the DockRMSD tool. Based on this value, the accuracy of the docking was evaluated.

According to Table 3, the ΔG values of nine of the ten molecules were found between -5.28 and -6.83 kcal/mol for CPT 2, -6.04 and -7.06 kcal/mol for β-tubulin.

Among these molecules, p-cymene exhibited the lowest binding affinities for CPT 2 and β-tubulin, with Ki values of 134.63 μM and 37.79 μM, respectively. The other eight molecules, aside from β-caryophyllene, displayed binding affinities similar to those of p-cymene. The top three major components identified were carvacrol, p-cymene, and γ-terpinene. Due to their small size, these three monoterpenes are predicted to have limited chemical interactions in inhibiting antinematodal targets. Figure 4 illustrates the structures of these three molecules. In silico calculations indicate that sesquiterpenes, which are larger than monoterpenes, demonstrate improved inhibition results against these target proteins (β-tubulin and CPT 2) and are therefore more promising as potential drug candidates.

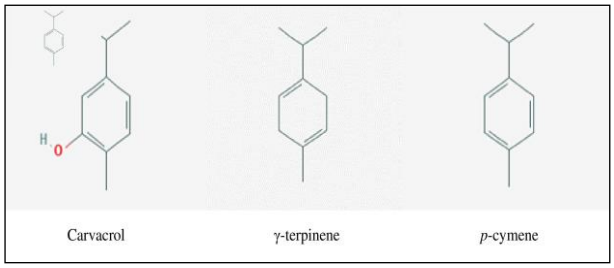

Figure 4: 2D structure of three major compounds in OEO

The docking simulation results indicated that β-caryophyllene exhibited the highest binding affinity for both the CPT 2 enzyme and β-tubulin among the ten molecules isolated from the essential oil of oregano (OEO). The ΔG and Ki values were -8.08 kcal/mol and 1.19 μM for CPT 2, and -8.27 kcal/mol and 873.99 nM for β-tubulin, respectively.

It was found that β-caryophyllene was surrounded by four apolar residues (ALA615, ALA613, LEU592, and VAL597) in the active site of the CPT 2 enzyme and formed electrostatic interactions with these residues (Figure 5 (a) and (b)).

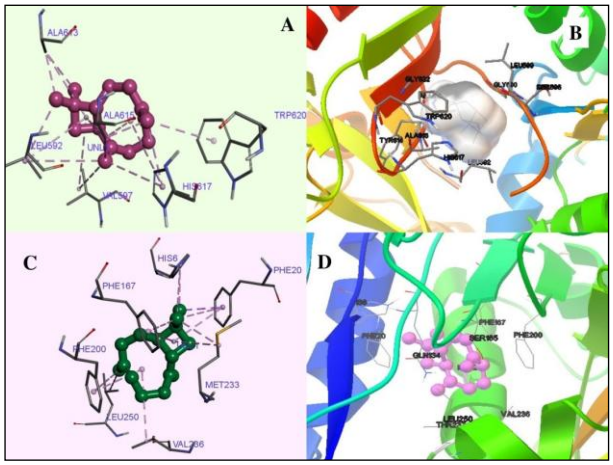

Figure 5: (**a**) β-caryophyllene and CPT 2 enzyme interactions 3D representation, (**b**) Secondary structure of CPT 2 with rainbow colour. β-caryophyllene in the surface area and surrounding with interacting residues of the CPT 2 enzyme. (**c**) β-caryophyllene and β-tubulin interactions 3D representation, (**d**) Secondary structure of β-tubulin's binding site. Pink balls and sticks represent β-caryophyllene.

The nine-membered cyclo-structure of β-caryophyllene effectively facilitated aromatic interactions with HIS617

and TRP620. Additionally, the following residues formed van der Waals interactions with the compound: SER590, GLY600, TYR614, CYS623, LEU599, GLY622, and SER598, as illustrated in Figure 6 (a).

