

## Determination of antifungal and antimicrobial properties of cream enriched with oils of different medicinal aromatic herbs

### Farklı tıbbi aromatik bitkilere ait yağlar ile zenginleştirilmiş kremlerin antifungal ve antimikrobiyal özelliklerinin belirlenmesi

Beril ATMACA<sup>1\*</sup>, Tuğba DERİCİ<sup>1</sup>, Tolga DURAN<sup>1</sup>, Ahmet ATMACA<sup>1</sup>, İbrahim SAVAS<sup>1</sup>

<sup>1</sup>Mercan Kimya San. Ve Tic. A.Ş. Denizli, Turkey.

beril.emek@mercankimya.com.tr, tugba.derici@mercankimya.com.tr, tolga.duran@mercankimya.com.tr, ahmet.atmaca@mercankimya.com.tr, ibrahim.savas@mercankimya.com.tr

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#### Abstract

The need for therapeutic aromatic herbs is expanding as a result of the emergence of worldwide antibiotic resistance. The literature on the advantages of using infusions or essential oils from these plants is growing. For decades, thyme, sage, and lavender have been popular sources of essential oils and are commonly employed in modern medicine. The goal was to create a cream formulation to facilitate the application of these oils, with the hope that it would be favored in terms of dosage and sensory acceptability. In this case, Lavender, Sage, and Thyme oils were incorporated into cream formulations in advance. Antimicrobial and antifungal actions of essential oils at 5% and 10% ratios were tested for this purpose, and then they were added to the cream formulation, and the same study was repeated. This study also considered stability and consumer acceptance. The findings revealed that lavender essential oil had no impact at any dosage, while sage and thyme essential oils exhibited a selective effect. Lavender and sage showed little heterogeneity in terms of stability in cream formulations; however, thyme oil was deemed unacceptable not only in terms of texture but also in sensory aspects. Clearly, the search for new medications continues, and antimicrobial resistance caused by each new formulation is a global issue. Although resistance to essential oils has not yet been recorded, research is being conducted with the hope that they might offer a solution to global resistance-related illnesses. In this context, the findings of this study, encompassing not only a cream formulation but also data on the use of essential oils with a guiding methodology in the literature, are amenable to clinical investigation.

**Keywords:** Medicinal plants, Essential oils, cream, antifungal, stabilization

#### Öz

Küresel antibiyotik direncinin tetiklenmesine bağlı olarak tıbbi aromatik bitkilere yönelim artmaktadır. Söz konusu bitkilerin infüzyon veya esansiyel yağlarının kullanımından elde edilen faydalar literatürde giderek artmaktadır. Kekik, adaçayı ve lavanta popüler esansiyel yağ kaynakları olarak yüzyıllardır bilinmekle beraber modern tıpta da yaygın kullanımı mevcuttur. Bu yağların kullanımında kolaylık olması için krem formülü dizayn edilmesi hedeflenmiştir. Böylece dozlama ve duyuşsal kabul edilebilirlik açısından tercih edilmesi sağlanmak istenmiştir. Bu kapsamda Lavanta, Adaçayı ve Kekik yağları önceden krem formülasyonu haline getirilmek istenmiştir. Bu amaçla % 5, 10 oranlarında esansiyel yağlarda antimikrobiyal ve antifungal etki çalışılmış daha sonra bunlar krem formülasyonuna eklenerek yeniden aynı çalışma yürütülmüştür. Bu çalışmada aynı zamanda stabilizasyon ve tüketici kabul edilebilirliği de göz önüne alınmıştır. Sonuçlara bakıldığında tüm dozlarda lavantanın etkisinin olmadığı buna karşılık adaçayı ve kekik esansiyel yağlarının seçkin etkisi olduğu gözlemlenmiştir. Krem formülasyonlarında lavanta ve adaçayı stabilizasyon açısından değişkenlik göstermezken, kekik yağı sadece tekstür açısından değil duyuşsal olarak da kabul edilebilirlik sınırının dışındaydı. Aşikârdır ki, yeni ilaç arayışları devam etmekte olup elde edilen her yeni formülün tetiklediği antimikrobiyal direnç küresel bir problemdir. Esansiyel yağlara karşı direnç tetiklenmesi henüz rapor edilmemiş olup, küresel dirençli enfeksiyonlara bir çıkış olabileceği düşüncesi ile çalışmalar yaygınlaşmaktadır. Bu kapsamda, sadece bir krem formülasyonu değil aynı zamanda literatürde rehber bir yaklaşım ile esansiyel yağların kullanımına dair veri sunulmuş olan bu çalışmanın çıktılarını klinik süreçlerde değerlendirilmeye açtık.

**Anahtar kelimeler:** Tıbbi Aromatik Bitki, Esansiyel yağlar, Krem, Antifungal, Stabilizasyon

## 1 Introduction

Between 1978 and 1988, relevant publications on medicinal plants were published, building a list of 75 species in which the authors established the activity of the extract as well as both the scope of and the concepts behind this activity (Recio and others, 2005). The research revealed that phenolics are the most prevalent active chemical in these plants, which has more impact on survival of Gram positive bacteria (Pelletier and others, 1976).

