

# Investigating the antifungal, antioxidant, and antibacterial activities of *Ocimum basilicum* L. and *Mentha piperita* L. essential oils and their synergistic potentials with antibiotics

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## ABSTRACT

**Background and Aims:** This research focuses on assessing the antioxidant, antifungal and antibacterial properties of *Mentha piperita* Lamiaceae and *Ocimum basilicum* Lamiaceae essential oils and their potential synergistic effects with various antibiotics.

**Methods:** The study identifies the chemical composition of *M. piperita* and *O. basilicum* essential oils by employing gas chromatography-mass spectrometry (GC-MS), cupric reducing antioxidant capacity (CUPRAC), and 2,2-Diphenyl-1-picrylhydrazyl (DPPH) methods to identify antioxidant activity. The study also uses the minimal inhibitory concentration (MIC) method for the antibacterial and antifungal activity tests.

**Results:** The main constituents of *M. piperita* are menthol (51.89%), L-menthone (17.81%), L-menthol (10.17%), and menthyl acetate 6.29%. The main constituents of *O. basilicum* are 65.51% estragole, 18.51% L-linalool, 2.69% bisabolene, and 2.66% trans-4-methoxycinnamaldehyde. With regard to the DPPH method, IC<sub>50</sub> values of 0.028 and 0.019 were found for *M. piperita* and *O. basilicum*, respectively, based on the inhibition values. The results for the CUPRAC method indicate *O. basilicum* to show more antioxidant activity than *M. piperita*. According to the MICs, the essential oils are effective against bacteria at 1:4-1:16 dilutions, while the MIC values for the oil mixture (1:1) are significantly lower at a dilution of up to 1:2048. When combining the oils combined with the antibiotics (i.e., tobramycin and ceftazidime), they provide a synergistic activity against *Staphylococcus epidermidis*, *Escherichia coli*, and *Klebsiella pneumoniae*. The antifungal activity tests reveal no sufficient activity against the mold *Aspergillus niger*, while a limited effect was observed against the yeast *Candida albicans*.

**Conclusion:** The results show that the studied essential oils, especially their mixture at a 1:1 ratio, could be a good treatment option either alone or as a drug adjuvant due to their antibacterial and antioxidant properties.

**Keywords:** Antibacterial, antioxidant, essential oil, *Mentha piperita* L., *Ocimum basilicum* L., antibiotics

## INTRODUCTION

The widespread use of antibiotics has triggered resistance in sensitive bacteria, leading to the ineffectiveness of antibiotics (Liu et al., 2017; Stanojevic et al., 2017). To overcome the increased antibiotic resistance in pathogenic bacterial strains, more effective antimicrobial agents must be developed. Plants are prevalent sources for new antibacterial agents, and many essential oils have been found to be effective against microorganisms (Jalal, El Atki, Lyoussi, & Abdellaoui, 2015; Marwa, Fikri-Benbrahim, Qu-Yahia, & Farah, 2017). *Mentha piperita* L. and *Ocimum basilicum* L., which belong to the Lamiaceae family, are among these essential oils and have antiviral (Sa-

harkhiz et al., 2012), antibacterial (Liu et al., 2017, Stanojevic et al., 2017), antifungal (Al-Maskri et al., 2011; Tullio, Roana, Scalas, & Mandras et al., 2019), and antioxidant (Al-Maskri et al., 2011, Aşkın & Kaynarca, 2020; Kizil, Hasimi, Tolan, Kilinc, & Yüksel, 2010) properties. Sometimes, different combinations of essential oils are able to provide higher efficacy against bacteria, resulting in lower doses and reduced toxic side effects (Gutierrez, Barry-Ryan, & Bourke, 2008; Clemente, Aznar, Silva, & Nerín, 2016).

Fungal infections are also a prevailing problem, and their treatment has become difficult due to resistant strains (Limon, Skalski, & Underhill, 2017; Hay, 2006). Plants with high an-

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tifungal activity can reduce the resistance of these strains and can also be used as alternative treatment agents (Stanojevic et al., 2017). Nonetheless, antifungal activity has not been a commonly used method or research topic (Letessier, Svoboda, & Walters, 2001). Antioxidants are essential chemicals that stop reactive free radicals from initiating and escalating oxidative processes (Ismail, Marjan, & Foong, 2004). High phenolic plants can also be shown as powerful antioxidants (Akyuz, Şahin, Islamoglu, Kolayli, & Sandra, 2014).

Antibiotic resistance has become a worldwide public health concern due to the ongoing appearance of new bacterial strains that are resistant to antibiotics, reduced the effectiveness of antibiotics and necessitated the use of more costly therapies when infections become untreatable by initial antimicrobials (Langeveld, Veldhuizen, & Burt, 2014). One of the most effective strategies to fight antibiotic resistance is to combine antibiotics with natural substances such as essential oils. The objectives of this combination are to reduce microbial toxicity and antibiotic resistance while producing synergistic antibacterial activities (Ju et al., 2020). However, limited data are still found regarding the antibacterial activities of *M. piperita* and *O. basilicum* alone or as a mixture of them combined with antibiotics against multidrug-resistant bacteria.

This study first obtained the chemical constituents of *M. piperita* and *O. basilicum* essential oils using gas chromatography and established their antioxidant characteristics. Subsequently, the study identified these oils' antimicrobial properties against common infectious bacteria and fungi both on their own as well as mixed together. Investigating potential synergistic antibacterial interactions between the oils and certain antibiotics (such as ciprofloxacin, tobramycin, ceftazidime, and meropenem) is another goal of the study.

## MATERIALS AND METHODS

### Chemicals

#### Essential oils

*M. piperita* and *O. basilicum* essential oils were supplied from the commercial market. For the antioxidant activity assays, readily available essential oils of *M. piperita* and *O. basilicum* were diluted to 1:10 using Polysorbate 80. The other parts of the study used the essential oils undiluted.

### Reagents

For the 2,2-diphenyl-1-picrylhydrazyl (DPPH) method, 1 mM:50 mL of DPPH radical solution was prepared according to Torres-Martínez et al. (2018). Due to the DPPH solution's light sensitivity, aluminum foil was used as a cover, and the solution was kept at +4°C in a dark environment. A fresh solution was prepared daily for the experiments. For the cupric ion reducing antioxidant capacity (CUPRAC) method, 10<sup>-2</sup> M copper (II) chloride (CuCl<sub>2</sub>), 7.5 × 10<sup>-3</sup> M neocuprine (Nc), and 1 M ammonium acetate (NH<sub>4</sub>Ac) solutions were prepared ac-

ording to Apak, Güçlü, Özyürek, & Karademir, (2004). Due to the Nc solution's light sensitivity, it was also stored wrapped in aluminum foil. A 10<sup>-4</sup> M gallic acid solution was also prepared.