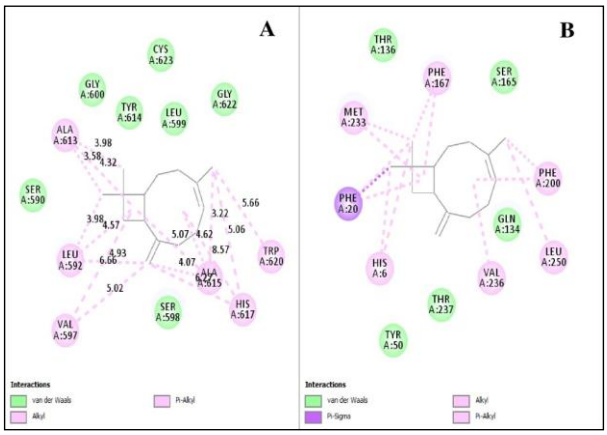

Figure 6: Molecular interactions of β-caryophyllene and residues in the active sites of proteins. (a) 2D representation of interactions with the CPT 2 enzyme. (b) 2 D representation of chemical interactions with β-tubulin. Pink imaginary lines represent alkyl and π-alkyl bonds; purple lines are π-sigma bonds; green spheres are van der Waals interactions.

Interactions of β-caryophyllene with β-tubulin involve several key residues. The tetracyclic portion of the ligand and its methyl groups create π-sigma interactions with PHE20, π-alkyl interactions with PHE167 and MET233, and alkyl bonds with HIS6, positioned opposite the methyl groups (Figure 5 (c) and (d)). These tetracyclic rings and methyl groups form an aromatic sandwich between residues PHE20 and PHE167. PHE200, one of the closest residues to the nine-carbon core, engages in both aromatic and π-alkyl interactions with the methyl group at the fourth position. Additionally, the hydrophobic amino acids LEU250 and VAL236 contribute alkyl interactions with the ligand.

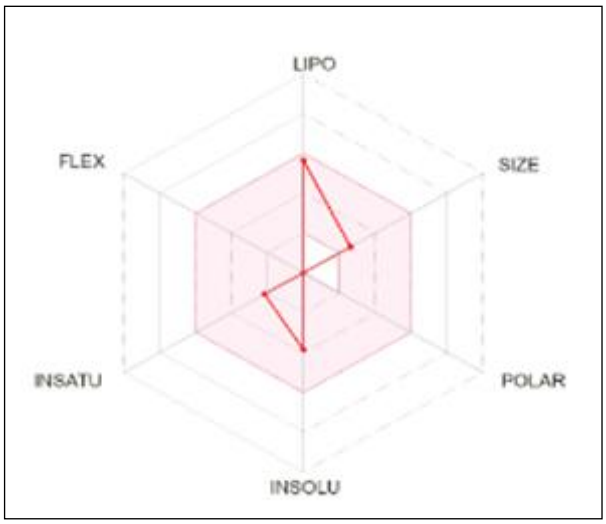

Figure 7: Radar plot of β-caryophyllene.

Figure 6 illustrates that THR136, THR237, TYR50, SER165, and GLN134 all participate in van der Waals interactions with β-caryophyllene in β-tubulin. According to the Swiss ADME analysis of β-caryophyllene's pharmacokinetics, the compound exhibits low gastrointestinal absorption, does not penetrate the blood-brain barrier, and does not inhibit CYP1A2, CYP2D6, or CYP3A4 enzymes. However, it can inhibit CYP19 and CYP2C9 enzymes. β-caryophyllene aligns with Ghose, Veber, and Egan's rules regarding bioavailability but does not meet Muegge's criteria. It satisfies four of Lipinski's five rules as per the SwissADME web server. The radar plot indicates that the molecular properties of β-caryophyllene fall within acceptable limits for oral administration (Figure 7), suggesting that this molecule merits further investigation as a potential drug candidate.

