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Wild Oregano Oil as a Potent Natural Antimicrobial Agent: Formulations of Oil-in-Water Emulsions

Amra Bratovcic[1*], Amela Becirovic[2]

[1]Faculty of Technology, University of Tuzla, Tuzla, Bosnia and Herzegovina
[2]Institute of Microbiology, Polyclinic for Laboratory Diagnostics, University Clinical Center Tuzla, Tuzla, Bosnia and Herzegovina
Email: *amra.bratovcic@untz.ba

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Abstract

The search for natural antimicrobial agents has gained increasing attention in cosmetics and therapeutics. This study evaluated the antimicrobial potential of wild oregano oil (*Origanum vulgare*), almond oil, and lavender macerate oil, as well as their incorporation into natural oil-in-water (O/W) cosmetic emulsions. Lavender macerate was prepared by soaking lavender flowers in almond oil for two months. Antimicrobial activity was tested against *Escherichia coli* (ATCC 25922), *Staphylococcus aureus* (ATCC 25923), and *Candida albicans* (ATCC 90028). Wild oregano oil exhibited potent, broad-spectrum antimicrobial and antifungal activity, completely inhibiting all tested strains, whereas almond oil and lavender macerate showed no detectable activity. FTIR analysis confirmed the presence of characteristic ester and alkyl functional groups. Based on literature data, Carvacrol can be identified as the key bioactive compound responsible for the strong antimicrobial effects. Eleven O/W emulsions were formulated using almond oil, lavender macerate, jojoba oil, and wild oregano oil. While all emulsions were sterile, their antibacterial activity was limited. Emulsions (E7, E8, E9, E10, and E11) containing almond oil above 15% in combination with Tween 20 and Lanette O (2:1) showed modest inhibition zones (2 - 4 mm) against *S. aureus*, but no activity against *E. coli*, suggesting that the observed effects likely arose from the surfactant system rather than the oil itself. Emulsions containing jojoba oil appeared to suppress antibacterial effects, highlighting the critical role of formulation in determining antimicrobial performance. These findings confirm wild oregano oil as a highly effective natural antimicrobial agent and emphasize that both

intrinsic bioactivity and formulation factors govern the efficacy of cosmetic emulsions. The study provides a foundation for developing safe, natural, and potent antimicrobial formulations for applications in cosmetics, food preservation, and alternative therapeutics.

Keywords

Wild Oregano Oil, Antimicrobial Activity, Oil-in-Water Emulsions, Carvacrol, Formulations

1. Introduction

Emulsions are mixtures in which one phase is dispersed in another, and the system is stabilized by an emulsifier. When an emulsion contains a higher proportion of water, the oil is dispersed in the water. This type of emulsion is known as an oil-in-water emulsion (O/W) (See **Figure 1**). Because they contain less oil, these emulsions are considered "lighter". Such creams are sometimes referred to as moisturizing creams and are pleasant for oily skin as "day" creams. The proportion of the oil phase is lower in this type of emulsion. It is usually around 20% - 25%, but it can vary from extremely low oil-phase contents (1% - 2%) up to 30%.

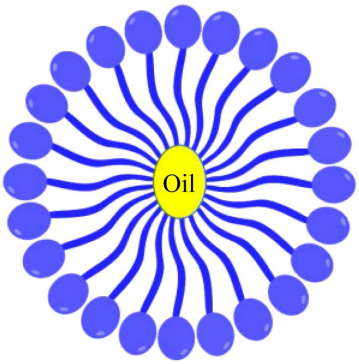


Figure 1. Oil-in-water (O/W) emulsion.

A high content of saturated and unsaturated fatty acids (including oleic, linoleic, lauric and palmitic acid) is fatty acids that give emollient properties when incorporated into topical formulations [1]. Polyunsaturated fatty acids have been used in the production of cosmetics to improve the appearance and health of the skin. Creams enriched with linoleic acid are especially related to reduction of dryness and problems of desquamation, thus providing brightness and softness to the skin [2]. The usage of natural components, including herbal or natural oils, has become more popular in the health and beauty business in recent times due to their low toxicity profile [3]. Certain oils, like jojoba oil, are better than others (such as castor, olive oil, etc.) because they resemble the sebum on human skin

both structurally and chemically [2] [3].

2. Natural Oils

2.1. Jojoba Oil

Jojoba oil is derived from the *Simmondsia chinensis* jojoba seed plant, commonly referred to as desert gold. It is primarily composed of straight-chain monoesters in the C20 - C22 range, with two double bonds present at the ends of the alcoholic and acidic ester linkages. The jojoba plant is native to South-Western North America, and its oil is used in food, medicine, cosmetics, lubricants, electrical insulators, and plasticizers. The oil's wide range of uses in the medical and pharmaceutical industries can be attributed to its special qualities. It is used to treat a variety of skin conditions, such as eczema, seborrheic dermatitis, acne, sores, and inflammation, as well as to make penicillin-G and act as an anesthetic for severe pain [4]. Jojoba oil has anti-aging properties that prevent the emergence of fine lines on the skin. It also stimulates the production of collagen, which leads to cutaneous repair and rejuvenation [5]. Jojoba oil has a refractive index of 1.46, is highly stable and resistant to oxidation both under normal and extreme temperature conditions [6]. The distinctive aroma of medicinal and aromatic plants comes from their essential oils, which are volatile substances with complex chemical compositions. These oils contain various terpene compounds such as alcohols like geraniol and α-bisabolol, ketones like menthone and p-vetivone, aldehydes like citronellal and sinensal, esters like γ-terpinyl acetate and cedryl acetate, and phenols like thymol. They also include non-terpene compounds and aromatic phenylpropanoid derivatives such as eugenol, cinnamaldehyde, and safrole. As secondary metabolites, essential oils play important roles in plants by protecting them from viruses, bacteria, fungi, insects, and herbivores, as well as by attracting pollinators [7]. Essential oils, originally discovered in traditional medicine, contain terpenoids as the main components found in lavender essential oil (Lavandula dentata) and nutmeg essential oil (Myristica fragrans). These are complex mixtures of compounds with significant pharmacological properties that are sensitive to high temperatures, light, and the presence of oxygen. Although primarily used in food and fragrances, they represent compounds with antioxidant, antimicrobial, anti-inflammatory, antitumor, hypoglycemic, anticonvulsant, and insecticidal properties. However, several factors must be considered that hinder their application and biological activity. Low water solubility often limits production and sustainability since they are hydrophobic and exhibit high oil/water distribution coefficients. This limitation restricts their incorporation into hydrophilic products, such as cosmetics. The application of essential oils as a natural antimicrobial agent and their effectiveness in preserving food are currently subjects of extensive research and growing consumer demand. However, the loss of bioactivity of essential oils due to variable environmental conditions is a significant drawback for their practical application. The stability of oils decreases due to oxidation, light exposure, and high temperatures [8].

