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In vitro Anti-*Trichomonas vaginalis* Activity of Cyprus Endemic Plant *Origanum majorana* Essential Oil and Synergistic Effect with Metronidazole

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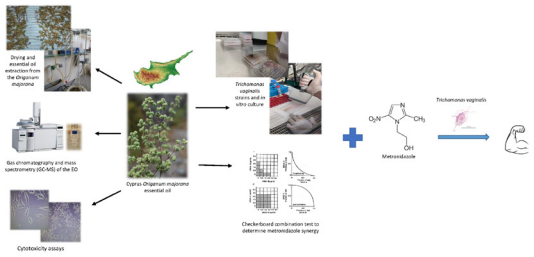
Abstract: Currently, the treatment of *Trichomonas vaginalis* (*T. vaginalis*) typically involves the use of 5-nitroimidazoles (such as metronidazole and tinidazole). However, an increasing failure in treatment is observed due to resistance developed to these drugs. For this reason, alternative drugs have been investigated, especially by using natural products. In our study, the anti-*T. vaginalis* activity of the *Origanum majorana* essential oil (OMEO), which is a Cyprus endemic plant, and its synergistic effect with metronidazole were investigated. The essential oil was extracted through hydrodistillation of the dried flowering tops of the plant. Gas chromatography and mass spectrometry (GC-MS) analyses were performed using the Agilent 5975 GC-MSD system. L929 mouse fibroblast cell line was used to determine cytotoxic activity. Two clinical strains and one metronidazole-resistant *T. vaginalis* standard strain were used. LC_{50}, and MLC (minimum lethal concentration) values of OMEO and metronidazole were determined by the broth microdilution method in vitro in aerobic and anaerobic conditions. The combination of OMEO with metronidazole was investigated against all strains by the checkerboard method. The major compounds in the OMEO content were determined as cis-sabinene hydrate (29.1%) and terpinen-4-ol (19.6%). In cytotoxic analyses, it was observed that the cell viability remained stable at low doses. OMEO is effective against all three *T. vaginalis* strains. There is a significant difference between the IC_{50} averages at the 24th and 48th hours (333.03 µg/mL and 226.43 µg/mL, respectively) in aerobic conditions (p=0.003). In addition, there is a statistically significant relationship between the results of the 24th and 48th hours (348.77 µg/mL and 238.80 µg/mL, respectively) in the anaerobic conditions (p=<0.0001). In general, OMEO has been shown to have a synergistic effect with metronidazole. In conclusion, we believe that OMEO is a potential natural agent that can be particularly used in the treatment of protozoan infections, including *T. vaginalis*.



Keywords: *Trichomonas vaginalis*, *Origanum majorana*, essential oil, metronidazole, synergy

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1 Introduction

Trichomonas vaginalis (*T. vaginalis*) is a sexually transmitted flagellated protozoan that causes urogenital trichomoniasis in men and women[1, 2]. Trichomoniasis is the most common non-viral sexually transmitted infection (STI). In 2020, the World Health Organization (WHO) reported that the number of trichomoniasis cases globally was 156.3 million (73.7 million in females, 82.6 million in males), and it was estimated that Africa accounted for 12% of all cases worldwide. Approximately one third of new infections occur in the WHO African Region, followed by the Region of the Americas[3, 4]. The infection is usually asymptomatic; however, when symptoms occur, women experience a foul-smelling yellowish, or greenish vaginal discharge, and vulvovaginal pain and itching[1, 2]. Rarely, symptoms such as urethral discharge, dysuria, urethritis, epididymitis, and prostatitis are encountered in men[5]. In females, studies conducted have proven that trichomoniasis is associated with complications such as pelvic diseases, cervical erosion, cervical cancer, and poor birth outcomes. In males, its associated with decreased sperm motility, and the infection increases the risk of human immunodeficiency virus (HIV) transmission for both gender[4,6].

In the treatment of trichomoniasis, 5-nitroimidazole (metronidazole and tinidazole) therapeutic agents approved by the Food and Drug Administration (FDA) are currently used[7, 8]. However, due to the side effects of these drugs and increased resistance to these drugs, infections that do not respond to treatment are observed[7]. Many side effects of these drugs have been described, such as potential carcinogens, mutagenicity and embryogenicity. Common side effects include headache, glossitis, urticaria, itching, dizziness, nausea, dry mouth, a bitter metallic taste in the mouth, and vomiting[9].

Although metronidazole is generally effective in the treatment of *T. vaginalis* infections, in vitro resistance and clinical failures are widely reported[1]. These treatment failures are particularly concerning in countries with limited resources where alternatives are not available[1]. Therefore, alternative treatment options for the treatment of trichomoniasis have been investigated for a long time[2]. So, it is clear that the development for new and effective drugs against *T. vaginalis* is necessary[10].

Essential oils (EOs) obtained from medicinal plants by distillation are complex hydrophobic liquids and contain volatile aromatic constituents of plants. It is known that these EOs have been used as natural treatment products for centuries. These EOs, which contain many biologically active substances, have antibacterial, antifungal, antiviral, antiprotozoal, and antioxidant properties[11]. Plants are widely used as a source for new drug discoveries. Such natural products constitute a rich source of active compounds that represent a promising alternative for the treatment of trichomoniasis[12]. The antiprotozoal effects of plants have been proven with examples such as emetine, quinine and artemisinin obtained from *Cephaelis ipecacuhana*, *Cinchona* species and *Artemisia annua*, respectively[9].

In our study, it was aimed to investigate the in vitro anti-*T. vaginalis* activity of the EO extracted from the Cyprus endemic plant *Origanum majorana* (*O. majorana*) L. var. *tenuifolium* Weston and examine its synergistic effect with metronidazole, which is frequently used in treatment of *T. vaginalis* infections.

