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Determination of Antimicrobial Activity in Commercially Available Oregano Essential Oil Samples

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Abstract

In this study, the antimicrobial efficacy of four commercial brands of oregano (*Origanum vulgare*) essential oil, sourced from four different vendors (pharmacy, e-commerce, herbalist, and supplier), was evaluated and compared according to their place of purchase. The research utilized standard strains, including Gram-negative bacteria *Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853; Gram-positive bacteria *Staphylococcus aureus* ATCC 29213 and *Bacillus cereus* ATCC 14579; and fungi *Candida albicans* ATCC 10231 and *Candida tropicalis* DSM 11953. Antimicrobial activity was determined using the Minimum Inhibitory Concentration (MIC) method. Among the four brands tested at ten different concentrations on four bacterial and two fungal strains, the thyme essential oil sourced from the pharmacy demonstrated the highest level of antimicrobial activity. Based on our experimental results, the essential oils demonstrated the strongest inhibitory activity against *Candida albicans*, whereas *Staphylococcus aureus* exhibited the highest resistance to the tested oils.. All tested concentrations of essential oil samples allowed the growth of *S. aureus*. These findings indicate that essential oils sold in the market and online, intended for therapeutic purposes, vary significantly in quality. Economic incentives may lead to adulteration of these oils, underscoring the importance of using plant material from the correct species and considering the geographical conditions that influence the plant's chemical composition. The superior antimicrobial efficacy of the pharmacy-sourced thyme essential oil highlights the need to rely on trained health professionals when selecting products for health-related purposes.

Key Words: Antimicrobial activity, origanum, thyme, essential oil

1. Introduction

Essential oils are aromatic mixtures containing numerous volatile compounds produced by specialized cells within various plant organs such as flowers, leaves, fruits, and bark. These oils are stored in oil ducts, resin channels, glands, or glandular hairs and can be extracted via water or steam distillation. The variety and amount of phytochemicals found in essential oils vary depending on many parameters such as the method of obtaining the essential oil, the geography where the plant grows, climate conditions, and the time the plant is collected (Bakkali et al., 2008; Kürekçi & Sakin, 2017; Uçar et al., 2015). Highly fragrant, often colorless, and volatile at room temperature, essential oils have been historically utilized in traditional medicine due to their distinctive scents and pharmacological effects. Applications of essential oils include antiseptic use, perfumery, mummification, food preservation, and treatment as analgesics, sedatives, spasmolytics, anti-inflammatories, local anesthetics, and antimicrobials (Bakkali et al., 2008).

The Labiateae (Lamiaceae) family, long valued in medicine, cooking, and as a food source, is of particular economic importance and known colloquially as the "mint family." Genera within this family, especially those containing terpenes, phenolic acids, and flavonoids, exhibit notable pharmacological activities (Ersoy, 2009; Lambert et al., 2001). Thyme, belonging to the Lamiaceae family, is a perennial or shrubby herb known for its distinct aroma, attributed to compounds such as thymol and carvacrol. Native to the Mediterranean region, thyme species are mainly found along the Aegean and southern coasts of Turkey, with 40 different species worldwide, 14 of which are endemic to Turkey. In Turkey, the term “thyme” refers generally to various species within the Lamiaceae family, especially those rich in carvacrol or thymol. The similarity in aroma among these species has led to the common nomenclature of “thyme” across different regions. Relevant genera include *Origanum*, *Thymbra*, *Satureja*, and *Thymus* from the Lamiaceae family, with *Lippia graveolens* being notable outside Turkey. These plants thrive in shallow, warm, moist, and fertilized soilsin different parts of the world (Başer, 2022; Demirçakmak, 1994; Stahl-Biskup & Saez, 2002). Thyme oil has applications as an antihypertensive, digestive aid, analgesic, antibacterial, antifungal, antihelmintic, and, topically, for halitosis, infections, sprains, arthritis, cramps, cellulitis, and acne, and it is also used in soaps, the food industry, and cosmetics (Sadıkoğlu, 2005).

In recent years, the rise of antibiotic-resistant microorganisms has complicated the treatment of infectious diseases. Resistance to existing antibiotics has fueled interest in exploring the efficacy of medicinal plant extracts and essential oils with antimicrobial properties against pathogenic microorganisms (Aslan et al., 2021).

In this study, we planned to assess the antimicrobial activity of thyme essential oils from four different sources (pharmacy, e-commerce, herbalist, and supplier) on bacterial strains *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Bacillus cereus*, and fungal strains *Candida albicans* and *Candida tropicalis*, using the MIC method.

2. Materials and Methods

2.1. Materials

Four *Origanum vulgare* essential oils were obtained from different vendors, pharmacies, e-commerce, herbalists, and suppliers. All the samples were pure and didn’t contain any carreer oil. To ensure unbiased analysis, each brand was coded with a letter designation. Samples were labeled as A, B, C, and D for evaluation. The origin and codes for the essential oils used in this study are detailed in Table 1.