### Microbial Strains

The American Type Culture Collection (ATCC) standard strains of gram-positive bacteria *Staphylococcus aureus* (ATCC 29213) and *S. epidermidis* (ATCC 12228) and gram-negative bacteria *Pseudomonas aeruginosa* (ATCC 27853), *Escherichia coli* (ATCC 25922), *Klebsiella pneumoniae* (ATCC 4352), and *Proteus mirabilis* (ATCC 14153) were used for identifying the antibacterial activities. The yeast *Candida albicans* (ATCC 10231) and a spore suspension of the mold *Aspergillus niger* (ATCC 16404) were used in the antifungal tests.

### Culture Media

Cation-adjusted Mueller-Hinton broth (CAMHB, Difco Laboratories) and Roswell Park Memorial Institute (RPMI) -1640 medium (Sigma) buffered to pH 7.0 were used for the antimicrobial activity tests for the bacteria and fungi, respectively.

### Antimicrobial agents

The antibiotics tobramycin, ceftazidime, ciprofloxacin, and meropenem and the antifungal fluconazole were obtained from Sigma-Aldrich (St Louis, MO, USA). Excluding the meropenem, 1280 µg/mL stock solutions were prepared in accordance with the manufacturer's instructions and kept at -80°C. The meropenem solutions were prepared daily.

### Gas Chromatography

The characterization of the essential oils was accomplished by Thermo Scientific GC TRACE 1300 using an MS detector TSO 8000 Evo and a Thermo Scientific Tr-5MS chromatographic column (length = 30 m, inner diameter (ID) = 0.25 mm, film thickness = 25 µm). The carrier gas the study used was helium (flow rate = 1.0 mL/min). The MS conditions were: ionization voltage = 70 eV; emission current = 40 mA; acquisition and scan range = 35–450 amu, and sampling rate = 1.0 scan/s. The inlet temperature = 250°C, and the oven temperature was programmed to remain at 80°C for four minutes, rise to 220°C over 30 min, and finally rise to 320°C over five minutes. The split ratio was 100:1, the injection volume was 1 µL, and the interface temperature was 320°C.

The composition of the selected essential oils was determined based on their retention time (RT) by comparing their mass spectral fragmentation patterns with the ones existing in the MS library (i.e., flavor2.hp, Wiley9, mainlib, replib, mist\_ri). The GC peak area without correction factor was the basis for calculating the constituents' relative concentrations (%).

## Antioxidant Activity

The antioxidant activities of the essential oils were assessed by employing the DPPH and CUPRAC methods.

### DPPH Antioxidant Assay

The study adhered to Torres-Martínez et al.'s (2018) method. To check the absorbance values, five tubes were prepared for each essential oil sample. 0.6 mL of the DPPH radical solution was placed into each tube, and 0.02, 0.04, 0.06, 0.08, and 0.1 mL of essential oils were added to the respective tubes. These mixtures were then filled to 6 mL with methanol. The incubation time was 30 minutes in a dark environment at room temperature. For the blank solution, 5.4 mL of methanol was added to 0.6 mL of the DPPH radical solution and incubated for 30 minutes in a dark environment at room temperature. At the end of the 30 minutes, the optic densities of both the samples and the blank solution were taken at 517 nm.

For the DPPH experiment, the inhibition value should be calculated to understand the antioxidant values. Inhibition values of *M. piperita* and *O. basilicum* essential oils were calculated using Equation 1, where  $A_{DPPH}$  = DPPH absorbance value of the blank and  $A_{extract}$  = the absorbance value of the sample.

$$\%inhibition = [(A_{DPPH} - A_{extract})/A_{DPPH}] \times 100 \quad (1)$$

The IC50 value is a concentration of the antioxidant substance that inhibits 50% of the DPPH radical in the environment. It is indirectly proportional to the antioxidant activity, which means that smaller values have higher antioxidant activity (Molyneux, 2004). For the IC50 values, the sample volumes that provide 50% inhibition of the radical are calculated from the acquired graph formulas ( $y=ax+b$ ), where  $a$  is the slope and  $b$  is the y-intercept (Equation 2).

$$50\% = a \times (\text{sample volume}) + b \quad (2)$$

### CUPRAC Antioxidant Assay

Based on Apak et al. (2004), the CUPRAC method was applied with respect to antioxidant activity. To check the absorbance values, five tubes were prepared for each essential oil sample. 1 mL of each of the prepared solutions was added to each tube, with a total of 3 mL of solution being obtained: 1 mL of Na, 1 mL of  $\text{CuCl}_2$ , and 1 mL of  $\text{NH}_4\text{Ac}$ . Consequently, different volumes of the essential oils were added to 0.2, 0.4, 0.6, 0.8 and 1 mL tubes, respectively. These mixtures were then filled to 4.1 mL with distilled water. The incubation time was 30 minutes in a dark environment at room temperature. The same procedure was applied to the gallic acid to be used for the comparison. For the blank solution, 1 mL of each of the prepared solutions was added, and a total of 3 mL of solution was obtained: 1 mL of Na, 1 mL of  $\text{CuCl}_2$ , and 1 mL of  $\text{NH}_4\text{Ac}$ . The solution

was filled to 4.1 mL by adding 1.1 mL of distilled water, then it was incubated in a dark environment at room temperature for 30 minutes. Following the incubation, the samples were taken, and the absorbance values of both the samples and the blank solution were examined at a wavelength of 450 nm in the UV-VIS spectrophotometer. The gallic solution was used to compare the results from the *M. piperita* and *O. basilicum* essential oils regarding the CUPRAC method.

### Determining the antimicrobial activities

The antibacterial and antifungal activities of *M. piperita* and *O. basilicum* were tested alone as well as a 1:1 mixture of the oils using the microbroth dilution technique. The minimum inhibitory concentration (MIC) values were then determined according to the Clinical and Laboratory Standards Institute (CLSI, 2006, 2000). Two-fold serial dilutions of the oils were prepared in CAMHB for the bacteria and the RPMI-1640 medium for the fungi in 96 U-shaped microtiter plates. Each well was inoculated with 50  $\mu\text{L}$  of fresh broth cultures, which yielded  $5 \times 10^5$  cfu/mL for the bacteria and  $5 \times 10^3$  cfu/mL for fungi. The plates were covered with plastic bags to avoid drying and incubated for 18-24 hrs. at 37°C for the bacteria, for 48 hrs. at 35°C for the *C. albicans*, and for 48-72 hrs. at 25°C for *A. niger*. The MIC values are the lowest concentrations of essential oils that inhibit the visible growth of microorganisms. The reference antibiotic and antifungals were ciprofloxacin and fluconazole, respectively.

### Determining the combined effects of essential oils and antibiotics

To determine the antibacterial activities of oils in combination with the antibiotics, the antibiotics' MIC values were tested both alone and in combination with *M. piperita*, *O. basilicum*, and a 1:1 mixture of the two oils. Two-fold dilutions of the antibiotics between 64-0.062  $\mu\text{g/mL}$  in CAMHB were prepared, and the oils were added to the corresponding wells of the plates to give a final concentration of 5% for the pure oils. To do this, the antibiotics tobramycin, ceftazidime, ciprofloxacin, and meropenem were used against the bacteria that are sensitive to the essential oils in accordance with their MIC values (CLSI, 2006; Andrews, 2001).