2 Materials and Methods

2.1 Collection of *Origanum majorana* plant

Aerial parts of *O. majorana* plant were collected in March, 2022, from Korucam (Kormakitis) region of Northern Cyprus (Y: 500934.25, X: 3914211.76) (35°21'25.7"N, 33°00'36.9"E) (**Fig. 1**). It was air-dried under the shade and determined

Fig. 1 Coordinates of collected plant materials.

using Flora of Cyprus[13] by Prof. Dr. K. Hüsnü Can Başer. The voucher specimen was kept at the Near East University (NEU) Herbarium Centre (NEUN 6895). The aerial parts of other collected plant materials were dried in a dark place and cut into small pieces with sterile scissors to increase the oil yield and the EO was obtained at NEU, Faculty of Pharmacy, Department of Pharmacognosy.

2.2 Isolation of the essential oil

Air-dried leaves of *O. majorana* (100 g) were hydro-distilled with 1 L distilled water for three (3) hours using the Clevenger apparatus. The resulting oil was collected in amber vials and the yield was calculated on a dry basis and the density of the essential oil was calculated with the help of a pycnometer. The essential oil was stored at +4˚C until the analysis.

2.3 Essential oil analyses

Gas chromatography and mass spectrometry (GC-MS) analyses were performed using the Agilent 5975 GC-MSD system. Helium gas (0.8 mL/min) was used as the carrier gas in the Innowaks FSC column (60 m x 0.25 m film thickness). For gas chromatography, the oven temperature was programmed to 220°C by keeping the temperature at 60°C for 10 minutes and then increasing it by 1°C per minute. The partition ratio was set to 40:1 and the injector temperature was adjusted to 250°C. The mass range was recorded at *m/z* 35 and 450 and the mass spectra were recorded at 70 eV[14].

Gas chromatography analysis was performed by using the Agilent 6890N GC system. The temperature of the flame ionization detector (FID) was set to 300°C. To achieve the same elution order by gas chromatography and mass spectrometry, a replica of the same column was simultaneously auto-injected under the same conditions. The percentage of separated compounds was calculated from the FID chromatograms. "Baser Library of Essential Oil Constituents" was used to identify the separated substances[14].

2.4 Cell line and cytotoxicity assays

The L929 mouse fibroblast cell line (ATCC/American Type Culture Collection, USA) was used to detect the cytotoxic activity of *O. majorana* essential oil (OMEO). 10% heat-inactivated fetal bovine serum (Capricorn Scientific, FBS-11B), 1% penicillin/streptomycin (Biochrom, A2213), and 1% glutamine (EMD Millipore, K0282) were added to the DMEM (Dulbecco's Modified Eagle Medium) (Biological Industries, 01-050-1A) medium, and cells proliferated in 5% CO_2 environment were treated with OMEO at 37°C, and the cytotoxic activity was determined by using MTT (3-(4.5-dimethylthiazol-2-yl)-2.5-diphenyltetrazolium bromide) kit (Glentham Code, GT3156)[12].

MTT is based on colorimetric measurement of the reduction of 3-(4.5-dimethylthiazol-2-yl)-2.5 diphenyltetrazolium bromide, which is a purple formazan product that is reduced by living cells. OMEO was prepared as to be 100 mg/mL by using dimethylsulfoxide (DMSO, Sigma-Aldrich), and diluted in a culture medium at five different concentrations (5 µg/mL, 10 µg/mL, 25 µg/mL, 50 µg/mL, and 100 µg/mL). L929 mouse fibroblast cells were added to DMEM medium and cultured in a 96-well plate as 100 µl ($5x10^3$/ml cell density) per well. While the negative control contained no cells and the EO, the positive control contained only cultured cells. OMEO dilutions were repeated three times and incubated in the cell line for 24-48 hours. After incubation, the MTT solution was warmed to 37°C and then 10 µl was added to each well. After being incubated in a 5% CO_2 environment at 37°C for 4 hours, 200 µl of DMSO was added to dissolve the formazan salts. Absorbance was measured at 570 nm with a spectrophotometer (Versa Max, Molecular Device, Sunnyvale, USA). All experiments were repeated 3 times[14].

2.5 *Trichomonas vaginalis* strains and in vitro culture

Two clinical strains (*T. vaginalis* BAUN-TV66 and *T. vaginalis* BAUN-TV78), and one metronidazole-resistant *T. vaginalis* standard strain (*T. vaginalis* ATCC 50143) were used in the study. Parasite codes are given according to the institution abbreviation and the order of isolation from patients. Both clinical isolates were studied using the in vitro broth microdilution method and were found sensitive to metronidazole. Clinical isolates were growth by inoculating TYM (trypticase, yeast extract, maltose) medium with vaginal swabs sent to Balıkesir University, Health Practice and Research Hospital, Microbiology Laboratory. The medium content includes trypticase, maltose, yeast extract, *l*-ascorbic acid, *l*-cyctein HCL, agar agar and pH regulators (K_2HPO_4-KH_2PO_4). The pH of the medium was adjusted to 6. Clinical isolates were sub-cultured until they entered the logarithmic phase and were kept in the parasite bank of Manisa Celal Bayar University, Department of Medical Parasitology[15]. Before the study, two clinical metronidazole sensitive strains and one metronidazole-resistant standard strain, which were preserved in the parasite bank, were resuscitated by inoculation into the TYM medium and subpassages were performed at 37°C for 48 hours until they entered the logarithmic phase.

Before utilization, 100 IU/mL streptomycin, 100 IU/mL penicillin, and horse serum were added to the TYM medium so that the total volume would be 2 mL. *T. vaginalis* strain was passaged five times in media containing TYM, antibiotics (penicillin+streptomycin) and horse serum, and then passaged three more times in media without antibiotics.