2.2. Almonds

Almonds (*Prunus dulcis*) are one of the most widely produced nuts worldwide. They are highly valued for their taste, numerous nutritional compounds (lipids, proteins, vitamin E, and polyphenols), and versatility in various productions [9] [10]. Almond oil is rich in fatty acids, proteins, and carbohydrates. Due to its abundance of vitamins and minerals, it is widely used in medicine for health purposes, such as the complex of B vitamins and zinc content found in almond oil, helping maintain healthy skin [11] [12].

2.3. Lavandula

The Lavandula genus belongs to the Lamiaceae family, consisting of many plants and shrubs known for their medicinal or culinary use [13]. The presence of essential oils (EO) makes them valuable plants and spices, holding great importance in the pharmaceutical, food, and cosmetic industries [14]. The use of lavender essential oils in the pharmaceutical industry is significantly limited due to their suboptimal physicochemical characteristics, such as limited water solubility [15]. The pleasant scent of lavender is mainly attributed to the content of monoterpenoids, concentrated mainly in the flowers and other upper parts of the plant. Lavender oils highly valued in cosmetics contain higher concentrations of linalyl acetate and linalool, and low concentrations of camphor [14]. In traditional medicine, lavender is used to treat many ailments and possesses antioxidant, anti-inflammatory, sedative, antidepressant, spasmolytic, anticholinesterase, antifungal, and antibacterial properties [16]. It is known as a therapeutic agent used to alleviate symptoms of psychological stress, insomnia, and digestive disorders, especially in aromatherapy, neuralgia, and as an antiseptic [17]-[19].

2.4. Oregano

Plants from the **Lamiaceae** family, such as *Origanum vulgare* (oregano), are rich in compounds with strong **antioxidant** and **antimicrobial** effects. Oregano is widely used in **medicine, cosmetics, food preservation,** and **traditional healing**. Studies show that **oregano essential oil**, high in **carvacrol** and **thymol**, offers significant **health benefits**, including **anti-inflammatory, anticancer,** and **antimicrobial** properties, making it a valuable natural ingredient for therapeutic and nutritional use [20]. The thymol present in the oregano oil causes odor. Carvacrol and thymol are phenolic compounds known for their strong antimicrobial properties and have been widely investigated for their capacity to suppress the growth of different bacterial species. Each compound is thought to act against bacteria in a comparable way, and when combined, they can produce a synergistic effect [21]. Carvacrol and thymol are strong antimicrobial compounds effective against both Gram-positive and Gram-negative bacteria. Their antimicrobial activity is believed to result from disrupting bacterial cell membranes, causing cell lysis and the leakage of internal materials. These substances can also inhibit efflux pumps, prevent or break down biofilms, and reduce bacterial motility and membrane

ATPase activity. Furthermore, when combined with traditional antibiotics, they often show additive or synergistic effects, which can help address the challenge of antibiotic resistance [22].

Carvacrol, a monoterpenoid phenol and the principal active compound in wild oregano oil (*Origanum vulgare*), is primarily responsible for the strong antimicrobial and antifungal effects observed in this study. The complete inhibition of *Escherichia coli* (ATCC 25922), *Staphylococcus aureus* (ATCC 25923), and *Candida albicans* (ATCC 90028) by wild oregano oil can be attributed to the high concentration of carvacrol, in contrast to the absence of activity in almond and lavender macerate oils. Carvacrol exerts its antimicrobial action by disrupting the integrity of microbial cell membranes, leading to the leakage of essential intracellular components and subsequent cell death. The FTIR spectrum of wild oregano oil revealed characteristic O-H and C-H stretching vibrations, consistent with the presence of phenolic structures such as carvacrol. These findings are in agreement with previous studies reporting the broad-spectrum antibacterial and antifungal activity of carvacrol and its synergistic effects when combined with other antimicrobial agents. Overall, the results confirm that carvacrol is the key bioactive constituent responsible for the potent antimicrobial activity of wild oregano oil, supporting its potential application as a natural preservative and therapeutic agent. Thymol and carvacrol are the primary active compounds in oregano essential oil and are known for their potent antimicrobial properties. Structurally, thymol is very similar to carvacrol (**Figure 2**). Both thymol and carvacrol have the same molecular formula, $C_{10}H_{14}O$, but they differ in the positions of their functional groups on the benzene ring.

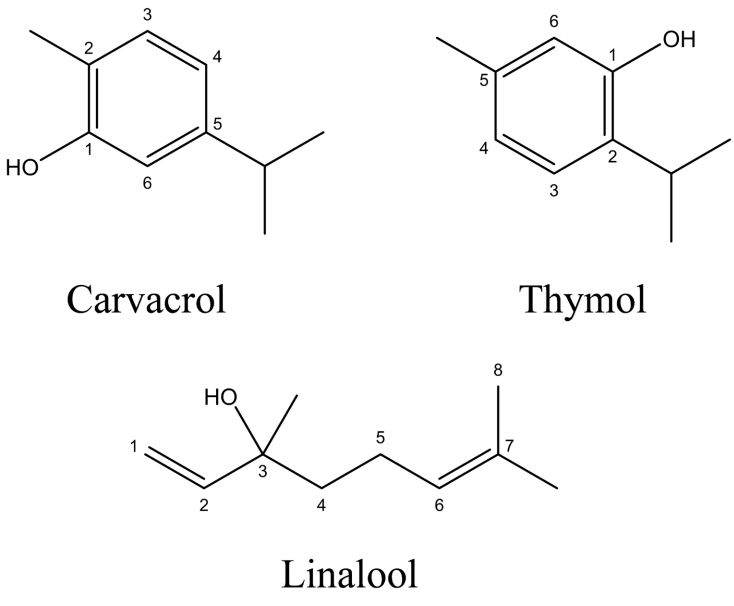


Figure 2. Chemical structures of carvacrol, thymol and linalool.