## RESULTS

### Gas chromatography results

Figure 1 presents the GC-MS chromatogram of *M. piperita* and *O. basilicum*, and Table 1 provides the compositions of the essential oils. The most represented compounds for *M. piperita* are monoterpenes, which constitute 86.61 % of this oil's total components. Based on the results, menthol can be stated as the most represented component (51.89%), followed by L-menthone (17.81%), L-menthol (19.17%), and

menthyl-acetate (6.29%). The most represented compounds for *O. basilicum* are phenylpropenes at 65.51% and monoterpenes at 18.51% of the total oil composition. For *O. basilicum*, estragole is the most represented component (65.51%), followed by L-linalool (18.51%), bisabolene (2.69%), and trans-4-methoxycinnamaldehyde (2.66%).

### Antioxidant activity results

The DPPH and CUPRAC methods were employed to identify antioxidant activity. Inhibition values were calculated according to the acquired absorbance values (Table 2). According to the results obtained from the DPPH method, the highest and lowest inhibition values were determined as 54.38 and 9.48 for *M. piperita* and as 40.48 and 13.30 for *O. basilicum*. The CUPRAC method was employed to establish the effects of the concentration of antioxidant compounds in the diluted *O. basilicum* and *M. piperita* essential oils on inhibiting the DPPH radical and the effect from antioxidant compounds being absorbed in diluted oils and Gallic acid (Figures 2-3). The IC50 value was calculated as 0.019 for *O. basilicum*, and 0.028 for *M. piperita*. The results indicate *M. piperita* and *O. basilicum* to indeed show antioxidant activity. When comparing the two oils, *O. basilicum* shows higher antioxidant activity than the *M. piperita* essential oil.

### Antimicrobial activity results

The *in vitro* antimicrobial activities of the studied essential oils against bacteria and fungi were evaluated using the CLSI criteria, with Table 3 summarizing the MIC values. For the standardization of the study, the MIC values of ciprofloxacin and fluconazole were also determined against bacteria and fungi, respectively, and the results were found to be within the quality control limits reported by the CLSI (2014). In the antifungal activity assays, neither *M. piperita* and *O. basilicum* individually nor their mixture showed any activity against the mold *A. niger*, while they showed limited activity against the yeast *C. albicans* (Table 3). Hence, no significant antifungal activity was detected from the essential oils.

When identifying their antibacterial activity, while the *O. basilicum* essential oil showed higher activity against *E. coli* and *K. pneumonia* at respective dilutions of 1:32 and 1:16, the *M. piperita* essential oil was more effective against *S. epidermidis*, *E. coli*, and *K. pneumonia* at the respective dilutions of 1:8, 1:8, and 1:16. Similarly, the mixture of essential oils (1:1) was also effective, especially against the same bacteria; interestingly, this mixture increased the activities up to a dilution of 1:2048.

### Results from the combination of essential oils and antibiotics

Table 4 summarizes the MIC values of antibiotics combined with the essential oils individually and as an oil mixture against the more sensitive bacteria *S. epidermidis*, *E. coli*, and *K. pneumoniae*. According to these results, the studied essential oils have synergistic effects against *S. epidermidis*, *E. coli*, and *K. pneumoniae* when combined with ceftazidime, while the tobramycin or ciprofloxacin combinations showed synergistic activity against *E. coli* and *K. pneumoniae*. These results indicate that combining essential oils with the antibiotics increase their inhibitory effects against the selected bacteria.

### DISCUSSION

This study has investigated the antioxidant, antifungal and antibacterial activities of *M. piperita* and *O. basilicum* essential oils individually and as a 1:1 mixture. Furthermore, their combinations with selected antibiotics were also examined not only to advance the understanding of essential oil properties but to also highlight potential synergies with antibiotics for developing more effective strategies.

In line with prior research, the analysis results of the selected essential oils obtained through the DPPH and CUPRAC methods have identified antioxidant activity. The fact that the DPPH method lacks a standard value made comparing the antioxidant activities difficult (Deng, Cheng, & Yang, 2011). Likewise, due to not having a fixed value, the found values cannot be verified with a standard analysis. The DPPH radical is sensitive to light, oxygen in air, and pH. For this reason, various results are obtained from each iteration. Thus, the results cannot be compared with those from distinct studies (Sharma & Bhat, 2009). However, based on the absorbance and inhibition values found in the current and previous studies (Aşkın & Kaynarca, 2020; Kizil et al., 2010), both *M. piperita* and *O. basilicum* essential oils are concluded to have antioxidant activity, and this activity is expected to increase as the oil content increases. This study prepared samples at different concentrations and compared the results with respect to these concentrations. The inhibition values (Table 2) and the increase in IC50 values display the antioxidant activity of the studied oils. When comparing the IC50 values for the two oils, the antioxidant activity of the *O. basilicum* essential oil was higher than that of *M. piperita*, based on the IC50 value for the *O. basilicum* essential oil being lower. When considering the lack of a standard result using the DPPH method, the decision was made to observe antioxidant activity using the CUPRAC method. Total antioxidant capacity (TAC) was calculated using the measured absorbance values of the oils at different concentrations (Figure 3). Accordingly, antioxidant activity was observed for both essential oils using the DPPH and CUPRAC methods.

Previous studies have identified the *M. piperita* and *O.*



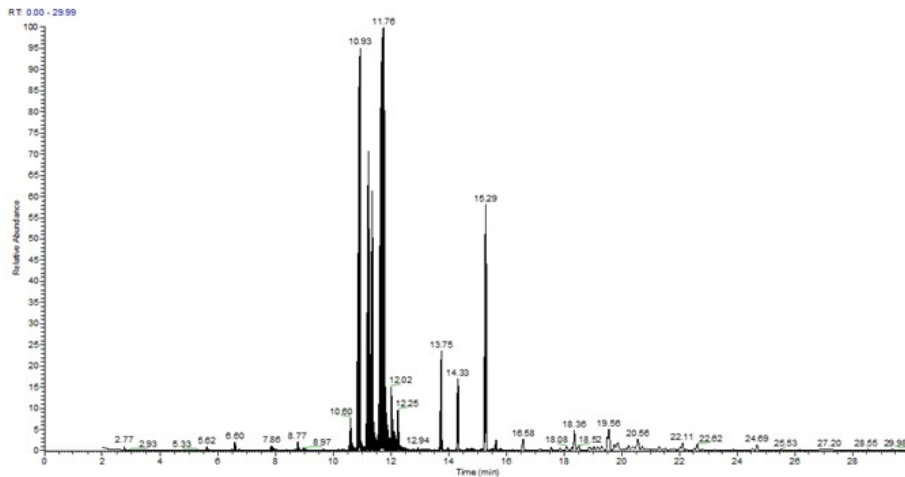


Figure 1a. GC-MS chromatogram of *M. piperita* L.