2.6 In vitro anti-trichomoniasis effect and metronidazole synergy

For all isolates, the LC_{50} and minimum lethal concentration (MLC) values of OMEO and metronidazole were determined in vitro by broth micro-dilution method in 96-well microplates[16]. The dilution range was prepared to be 64.000-31.25 µg/mL for OMEO, and 780-0.375 µM for metronidazole. After the trophozoites entered the logarithmic phase, viability was determined by counting them on the thoma slide using trypan blue. Trophozoites with a viability rate of more than 95% were used in the experiments. The amount of parasites was adjusted to $5x10^3$ parasites/mL using the thoma slide. Except for the negative control, $5x10^3$ parasites/mL trophozoites of *T. vaginalis* were added to each well, and the microplates were incubated at 37°C for 48 hours. At the end of incubation, the motility of *T. vaginalis* trophozoites was evaluated by direct examination under an inverted microscope, and their viability was evaluated on a thoma slide using trypan blue. For growth control (positive control), two wells containing *T. vaginalis* but not the substances tested were prepared. For contamination control (negative control), two wells to which the substances were tested and *T. vaginalis* were not added were studied. Plates were incubated at 37°C under both aerobic and anaerobic conditions. The anaerobic environment was provided using the GENbox brand (Biomerioux, France) kit, which was placed in the anaerobic jar and absorbed the oxygen in the environment. Additionally, an indicator strip was placed into the jar and it was checked that the environment reached anaerobic conditions. At the 24^{th} and 48^{th} hours of incubation, the motility of *T. vaginalis* trophozoites was evaluated under an inverted microscope, and their viability was evaluated on a thoma slide using trypan blue. The dilution in the last well, where approximately half of the *T. vaginalis* trophozoites were determined to be alive using trypan blue, was accepted as the LC_{50} value. On the other hand, the MLC value was determined by taking trophozoites from wells with immobile parasites and inoculating them in a new TYM medium, and checking for growth at the 24^{th} and 48^{th} hours to prove that the dormant parasites were not viable[2].

2.7 Checkerboard combination test

The combination of OMEO with metronidazole was investigated against all three *T. vaginalis* isolates using the checkerboard method[17]. Two 96-well microplates were used to determine the combination of OMEO with metronidazole. Serial dilutions of EO were made from top to bottom in the first microplate, while the serial dilutions of metronidazole were made from right to left in the second microplate. OMEO and metronidazole serial dilutions were adjusted to be 2-3 times below and 2-3 times above the LC_{50} value. Serial dilutions in the second microplate were transferred to the corresponding wells in the first microplate and wells containing all combinations of both substances were obtained. Except for the negative control, in 10^{th} column in microplates, $5x10^3$ parasite/mL trophozoites of *T. vaginalis* were added to each well. The 9^{th} columns in the plates represented the growth control (positive control) of the parasite without any drug or substance added (100% growth). The plates were incubated for 48 hours at 37°C and the viability of *T. vaginalis* trophozoites were evaluated under the invert microscope using with trypan blue, and fractional inhibitory concentration index (FICI) values were calculated. Interactions were interpreted as synergy if the detected FICI value was < 0.5, partial synergy if it was between 0.5-0.75, additive if it was between 0.75-1, indifferent if it was between 1-4, and antagonism if it was > 4.18 All studies were carried out by repeating them three times on separate days.

2.8 Statistical analyses

Statistical analysis of the data obtained was performed with Statistical Package for the Social Sciences (SPSS) Demo Version 22.0 (SPSS Inc., Chicago, IL, USA) program. One-way ANOVA was used to compare IC_{50} mean values between the groups and $p < 0.05$ values were considered statistically significant.

3 Results

3.1 *Origanum majorana* essential oil content

The OMEO yield calculated as 8.1% (v/w) on a dry basis. The chemical composition of the EO obtained from *O. majorana* used in our study is listed in **Table 1**. Accordingly, 29.1% of *cis*-sabinene hydrate has been identified as the major compound with the highest amount, followed by terpinen-4-ol with a rate of 19.6%.

3.2 Cytotoxicity analyses

L929 mouse fibroblasts were incubated with OMEO at concentrations of 5, 10, 25, 50, and 100 µg/mL for 24 and 48

Table 1 Essential oil composition of OMEO.

RRI	Compound	%
1032	α-Pinene	0.6
1035	α-Thujene	0.7
1076	Camphene	0.3
1118	ß-Pinene	tr
1132	Sabinene	5.2
1174	Myrcene	1.9
1176	α-Phellandrene	0.3
1188	α-Terpinene	5.7
1203	Limonene	1.9
1218	ß-Phellandrene	1.9
1255	γ-Terpinene	9.5
1280	*p*-Cymene	2.5
1290	Terpinolene	2.2
1474	*trans*-Sabinene hydrate	4.3
1553	Linalool	1.9
1556	*cis*-Sabinene hydrate	29.1
1571	*trans*-*p*-Menth-2-en-1-ol	1.2
1591	Bornyl acetate	1.5
1611	Terpinen-4-ol	19.6
1612	ß-Caryophyllene	0.7
1638	*cis*-*p*-Menth-2-en-1-ol	0.8
1689	*trans*-Piperitol	0.2
1706	α-Terpineol	5.8
1719	Borneol	1.1
1755	Bicyclogermacrene	0.5
1758	*cis*-Piperitol	0.4
2144	Spathulenol	0.2
	Total	100