Both compounds appear to increase the permeability of bacterial cell mem-

branes. In gram-negative bacteria, they can disrupt the outer membrane, release lipopolysaccharides, and enhance cytoplasmic membrane permeability to ATP. In studies with *Bacillus cereus*, carvacrol has been shown to form channels within the fatty acid chains of phospholipids, causing membrane expansion and destabilization [23]. This structural disruption increases membrane fluidity and passive ion leakage from the cytoplasm. The phenolic ring, with its electron instability, plays a critical role in the antibacterial action of these aromatic compounds. Consequently, thymol and carvacrol exhibit strong antibacterial activity against *Salmonella typhimurium* [24]. Their application as food preservatives is often limited due to their strong flavor and aroma. Ideally, these compounds could be used in foods at concentrations low enough to maintain antibacterial effectiveness while minimizing sensory changes.

3. Gram-Positive and Gram-Negative Bacteria

Bacteria are generally classified as Gram-positive or Gram-negative based on their cell wall structure. Gram-positive bacteria, such as *Staphylococcus aureus*, possess thick peptidoglycan layers with embedded teichoic acids, which provide rigidity, a net negative charge, and resistance to environmental stresses [25]. In contrast, Gram-negative bacteria, including *Escherichia coli*, have a thinner peptidoglycan layer and an additional outer membrane containing lipopolysaccharides (LPS). This outer membrane acts as a protective barrier, contributes to the negative surface charge, and can limit the penetration of antimicrobial agents. These structural differences are key to understanding why Gram-positive bacteria are often more susceptible to certain antimicrobials than Gram-negative bacteria, which can exhibit higher resistance due to the protective effect of their outer membrane [25].

4. Experimental Part

4.1. Chemicals

The following chemicals were used in this study: Tween 20 (Fluka) with a viscosity of 250 - 450 mPa·s at 25˚C and an HLB value of 16.7 ± 1.0; Lanette O (cetearyl alcohol); lavender macerate oil prepared at 8 g per 100 mL of almond oil; almond oil; cold-pressed jojoba oil (Maxivita); wild oregano oil (*Origanum minutiflorum*, Probotanic dietary supplement, 100% organic, pure and undiluted); glycerol; aloe vera gel (1:1); and Kem DHA (benzyl alcohol and dehydroacetic acid) as a preservative.

4.2. Accessories and Equipment

The following accessories and equipment were used in the study: disposable gloves; gas cylinder with burner; iridium probe; saline solution; McFarland standard or turbidometer for microbial suspension standardization; reference microbial strains including *Candida albicans* (ATCC 90028), *Escherichia coli* (ATCC 25922), and *Staphylococcus aureus* (ATCC 25923); Mueller-Hinton (MH) agar plates (90 mm diameter); incubator/thermostat with a temperature range of 37˚C

± 1˚C; sterile swabs; and an inhibition zone measuring device.

4.3. Emulsion Preparation Procedure

The oil phase contained, in addition to oils, the surfactant Tween 20 and the co-surfactant Lanette O (cetearyl alcohol, *i.e.*, a mixture of cetyl and stearyl alcohols). The co-surfactant was added to enhance emulsification efficiency. Co-surfactants act as effective thickeners and provide a smoother texture. They have a relatively high HLB value (15.5). Increasing the amount of co-emulsifier results in more viscous emulsions. The aqueous phase, in addition to water, contained glycerol and aloe vera gel (1:1). To prevent oil oxidation and microbial growth, Kem DHA (benzyl alcohol, dehydroacetic acid) was used as a preservative. Emulsifiers used for preparing oil-in-water (O/W) emulsions typically have HLB values in the range of 8 - 18. For emulsion preparation, three different phases were prepared and labeled as phases A, B, and C. Phase A was the oil phase, phase B was the aqueous phase, and phase C consisted of the preservative. The components of phases A and B were weighed into two larger containers, while Kem DHA (phase C) was weighed into a smaller container. The containers with phases A and B were placed in a water bath heated to 70˚C. Phase B was stirred thoroughly to dissolve the glycerol. Phase A was gently stirred to prevent solidification on the container walls and was mixed until Lanette O was completely dissolved in the oil phase. After 1 - 2 minutes in the water bath at 70˚C, Lanette O was fully dissolved. Both containers were then removed from the water bath. The aqueous phase was added to the oil phase while stirring with a glass rod for 1 - 2 minutes. Phase C was then added, and mixing with a hand mixer was started immediately. The mixture was homogenized for 5 minutes (Figure 3). The emulsion was poured into suitable containers and cooled in a refrigerator at 5˚C for 5 minutes. After cooling, the containers were sealed and stored at room temperature in a dark place for further characterization. All samples were sterile.

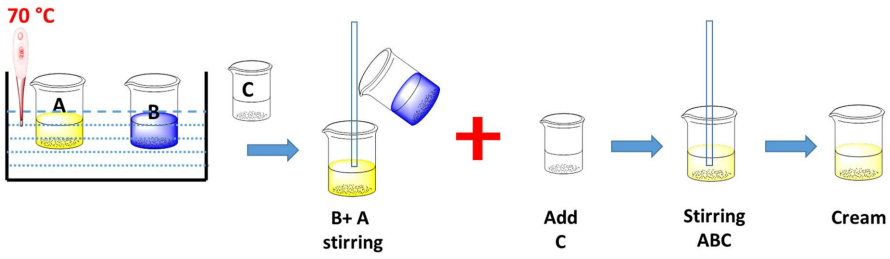


Figure 3. Schematic representation of emulsion synthesis.