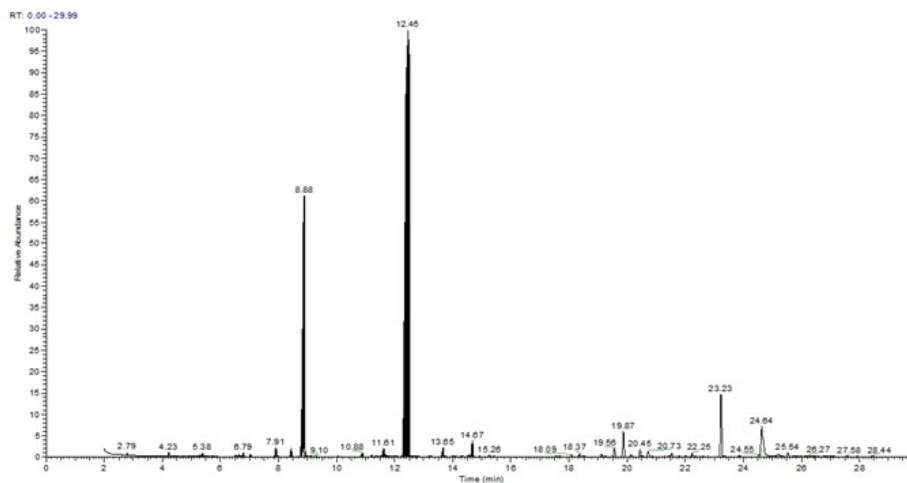


Figure 1b. GC-MS chromatogram of *O. basilicum* L.

*basilicum* essential oils to show antibacterial (Liu et al., 2017; Stanojevic et al., 2017) and antifungal (Al-Maskri et al., 2011; Tullio et al., 2019) activities against several microorganisms. When considering the antifungal activity assays, while limited activity occurred against *C. albicans*, no effect was found on *A. niger*. Although the antibacterial activity results are similar to those from previous studies, several reasons may exist for the lack of antifungal activity compared to other studies (Stanojevic et al., 2017; Al-Maskri et al., 2011). For example, the differences in antifungal activities might occur as a result of the diversities of fungi and/or due to the filamentous structure of *A. niger*, with essential oils perhaps being unable to interact with the cell components. The quality, purity, production method, and trademark of the studied oils might also be other

possible reasons for the lack of antifungal activity. On the other hand, this study's antibacterial activity results support other researchers' findings. While the *M. piperita* and *O. basilicum* essential oils individually show moderate antibacterial activity against *S. epidermidis*, *E. coli*, and *K. pneumoniae*, they had quite a valuable effect when combined together. Therefore, combining these two oils is considered to have a possible synergistic effect on the studied bacteria, with the mixture of *M. piperita* and *O. basilicum* essential oils perhaps being an alternative treatment against antibiotic resistant infectious agents.

The antimicrobial activities of the essential oils may have several mechanisms, and these mechanisms may affect various biochemical and structural functions such as cytoplasm,

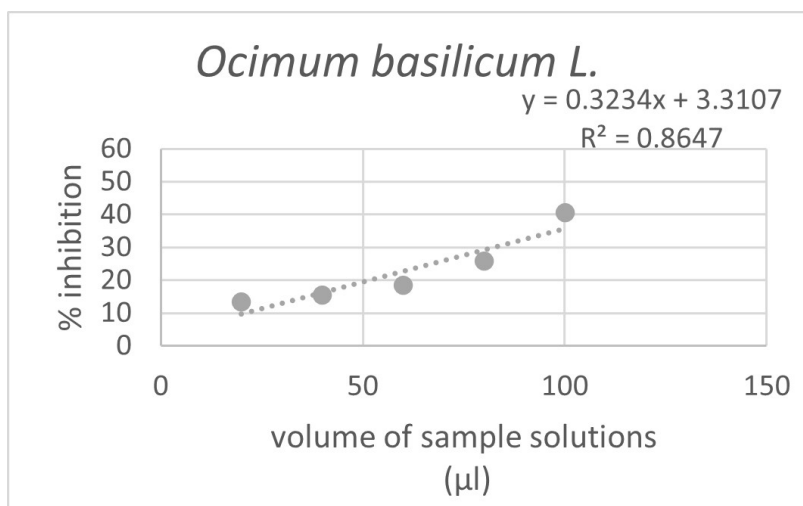
**Table 1.** The compositions of the essential oils identified by GC-MS analysis

<i>Mentha piperita</i>			<i>Ocimum basilicum</i>		
Compound Name	RT (min)	% Area	Compound Name	RT (min)	% Area
2-[(Phenylamino)caronyl]cyclohexanecarboxylic acid	5.07	0.00	3-Pyrrolidinecarboxylic acid, 5-oxo-1-(2-pyridinylmethyl)-	5.11	0.00
(E)-4-cyanopent-3-en-1-ol	5.33	0.01	2-[(Phenylamino)caronyl]cyclohexanecarboxylic acid	5.38	0.05
Acetyl bromide (CAS)	5.62	0.01	(E)-4-cyanopent-3-en-1-ol	5.57	0.00
2-[(Phenylamino)caronyl]cyclohexanecarboxylic acid	6.60	0.03	Acetyl bromide (CAS)	5.85	0.00
dl-Limonene	6.60	0.23	Benzenamine (CAS)	6.79	0.05
2-[(Phenylamino)caronyl]cyclohexanecarboxylic acid	6.99	0.00	3-Pyrrolidinecarboxylic acid, 5-oxo-1-(2-pyridinylmethyl)-	7.03	0.02
2-[(Phenylamino)caronyl]cyclohexanecarboxylic acid	7.46	0.00	Linalool Oxide (2)	7.91	0.38
1-Octanol (CAS)	7.86	0.27	2-[(Phenylamino)caronyl]cyclohexanecarboxylic acid	7.91	0.06
2-[(Phenylamino)caronyl]cyclohexanecarboxylic acid	7.87	0.03	Ethanone, 1-(methylenecyclopropyl)-	7.91	0.02
L-Linalool	8.77	0.39	Ethanone, 1-(methylenecyclopropyl)-	8.43	0.02
Propanoic acid, 2-(phenylmethoxy)-	10.60	0.03	Trans-Linalool Oxide	8.43	0.34
Propanoic acid, 2-(phenylmethoxy)-	10.93	0.11	2-[(Phenylamino)caronyl]cyclohexanecarboxylic acid	8.43	0.06
L-Menthone	10.93	17.81	1H-Pyrrole, 2-methyl-	8.65	0.01
Propanoic acid, 2-(phenylmethoxy)-	11.22	0.05	Hex-2-yn-4-one, 2-methyl-	8.88	0.86
Ethanone, 1-(methylenecyclopropyl)-	11.35	3.23	L-Linalool	8.88	18.52
Propanoic acid, 2-(phenylmethoxy)-	11.75	0.27	Diaminomaleonitrile	8.89	0.66
Menthol	11.76	51.89	Ethanone, 1-(methylenecyclopropyl)-	10.88	0.01
L-menthol	11.76	10.18	Cyclopentene, 1-(1-methylethyl)-	10.88	0.01
Bromoacetic acid, 2-tetrahydrofurylmethyl ester	12.52	0.01	Ethanone, 1-(methylenecyclopropyl)-	11.58	0.02
Bromonitromethane	13.74	0.09	L-(-)-Menthol	11.61	0.81
Pulegone	13.75	2.20	1-Heptyn-6-one	11.61	0.15
Phosphorocyanidous difluoride	14.32	0.20	Hex-2-yn-4-one, 2-methyl-	12.36	0.04
2-Cyclohexen-1-one, 3-methyl-6-(1-methylethyl)- (CAS)	14.33	1.61	Estragole	12.45	65.51
Phosphorocyanidous difluoride	15.28	1.36	Propanoic acid, 2-(phenylmethoxy)-, methyl ester	12.50	3.80
Menthyl acetate	15.29	6.30	N-Formylthiocarbamic acid	12.52	0.41
Cyclohexanol, 3-methyl-2-(1-methylethyl)-, acetate, (1 $\alpha$ ,2 $\alpha$ ,3 $\alpha$ )-	15.64	0.28	Bromonitromethane	13.65	0.03
Phosphorocyanidous difluoride	15.64	0.05	Z-Citral	13.65	0.35
Cyclohexane, 1-ethenyl-1-methyl-2-(1-methylethenyl)-4-(1-methylethylidene)-	16.58	0.25	Bromine azide	14.67	0.02
$\beta$ -Bourbonene	18.36	0.66	(E,E)-3,7-Dimethyl-1-(methoxymethoxy)-1,6-octadien-3-ol	14.67	0.64
beta-Caryophyllene	19.56	0.77	Phosphorocyanidous difluoride	15.26	0.01
Germacrene D	19.86	0.43	trans-Caryophyllene	19.56	0.36
$\gamma$ -Cadinene (CAS)	20.56	0.72	Trans-A-Bergamotene	19.87	1.11
$\beta$ -Cubebene	22.11	0.23	trans- $\beta$ -Farnesene	20.45	0.33
$\beta$ -cadinene	22.62	0.14	Bisabolene	23.23	2.70
(-)-Caryophyllene oxide	24.69	0.16	trans-4-Methoxycinnamaldehyde	24.64	2.66