RRI: Relative retention indices calculated against *n*-alkanes

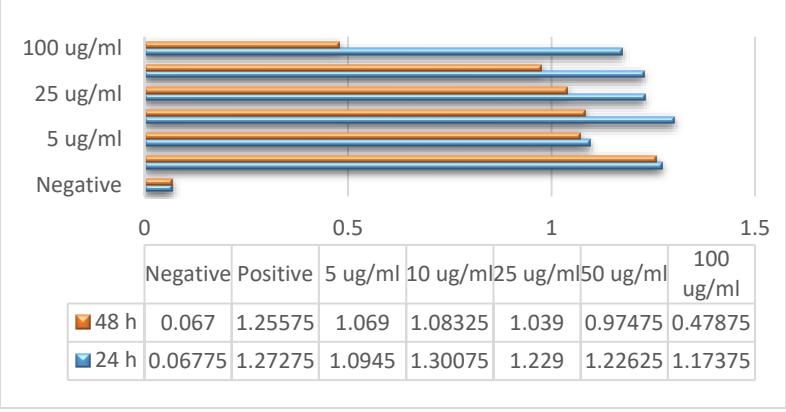

Fig. 2 Cytotoxic activity of OMEO against L929 mouse fibroblast cells (x: Absorbance at 570 nm, y: Concentration).

hours. It was observed that approximately half (50%) of the L929 mouse fibroblast cells incubated with OMEO at a concentration of 100 µg/mL, in particular, lost their viability at the end of 48th hours of incubation and the cell viability remained stable at low doses (**Fig. 2**)[11].

Table 2 MLC and IC_{50} values of *Origanum majorana* essential oil and metronidazole.

	T. vaginalis strains	Aerobic				Anaerobic			
		24. hour		48. hour		24. hour		48. hour	
		MLC	IC_{50}	MLC	IC_{50}	MLC	IC_{50}	MLC	IC_{50}
OMEO (µg/mL)	*T. vaginalis* BAUN-TV66	2000	311.1	1000	211.4	2000	343.4	1000	228.4
	T. vaginalis BAUN-TV78	2000	331.5	1000	243.7	2000	355.5	1000	239
	T. vaginalis ATCC 50143	2000	356.5	1000	224.2	2000	347.4	1000	249
	Mean	***2000***	***333.03***	***1000***	***226.43***	***2000***	***348.77***	***1000***	***238.8***
Metronidazole (µM)	*T. vaginalis* BAUN-TV66	390	41	97	12.7	195	34.4	97	12.2
	T. vaginalis BAUN-TV78	24	6	12	2.1	12	3.6	6	2.1
	T. vaginalis ATCC 50143	390	46.6	97	12	195	33.7	97	14.6
	Mean	***268***	***31.2***	***68.67***	***8.93***	***134***	***23.9***	***66.67***	***9.63***

IC_{50}: Half-maximal inhibitory concentration; **MLC:** Minimum lethal concentration; OMEO: *Origanum majorana* essential oil

3.3 Anti-*Trichomonas vaginalis* activity

The IC_{50} and MLC values of OMEO and metronidazole against *T. vaginalis* isolates at the end of 24 and 48 hours under aerobic and anaerobic conditions are presented in **Table 2** and the graphs in which IC_{50} values have been determined are shown in **Figs. 3**, **4**, **5** and **6**. It was observed that trophozoites were completely lysed at high EO concentrations. At concentrations close to the IC_{50} value, morphological changes such as loss of movement, rounded shape of the cell, and distortions on the membrane surfaces were observed. At low EO concentrations, where viability begins, the slowness of movement in the parasite cells and the presence of vacuoles in the cytoplasm were remarkable.
When the mean IC_{50} values at 24 hours of OMEO against *T. vaginalis* strains in aerobic and anaerobic conditions (333.03 µg/mL and 348.77 µg/mL, respectively) were compared, no statistical relationship was found (p=0.312). The same comparison was made for the results obtained at the 48th hour (226.43 µg/mL and 238.80 µg/mL, respectively) and no significant difference was observed (p=0.328). However, there is a significant difference between the IC_{50} mean values at the 24th and 48th hours (333.03 µg/mL and 226.43 µg/mL, respectively) in aerobic conditions (p=0.003). In addition, there is a statistically significant relationship between the results of the 24th and 48th hours (348.77µg/mL, and 238.80 µg/mL, respectively) in the anaerobic environment (p=<0.0001).
According to the FICI values obtained in the checkerboard test, synergy was detected against *T. vaginalis* BAUN-TV78 and *T. vaginalis* ATCC 50143 strains, and partial synergy was detected against *T. vaginalis* BAUN-TV66 isolate in the combination of OMEO and metronidazole (**Table 3**).

4 Discussion

T. vaginalis is one of the most common non-viral sexually transmitted infections (STIs) worldwide and causes serious health problems. Treatment is still limited to metronidazole and tinidazole. Due to the increasing incidence of resistance to these drugs, difficulties are experienced in the treatment, and therefore, the need to search for new therapeutic alternatives increases[7, 19]. To this end, new studies are being conducted by using both natural and synthetic products, and promising outcomes have been obtained[20]. The OMEO used in our study was found to be effective against all *T. vaginalis* isolates. In addition, when OMEO was used together with metronidazole, its effectiveness against *T. vaginalis* isolates increased. When the cytotoxic analyses of OMEO are evaluated, it is apparent that especially low doses do not have a toxic effect on mouse fibroblast cells.