DISCUSSION AND CONCLUSION

Out of the ten molecules tested for their inhibition properties using the *in silico* docking method, only β-caryophyllene exhibited a ΔG value lower than −8 kcal/mol. The remaining nine compounds did not show inhibition of the two proteins tested. This was likely due to their limited size and fewer side chains, which reduced the diversity and number of potential chemical interactions with the proteins. Although these molecules may have anthelmintic effects, it would be beneficial to test them against other anthelmintic protein targets using *in silico* modelling, as well as to investigate their effects *in vitro*.

The results suggest that the anthelmintic effect of these nine molecules operates through a pathway other than β-tubulin or CPT 2 inhibition. The low binding affinity observed may be attributed to the small size of these monoterpenes. In a previous study, sesquiterpene lactone and arteannuin-B demonstrated favorable binding affinity to the CPT 2 enzyme, facilitating numerous interactions (Karaman et al. 2024). Similarly, stigmasterol and fridelin showed potential to inhibit the CPT 2 enzyme, given their available free energy of binding. These two compounds possess larger cyclic cores (steroidal structures), allowing for multiple aromatic and electrostatic interactions with enzyme residues.

Therefore, having a larger core structure and hydroxyl groups in herbal compounds is crucial for inhibiting anthelmintic targets. Since β-caryophyllene is a sesquiterpene containing a cyclobutane ring and a nine-membered ring, it serves as a more suitable ligand for this type of inhibition compared to smaller monoterpenes (Gertsch et al. 2008).

β-Caryophyllene is an agonist of cannabinoid receptors, which belong to the group of G protein-coupled receptors (Francomano et al. 2019). Some of these G protein-coupled receptors are targets for anthelmintic drugs (Lacey 1990; Martin et al. 2014). Therefore, it is important to conduct docking experiments of β-caryophyllene with the anthelmintic target G protein-coupled receptors.

Molecular docking studies have demonstrated that β-caryophyllene interacts with the hydrophobic amino acid residues of CBR-2 (cannabinoid receptor) at the active site. These residues include VAL113, PHE117, ILE198, TRP258, and MET265. In our study, we found that β-caryophyllene also interacted with hydrophobic residues in the CPT 2 enzyme and β-tubulin. The key amino acid residues identified in the CPT 2 enzyme are TRP620, ALA613, LEU592, VAL597, and ALA615, while those in β-tubulin include GLY134, VAL236, LEU250, PHE200, PHE20, PHE167, and MET233.

Origanum species have been extensively studied for their effectiveness against nematodes and cestodes. For example, a study demonstrated that the essential oil of *O. vulgare* was effective against *Echinococcus granulosus* at a final concentration of 10 µg/mL (Pensel et al. 2014). After 72 days of incubation, the protoscolex of *E. granulosus* completely lost its vitality. However, our research showed

that not all nematodes in mice succumbed to the treatment with Origanum essential oil (OEO). This was likely because the treatment period was insufficient to achieve a significant antinematodal effect. Even if all nematodes were eliminated after a more extended treatment, it remained unclear whether the mice were still infected. Therefore, we could not conclusively determine the infection status of the mice, regardless of whether all nematodes had been killed. Additionally, using experimental animals to demonstrate the antiproliferative effect of OEO on *S. obvelata* eggs is inefficient, as OEO can irritate animals, even when diluted and added to their feed. Thus, in vitro studies are more appropriate for demonstrating the antiproliferative effects of OEO on nematodes.