Table 1. Formulation of emulsions of the first group.

Composition	E1	E2	E3	E4
Phase A	%	%	%	%
Tween 20	6.06341	5.980582	6.125271	6.010768
Lanette O	4.090978	4.009649	4.061764	4.13366

Continued

ML	8.035443	7.96228	0	0
GJ	7.016047	7.971451	14.96236	15.94307
W. Oregano	1.078561	0	1.040407	0
Phase B	%	%	%	%
Glycerol	2.999025	3.044518	3.050401	3.053088
Aloe V 1:1	5.038217	5.102574	5.002705	5.007081
D. water	65.0399	65.25728	65.16169	65.19583
Phase C	%	%	%	%
Kem DHA	0.638422	0.671664	0.595399	0.65651

*(**E**-denotes the emulsion, **ML**-Lavender macerate, **GJ**-Golden jojoba).

Table 2. Formulation of emulsions of the second group.

Composition	E5	E6	E7	E8
Phase A	%	%	%	%
Tween 20	6.107493	6.001672	6.035845	6.05879
Lanette O	4.038278	4.001644	4.004832	4.159748
A. O.	8.04657	8.018972	15.05521	16.02856
GJ	7.027655	7.987008	0	0
W. Oregano	1.023313	0	1.029105	0
Phase B				
Glycerol	3.021962	3.034587	3.057719	3.032192
Aloe V 1:1	5.02161	4.970091	5.054735	5.013757
D. water	65.123	65.37315	65.11041	65.07774
Phase C				
Kem DHA	0.590119	0.612873	0.65214	0.629217

***(A.O.**-Almond oil, **GJ**-Golden jojoba).

Table 3. Formulation of emulsions of the third group.

Composition	E9	E10	E11
Phase A	%	%	%
Tween 20	6.006397	6.115632	6.01062
Lanette O	4.493339	3.502933	3.998953
A.O.	15.97282	16.01921	16.03698
GJ	0	0	0
W. Oregano	0	0	0
Phase B			
Glycerol	3.083714	3.097518	3.07126
Aloe V 1:1	5.008719	5.023391	0
D. water	64.8168	65.55069	70.28524
Phase C			
Kem DHA	0.618218	0.690626	0.596945

Various formulations of the prepared emulsions are presented in Tables 1-3.

Figure 4 shows the appearance of emulsions (E9, E10, and E11) prepared according to the formulation described in Table 3.

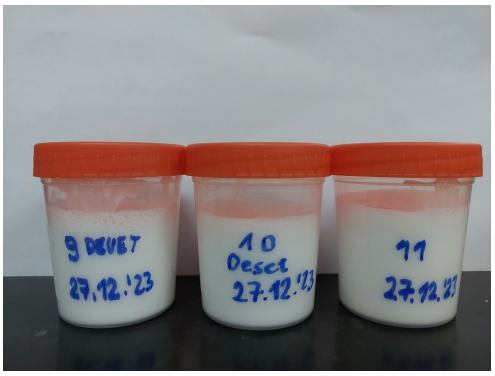


Figure 4. Almond oil-based emulsions without wild oregano oil and without jojoba oil (9, 10 and 11), and different Tween 20 and Lanette O ratio.

5. Results

5.1. Testing the Antibacterial Activity of Almond Oil, Lavender Macerate Oil and Wild Oregano Oil

From the pure bacterial culture of the reference strain *E. coli* (25922), which had previously grown on a solid medium, an inoculum was made in a physiological solution so that it contained 1 – 2 × 10^8 CFU/ml (0.5 McFarland). After 15 minutes, a sterile swab was dipped into the inoculum, and the entire surface of the Muller-Hinton agar was smeared with the swab in all three directions. The plate was left open for a few minutes so that the agar surface could dry. The procedure was repeated a total of 4 times (2 M-H agar with *E. coli* - one for almond oil and one for lavender macerate). Four drops of each oil were applied to the sown and dried surfaces of M-H agar. The inverted M-H substrates were left in a thermostat for incubation for 24 hours at 37˚C. After 24 hours, no zone of inhibition was formed around the seeded almond oil and lavender macerate oil droplets on both M-H agar plates, while complete elimination was recorded around the wild oregano oil droplet. Wild oregano oil was also tested for *S. Aureus* and its complete elimination was also recorded.

5.2. Testing the Antifungal Activity of Almond Oil, Lavender Macerate Oil and Wild Oregano Oil

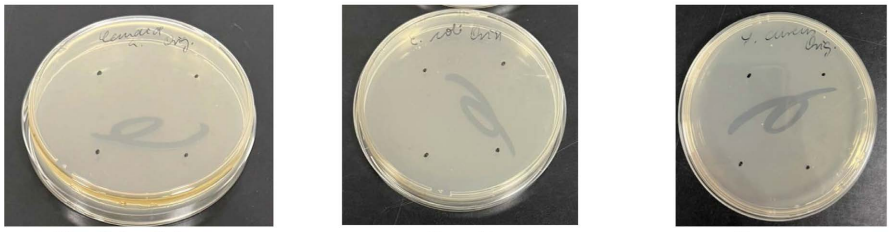
From a pure culture of the representative strain of *Candidae albicans* (ATCC 90028), which had previously grown on a solid medium, an inoculum was made in a saline solution containing 1 × 10^6 to 5 × 10^6 CFU/ml (0.5 MCFarland). After 15 minutes, a sterile swab was dipped into the inoculum, and the entire surface of the Muller-Hinton agar to which 2% glucose and methylene blue were added was smeared in all three directions. The plate was left open for a few minutes to allow the agar surface to dry. After that, 4 drops of each oil were applied to the seeded

and dried surfaces of M-H agar. The inverted M-H substrates were left in a thermostat for incubation for 24 hours at 37˚C. After 24 hours, there was no formation of an inhibition zone around the drops of seeded almond oil and lavender macerated oil on both M-H agars, while complete elimination was recorded around the drop of wild oregano oil. Antibacterial and antifungal properties of almond oil and lavender macerate oil are shown in Figure 5.

a) b)

Figure 5. Zone of inhibition for a) reference strain *E. coli* and b) Candida albicans for almond oil and lavender macerate oil.