Nitroimidazoles are anti-*T. vaginalis* drugs that inhibit hydrogen production, and among these, metronidazole is the most frequently used. Metronidazole, which becomes active by reducing the nitro group in anaerobic conditions, binds to the DNA of the parasite, disrupts the base sequences and therefore the double helix structure, and prevents DNA

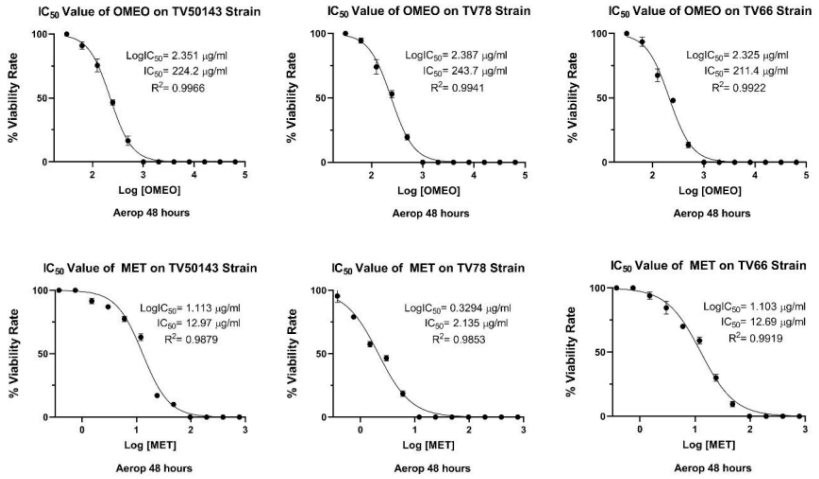

Fig. 3 IC_{50} values of OMEO and metronidazole on *Trichomonas vaginalis* strains (aerobic-24th hour).

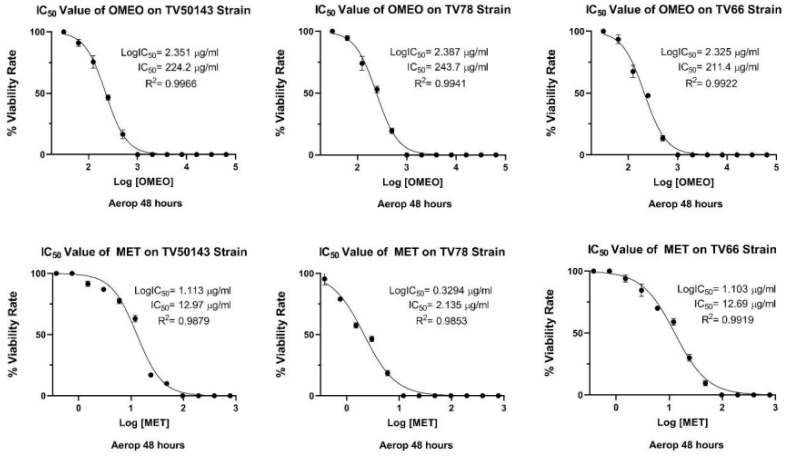

Fig. 4 IC_{50} values of OMEO and metronidazole on *Trichomonas vaginalis* strains (aerobic-48th hour).

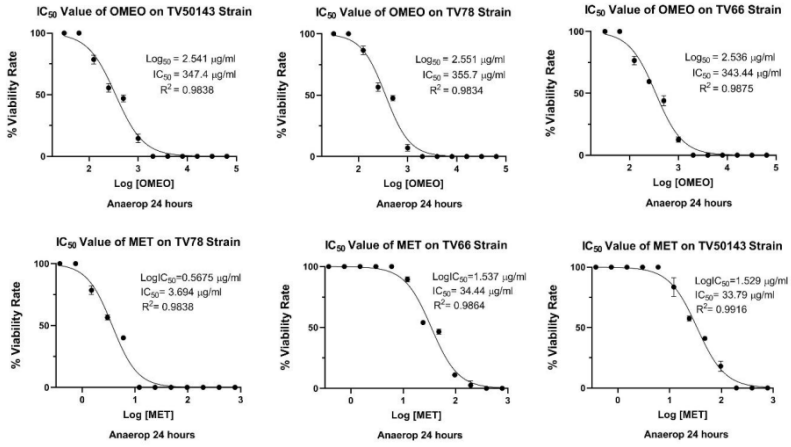

Fig. 5 IC_{50} values of OMEO and metronidazole on *Trichomonas vaginalis* strains (anaerobic-24th hour).

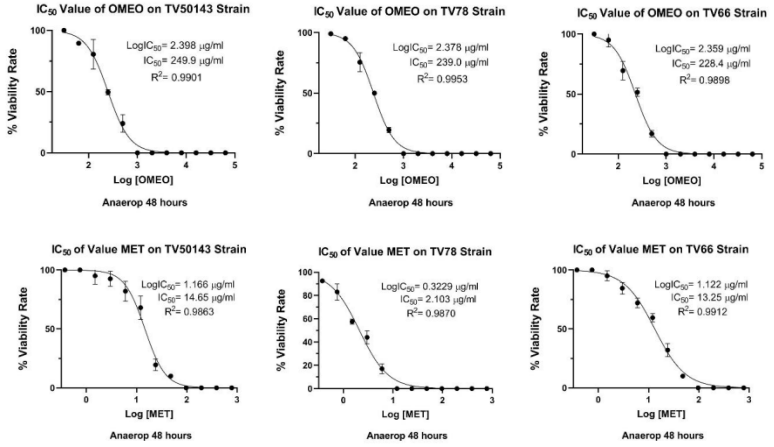

Fig. 6 IC_{50} values of OMEO and metronidazole on *Trichomonas vaginalis* strains (anaerobic-48th hour).

Table 3 OMEO/metronidazole synergy results.