Essential oils (EOs) are plant secondary metabolites that have a characteristic smell, flavor, or both. More than 17,500 plant species from several angiosperm families, including *Lamiaceae*, *Rutaceae*, *Myrtaceae*, *Zingiberaceae*, and *Asteraceae*, generate EOs, although only around 300 of them are marketed (Mérillon

and others, 2018). The chemical structures of the most prevalent molecules in essential oils have been linked to antifungal actions (Özcan and others, 2000).

Many studies on the antibacterial activities of different spices and compounds have been undertaken throughout the years. Sage, thyme, oregano, and savory are fragrant herbs made out of the leafy parts of plants. *Lamiaceae* family, and are commonly used in the preparation of meat, sausage, poultry, fish, and culinary products. They are mostly utilized in cuisines nowadays for their flavors and fragrances. Several spices and plants have been shown to have antibacterial properties (Özcan and others, 2000).

Thyme has been used as an antimicrobial, gastrointestinal system and with other health benefits and it is widely used

\*Corresponding author/Yazışılan Yazar

globally (Almanea and others, 2019). Thyme contains antibacterial characteristics, which is why it is frequently used to treat infections and disorders caused by bacteria and fungus, such as *Escherichia coli* (*E. coli*). According to research, thyme essential oil has the ability to combat antibiotic resistant strains of several types of bacteria. It has the ability to eliminate microorganisms both inside and outside the body. Thyme tea is also used to disinfect the skin and other surfaces (Firdous and others, 2020).

Lavender oil possesses anti-inflammatory, antiseptic, antibacterial, antifungal, antimicrobial, and antidepressant effects. This plant promotes urine production and improves digestion, reduces emotional tension and anxiety, heals burns and wounds and improves sleep, cures eczema and psoriasis, reduces acne and improves skin color. Lavender is also used in aromatherapy (Sharma and others, 2019).

Sage leaves and essential oil have carminative, antispasmodic, antiseptic, astringent, and antihydrotic effects. Monoterpenes (e.g.,  $\alpha$ - and  $\beta$ - thujone, 1,8-cineole, camphor), diterpenes (e.g., carnosic acid), triterpenes (oleanic and ursolic acids), and phenolic compounds (e.g., rosmarinic acid) are all examples of terpenes. Sage essential oil and preparations are used topically to treat inflammations and infections of the throat and mouth mucous membranes (stomatitis, gingivitis, and pharyngitis). The essential oil is used internally to alleviate dyspeptic symptoms and excessive perspiration (Raal and others, 2007; Abu-Darwish and others, 2013; Lamaison and others, 1991; Cuvelier and others, 1994; Lawrence, 1983).

For a satisfactory patient outcome, all significant fungal infections require proper antifungal medication. Because there are just a few classes of antifungal medications available, the establishment of resistance to single drug classes, and increasingly multidrug resistance, significantly complicates patient care. Drug-induced cellular stress increases adaptation, which contributes to breakthrough resistance. Drug exposure also contributes to the development of resistance. When a patient fails to respond or no longer responds to a medicine delivered at a regular dose, this is referred to as therapeutic failure. Various host, pharmacological, and microbial variables contribute to such failures; for example, individuals with a weakened immune system are more likely to fail to react to therapy because the antifungal medicine is fighting the infection without the aid of a powerful immune response (Perlin and others, 2017).

In this study different medicinal plants including Thyme, sage and lavender oils were obtained and added in to cream formulation to determine the antimicrobial and antifungal activity. Additionally, the stability of the formulation was also held to determine the shelf life of the product and consumer acceptability was taken into account.

## 2 Materials and methods

The materials used in the production of the cream in this study are given in Table 1.

Table 1. Ingredients and formulation of base cream.

Chemical Name	Phase	Usage Amount/Solubility
Cetyl Stearyl Alcohol 50/50	A	Usage amount: 1-10% oil based.
Glyceryl Monostearate - Gms 40 Se	A	Usage amount: 1-10% oil based.
Cetareth 25	A	Usage amount: 1-10% oil based
Dimethicone 100 cps	A	Hair Care : 0.5-40% Skin Care: 0.5-40% In Makeup Products: 0.5-30% Sun Care: 1-40% /Deodorants: 0.5-20%
Isopropyl Myristate	A	1-15%
Caprylic Capric Triglyceride	A	1-15%
Phenoxyethanol (and) Caprylyl glycol (and) Decylene glycol	D	0.6 - 1% Miscibility: Not miscible in water Miscible in alcohol and glycol max. recommended level
Glycerin	B	1-20%
Carbomer	C	Usage Amount: 1-2 % Solubility: Water Soluble
Deionized Water	B and C	
Vaseline	A	
Vegetable Oil	D	

### 2.1 Methods

- Characterization of medicinal aromatic herbs oils

The herb-extracted oils used in this study were analyzed for composition at the Batı Akdeniz Agricultural Research Institute laboratory. For this purpose, among the submitted oils, we examined the following groups (Table 2. and Table 3.) which are of particular importance for antibacterial efficacy. It is therefore included in cream formulations.

