Journal of Research in Pharmacy

Research Article

www.jrespharm.com

Antimicrobial synergy of *Lavandula angustifolia* L. and *Rosa damascene* L. essential oils: Preparation of herbal lip formulations

Bashar IBRAHIM[1] *, Ahmet Arif KURT[2,3], Buse ZEREN[4,5], Duygu TURKMEN[4,5], Şeyma AKGÜN[4], Ceren AKINCI[4], Hande YARMALI[4], Ismail ASLAN[6]

1 Department of Pharmaceutical Microbiology, Faculty of Pharmacy, Suleyman Demirel University, Isparta,23000, Turkey
2 Department of Pharmaceutical Technology, Faculty of Pharmacy, Suleyman Demirel University, Isparta,23000, Turkey
3 Research laboratory, R&D unit, Polisome R&D Pharmaceutical Industry Trade Company, Isparta, 32000, Turkey
4 Faculty of Pharmacy, Suleyman Demirel University, Isparta,23000, Turkey
5 Department of Pharmaceutical Technology, Faculty of Pharmacy, Izmir Kâtip Celebi University, Izmir, 35000,Turkey
6 University of Health Sciences Türkiye, Hamidiye Faculty of Pharmacy, Department of Pharmaceutical Technology, İstanbul, Türkiye ; ismail.aslan@sbu.edu.tr
* Corresponding Author. E-mail: basharibrahim@sdu.edu.tr (B.I.); Tel. +905343356199

Received: 13 March 2025 / Revised: 4 May 2025 / Accepted: 10 May 2025

ABSTRACT: Increasing concerns about the potential toxicity of heavy metals such as lead and various chemical compounds commonly found in synthetic lip care products have increased the demand for herbal alternatives. This study aimed to formulate a low-toxicity, natural, antimicrobial lip product using Isparta-grown *Lavandula angustifolia* L. (lavender) and *Rosa damascena* L. (rose) essential oils. Cosmetic grade oils, chitosan, lanolin, and ethylhexylglycerin were included. Gas chromatography determined essential oil components, while standard methods assessed the formulations' physicochemical properties (pH, viscosity, melting point, weight deviation). Furthermore, microbiological tests, safety limit analyses, and in vitro antimicrobial activity examinations were conducted for a comprehensive evaluation of the formulations. GC analyses revealed distinct component profiles, with rose oil mainly composed of beta-citronellol, geraniol, and nonadecane, while lavender oil featured linalool, linalyl acetate, and terpinen-4-ol. The developed LF-9 formulation successfully met the basic requirements by exhibiting homogeneity, an acceptable pH level, resistance to microbial contamination and stability maintained for six months. The strong antimicrobial activity of the formulation against *E. coli*, *B. cereus*, and *C. albicans*, especially due to the effect of lavender oil, was also confirmed by the relevant Minimum Inhibitory Concentration (MIC) values. These results demonstrate the potential of developing natural and safe lip care products by utilizing the antimicrobial properties of lavender and rose essential oils and position the LF-9 formulation as an effective natural alternative to synthetics due to its superior qualities.

KEYWORDS: *Rosa damascene* L; *Lavandula angustifolia* L; lip cream formulation; microbiological analysis; antimicrobial activity.

1. INTRODUCTION

"Cosmetics" refers to scientifically formulated products designed to enhance or maintain the aesthetic appearance of various areas, including the skin, hair, and body. These products encompass a wide range of categories, such as skin care (e.g., lotions, creams, masks), makeup (e.g., lipsticks, eye shadows, foundations), hair care (e.g., shampoos, conditioners, dyes), and personal care (e.g., deodorants, perfumes). The primary objectives of cosmetic products are to improve the appearance of skin issues, conceal existing imperfections, and enhance overall aesthetic appeal [1]. Increasing consumer demand and health/environmental consciousness are shifting the cosmetics industry toward botanical formulations based on plant-derived ingredients (oils, waxes) instead of synthetics. Scientific data support the benefits of these ingredients, such as

How to cite this article: Ibrahim B, Kurt AA, Akinci C, Yarmali H, Zeren B, Akgun S. Antimicrobial synergy *of Lavandula angustifolia* L. and *Rosa damascene* L. essential oils: Preparation of herbal lip formulations. J Res Pharm. 2025; 29(4): 1636-1652.

ISSN: 2630-6344

https://doi.org/10.12991/jrespharm.1657486

enhanced skin barrier function and anti-aging effects, owing to their antioxidant and antibacterial properties [2,3]. In this context, the growing environmental consciousness and healthy lifestyle trends have led consumers to gravitate toward personal care products with natural ingredients. This has significantly increased the market share of natural products [2].

Lip products are some of the most used cosmetics today. In addition to enhancing the aesthetic appeal of the lips, lip care products now also focus on promoting lip health. This shift has led to a rise in the availability of lip care products on the market that contain active medical ingredients. Research has shown that these active ingredients can help protect against bacterial infections [4].

Lavender and rose oils are reported in literature to have strong antimicrobial and antifungal properties [5]. Additionally, their pharmacological effects have been extensively studied, leading to their use in various fields, including medicine, cosmetics, and industry. The effectiveness of these oils can differ based on the types and quantities of active ingredients they contain [5,6].