Combination	*T. vaginalis* isolates	ΣFIKI	Interpretation
EO + Metronidazole	*T. vaginalis* BAUN-TV66	0.625	Partial synergy
	T. vaginalis BAUN-TV78	0.281	Synergy
	T. vaginalis ATCC 50143	0.375	Synergy

replication and transcription[2]. The antimicrobial action mechanisms of EOs are generally based on affecting the plasma membrane, cell wall, cell proliferation or protein synthesis[21]. Due to their hydrophobic properties, EOs are thought to act on cell plasma membranes or mitochondria lipids, functionally disrupting these structures by increasing the electrical conductivity and proton permeability of the membranes[22]. Therefore, due to the damage caused by the EO on the cell membrane, metronidazole may be easier to enter the cell. It is thought that the existence of a synergistic interaction between EO and metronidazole may be due to this mechanism. Moreover, in synergistic interactions, the concentrations of the drugs decrease by at least 4 times[23]. This may offer a positive approach to reducing drug toxicity and side effects. *O. majorana* (sweet marjoram) has been used for centuries as both an edible and medicinal herb[24]. In addition, this plant is widely used in traditional medicine in different countries[25]. Major components such as terpinen-4-ol, α- and γ-terpinene, linalool and carvacrol in the OMEO content are considered responsible for the antimicrobial activity of the oil[24]. In their study, Vera et al. determined that terpinen-4-ol (38.4%) and *cis*-sabinene hydrate (15.0%) were the major components with the highest rate in the OMEO content[26]. In the study conducted by Ramos et al., the major components in OMEO were determined as *cis*-sabinene hydrate (30.2%) and terpinen-4-ol (28.8%)[27]. In their study conducted in the Southern part of Cyprus, Novak et al. suggested that the OMEO content consists mainly of sabinyl compounds and α-terpineol[28]. In another study conducted on six *O. majorana* populations spread over the western part of the island of Cyprus, Karousou et al. found that OMEOs are rich in either *trans*-sabinene hydrate/terpinen-4-ol or α-terpineol/*trans*-sabinene hydrate[29]. Similarly, in our study, the major compounds in OMEO collected from Northern Cyprus were found to be *cis*-sabinene hydrate (29.1%) and terpinen-4-ol (19.6%), respectively. However, the *O. majorana* species grown in Turkey has a completely different component composition. In a study by Bağci et al., carvacrol was found to be the main component, but it was determined that chemical compositions in fresh and dried thyme differed. In the study using two different *O. majorana* species, carvacrol was detected at rates of 80.33% and 83.46% from dried plants and 56.39% and 69.49% from fresh plants[30]. In Cuba, Brazil, Hungary and Tunisia, it has been reported that the major components are terpinen-4-ol, γ-terpinene and linalool[27].

Studies have suggested that *O. majorana* ethanolic extract has therapeutic potential in the treatment of colon cancer[31]. In a study, it is emphasized that hydroethanolic extracts have the potential to prevent diseases by preventing excessive

production of free radicals and can be used as a natural antioxidant agent[32]. In addition, the antimicrobial activity of OMEO has been shown in various studies[32,33]. Hamida-Ben Ezzeddine et al. reported that OMEO had an antibacterial effect on 10 different bacteria[34]. In addition, the antibacterial and antifungal effects of OMEO have been proven in the study of Della Pepa et al.[35]. *O. majorana* aqueous extracts showed dose and time-dependent anti-*Blastocystis* activity[36] and marjoram was active against *Toxoplasma gondii*[33]. In a study conducted by Güler et al., the antimalarial activity of OMEO was demonstrated in an *in vivo* malaria model. In the same study, OMEO was given orally to mice infected with *Plasmodium berghei* and it was reported that the life span of the mice was significantly prolonged[14]. In another study, the effectiveness of OMEO on *Leishmania tropica* promastigotes was investigated in vitro and it was found to be effective at MIC 3.13 μg/mL, and LD_{50} 1.56 μg/mL concentrations[11]. The limitation of our study is that the cell line used was not a human cell line, but our study is valuable since there is no research has been found in the literature showing the anti-*T. vaginalis* efficacy of OMEO. However, in a study conducted by Bailen et al., it was reported that OMEO had good anti-*Trichomonas gallinae* activity. In this study, the major compounds in OMEO obtained by hydrodistillation were found to be 4-terpineol (29.7%) and γ-terpinene (10.7%)[37].

It is an important finding that our study not only demonstrates the effectiveness of OMEO against *T. vaginalis* but also its synergy with metronidazole. In addition to all these, it is clear that the major components in OMEO should be isolated separately and then their effectiveness against infectious agents should be tested both in vitro and in vivo. Thus, we can obtain more precise information about which component(s) may be responsible for the antimicrobial activity of the EO.

5 Conclusion

Our results show that the EO obtained from the Cyprus endemic plant *O. majorana* has anti-*T. vaginalis* activity. It also shows a synergistic effect with metronidazole, which is frequently used in the treatment of *T. vaginalis* infections. Synergistic interactions can contribute to the activity of drugs in two different ways: first, reducing the toxicity of highly toxic active substances by using lower doses, and second, saving existing drugs that cannot reach the effectiveness limit values and become resistant. Synergistic combinations may therefore show similar efficacy at lower, non-toxic doses and prevent the development of resistance resulting from monotherapy. Therefore, considering similar studies in the literature, we think that OMEO may be a potential new, natural and reliable agent that can be especially used in the treatment of protozoal infections. With further studies, it will be possible to culture the plant, make cell suspensions of the substances responsible for the activity, produce them in higher amounts under laboratory conditions with tissue culture, and design molecules that can selectively interact with enzymes if their activity mechanisms are determined. Active ingredient screenings performed in in vitro models do not fully reflect the *in vivo* environment in terms of obtaining results on a single and fixed plane. However, it can provide some insight in terms of antimicrobial effectiveness. Due to the difficulty of in vivo models and ethical concerns, in vitro screening should be performed beforehand and candidate molecules with low toxicity and high efficiency should be selected for in vivo studies. Moreover, it is clear that the antimicrobial activity of the relevant EO should be supported by in vivo studies.

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Conflict of Interest

The authors declare that they have no conflicts of interest.