Wild oregano oil completely eliminated the presence of all tested reference strains of *Candida albicans*, *E. coli* and *S. aureus* (Figure 6).



Candida albicans E. coli S. aureus

Figure 6. Zone of inhibition for reference strain *Candida albicans*, *E. coli* and *S. aureus* for wild oregano oil.

5.3. Microbial Testing of Emulsions

For the antimicrobial evaluation, selective CPS medium was diluted in two separate test tubes. One tube contained physiological saline with a *Staphylococcus aureus* colony, and the other contained physiological saline with an *Escherichia coli* colony. These suspensions were evenly spread onto Mueller-Hinton agar plates. Each of the 11 emulsions was applied as a single drop onto the agar surface. The plates were divided into quadrants, resulting in a total of three plates for testing the sensitivity of *S. aureus* and three plates for testing the sensitivity of *E. coli*. After 24 hours of incubation, the inhibition zones were measured on all test plates. Sterility of the samples was verified by inoculating each onto non-selective CPS medium. After 24 hours, no microbial growth was observed, indicating that all samples were sterile. For the main antimicrobial assay, two additional dilutions were prepared: 1) Physiological saline with a *Staphylococcus aureus* colony and 2) Physiological saline with an *E. coli* colony. These dilutions were evenly spread

on Mueller-Hinton agar plates, and each of the 11 emulsions was applied as a drop onto the plates. As before, the plates were divided into quadrants, with three plates used for testing *S. aureus* sensitivity and three for *E. coli*. After 24 hours of incubation, inhibition zones were measured. No inhibition zones were observed on the plates inoculated with *E. coli*. Plates inoculated with *S. aureus* showed no inhibition zones for emulsions 1 - 6. All emulsions from 7 to 11 (E7, E8, E9, E 10 and E11) exhibited inhibition zones of 2 - 4 mm against *S. aureus* (a Gram-positive spherical bacterium), while none of the emulsions showed activity against *E. coli*. All tested samples were sterile and microbiologically compliant. The results indicate that almond oil in emulsions in combination of Tween 20 and Lanette O (2:1) exhibits antibacterial activity against *S. aureus* only at concentrations above 15%, and it has no effect on *E. coli*.

5.4. FTIR Characterization

FTIR of lavender macerate oil, almond oil and wild oregano oil are shown in Figures 7-9. Distinct peaks corresponding to O-H, C = O, and C-H stretching are observed, reflecting the unique chemical compositions of each oil. In Figures 7-9 are shown informations about the functional groups present in a lavender macerate oil, almond oil and wild oregano oil. It can be observed peaks around 3200 - 3600 **cm^{-1} for wild oregano oil** which usually indicates O-H (alcohol, phenol) stretching. Peaks near 2850 - 2950 **cm^{-1}** are typical for C-H stretching in alkanes (sp^3 C-H). Sharp strong peak at 1743 **cm^{-1}** is characteristic of a carbonyl group (C = O), so it comes from **esters**. Presence of the peak at 1463 cm^{-1} can indicate **C = C stretching** (aromatic ring) or **C-H bending. Peak at 1377 cm^{-1}** indicate **methyl group bending (CH_3)** or other alkyl groups. Peaks between 1000 - 1300 **cm^{-1}** indicate **C-O stretching**, typical for **esters**. In the **fingerprint region, below 1000 cm^{-1}** peaks are specific to each molecule and often indicate **C-H bending in long chains.** The spectrum is consistent with a molecule that has **long alkyl chains with an ester functional group**, such as a fatty acid ester.

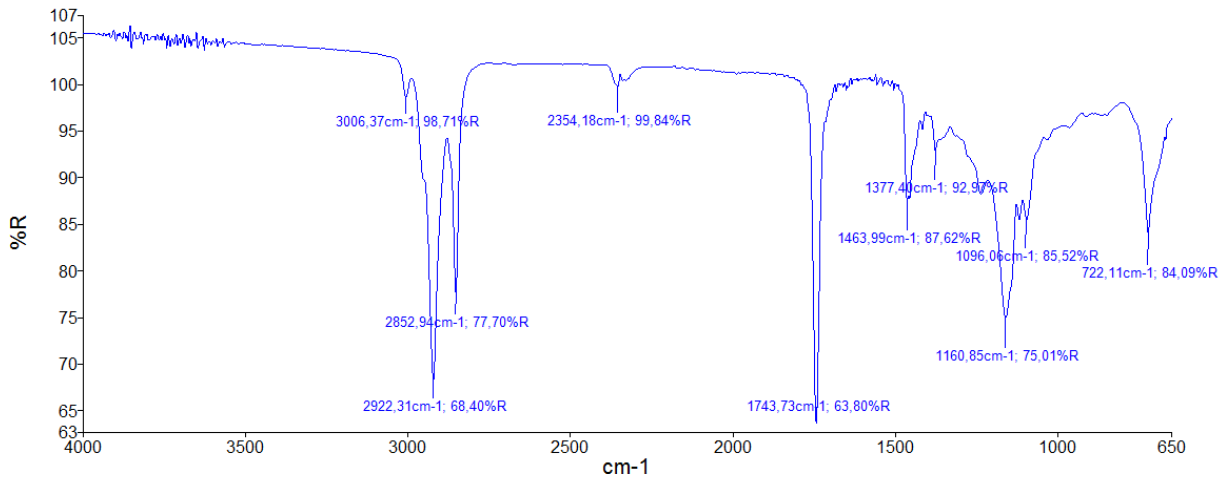


Figure 7. FTIR of lavender macerate oil.