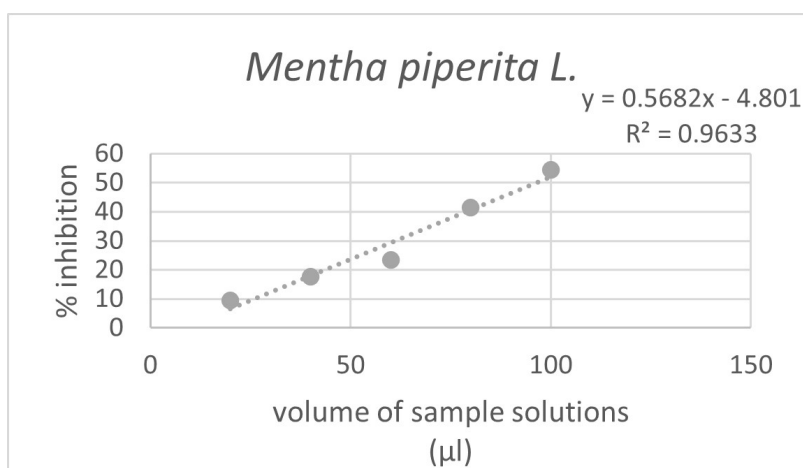
**Table 2.** Inhibition values of the essential oils in different amounts (DPPH).

Volume	Inhibition %	
	<i>Mentha piperita L.</i>	<i>Ocimum basilicum L.</i>
0.02mL	9.475	13.301
0.04mL	17.669	15.502
0.06mL	23.431	18.469
0.08mL	41.485	25.837
0.1mL	54.383	40.478

enzyme system, and protein structure. These oils are able to change the permeability of membrane proteins as well as their functions and can also adhere to the bacterial cell wall and interact with the proteins, disrupting their regular functions (Johnson-Henry, Hagen, Gordonpour, Tompkins, & Sherman, 2007). These effects are also thought to be higher against Gram-positive bacteria compared to Gram-negative bacteria (Nazzaro, Fratianni, Martino, Coppola, & De Feo, 2013). This may be a result of their very different cell wall structures. The two-layer cell wall structure of the Gram-negative bacteria possible does not easily permit the penetration of drugs, antibiotics, phenolic compounds (such as thymol, carvacrol, and eugenol), and essential oils; meanwhile, the Gram-positive bacteria has an uncomplicated cell wall (Trombetta et al., 2005). Slightly dif-



**Figure 2a.** The effect of the concentration of antioxidant compounds in diluted *Ocimum basilicum* L. essential oils on the inhibition of DPPH radical.

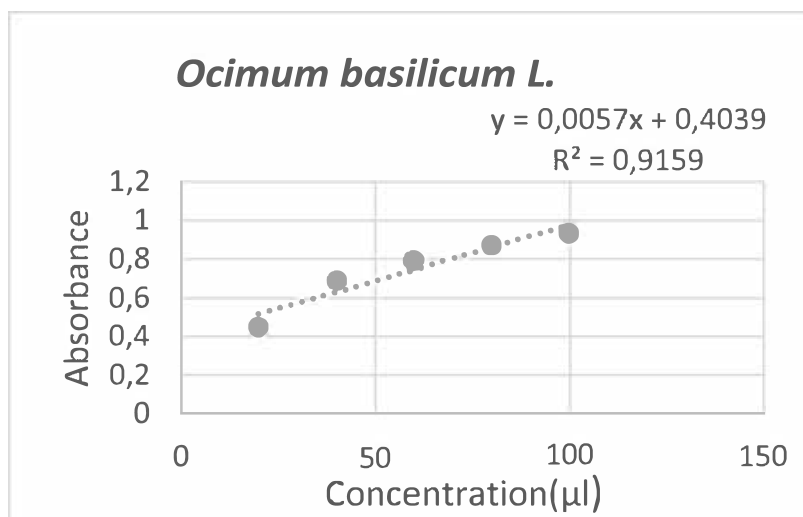


**Figure 2b.** The effect of the concentration of antioxidant compounds in diluted *Mentha piperita* L. essential oils on the inhibition of DPPH radical.