- Determination of the cream's optimal mix formulation

Three different formulas have been developed to ensure consumer satisfaction and the oils derived from the aromatic herbs used can be extracted at minimum speed and maximum power. Shown in Table 1. A formula has been identified that minimizes interactions with oils derived from medicinal

aromatic herbs, yet does not lose potency (COSMER Kimya San. Ve Tic. A.Ş. Yayınları; 2020).

• Creation of cream formulation

When making the cream, it was produced in four separate stages. These phases are outlined in Table 1. Here are the relevant steps: Phase A is heated to 75-80 °C through mixing. Phase B is also heated to 75-80 °C. Slowly add Phase A to Phase B using a homogenizer. The temperatures of the two phases should match during this addition. The homogenizer should be turned off, allowing the temperature to drop to about 40 °C. (At this point, the mixer and anchors should be running.) Phase C is created by dissolving carbomer in a specified amount of water, and when the temperature drops to 40 °C, Phase C is added. Add the final Phase D and mix for an additional 15 minutes. Oils derived from medicinal aromatic plants are added to the specified cream formula at concentrations of 1%, 5%, and 10%, respectively. The prepared creams were placed in sterile single-use 40-gram plastic containers and stored at room temperature for further analysis.

• Antifungal and antimicrobial tests

The microorganisms to be tested were incubated in trypticase soy broth for two hours at 37 °C. Once turbidity developed, a standard turbidity was achieved by adjusting to McFarland 0.5 (eight log colony forming units/mL). A sample from this suspension was taken using a sterile swab and then inoculated onto the surface of Mueller-Hinton agar. Subsequently, discs containing different oils or creams were placed onto the agar surface using sterile forceps. Care was taken to ensure a distance of 22 mm between the discs and 14 mm from the edge of the petri dish to prevent overlapping of the zones to be formed. The media were then incubated for 18-24 hours at 27-35 °C, and the resulting inhibition zones were measured using a ruler.

Table 2. Antimicrobial effects of pure oils

	Essential Oils	Sage Oil	Lavender Oil	Thyme Oil 5%	Thyme Oil 10%	Sage Oil 5%	Sage Oil 10%
Enterobacteriaceae	<i>Salmonella Enteritidis</i>	8	2	12	12	10	14
	<i>Campylobacter jejuni</i>	11	3	13	11	13	15
	<i>Shigella flexneri</i>	6	2	12	11	4	7
	<i>Cronobacter sakazakii</i>	7	2	12	10	9	14
	<i>E.coli</i> O157	8	1	11	12	12	9
	<i>Enterococcus faecalis</i>	9	3	9	9	5	11
	<i>Vibrio parahaemolyticus</i>	8	1	10	10	15	12

	Essential Oils	Sage Oil	Lavender Oil	Thyme Oil 5%	Thyme Oil 10%	Sage Oil 5%	Sage Oil 10%
Coliform	<i>Escherichia coli</i>	10	5	13	10	10	12
	<i>Klebsiella pneumoniae</i>	9	4	12	13	9	15
	<i>Enterococcus faecium</i>	8	4	12	9	12	14
Antibiotic Resistant	Vancomycin resistant <i>Enterococcus faecium</i>	8	2	13	9	9	15
	Methicillin resistant <i>S. aureus</i>	10	3	11	12	4	8
Sporeformers	<i>Bacillus subtilis</i>	7	4	14	14	3	4
	<i>Bacillus cereus</i>	7	3	10	11	5	6
	<i>Geobacillus stearothermophilus</i>	6	2	10	12	4	5
Soilborne	<i>Listeria monocytogenes</i>	9	4	13	13	5	9
	<i>Pseudomonas aeruginosa</i>	10	1	10	12	12	8
	<i>Proteus mirabilis</i>	11	2	12	13	12	11
	<i>Acinetobacter baumannii</i>	10	3	10	13	9	13
Toxigenic bacteria	<i>Staphylococcus aureus</i>	11	2	13	11	15	12
	<i>Clostridium perfringens</i>	5	3	9	9	4	5
Toxigenic fungus	<i>Aspergillus brasiliensis</i>	6	1	10	11	12	9
	<i>Aspergillus niger</i>	6	1	10	10	9	12
	<i>Penicillium commune</i>	7	1	9	9	12	9
	Yeast	<i>Saccharomyces cerevisiae</i>	7	1	14	11	13

\*Units are mm.