2.2. Microorganisms

The microorganism strains used in the antimicrobial activity studies were obtained from the Microbiology Laboratory Collection at the Faculty of Pharmacy, Sivas Cumhuriyet University. In this study, standard strains of *Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853 (Gram-negative bacteria); *Staphylococcus aureus* ATCC 29213 and *Bacillus cereus* ATCC 14579 (Gram-positive bacteria); and *Candida albicans* ATCC 10231 and *Candida tropicalis* DSM 11953 (fungi) were used (Table 2). Blood agar (MERCK) was used to prepare cultures of microorganisms, while Mueller Hinton Broth (MHB) (MERCK) was used as the liquid medium for bacteria, and Sabouraud Dextrose Broth (SDB) (MERCK) for yeasts in the microdilution test.

2.3. Antimicrobial Activity Analysis

The antimicrobial activity was determined using the Microdilution Broth method. Bacterial strains were incubated for 24 hours at 37°C, while yeast strains were incubated for 48 hours at 27°C, prepared from stock cultures. After incubation, single colonies were selected, and the inoculum density was adjusted to a 0.5 McFarland standard turbidity (approximately 1×10^8 CFU/mL) in Tryptic Soy Broth (MERCK) and inoculated into Mueller Hinton Broth (MHB) for bacteria.

2.4. Minimum Inhibitory Concentration (MIC)

The experiments were conducted using U-bottomed 96-well microplates. Stock solutions of essential oil samples were prepared at a 100% concentration. A total of 90 µL of culture medium was added to the first row of wells, while 50 µL was added to the remaining wells. The culture medium was MHB for bacteria and SDB for yeasts. Ten microliters of each essential oil were added to the first row and mixed well using a micropipette. Serial two-fold dilutions were performed across the plate (100%, 50%, 25%, 12.5%, 6.25%, 3.125%, 1.562%, 0.781%, 0.391%, and 0.195% concentrations). A 50 µL sample from the 10th well was discarded, while 50 µL of culture medium was added to the 11th well as a sterility control, and 50 µL of bacterial or yeast inoculum was added to the 12th well as a growth control. Bacteria and yeasts, adjusted to McFarland 0.5 turbidity (5×10^5 CFU/mL for bacteria and $0.5–2.5\times10^3$ CFU/mL for yeasts), were added at 50 µL per well (Pfaller et al., 2012; Tullio et al., 2007). The plates were incubated at 37°C for 24 hours. After two hours at 37°C, the first well showing turbidity was considered the MIC value. MIC results were classified as effective (MIC <100 µg/mL), moderate (100 <MIC ≤625 µg/mL), or weak (MIC >625 µg/mL), as per reference sources (Awouafack et al., 2013; Djeussi et al., 2013).

3. Results and Discussion

3.1. Antimicrobial Activities of Essential Oils

The antimicrobial activities of essential oils were evaluated using *S. aureus* and *B. cereus* as Gram-positive bacteria; *E. coli* and *P. aeruginosa* as Gram-negative bacteria; and *C. albicans* and *C. tropicalis* as fungi via the Microdilution Broth Method.

3.2. Minimum Inhibitory Concentration (MIC)

Antimicrobial test results for the four different commercially obtained samples are presented in Table 3.

According to the results of our study, Sample A exhibited the highest antimicrobial effect against *E. coli* and demonstrated moderate effectiveness (0.1 < MIC ≤ 0.625) for *E. coli* (Djeussi et al., 2013). For *P. aeruginosa*, Samples A and B showed the strongest antimicrobial effects, while Sample C showed no effect, with bacterial growth observed at all concentrations. Sample D inhibited bacterial growth at 100% concentration. For *S. aureus*, none of the thyme essential oils exhibited MIC values, with bacterial growth observed across all concentrations. For *B. cereus,* Samples A and B showed bacterial growth at all concentrations, and no MIC value was observed. However, Sample D displayed the highest antimicrobial effect against *B. cereus*, showing moderate effectiveness (0.1 < MIC ≤ 0.625) for this microorganism (Djeussi et al., 2013). Regarding *C. albicans*, Samples A and D had equal MIC values, indicating a higher antimicrobial effect against *C. albicans* compared to other essential oils, with moderate effectiveness against *C. albicans* (0.1 < MIC ≤ 0.625) (Djeussi et al., 2013). For *C. tropicalis*, Sample A demonstrated moderate effectiveness (0.1 < MIC ≤ 0.625) (Djeussi et al., 2013). whereas Samples B, C, and D showed weak activity against *C.*

tropicalis (MIC > 0.625 mg/mL) (Djeussi et al., 2013).

A recent study determined the antimicrobial effects of essential oils from *Origanum vulgare* (oregano), *Salvia officinalis* (sage), *Rosmarinus officinalis* (rosemary), and *Mentha piperita* (peppermint) on *B. cereus*, *E. coli*, *Salmonella typhimurium*, and *S. aureus* using the microdilution method. The study found *E. coli* to be the most sensitive bacterium to essential oils, while *S. typhimurium* was the most resistant. *Origanum vulgare* essential oil demonstrated the highest antimicrobial activity (Kemer et al., 2022), results which align well with our findings. Additionally, the biological activity of *Origanum vulgare* essential oil obtained from samples collected in Erzurum was studied on both Gram-positive (+) and Gram-negative (+) bacteria. This essential oil showed the strongest effect against *Clavibacter michiganense*, while the weakest activity was observed against *Enterococcus faecalis*, *S. aureus*, and *Streptococcus pyogenes* (Şahin et al., 2004).