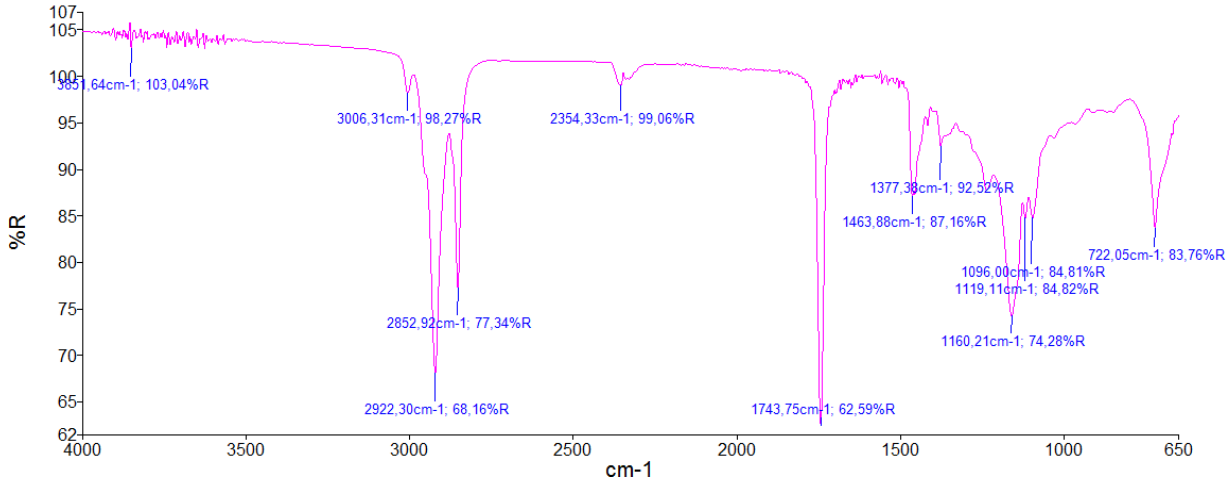


Figure 8. FTIR of almond oil.

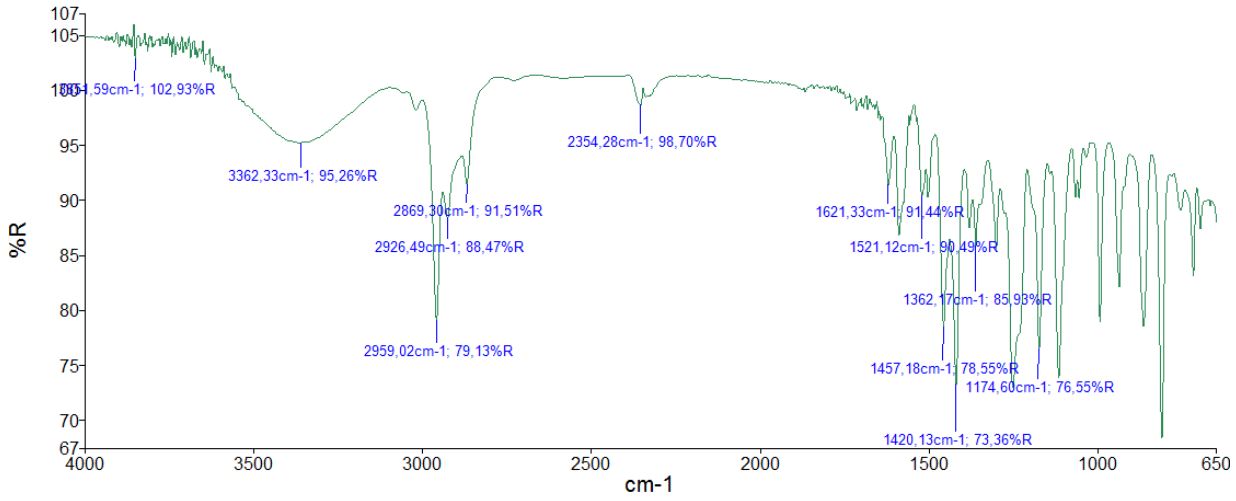


Figure 9. FTIR of wild oregano oil.

6. Discussion

Terpenoids are the main active compounds in nutmeg (Myristica fragrans) and lavender (Lavandula dentata) essential oils, traditionally recognized in medicinal practices. They exhibit antibacterial, anti-inflammatory, anticancer, hypoglycemic, anticonvulsant, and insecticidal activities, although their primary applications remain in food and fragrance industries. The antibacterial activity of essential oils is largely attributed to their ability to disrupt microbial cell membranes [26].

The outer membrane of bacteria serves as a protective barrier, contributes to the negative surface charge, and can limit the penetration of antimicrobial agents. These structural differences help explain why Gram-positive bacteria are often more susceptible to certain antimicrobials, whereas Gram-negative bacteria can exhibit higher resistance due to the additional protective effect of their outer membrane.

In this study, wild oregano oil (*Origanum vulgare*) demonstrated significant antimicrobial activity against a range of pathogens, including *Escherichia coli*, *Staphylococcus aureus*, and *Candida albicans*. This activity is likely attributable to its major bioactive compounds, carvacrol and thymol.

Tao *et al.* (2025) [27] found that carvacrol, the primary bioactive compound in oregano oil, exhibits potent antibacterial effects. Studies have reported minimum inhibitory concentrations (MICs) for carvacrol against *E. coli* and *S. aureus* in the range of 0.005 - 0.04 mg/mL. The mechanism of action involves disruption of the bacterial cell membrane, leading to leakage of cellular contents and cell death. Additionally, oregano oil has shown synergistic effects when combined with antibiotics like tobramycin, enhancing its efficacy against resistant strains. Oregano oil also exhibits antifungal properties, particularly against *C. albicans*. Research indicates that the combination of oregano oil and vitamin C significantly reduces MIC and minimum fungicidal concentrations (MFCs) against *C. albicans*, suggesting a synergistic effect. This combination enhances the antifungal activity, potentially offering a therapeutic approach for fungal infections [28]. This study demonstrates that wild oregano oil (*Origanum vulgare*) exhibits strong antimicrobial and antifungal activity, attributable to its high carvacrol content, which disrupts microbial cell membranes and leads to cell death. In contrast, almond oil and lavender macerate oil showed no significant antimicrobial effects, likely due to their limited water solubility and sensitivity to heat, light, and oxygen. Emulsions containing almond oil above 15% combined with Tween 20 and Lanette O (2:1) displayed only limited activity against *Staphylococcus aureus*, with no effect on *Escherichia coli*. We hypothesized that the limited antibacterial effect arises from the surfactant system rather than from the oil itself. The future studies should include an appropriate control emulsion without active compounds to directly assess and confirm the influence of the surfactant system on antibacterial activity. Furthermore, we assume that emulsions containing jojoba oil may suppress antibacterial effects due to potential interactions between jojoba oil and the surfactant system, which could influence the availability or activity of the bioactive compounds. The absence of activity against *E. coli*, a Gram-negative bacterium, highlights the protective role of its outer membrane. These findings underscore that the antimicrobial efficacy of emulsions depends both on the intrinsic activity of bioactive oils, such as wild oregano oil, and on formulation factors, emphasizing the need to carefully design natural formulations for effective antimicrobial performance.