Green: Sensitive (> 5 mm)

Yellow: Intermittans (5 mm)

Red: Resistant (< 5 mm)

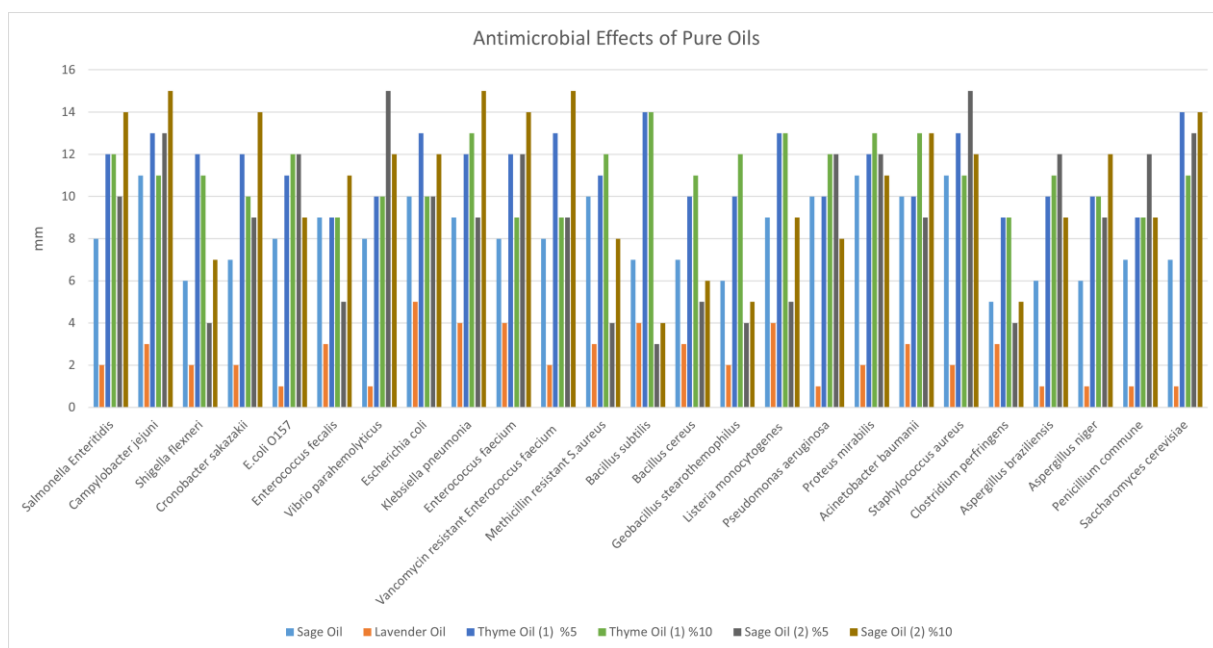


Figure 1. Antimicrobial effects of pure oils

Table 3. Antimicrobial effects of cream formulations

Cream Formulations	Enterobacteriaceae						Coliform			Antibiotic resistant		Sporeformers		Soilborne			Toxigenic bacteria		Toxigenic fungus		Yeast				
	<i>Salmonella Enteritidis</i>	<i>Campylobacter jejuni</i>	<i>Shigella flexneri</i>	<i>Cronobacter sakazakii</i>	<i>E.coli</i> O157	<i>Enterococcus faecalis</i>	<i>Vibrio parahaemolyticus</i>	<i>Escherichia coli</i>	<i>Klebsiella pneumoniae</i>	<i>Enterococcus faecium</i>	<i>Vancomycin resistant Enterococcus faecium</i>	<i>Methicillin resistant S.aureus</i>	<i>Bacillus subtilis</i>	<i>Bacillus cereus</i>	<i>Geobacillus stearothermophilus</i>	<i>Listeria monocytogenes</i>	<i>Pseudomonas aeruginosa</i>	<i>Proteus mirabilis</i>	<i>Achromobacter baumannii</i>	<i>Staphylococcus aureus</i>	<i>Clostridium perfringens</i>	<i>Aspergillus brasiliensis</i>	<i>Aspergillus niger</i>	<i>Penicillium commune</i>	<i>Saccharomyces cerevisiae</i>
Cream formula with lavender oil (5%)	4	4	1	2	4	4	4	3	4	1	2	1	3	3	1	4	3	4	4	1	4	2	1	2	2
Cream formula with lavender oil (10%)	2	2	5	2	1	5	3	4	1	1	2	4	1	5	3	5	3	1	4	2	1	1	2	1	2
Cream formula with sage oil (5%)	7	4	5	3	6	3	7	5	7	4	3	7	3	4	6	5	5	4	6	7	5	4	4	6	5
Cream formula with sage oil (10%)	6	6	5	5	8	8	6	6	7	7	7	6	5	4	9	5	7	6	7	7	6	7	6	6	8
Cream formula with thyme oil (5%)	7	5	8	4	6	11	4	5	9	8	11	2	1	3	2	4	11	9	8	6	4	8	9	10	6
Cream formula with thyme oil (10%)	9	5	6	5	11	4	7	10	4	5	7	2	2	4	2	5	9	9	6	10	5	11	7	6	10
Control Sample	2	1	1	1	3	1	2	2	2	1	3	3	2	3	3	2	1	2	1	1	3	1	2	1	1

\*Units are mm.