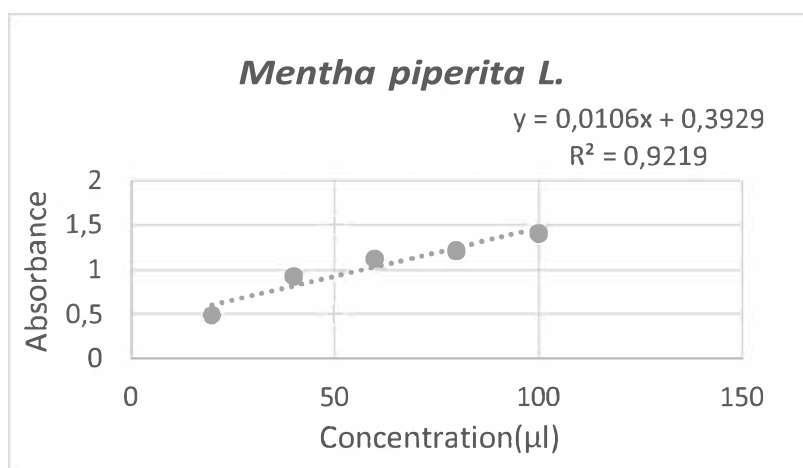
**Table 3.** MIC values (dilution ratios) of the studied essential oils against various microorganisms.

Samples	Microorganisms							
	Bacteria						Fungi	
	S.a	S.e	P.a	E.c	K.p	P.m	C.a	A.n
<i>Ocimum basilicum</i> L.	1:4	1:4	1:4	1:32	1:16	1:4	1:2	-
<i>Mentha piperita</i> L.	1:4	1:8	1:4	1:8	1:16	1:4	1:2	-
Mixture of them (1:1)	1:2	1:1024	1:2	1:256	1:2048	1:8	1:4	-

\*MICs are given for the dilutions of the pure essential oils, \*\*S.a = *S.aureus*; S.e = *S.epidermidis*; P.m = *P.mirabilis*; E.c = *E.coli*; K.p = *K.pneumoniae*; P.a = *P.aeruginosa*; C.a = *C.albicans*; A.n = *A. niger*



**Figure 3a.** The effect of substances on absorbance at different concentrations for *O. basilicum L.* (CUPRAC method).



**Figure 3b.** The effect of substances on absorbance at different concentrations for *M. piperita L.* (CUPRAC method).

ferent from most other studies, the *M. piperita* and *O. basilicum* essential oils in this study affected both Gram-positive and Gram-negative bacteria. In addition, when comparing the antibacterial activities of *M. piperita* and *O. basilicum* essential oils with each other, *O. basilicum* was observed to show higher activity than *M. piperita*.

The antibacterial and antioxidant activities of essential oils generally come from their active terpene molecules (Poonkodi, 2016; Ouakouak, Chohra, & Denane, 2015), such as thyme, eugenol, and linalool, which are constituents of *M. piperita* and *O. basilicum* (Cox-Georgian, Ramadoss, Dona, & Basu, 2019). On the other hand, menthol as a phenolic monoterpene also shows antimicrobial affects (Saharkhiz et al., 2012), and the antibacterial activities of *M. piperita* may be due to its menthol composition (İşcan, Kirimer, Kürkcüoğlu, Başer, & Demirci, 2002). Aside from these, the linalool component was

also shown to have antioxidant, antibacterial, and antifungal effects (Hussain, Anwar, Sherazi, & Przybylski, 2008); therefore, the activities of *O. basilicum* may be due to linalool. When determining the chemical compositions of the essential oils using gas chromatography, this study found monoterpenes to make up 86.61% of the total components in the *M. piperita* essential oil. However, phenylpropanes make up 65.51% and monoterpenes 18.51% of *O. basilicum*'s overall oil composition. These results support the fact that these essential oils have antioxidant and antimicrobial properties.

Previous studies have identified combining antibiotics and essential oils to have potential synergistic effects (Fadli et al., 2012; Rosato, Vitali, Laurentis, Armenise, & Milillo, 2007) against some resistant bacteria. For instance, a study done with cinnamon essential oil concluded that essential oils could be used as an alternative therapeutic application (El Atki et al.,



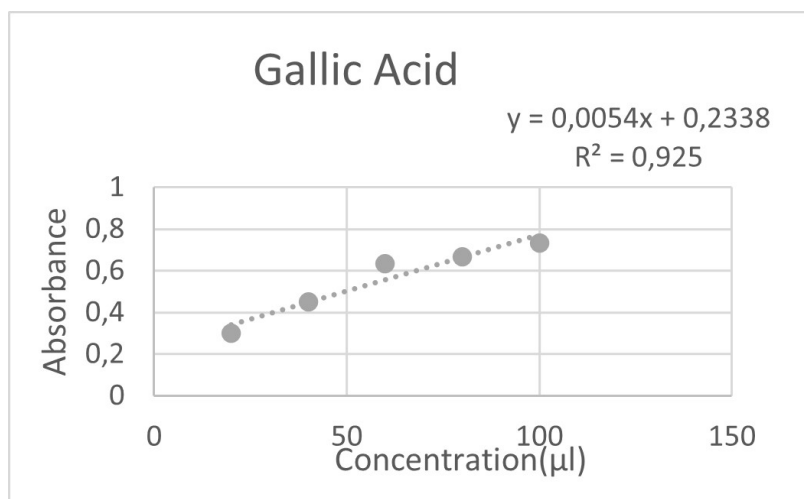


Figure 3c. The effect of substances on absorbance at different concentrations for Gallic acid (CUPRAC method)

Table 4. MIC values (µg/mL) of antibiotics with essential oils against various microorganisms

Samples	Microorganisms		
	<i>S. epidermidis</i>	<i>E. coli</i>	<i>K. pneumoniae</i>
<b>Ciprofloxacin</b>	0.062	0.25	0.25
Ciprofloxacin + <i>Ocimum basilicum</i> L.	≤ 0.062	<b>0.062</b>	<b>0.062</b>
Ciprofloxacin + <i>Mentha piperita</i> L.	≤ 0.062	<b>0.062</b>	<b>0.062</b>
Ciprofloxacin + Oil Mixture	≤ 0.062	<b>0.062</b>	<b>0.062</b>
<b>Tobramycin</b>	0.125	0.5	0.25
Tobramycin + <i>Ocimum basilicum</i> L.	≤ <b>0.062</b>	<b>0.062</b>	<b>0.062</b>
Tobramycin + <i>Mentha piperita</i> L.	≤ <b>0.062</b>	<b>0.062</b>	<b>0.062</b>
Tobramycin + Oil Mixture	≤ <b>0.062</b>	<b>0.062</b>	<b>0.062</b>
<b>Ceftazidime</b>	0.5	0.25	1
Ceftazidime + <i>Ocimum basilicum</i> L.	<b>0.062</b>	<b>0.062</b>	<b>0.062</b>
Ceftazidime + <i>Mentha piperita</i> L.	<b>0.062</b>	<b>0.062</b>	<b>0.062</b>
Ceftazidime + Oil Mixture	<b>0.062</b>	<b>0.062</b>	<b>0.062</b>
<b>Meropenem</b>	0.125	0.125	0.062
Meropenem + <i>Ocimum basilicum</i> L.	≤ <b>0.062</b>	≤ <b>0.062</b>	≤ 0.062
Meropenem + <i>Mentha piperita</i> L.	≤ <b>0.062</b>	≤ <b>0.062</b>	≤ 0.062
Meropenem + Oil Mixture	≤ <b>0.062</b>	≤ <b>0.062</b>	≤ 0.062