Castro et al. (2013) found that *Origanum* extract had a significant effect on the egg hatching of *Haemonchus contortus*, with the impact varying according to the dose used. The authors concluded that a low concentration of *Origanum vulgare* (10 mg/ml) exhibited ovicidal activity, making it a potential treatment for helminthiasis (Pensel et al. 2014). In a separate study, Andre et al. (2016) demonstrated that carvacrol and carvacryl acetate inhibited the larval development of *H. contortus* at doses of 0.8, 1.0, and 2.0 mg/kg, showing efficacies of 89.3%, 97.7%, and 100%, respectively, as evidenced by larval development and egg-hatching tests. They noted that carvacryl acetate was less toxic than carvacrol and was a more effective anthelmintic both in vitro and in vivo against *H. contortus*, a common nematode affecting sheep (Andre et al. (2016). According to Ozcan et al. (2012), the analysis of *Origanum vulgare* subsp. *hirtum* leaves collected from two regions of Türkiye (Büyükeceli and Delikkaya locations of South Anatolia) showed the presence of twenty-eight compounds described with GC-MS. *Origanum* essential oil was found to be composed of oxygenated monoterpenoids and monoterpenoid hydrocarbons, with the majority of them being carvacrol (ranging from 51.4% to 63.3%), linalool (ranging from 28.3% to 2.3%), γ-terpinene (ranging from 6.0% to 9.9%), and p-cymene (ranging from 4.8% to 8.7%) (Ozcan et al. 2012). In a recent research conducted in Tokat province of Türkiye; Erenler et al. (2023) detected the percentage rate of major compounds in *O. vulgare* L. essential oil as 90.4%, 4.21%, and 1.19% for carvacrol, *p*-cymene, and γ-terpinene, respectively. In our study, we observed lower rates of certain compounds compared to previous findings. Specifically, carvacrol was present at 84.0%, while linalool was detected in trace amounts, γ-terpinene at 3.5%, and p-cymene at 4.6%. It is essential to recognize that the composition of active compounds in the same species can vary due to environmental factors such as location, soil type, climate zone, and harvesting time.

Additionally, we noted a steady decrease in the number of parasite eggs in mice fed soy oil, which continued up to day 14. This reduction may be attributed to the laxative effect of soy oil or its potential anthelmintic properties.

On the other hand, *Origanum vulgare* subsp. *hirtum* has been shown to reduce parasite eggs; however, its effects are not long-lasting and require continuous use. A low-dose, repeatable treatment using oregano essential oil (OEO) is recommended, as it has demonstrated effectiveness even at low doses. Further research is needed to explore the anthelmintic effects of carvacrol on other drug target proteins through molecular docking studies.

β-Caryophyllene is a compound found in several plants, including basil (*Ocimum* spp.), cinnamon (*Cinnamomum verum*), and cloves (*Syzygium aromaticum*), as well as in oregano (*Origanum* spp.) (Karaman 2022). These plants are believed to have anthelmintic properties, which can help eliminate parasitic worms. Recent studies predicted that β-caryophyllene may possess anthelmintic effects, and this was demonstrated for the first time through molecular modelling.

Extracts derived from specific plant parts can offer various benefits when utilised in appropriate doses for treatment. They can enhance the immune system of animals through natural means, aiding in the prevention of recurrent helminthic infections due to their antioxidant properties. Additionally, these extracts can help inhibit the development of drug resistance in parasites affecting animals, thereby reducing the dependency on synthetic medications for treatment. Given these advantages, it is increasingly important to explore natural components in the development of anthelmintic drugs.

In this study, we have concluded that *Origanum vulgare* subsp. *hirtum* grown in Türkiye has a high concentration of carvacrol (84.0%), which makes it apotentiallyeffective therapeutic herb. When mice were treated with a 600 mg/kg dose of OEO, it resulted in a 95.48% reduction in the number of *S. obveleta* eggs on the 14th day post-treatment. The docking results suggest that β-caryophyllene may contribute to the antinematodal effect of *Origanum* essential oil.So, this study can be considered a preliminary study, and future studies should administer OEO to experimental animals using taste-masking methods, controlled-release systems, or oral gavage.

CONFLICTS OF INTEREST

The authors report no conflicts of interest.

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AUTHOR CONTRIBUTIONS

Idea/Concept: DK
Supervision/Consultancy: MK, AOG
Data Collection and/or Processing: DK, OG
Analysis and/or Interpretation: DK, MK
Writing the Article: DK, OG
Critical Review: OG, AOG

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