Green: Sensitive (> 5 mm)

Yellow: Intermitans (5 mm)

Red: Resistant (< 5 mm)

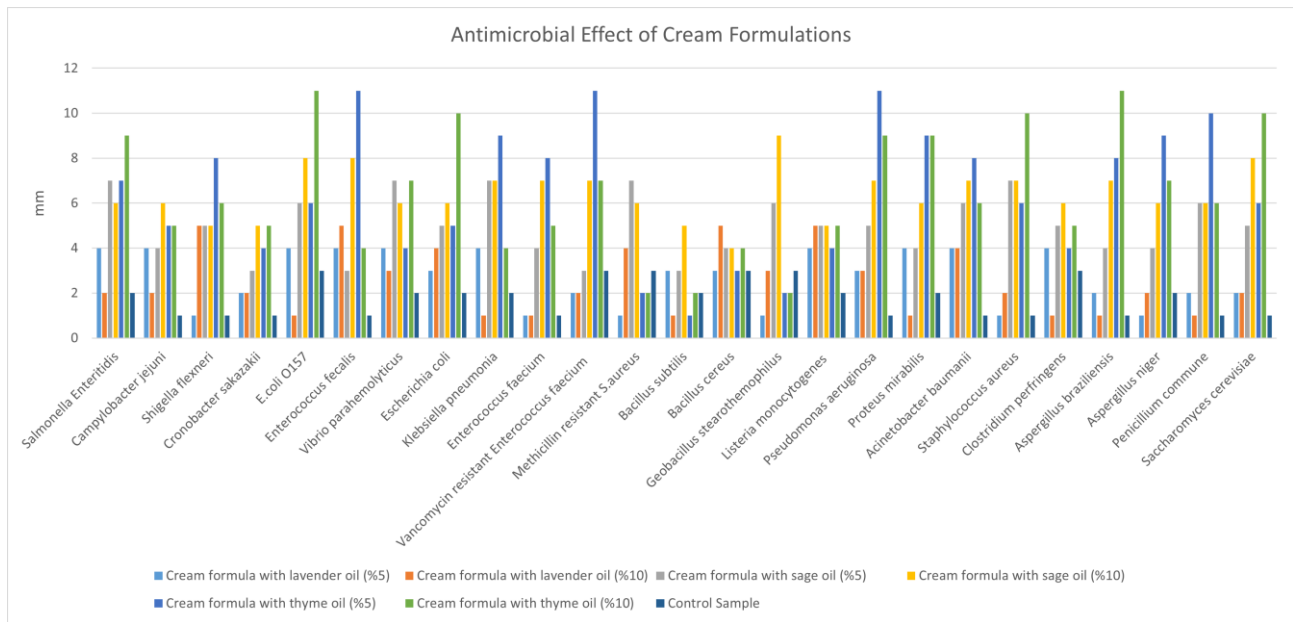


Figure 2. Antimicrobial effects of cream formulation

• Stabilization and homogenization

The visual acceptance of the cream was checked. For this purpose, the oils were checked for viscosity, color, odor and appearance.

In addition, the cream was checked at regular intervals using Fourier transform infrared spectroscopy (FTIR) and the parameters used here were recorded and set directly without prior preparation. The spectra obtained were evaluated together for all samples and differences in intervals (0-1-2-3) were recorded.

• Statistical Analysis

Hypotheses were formulated for statistical testing:

Null hypothesis (H0): There is no significant difference in the antimicrobial effect among thyme, lavender, and sage oils.

Non-parametric statistical tests were used due to single replicates:

The Kruskal-Wallis test was performed to compare the mm diameter measurements of the inhibitory zones between the three oils.

### 3 Results

The objective of this study was to formulate creams enriched with oils derived from different medicinal and aromatic herbs in varying quantities. The composition and chemistry of thyme oil and sage oil used in this context are presented in Table 4 and Table 5, respectively. The analyses were conducted at Batı Akdeniz Agricultural Research Institute. No characterization process was conducted in relation to the antimicrobial results obtained with lavender oil.

Table 4. The composition of theme oil

Bileşen Adı	Miktar(%)	Bileşen Adı	Miktar(%)
$\alpha$ -Pinen	0.45	linalil asetat	0.19
$\alpha$ -Thujene	1.51	terpinen-4-ol	0.78
Myrcene	2.07	$\beta$ -Karyofilen	0.51
$\alpha$ -Phellandrene	0.26	Borneol	0.33
$\alpha$ -terpinen	1.53	$\beta$ -Bisabolen	0.73
$\gamma$ -Terpinen	6.12	karyakril asetat	0.27
p-Cymene	3.37	karyofilen oksit	0.12
trans-Sabinene hidrat	0.40	timol	0.29
linalool	0.57	Karyakrol	80.29
cis-Sabinene hidrat	0.21		

Table 5. The composition of sage oil

Bileşen Adı	Miktar(%)	Bileşen Adı	Miktar(%)
$\alpha$ -Pinen	1.95	$\alpha$ -Thujone	23.88
$\alpha$ -Thujene	1.02	$\beta$ -Thujone	4.38
kamfen	2.52	kafur	13.26
$\beta$ -Pinen	3.46	Bornil asetat	0.98
Sabinen	0.76	Terpinen-4-ol	0.52
Myrcene	1.85	$\beta$ -Karyofilen	8.48
$\alpha$ -terpinen	0.75	$\alpha$ -Humulen	1.90
limonen	2.53	Borneol	0.49
1-8-Sineol	20.07	viridiflokol	1.19
$\gamma$ -Terpinen	2,20	Karyakrol	5.50
p-Cymene	1.45	Tanımsız	0.32
terpinolen	0.54		