2019), and another study established the *M. piperita* essential oil as having a synergistic effect with certain antibiotics (Talei, Mohammadi, Bahmani, & Kopaei, 2017). Even though

ciprofloxacin combined with the oils did not significantly inhibit *S. epidermidis* in this study, they did lower the MIC values and increase the antibacterial activities of other antibiotics,

with ceftazidime in particular having a higher inhibitory effect against *S. epidermidis*. While the MIC value of ceftazidime against *S. epidermidis* was 0.5 µg/mL, it was 0.062 µg/mL when combined with the oils. Similarly, the *M. piperita* and *O. basilicum* essential oils increased the inhibitory effects of all studied antibiotics against *E. coli*, with the oils showing more effective results when used with tobramycin in particular, increasing the inhibitory effect of antibiotics by lowering the MIC values from 0.5 to 0.062 µg/mL. Against *K. pneumoniae*, however, the antibiotic-essential oil combinations were unable to change the meropenem MICs but significantly increased the ceftazidime antibiotic activity. On its own, ceftazidime's MIC was 1 µg/mL against *K. pneumoniae*, but this decreased to 0.062 µg/mL when combined with the essential oils.

Even though the MIC determination is still the gold standard for testing the antimicrobial activities of compounds, some molecules have enhancer activities, and synergistic interactions with some antibiotics. While the microbroth checkerboard method has high throughput and is the basic technique for determining antimicrobial combinations, determining MIC alongside the presence of a fixed concentration enhancer, similar to this study, can also be preferred as a fast and simpler preliminary screening test. If a MIC value decreases four-fold through the combination, that combination can be said to create a synergistic effect (Rand, Houck, Brown, & Bennett, 1993). Therefore, the *M. piperita* and *O. basilicum* essential oils can be said to have synergistic interaction with antibiotics against the studied bacteria.

To the best of this study's knowledge, no such study has been done before with the mixture of the two studied essential oils. While these oils did not have significant effects on all the bacteria studied herein, they did show a synergistic effect with several of the antibiotics. For example, the mixture of ciprofloxacin and the 1:1 oil mixture created a synergistic effect, reducing the MIC values of the antibiotics against *E. coli* and *K. pneumoniae*; however, no clear reduction was observed for *S. epidermidis*. Similarly, the synergistic effect of meropenem and the combined oils increased the inhibitory effect of the antibiotic against *S. epidermidis* and *E. coli*; however, the expected decrease against *K. pneumoniae* MIC values was unobservable. Also, when tobramycin or ceftazidime are combined with *M. piperita* or *O. basilicum*, each combination can provide much more effective antibacterial activity. According to these results, the mixture of *M. piperita* and *O. basilicum* essential oils, whether alone or as a 1:1 combination with antibiotics, gives promising, natural, and environmentally friendly alternative antibacterial and antioxidant treatment strategies for clinics and the pharmaceutical industry.

## CONCLUSION

This present study has determined the *M. piperita* and *O. basilicum* essential oils to exhibit antioxidant activities. These

oils also have antibacterial effects, with the mixture of these two oils increasing this effect. Meanwhile, the oils showed no significant antifungal activity. Furthermore, the combination of these two essential oils with certain antibiotics also showed synergistic effects against specific bacteria. In conclusion, although experiments need to be conducted on more types and greater numbers of microorganisms, the *M. piperita* and *O. basilicum* essential oils, especially at a 1:1 mixture, could provide a good treatment option individually or as a drug adjuvant with their antibacterial and antioxidant activities.

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## REFERENCES

- Akyuz, E., Şahin, H., Islamoğlu, F., Kolaylı, S., & Sandra, P. (2014). Evaluation of phenolic compounds in *Tilia rubra* subsp. *caucasica* by HPLC-UV and HPLC-UV-MS/MS. *International journal of food properties*, 17(2), 331-343.
- Al-Maskri, A. Y., Hanif, M. A., Al-Maskari, M. Y., Abraham, A. S., Al-sabahi, J. N., & Al-Mantheri, O. (2011). Essential oil from *Ocimum basilicum* (Omani Basil): a desert crop. *Natural product communications*, 6(10), 1934578X1100601020.
- Andrews J. M. (2001). Determination of minimum inhibitory concentrations. *The Journal of antimicrobial chemotherapy*, 48(48-71).
- Apak, R., Güçlü, K., Özyürek, M., & Karademir, S. E. (2004). Novel total antioxidant capacity index for dietary polyphenols and vitamins C and E, using their cupric ion reducing capability in the presence of neocuproine: CUPRAC method. *Journal of agricultural and food chemistry*, 52(26), 7970-7981.
- Aşkın, B., & Kaynarca, G. B. (2020). Determination of Antioxidant Properties and Composition of Rosemary and Thyme Essential Oils. *Turkish Journal of Agriculture-Food Science and Technology*, 8(10), 2105-2112.