From a practical formulation perspective, these findings demonstrate that the mere incorporation of a potent antimicrobial agent does not guarantee antimicrobial efficacy in an emulsion system. The overall performance is strongly formulation-dependent, and careful optimization of the entire emulsion matrix including the selection of oils, surfactants, and their potential interactions is critical to preserve the bioactivity of the active compound and to ensure its effective delivery. This highlights the importance of rational formulation design in cosmetic and therapeutic applications, where inappropriate excipient choices may significantly

attenuate the intended antimicrobial effect.

7. Conclusion

This study demonstrates that wild oregano oil (*Origanum vulgare*) possesses strong antibacterial and antifungal activity against *Escherichia coli*, *Staphylococcus aureus*, and *Candida albicans*, primarily due to its major bioactive compound, carvacrol. In contrast, almond oil and lavender macerate oil showed no significant antimicrobial activity, although they retain therapeutic and cosmetic value. Emulsion testing revealed that only emulsions (E7, E8, E9, E10, and E11) containing almond oil at concentrations above 15% in combination of Tween 20 and Lanette O (2:1) exhibit exhibited limited antibacterial activity against *S. aureus*, with no effect on *E. coli*. All tested samples were sterile and microbiologically compliant. These findings highlight the potential of wild oregano oil as a natural antimicrobial and antifungal agent for applications in food preservation, cosmetics, and alternative therapeutics.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

References

[1] Sousa, G.D., Kishishita, J., Aquino, K.A.S., Presgrave, O.A.F., Leal, L.B. and Santana, D.P. (2016) Biopharmaceutical Assessment and Irritation Potential of Microemulsions and Conventional Systems Containing Oil from *Syagrus cearensis* for Topical Delivery of Amphotericin B Using Alternative Methods. *AAPS PharmSciTech*, **18**, 1833-1842. https://doi.org/10.1208/s12249-016-0663-3

[2] Boucetta, K.Q., Charrouf, Z., Derouiche, A., Rahali, Y. and Bensouda, Y. (2014) Skin Hydration in Postmenopausal Women: Argan Oil Benefit with Oral and/or Topical Use. *Menopausal Review*, **5**, 280-288. https://doi.org/10.5114/pm.2014.46470

[3] Chanchal, D. and Swarnlata, S. (2008) Novel Approaches in Herbal Cosmetics. *Journal of Cosmetic Dermatology*, **7**, 89-95. https://doi.org/10.1111/j.1473-2165.2008.00369.x

[4] Kim, Y., Lee, M.S., Yang, Y.S. and Hur, M. (2011) Self-Aromatherapy Massage of the Abdomen for the Reduction of Menstrual Pain and Anxiety during Menstruation in Nurses: A Placebo-Controlled Clinical Trial. *European Journal of Integrative Medicine*, **3**, e165-e168. https://doi.org/10.1016/j.eujim.2011.08.007

[5] Adejokun, D.A. and Dodou, K. (2020) A Novel Method for the Evaluation of the Long-Term Stability of Cream Formulations Containing Natural Oils. *Cosmetics*, **7**, Article 86. https://doi.org/10.3390/cosmetics7040086

[6] Kubitschek-KM, A.R. and Zero, J.M. (2014) Development of Jojoba Oil (*Simmondsia chinensis* (Link) CK Schneid.) Based Nanoemulsions. *Latin American Journal of Pharmacy*, **33**, 459-463.

[7] Dhifi, W., Bellili, S., Jazi, S., Bahloul, N. and Mnif, W. (2016) Essential Oils' Chemical Characterization and Investigation of Some Biological Activities: A Critical Review. *Medicines*, **3**, Article 25. https://doi.org/10.3390/medicines3040025

[8] Cossetin, L.F., Garlet, Q.I., Velho, M.C., Gündel, S., Ourique, A.F., Heinzmann, B.M., *et al.* (2021) Development of Nanoemulsions Containing *Lavandula dentata* or

Myristica fragrans Essential Oils: Influence of Temperature and Storage Period on Physical-Chemical Properties and Chemical Stability. *Industrial Crops and Products*, **160**, Article ID: 113115. https://doi.org/10.1016/j.indcrop.2020.113115

[9] Furlan Goncalves Dias, F., Huang, Y., Schauer, J., Barile, D., Van de Water, J. and Leite Nobrega de Moura Bell, J.M. (2023) Effects of Protease-Assisted Aqueous Extraction on Almond Protein Profile, Digestibility, and Antigenicity. *Current Research in Food Science*, **6**, Article ID: 100488. https://doi.org/10.1016/j.crfs.2023.100488

[10] Li, J.N., Henning, S.M., Thames, G., Bari, O., Tran, P.T., Tseng, C., *et al.* (2021) Almond Consumption Increased UVB Resistance in Healthy Asian Women. *Journal of Cosmetic Dermatology*, **20**, 2975-2980. https://doi.org/10.1111/jocd.13946

[11] Rinaldi, F., Hanieh, P.N., Maurizi, L., Longhi, C., Uccelletti, D., Schifano, E., *et al.* (2022) Neem Oil or Almond Oil Nanoemulsions for Vitamin E Delivery: From Structural Evaluation to *in Vivo* Assessment of Antioxidant and Anti-Inflammatory Activity. *International Journal of Nanomedicine*, **17**, 6447-6465. https://doi.org/10.2147/ijn.s376750