There is no significant difference between compositions ( $p < 0.05$ )

On the contrary there is significant difference between antimicrobial effect of each oil ( $p < 0.05$ )

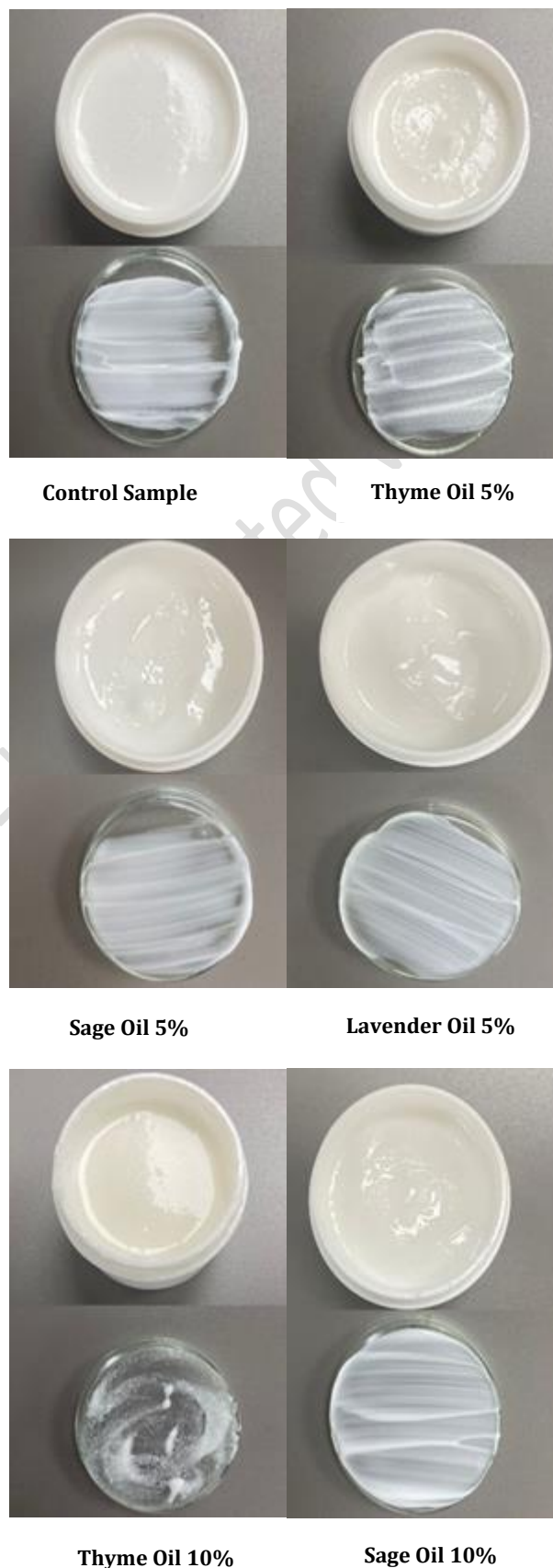
Various ratios of ingredients were used to make creams from oils obtained from characteristic herbs. This was spiked with 1, 5 and 10% oil and further analysis was performed accordingly. The formulation in question was created by simulating a product commonly offered on the market. Once manufactured and stabilized, the products were subjected to antibacterial, antifungal, and Fourier Transform Infrared (FTIR) spectroscopy at specified frequencies.

Antibacterial and antifungal assays found all oils to be active against *E. coli* at concentrations of at least 5 mm. In general, lavender oil has been observed to inhibit only 5 mm *E. coli*, and no other effects have been documented. Therefore, lavender oil is much more tolerable sensory, but has no antibacterial and antifungal activity was excluded from the study because there was no thyme oil exhibited potent antibacterial and antifungal properties when viewed in its pure form or at various concentrations. In this context, it has been observed that sage oil, in contrast to thyme oil, has little or no effect on spore-forming microorganisms is shown.

The antimicrobial and antifungal analyses conducted on cream formulations indicated that the blank formulation had no effect. The supplementation with thyme and sage essential oils exhibited significant antimicrobial and antifungal effects, with the dose of sage oil triggering a noteworthy change in the observed effects. Moreover, lavender oil demonstrated no effect, which is consistent with the results obtained from the raw material analysis. It is evident that thyme oil does not affect spore formers; conversely, sage oil at 10% exhibited significance by decreasing spore-forming bacteria. The cumulative results suggest that thyme oil alone possesses potential effects on microorganisms; however, its effectiveness decreases when incorporated into a cream formulation. Contrary to expectations, sage oil exhibited no significant decrease in its antimicrobial and antifungal potential. This finding is a significant outcome of these analyses.

The last step of the cream formulation analysis was stabilization. After preparing the formulations it was observed that the formulation of 5 and 10% of thyme oil were not homogeneous. Figure 3. shows detailed texture of cream of concern. Not only the homogeneity but also the sensory characteristics were not acceptable as a stinging smell of thyme occurs. As the stability of thyme oil enriched cream deteriorated sage formulations were more stabilized and were more homogenized by days. Lavender including samples were all homogeneous.