- Clemente, I., Aznar, M., Silva, F., & Nerín, C. (2016). Antimicrobial properties and mode of action of mustard and cinnamon essential oils and their combination against foodborne bacteria. *Innovative Food Science & Emerging Technologies*, 36, 26-33.
- Clinical and Laboratory Standards Institute (CLSI). (2014). Performance standards for antimicrobial susceptibility testing; 2th informational supplement. M100-S24. Wayne, PA: CLSI.
- Clinical and Laboratory Standards Institute (CLSI). (2000). Reference Method for Broth Dilution Antifungal Susceptibility Testing of Yeasts; Approved Standard M27-A NCCLS, Wayne, PA; CLSI..
- Clinical and Laboratory Standards Institute (CLSI). (2006). Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically: Approved Standard M7-A5. Wayne, PA: CLSI.
- Cox-Georgian, D., Ramadoss, N., Dona, C., & Basu, C. (2019). Therapeutic and medicinal uses of terpenes. In *Medicinal Plants* (pp. 333-359). Springer, Cham.
- Deng, J., Cheng, W., & Yang, G. (2011). A novel antioxidant activity index (AAU) for natural products using the DPPH assay. *Food Chemistry*, 125(4), 1430-1435.
- El Atki, Y., Aouam, I., El Kamari, F., Taroq, A., Nayme, K., Timinouni, M., ... & Abdellaoui, A. (2019). Antibacterial activity of cinnamon essential oils and their synergistic potential with antibiotics. *Journal of Advanced Pharmaceutical Technology & Research*, 10(2), 63.
- Fadli, M., Saad, A., Sayadi, S., Chevalier, J., Mezrioui, N. E., Pagès, J. M., & Hassani, L. (2012). Antibacterial activity of *Thymus maroccanus* and *Thymus broussonetii* essential oils against nosocomial infection-bacteria and their synergistic potential with antibiotics. *Phytomedicine*, 19(5), 464-471.
- Gutierrez, J., Barry-Ryan, C., & Bourke, P. (2008). The antimicrobial efficacy of plant essential oil combinations and interactions with food ingredients. *International Journal of Food Microbiology*, 124(1), 91-97.
- Hay, R. J. (2006). Fungal infections. *Clinics in Dermatology*, 24(3), 201-212.
- Hussain, A. I., Anwar, F., Sherazi, S. T. H., & Przybylski, R. (2008). Chemical composition, antioxidant and antimicrobial activities of basil (*Ocimum basilicum*) essential oils depends on seasonal variations. *Food Chemistry*, 108(3), 986-995.
- Ismail, A., Marjan, Z. M., & Foong, C. W. (2004). Total antioxidant activity and phenolic content in selected vegetables. *Food chemistry*, 87(4), 581-586.
- İşcan, G., Kirimer, N., Kürkcüoğlu, M., Başer, H. C., & Demirci, F. (2002). Antimicrobial screening of *Mentha piperita* essential oils. *Journal of Agricultural and Food Chemistry*, 50(14), 3943-3946.
- Jalal, Z., El Atki, Y., Lyoussi, B., & Abdellaoui, A. (2015). Phytochemistry of the essential oil of *Melissa officinalis* L. growing wild in Morocco: Preventive approach against nosocomial infections. *Asian Pacific Journal of Tropical Biomedicine*, 5(6), 458-461.
- Johnson-Henry, K. C., Hagen, K. E., Gordonpour, M., Tompkins, T. A., & Sherman, P. M. (2007). Surface-layer protein extracts from *Lactobacillus helveticus* inhibit enterohaemorrhagic *Escherichia coli* O157: H7 adhesion to epithelial cells. *Cellular Microbiology*, 9(2), 356-367.
- Ju, J., Xie, Y., Yu, H., Guo, Y., Cheng, Y., Qian, H., & Yao, W. (2022). Synergistic interactions of plant essential oils with antimicrobial agents: a new antimicrobial therapy. *Critical Reviews in Food Science and Nutrition*, 62:7, 1740-1751.
- Kizil, S., Hasimi, N., Tolan, V., Kilinc, E., & Yuksel, U. (2010). Mineral content, essential oil components and biological activity of two mentha species (*M. piperita* L., *M. spicata* L.). *Turkish Journal of Field Crops*, 15(2), 148-153.
- Langeveld, W.T., Veldhuizen E.J.A., & Burt S.A. (2014). Synergy between essential oil components and antibiotics: a review, *Critical Reviews in Microbiology*, 40:1, 76-94.
- Letessier, M. P., Svoboda, K. P., & Walters, D. R. (2001). Antifungal activity of the essential oil of hyssop (*Hyssopus officinalis*). *Journal of Phytopathology*, 149(11-12), 673-678.
- Limon, J. J., Skalski, J. H., & Underhill, D. M. (2017). Commensal fungi in health and disease. *Cell host & microbe*, 22(2), 156-165.
- Liu, Q., Meng, X., Li, Y., Zhao, C. N., Tang, G. Y., & Li, H. B. (2017). Antibacterial and antifungal activities of spices. *International Journal of Molecular Sciences*, 18(6), 1283.
- Marwa, C., Fikri-Benbrahim, K., Ou-Yahia, D., & Farah, A. (2017). African peppermint (*Mentha piperita*) from Morocco: Chemical composition and antimicrobial properties of essential oil. *Journal of Advanced Pharmaceutical Technology & Research* 8(3), 86.
- Molyneux, P. (2004). The use of the stable free radical diphenylpicrylhydrazyl (DPPH) for estimating antioxidant activity. *Songklanakarinn Journal of Science and Technology* 26(2), 211-219.
- Nazzaro, F., Fratianni, F., De Martino, L., Coppola, R., & De Feo, V. (2013). Effect of essential oils on pathogenic bacteria. *Pharmaceuticals*, 6(12), 1451-1474.
- Ouakouak, H., Chohra, M., & Denane, M. (2015). Chemical composition, antioxidant activities of the essential oil of *Mentha pulegium* L., South East of Algeria. *International Letters of Natural Sciences*, 39.
- Poonkodi, K. A. T. H. I. R. V. E. L. (2016). Chemical composition of essential oil of *Ocimum basilicum* L.(Basil) and its biological activities-an overview. *Journal of Critical Reviews*, 3(3), 56-62.
- Rand, K. H., Houck, H. J., Brown, P., & Bennett, D. (1993). Reproducibility of the microdilution checkerboard method for antibiotic synergy. *Antimicrobial Agents and Chemotherapy*, 37(3), 613-615.
- Rosato, A., Vitali, C., De Laurentis, N., Armenise, D., & Milillo, M. A. (2007). Antibacterial effect of some essential oils administered alone or in combination with Norfloxacin. *Phytomedicine*, 14(11), 727-732.
- Saharkhiz, M. J., Motamedi, M., Zomorodian, K., Pakshir, K., Miri, R., & Hemyari, K. (2012). Chemical composition, antifungal and antibiofilm activities of the essential oil of *Mentha piperita* L. *International Scholarly Research Notices*, 2012.
- Sharma, O. P., & Bhat, T. K. (2009). DPPH antioxidant assay revisited. *Food Chemistry*, 113(4), 1202-1205.
- Stanojevic, L. P., Marjanovic-Balaban, Z. R., Kalaba, V. D., Stanojevic, J. S., Cvetkovic, D. J., & Cakic, M. D. (2017). Chemical composition, antioxidant and antimicrobial activity of basil (*Ocimum basilicum* L.) essential oil. *Journal of Essential Oil Bearing Plants*, 20(6), 1557-1569.
- Talei, G. R., Mohammadi, M., Bahmani, M., & Kopaei, M. R. (2017). Synergistic effect of *Carum copticum* and *Mentha piperita* essential oils with ciprofloxacin, vancomycin, and gentamicin on Gram-negative and Gram-positive bacteria. *International Journal of Pharmaceutical Investigation*, 7(2), 82.
- Torres-Martínez, R., García-Rodríguez, Y. M., Ríos-Chávez, P., Saavedra-Molina, A., López-Meza, J. E., Ochoa-Zarzosa, A., & Garciglia, R. S. (2017). Antioxidant activity of the essential oil and its major terpenes of *Satureja macrostema* (Moc. and Sessé ex Benth.) Briq. *Pharmacognosy Magazine*, 13(Suppl 4), S875.
- Trombetta, D., Castelli, F., Sarpietro, M. G., Venuti, V., Cristani, M., Daniele, C., ... & Bisignano, G. (2005). Mechanisms of an-

tibacterial action of three monoterpenes. *Antimicrobial Agents and Chemotherapy*, 49(6), 2474-2478.

Tullio, V., Roana, J., Scalas, D., & Mandras, N. (2019). Evaluation of the antifungal activity of *Mentha x piperita* (Lamiaceae) of Pancalieri (Turin, Italy) essential oil and its synergistic interaction with azoles. *Molecules*, 24(17), 3148.

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