[12] Faghihi, A., Zohalinezhad, M.E. and Najafi Kalyani, M. (2022) Comparison of the Effects of Abdominal Massage and Oral Administration of Sweet Almond Oil on Constipation and Quality of Life among Elderly Individuals: A Single-Blind Clinical Trial. *BioMed Research International*, **2022**, Article ID: 9661939. https://doi.org/10.1155/2022/9661939

[13] Crișan, I., Ona, A., Vârban, D., Muntean, L., Vârban, R., Stoie, A., *et al.* (2023) Current Trends for Lavender (*Lavandula angustifolia* Mill.) Crops and Products with Emphasis on Essential Oil Quality. *Plants*, **12**, Article 357. https://doi.org/10.3390/plants12020357

[14] Habán, M., Korczyk-Szabó, J., Čerteková, S. and Ražná, K. (2023) Lavandula Species, Their Bioactive Phytochemicals, and Their Biosynthetic Regulation. *International Journal of Molecular Sciences*, **24**, Article 8831. https://doi.org/10.3390/ijms24108831

[15] Miastkowska, M., Sikora, E., Kulawik-Pióro, A., Kantyka, T., Bielecka, E., Kałucka, U., *et al.* (2023) Bioactive *Lavandula angustifolia* Essential Oil-Loaded Nanoemulsion Dressing for Burn Wound Healing. *In Vitro* and *in Vivo* Studies. *Biomaterials Advances*, **148**, Article ID: 213362. https://doi.org/10.1016/j.bioadv.2023.213362

[16] D'Auria, F.D., Tecca, M., Strippoli, V., Salvatore, G., Battinelli, L. and Mazzanti, G. (2005) Antifungal Activity of *Lavandula angustifolia* Essential Oil Against *Candida albicans* Yeast and Mycelial Form. *Medical Mycology*, **43**, 391-396. https://doi.org/10.1080/13693780400004810

[17] Dobros, N., Zawada, K.D. and Paradowska, K. (2022) Phytochemical Profiling, Antioxidant and Anti-Inflammatory Activity of Plants Belonging to the Lavandula Genus. *Molecules*, **28**, Article 256. https://doi.org/10.3390/molecules28010256

[18] Batiha, G.E., Teibo, J.O., Wasef, L., Shaheen, H.M., Akomolafe, A.P., Teibo, T.K.A., *et al.* (2023) A Review of the Bioactive Components and Pharmacological Properties of Lavandula Species. *Naunyn-Schmiedeberg's Archives of Pharmacology*, **396**, 877-900. https://doi.org/10.1007/s00210-023-02392-x

[19] Firoozeei, T.S., Feizi, A., Rezaeizadeh, H., Zargaran, A., Roohafza, H.R. and Karimi, M. (2021) The Antidepressant Effects of Lavender (*Lavandula angustifolia* Mill.): A Systematic Review and Meta-Analysis of Randomized Controlled Clinical Trials. *Complementary Therapies in Medicine*, **59**, Article ID: 102679. https://doi.org/10.1016/j.ctim.2021.102679

[20] Nurzyńska-Wierdak, R. and Walasek-Janusz, M. (2025) Chemical Composition, Bi-

ological Activity, and Potential Uses of Oregano (*Origanum vulgare* L.) and Oregano Essential Oil. *Pharmaceuticals*, **18**, Article 267. https://doi.org/10.3390/ph18020267

[21] Zhou, F., Ji, B., Zhang, H., Jiang, H., Yang, Z., Li, J., *et al.* (2007) Synergistic Effect of Thymol and Carvacrol Combined with Chelators and Organic Acids against Salmonella Typhimurium. *Journal of Food Protection*, **70**, 1704-1709. https://doi.org/10.4315/0362-028x-70.7.1704

[22] Kachur, K. and Suntres, Z. (2019) The Antibacterial Properties of Phenolic Isomers, Carvacrol and Thymol. *Critical Reviews in Food Science and Nutrition*, **60**, 3042-3053. https://doi.org/10.1080/10408398.2019.1675585

[23] Ultee, A., Kets, E.P.W., Alberda, M., Hoekstra, F.A. and Smid, E.J. (2000) Adaptation of the Food-Borne Pathogen Bacillus Cereus to Carvacrol. *Archives of Microbiology*, **174**, 233-238. https://doi.org/10.1007/s002030000199

[24] Ultee, A., Bennik, M.H.J. and Moezelaar, R. (2002) The Phenolic Hydroxyl Group of Carvacrol Is Essential for Action against the Food-Borne Pathogen *Bacillus cereus*. *Applied and Environmental Microbiology*, **68**, 1561-1568. https://doi.org/10.1128/aem.68.4.1561-1568.2002

[25] Tetteh, J.N.A., Matthäus, F. and Hernandez-Vargas, E.A. (2020) A Survey of Within-Host and Between-Hosts Modelling for Antibiotic Resistance. *Biosystems*, **196**, Article ID: 104182. https://doi.org/10.1016/j.biosystems.2020.104182

[26] Bratovcic, A. (2024) Antibacterial Properties of Nanoemulsions Based on Almond or Lavender Essential Oils. *Acta Scientifci Nutritional Health*, **8**, 8-15. https://doi.org/10.31080/asnh.2024.08.1406

[27] Tao, L., Liang, Y., Xia, Z., Wang, X., Wang, X., Chao, Z., *et al.* (2025) Antibacterial Activities of Oregano Essential Oils and Their Active Components. *Frontiers in Pharmacology*, **16**, Article ID: 1579283. https://doi.org/10.3389/fphar.2025.1579283

[28] Stamova, S., Ermenlieva, N., Tsankova, G., Nikolova, S.P. and Georgieva, E. (2025) Synergistic Antimicrobial Activity of Essential Oils and Vitamin C: Mechanisms, Molecular Targets and Therapeutic Potential. *Applied Sciences*, **15**, Article 4294. https://doi.org/10.3390/app15084294