The visual analysis of formulations is given in Figure 3.





Lavender Oil 10%

Figure 3. The visual analysis of formulations

FTIR analysis revealed no significant effects in any of the samples. It should be noted that the transmittance percentage (T%) can vary with each oil ingredient, but the repeatability of the results obtained from the enriched samples was low. The changes observed over the course of days were also non-repeatable, which could lead to confusion when determining the appropriate cream formulation. As a result, the FTIR analysis results were presented in Figure 4, but were not considered conclusive in determining the correct cream formulation.

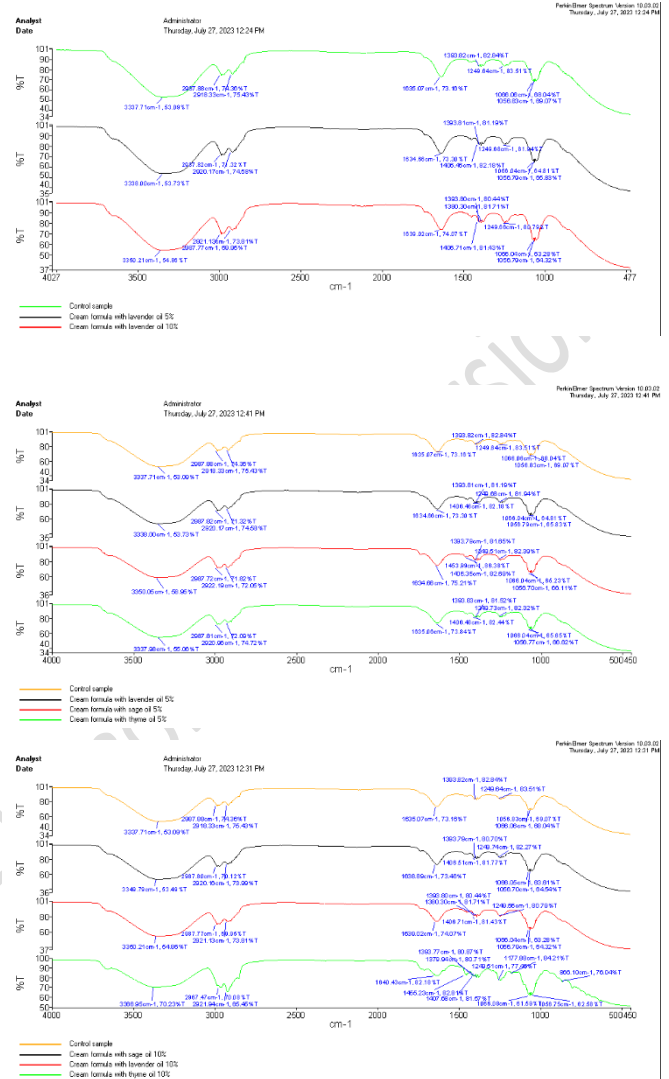


Figure 4. FTIR analysis and spectra obtained

## 4 Discussion

Medicinal herbs have been recognized for ages due to their significant effects on health. In this context, numerous plants, including bearberry (*Arctostaphylos uva-ursi*) and cranberry juice (*Vaccinium macrocarpon*), have been reported to be used as medicinal herbs in treating urinary tract infections. Garlic (*Allium sativum*) and tea tree (*Melaleuca alternifolia*) are also notable in this regard. These plants are not only used for infusions, but their essential oils (EOs) are also employed for their health benefits. EOs are produced by over 17,500 plant species across various angiosperms of *B. Lamiaceae*, *Rutaceae*, *Myrtaceae*, *Zingiberaceae*, *Asteraceae*. However, only about 300 of them have been commercialized. With this hypothesis in mind, we designed a cream enriched with sage, lavender, and thyme for their antimicrobial and antifungal benefits (Heinrich and others, 2004; Mérillon and others, 2018).

Numerous studies have demonstrated that medicinal oils have a significant impact on fungi and microorganisms (Roller and others, 2009; Sienkiewicz and others, 2011; Abu-Darwish and others, 2013; Longaray Delamare and others, 2007; El Astal and others, 2005).

Lavender oil was found to have no effect on microorganisms. Additionally, it was reported that all four types of lavender oils inhibited the growth of both MSSA and MRSA through direct contact but not in the vapor phase. Inhibition zones ranged from 8 to 30 mm in diameter at oil doses ranging from 1 to 20  $\mu$ L, respectively, demonstrating a dose response. The authors used four different species: *Lavandula angustifolia*, *Lavandula latifolia*, *Lavandula stoechas*, and *Lavandula luisieri*. In our study, we utilized the essential oil of *L. intermedia*, which could potentially impact the results obtained and account for the differences between the two studies (Roller and others, 2009).

Thyme oil was significantly antimicrobial and antifungal in all supplementation levels. In a study it was reported that the results of experiments showed that the oil from *Thymus vulgaris* exhibited extremely strong activity against all of the clinical strains. Thyme oil demonstrated a good efficacy against antibiotics resistant strains of the tested bacteria (Sienkiewicz and others, 2011). This data represented supports the hypothesis of thyme antimicrobial activity.