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References

1) Mtshali, A.; Ngcapu, S.; Govender, K.; Sturm, A.W.; Moodley, P. et al. In vitro effect of 5-nitroimidazole drugs against *Trichomonas vaginalis* clinical isolates. *Microbiol Spectr.* **10**, e0091222 (2022). doi.org/10.1128/spectrum.00912-22

2) Aksoy Gökmen, A.; Girginkardeşler, N.; Kilimcioğlu, A.A.; Şirin, M.C.; Özbilgin, A. In vitro susceptibility of *Trichomonas vaginalis* to metronidazole, ornidazole and proton pump inhibitors pantoprazole and esomeprazole. *Mikrobiyol Bul*. **50**, 133-139 (2016). doi.org/10.5578/mb.10241
3) World Health Organization (WHO) 2021: Global progress report on HIV, viral hepatitis and sexually transmitted infections, 2021. World Health Organization, Geneva, Switzerland. Available from: https://www.who.int/publications/i/item/9789240027077
4) World Health Organization (WHO). Trichomoniasis. Access date: 21.11.2023. Available from: https://www.who.int/news-room/fact-sheets/detail/trichomoniasis
5) ahmoudvand, H.; Babparva, E.; Baharvand, Z.; Salehi Lalehmarzi, H. Anti-*Trichomonas vaginalis* activities and apoptotic effects of some Iranian medical plants. *Trop Biomed*. **35**, 347-353 (2018).
6) Mercer, F.; Johnson, P.J. *Trichomonas vaginalis*: Pathogenesis, symbiont interactions, and host cell immune responses. *Trends Parasitol*. **34**, 683-693 (2018). doi.org/10.1016/j.pt.2018.05.006
7) Trein, M.R.; Oliveira, L.R.E.; Rigo, G.V.; Rocha Garcia, M.A.; Petro-Silveira, B. et al. Anti-*Trichomonas vaginalis* activity of chalcone and amino-analogues. *Parasitol Res*. **118**, 607-615 (2019). doi.org/10.1007/s00436-018-6164-4
8) Centers for disease control and prevention (CDC) 2021. Sexually Transmitted Infections Treatment Guidelines. Trichomiasis, Treatment. Available from: https://www.cdc.gov/std/treatment-guidelines/trichomoniasis.htm
9) Mirzaei, F.; Raissi, V.; Teimouri, A.; Mousavi, P.; Mohaghegh, M.A. et al. Anti-*Trichomonas vaginalis* Activity of ethanolic extracts of *Medicago sativa* and *Satureja hortensis*, in vitro study. *Int. J. Med. Lab.* **6**, 166-171 (2019). doi.org/10.18502/ijml.v6i3.1397
10) Kulyashova, L.B.; Roschina, N.; Nikitina, T.V.; Soultanov, V.S. Anti-protozoal activity of conifer green needle complex Against *Trichomonas vaginalis*. *Nat. Prod. Commun.* **14**, 147-150 (2019). doi.org/10.1177/1934578X1901400138
11) Güler, E.; Özbilgin, A.; Çavuş, İ.; Baddal, B.; Etikan, İ. et al. *In vitro* Anti-Leishmanial Activity of Essential Oils Extracted from Plants Growing in Northern Cyprus Against *Leishmania tropica*. *Turkiye Parazitol Derg*. **45**, 101-107 (2021). doi.org/10.4274/tpd.galenos.2021.6888
12) Bitencourt, F.G.; de Brum Vieira, P.; Meirelles, L.C.; Rigo, G.V.; da Silva, E.F. et al. Anti-*Trichomonas vaginalis* activity of ursolic acid derivative: a promising alternative. *Parasitol. Res.* **117**, 1573-1580 (2018). doi.org/10.1007/s00436-018-5839-1
13) Meikle, R.D. Flora of Cyprus 2. The Bentham-Moxon Trust (1985).
14) Güler, E.; Özbilgin, A.; Becer, E.; Hanoğlu, A.; Şanlıdağ, T. An endemic plant of Cyprus, *Origanum majorana*: Is it a new alternative natural product for Malaria treatment?. *Mikrobiyol. Bul.* **54**, 463-78 (2020). doi.org/10.5578/mb.69705
15) McMillan, A. Laboratory diagnostic methods and cryopreservation of Trichomonads. in *Trichomonads Parasitic in Humans* (Honigsberg, B.M. ed.), Spinger, New York. pp. 297-310 (1990).
16) Clinical and Laboratory Standards Institute (CLSI). Performance standards for antimicrobial susceptibility testing; twenty-fourth informational supplement M100-S24, 2014; Vol. **34** No. 1, 950 West Valley Road, Suite 2500 Wayne, PA 19087 USA.
17) Eliopoulos, G.; Moellering, R.C. Antimicrobial Combinations. in Antibiotics in Laboratory Medicine (Lorian, V. ed.), 4th ed. Williams & Wilkins Co., Baltimore, pp. 330-396 (1996).
18) Li, Y.J.; Pan, C.Z.; Zhao, Z.W.; Zhao, Z.X.; Chen, H.L. et al. Effects of a combination of amlodipine and imipenem on 42 clinical isolates of *Acinetobacter baumannii* obtained from a teaching hospital in Guangzhou, China. *BMC Infect. Dis*. **13**, 548 (2013). doi.org/10.1186/1471-2334-13-548
19) Alves, M.S.D.; das Neves, R.N.; Sena-Lopes, A.; Domingues, M.; Casaril, A.M. et al. Antiparasitic activity of furanyl N-acylhydrazone derivatives against *Trichomonas vaginalis*: in vitro and in silico analyses. *Parasites Vectors* **13**, 59 (2020). doi.org/10.1186/s13071-020-3923-8
20) Rigo, G.V.; Petro-Silveira, B.; Devereux, M.; McCann, M.; Dos Santos, A.L.S. et al. Anti-*Trichomonas vaginalis* activity of 1,10-phenanthroline-5,6-dione-based metallodrugs and synergistic effect with metronidazole. *Parasitology* **146**, 1179-1183 (2019). doi.org/10.1017/S003118201800152X
21) Sikkema, J.; de Bont, J.A.; Poolman, B. Mechanisms of membrane toxicity of hydrocarbons. *Microbiol Rev*. **59**, 201-222 (1995). doi.org/10.1128/mr.59.2.201-222.1995
22) Kozlowska, M.; Laudy, A.E.; Starosciak, B.J.; Napiorkowski, A.; Chomicz, L. et al. Antimicrobial and antiprotozoal effect of Sweet Marjoram (*Origanum majorana* L.). *Acta Sci. Pol. Hortorum Cultus* **9**, 133-141 (2010).
23) Gómara-Lomero, M.; López-Calleja, A.I.; Rezusta, A.; Ainsa, J.A.; Ramón-Garcia, S. In vitro synergy screens of FDA-approved drugs reveal novel zidovudine- and azithromycin-based combinations with last-line antibiotics against *Klebsiella pneumoniae*. *Sci. Rep.* **13**, 14429 (2023). doi.org/10.1038/s41598-023-39647-9