In the study by Saraç and Uğur (2008), essential oils from *O. onites*, *S. thymbra*, and *O. vulgare* were tested against bacterial strains with multiple antibiotic resistance, with the highest antibacterial activity observed in *O. onites* essential oil (Sarac & Ugur, 2008). In another study by Oral et al. (2010), the antimicrobial and antibiofilm activities of *O. onites* essential oil were examined on *Staphylococcus aureus*, *Staphylococcus lugdunensis*, *Staphylococcus haemolyticus*, *Staphylococcus sciuri*, and *Escherichia coli*. The results indicated that the essential oil could be an effective natural antimicrobial agent for controlling these microorganisms (Bilge Oral et al., 2009). Boruga and colleagues studied the antimicrobial activity of *Thymus vulgaris* and found that its essential oil exhibited strong antimicrobial activity against several bacteria, including *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Staphylococcus aureus*, and *Enterococcus faecalis* (Jianu, 2014).

Ertürk et al. (2010) conducted a study comparing the antimicrobial effects of oregano and peppermint essential oils on bacteria and yeasts, using 12 Gram (+) bacteria, 8 Gram (-) bacteria, 1 mycobacterium, and 7 yeast strains. They employed the disk diffusion method to determine the antimicrobial effects of commercially obtained oregano and peppermint essential oils. The results indicated that both commercial oregano and peppermint essential oils exhibited strong antimicrobial activity against many microorganisms. Notably, oregano essential oil showed a strong antimicrobial effect against all tested microorganisms except for *Pseudomonas aeruginosa* (Ertürk et al., 2010). Balkan et al. (2016) investigated the antimicrobial effects of ozone, St. John's wort, rose, and oregano oils against microorganisms including *Proteus vulgaris*, *Escherichia coli*, *Proteus mirabilis*, *Enterococcus spp.*, *Candida albicans*, *Stenotrophomonas maltophilia*, *Acinetobacter baumannii*, *Streptococcus spp.*, *Staphylococcus aureus*, and *Citrobacter freundii*. The zone diameters were measured using the disk diffusion method, and the antimicrobial activities of the oils were determined. According to the study results, oregano oil was found to have significantly greater antimicrobial effects than rose, ozone, and St. John's wort oils (Balkan et al., 2016). In another study, the antimicrobial activity of commercially sold *Origanum onites* essential oil was investigated against eight bacteria and two yeast strains using disk diffusion and dilution methods. Hospital-derived *E. coli* isolates that produce beta-lactamase were used in the study. The results showed that *O. onites* essential oil exhibited antimicrobial activity against all standard strains and inhibited the microbial growth of beta-lactamase producing positive *E. coli* isolates (Kaskatepe et al., 2017). Our study also observed effective inhibition against *E. coli* bacteria.

When comparing our findings with the literature, it is observed that the antimicrobial activity of the different commercial brands of *Origanum vulgare* essential oils tested is in parallel with our results, although some differences are also noted. These differences indicate that not all essential oils available for sale in the market and online for therapeutic purposes are of the same quality. This discrepancy may be attributed to economic concerns that can lead to the adulteration of essential oils. Furthermore, it is essential that the plant used for oil extraction belongs to the correct species, and the geographical conditions where the plant is grown can influence its chemical composition.

According to the results of our study, the most effective microorganism against the essential oils of samples A, B, C, and D was identified as *C. albicans*, while the most resistant microorganism against these essential oils was found to be *Staphylococcus aureus*. The sample with the highest antimicrobial activity was identified as sample A, which is the *Origanum vulgare* essential oil obtained from the pharmacy.

Table 1. Source and codes of essential oils used in the study

Sample Code	Source
A	Pharmacy
B	E-commerce
C	Herbalist
D	Manufacturer

Table 2. Bacterial and fungal strains used in antimicrobial activity experiments

Microorganism	Strain Number
E. coli	ATCC 25922
P. aeruginosa	ATCC 27853
S. aureus	ATCC 29213
B. cereus	ATCC 14579
C. albicans	ATCC 10231
C. tropicalis	DSM 11953

Table 3. MIC values (µg/mL) of different commercial thyme essential oils

Microorganism	Sample Code / MIC Values			
	A	B	C	D
E. coli	0.195	0.781	12.5	1.562
P. aeruginosa	50	50	-	100
S. aureus	-	-	-	-
B. cereus	-	-	1.562	0.391
C. albicans	0.195	0.391	0.781	0.195
C. tropicalis	0.391	1.562	1.562	12.5

4. Conclusion

Consumers using essential oils therapeutically should be informed on appropriate purchase and storage conditions. Essential oils, sensitive to temperature and light, should be stored in dark glass bottles in cool environments to prevent compositional changes. Products intended for health purposes should be purchased from pharmacies where trained health professionals are available.

Author Contribution

The authors declare that they have contributed equally to the article.

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Conflicts of Interest

The authors of the articles declare that they have no conflict of interest.

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