Sage was found to have effect on microorganism. This was also reported in other studies (Abu-Darwish and others, 2013; Longaray Delamare and others, 2007; El Astal and others, 2005). Additionally, it was reported that the essential oil content of *Salvia officinalis* is strongly impacted by genetic and environmental variables such as organ age, climatic conditions, and seasonality (Farhat and others, 2009).

The cream formulation was preferred due to slow releasing of antifungal agents on fungus and bacteria. Cream was found to be more user friendly. The formulation was selected according to commercial feedbacks (COSMER Kimya San. Ve Tic. A.Ş. Yayınları; 2020).

The antimicrobial and antifungal effects were accepted to be due to the chemical compositions of EOs. The sage essential oil composition was given in standard ISO 9909 as seen below:  $\alpha$ -thujone (18.0–43.0%),  $\beta$ -thujone (3.0–8.5%), 1,8-cineole (5.5–13.0%), camphor (4.5–24.5%), camphene (1.5–7.0%),  $\alpha$ -humulene (0–12.0%),  $\alpha$ -pinene (1.0–6.5%), limonene (0.5–3.0%), linalool (free and esterified (1.0% maximum)), and bornyl acetate (2.5% max) (ISO 9909, 1997). These data were similar to ours however it should be taken into account that seasonality and species level can affect the amounts of these chemicals. In a study the researchers reported that 1,8-cineole continuously diminishes throughout a vegetative phase, camphor peaks in the middle of the vegetative period and  $\alpha$ - and  $\beta$ -thujones progressively rise during the vegetative period (Grausgruber-Groger and others, 2012).

The final step in the analysis of cream formulations was stabilization. After preparing the formulations, it was observed that the 5% and 10% thyme oil formulations were not homogeneous. Not only was the homogeneity affected, but the sensory properties were also deemed unacceptable due to the development of the pungent odor of thyme. As the thyme oil-enriched cream became less stable, the sage formulation became increasingly stable and more uniform from day to day. All lavender-containing samples exhibited uniformity. After the tests, it was determined that the stabilization and consumer acceptance of thyme were not feasible due to the changing nature of the cream. In a stability study, it was reported that generating stable emulsions of thyme essential oil is challenging due to one or more destabilization processes, such as flocculation, coalescence, Ostwald ripening, or gravitational separation. In the same study, various surfactants with different hydrophilic-lipophilic balances (HLBs) were

examined, including ethoxylated sorbitan esters (Tween 20, Tween 60, and Tween 80) and sucroesters (SP 70, Appyclean 6548, and Appyclean 6552). The study demonstrated that welan gum, used as a stabilizer, proved more efficient than other stabilizers. The data presented by the authors showed similarities to our study; however, significant flocculation and deterioration were observed, which were anticipated to be unacceptable to consumers. This discrepancy might be attributed to the stabilizer chosen for the cream formulation (Martin and others, 2018). In another study aimed at exploring prospective antimicrobial delivery methods, thyme oil-in-water nanoemulsions (pH 3.5) were created. Due to the relatively high-water solubility of thyme oil, the nanoemulsions exhibited. Due to the relatively high-water solubility of thyme oil, the nanoemulsions exhibited pronounced instability, including droplet development and phase separation, attributed to Ostwald ripening (Chang and others, 2012).

## 5 Conclusion

This study was designed to demonstrate the antimicrobial and antifungal effects of different essential oils (EOs) in cream formulations. The study yielded data indicating that lavender oil has no significant effect on bacteria and fungi, whereas thyme oil exhibited significant antimicrobial and antifungal properties. However, the stabilization of the thyme-enriched cream was deemed unacceptable due to significant visual changes, including phase separation and sensory defects. The last EO examined was sage oil, which not only demonstrated antimicrobial and antifungal properties but also possessed an acceptable texture and sensory effect.

If antimicrobial and antifungal natural agents are needed, due to increasing numbers of antimicrobial resistance by chemical agents, sage EOs could be a good choice to be applied. The escalating resistance brought about by antibiotics and antifungals is a significant global concern. It is crucial to consider that natural EOs do not induce resistance in microorganisms of concern. Cream formulation is more user friendly due to controlled viscosity and sensory properties. EOs generally regarded as safe and they have numerous advantages. Sage EOs may have an additional positive effect on health. Global studies have a tendency to use natural substances which also can reach a new approach or technologic aspect with sage EOs. We recommend more studies to be held on the area of stability for natural EOs cream formulations. In future studies additional health aspects should be taken into account for EOs.

## 6 Author contribution statements

In the scope of this study, the Author 1 and Author 2 in the formation of the idea, the design and the literature review; Author 3 in the assessment of obtained results, supplying the materials used and examining the results; the Author 4 and Author 5 the spelling and checking the article in terms of content were contributed.

## 7 Ethics committee approval and conflict of interest statement

There is no need to obtain permission from the ethics committee for the article prepared.



There is no conflict of interest with any person / institution in the article prepared.

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