24) Ambrosio, C.M.S.; Contreras-Castillo, C.J.; Da Gloria, E.M. In vitro mechanism of antibacterial action of a citrus essential oil on an enterotoxigenic *Escherichia coli* and *Lactobacillus rhamnosus*. *J. Appl. Microbiol.* **129**, 541-553 (2020). doi.org/10.1111/jam.14660
25) Bina, F.; Rahimi, R. Sweet Marjoram: A review of ethnopharmacology, phytochemistry, and biological activities. *J. Evid. Based Complementary Altern. Med.* **22**, 175-185 (2017). doi.org/10.1177/21565872166507
26) Vera, R.R.; Chane-Ming, J. Chemical composition of the essential oil of marjoram (*Origanum majorana* L.) from Reunion Island. *Food Chem.* **66**, 143-145 (1999).
27) Ramos, S.; Rojas, L.B.; Lucena, M.E.; Meccia, G.; Usubillaga, A. Chemical composition and antibacterial activity of *Origanum majorana* L. essential oil from the Venezuelan Andes. *J. Essent. Oil Res.* **23**(5), 45-49 (2011). doi.org/10.1080/10412905.2011.9700481
28) Novak, J.; Lukas, B.; Franz, C.M. The essential oil composition of wild growing sweet marjoram (*Origanum majorana* L., Lamiaceae) from Cyprus-Three Chemotypes. *J. Essent. Oil Res.* **20**, 339-341 (2008). doi.org/10.1080/10412905.2008.9700026
29) Karousou, R.; Efstathiou, C.; Lazari, D. Chemical diversity of wild growing *Origanum majorana* in Cyprus. *Chem. Biodivers.* **9**, 2210-2217 (2012). doi.org/10.1002/cbdv.201100375
30) Bağci, Y.; Kan, Y.; Doğu, S.; Çelik, S.A. The essential oil compositions of *Origanum majorana* L. Cultivated in Konya and collected from Mersin-Turkey. *Indian J. Pharm. Educ. Res.* **51**, 463-469 (2017). doi.org/10.5530/ijper.51.3s.68
31) Benhalilou, N.; Alsamri, H.; Alneyadi, A.; Athamneh, K.; Alrashed, A. et al. *Origanum majorana* Ethanolic Extract Promotes Colorectal Cancer Cell Death by Triggering Abortive Autophagy and Activation of the Extrinsic Apoptotic Pathway. *Front Oncol.* **9**, 795 (2019). doi.org/10.3389/fonc.2019.00795
32) Tripathy, B.; Satyanarayana, S.; Khan, K.A.; Raja, K.; Mohanty, C. Phytochemical screening and antifungal activity of ethanol and petroleum-ether leaf extracts of *Origanum Majorana*. *Int. J. Pharma. Res. Health Sci.* **4**, 1320-1323 (2019). doi.org/10.21276/ijprhs.2016.04.14
33) Elezab, S.T.; Soliman, A.F.; Nishikawa, Y. Effect of some plant extracts from Egyptian herbal plants against *Toxoplasma gondii* tachyzoites in vitro. *J. Vet. Med. Sci.* **83**, 100-107 (2021). doi.org/10.1292/jvms.20-0458
34) Ezzeddine, N.B.H.B.; Abdelkefi, M.M.; Aissa, R.B.; Chaabouni, M.M. Antibacterial screening of *Origanum majorana* L. Oil from Tunisia. *J. Essent. Oil Res.* **13**, 295-297 (2001). doi.org/10.1080/10412905.2001.9699698
35) Della Pepa, T.; Elshafie, H.S.; Capasso, R.; De Feo, V.; Camele, I. et al. Antimicrobial and phytotoxic activity of *Origanum heracleoticum* and *O. majorana* essential oils growing in Cilento (Southern Italy). *Molecules* **24**, 2576 (2019). doi.org/10.3390/molecules24142576
36) Paudel, P.N.; Satyal, P.; Satyal, R.; Setzer, W.N.; Gyawali, R. Chemical composition, enantiomeric distribution, antimicrobial and antioxidant activities of *Origanum majorana* L. essential oil from Nepal. *Molecules* **27**, 6136 (2022). doi.org/10.3390/molecules27186136
37) Bailén, M.; Díaz-Castellanos, I.; Azami-Conesa, I.; Fernández, S.A.; Martínez-Díaz, R.A. et al. Anti-*Trichomonas gallinae* activity of essential oils and main compounds from Lamiaceae and Asteraceae plants. *Front. Vet. Sci.* **9**, 981763 (2022). doi: 10.3389/fvets.2022.981763