Numerous studies have demonstrated that lavender and rose plants possess therapeutic potential in treating bacterial infections. The rose plant exhibits a wide array of pharmacological activities, including anti-inflammatory, antiviral, antimicrobial, analgesic, and antibacterial effects. These qualities position lavender and rose as promising candidates for various therapeutic applications. Native to Isparta, both lavender and rose have been recognized for their medicinal benefits for centuries. Lavender oil has garnered significant attention due to its antibacterial properties, attributed to compounds like linalool and linalyl acetate that it contains [7,8].

This study aims to formulate a non-toxic, daily-use herbal lip balm by utilizing the antimicrobial properties of *Lavandula angustifolia* L. and *Rosa damascena* L. plants grown in the Isparta region. The study also includes the identification of volatile components in rose oil samples by GC analysis and the evaluation of the physicochemical properties of the formulations.

2. RESULTS

The various lip formulas developed underwent thorough testing, focusing on their physical and chemical properties, stability over time, and the potential for harboring harmful microorganisms, particularly in the lip area. After conducting microbiological tests, it was confirmed that all formulas complied with established microbial limits. Following a comprehensive examination and safety assessment of the results, it was determined that the formula designated as LF-9 outperformed the other eight formulas in terms of appearance, fluidity (consistency), and durability, making it the most suitable candidate.

2.1. Physicochemical Test Results

The lip formulations were subjected to comprehensive analyses encompassing their physicochemical attributes, organoleptic characteristics, pH values, and stability profiles. After the execution of these analyses, the data acquired were evaluated, culminating in the identification of the most suitable candidate formulation from among the evaluated formulations

2.2. Weight Variance Determination Results

The assessment of weight variation in the lip formulations was conducted following the methodology outlined in the British Pharmacopoeia (BP) 2002 [9]. In this evaluation, twenty samples were taken from each formulation, and multiple measurements were performed. This approach allowed us to determine weight variation values for all nine formulations. As a result of the analysis, it was found that none of the formulations exceeded the 5% weight variation limit specified in the BP. In addition, Analysis of Variance (ANOVA) showed that there were statistically significant differences in mean weight variations among the nine formulations ($p < 0.01$), suggesting different levels of manufacturing consistency between the groups. A comprehensive summary of the weight variation data is presented in Table 2.

2.3. Appearance Control

Within the scope of the appearance assessment, no evidence of crystallization was observed on the outer surfaces of any of the lip formulations, nor was any indication of subsidence or void formation detected in their transverse or longitudinal sections. After the conducted evaluations, it was determined that all

formulations exhibited a homogeneous and flawless appearance (Figure 1).

Figure 1. Appearance of LF9 formulation in lips molds and cream boxes

2.4. Melting Point Result

Following the melting point analyses of the lip formulations, it was determined that the LF-9 formulation, which incorporates a combination of witepsol and beeswax, melts at 37 °C and maintains its physical integrity under ambient conditions. While the melting temperatures of other formulations such as LF-1, LF-2, LF-3, LF-4, LF-5, and LF-7 were observed to be below 37 °C, the LF-6 formulation was found to melt at about 39 ± 1 °C (Figures 2, 3). ANOVA confirmed that these melting point differences among the formulations were statistically significant

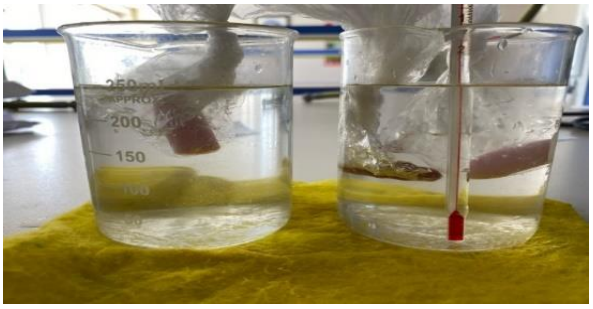

Figure 2. Melting Point Test Of Lips Formulations

Table 1, depicting the melting points of the different formulations labeled from LF-1 to LF-9, demonstrates a range of melting points from below 37°C to 39°C, with the majority of formulations exhibiting a melting point below 37°C.

Table 1. Melting point analysis of lip formulations

Formulation	Melting Point (°C)	Integrity under ambient conditions
LF-1	< 37	Preserved
LF-2	< 37	Preserved
LF-3	< 37	Preserved
LF-4	< 37	Preserved
LF-5	< 37	Preserved
LF-6	39 ± 1	Preserved
LF-7	< 37	Preserved
LF-8	< 37	Preserved
LF-9	36	Preserved

Weight deviations across the formulations labeled LF-1 through LF-9 exhibit variability. Formulation LF-9 presented the minimum weight deviation (4.11 g), whereas LF-6 demonstrated the maximum deviation (4.25 g) (Table 2, Figure 3).

Table 2. Analysis results of lip formulations: Weight deviation, melting temperature, breaking strength, and appearance

Formulations	Weight deviation(gr)	Melting temperature (^{0}C)	Breaking strength(N)	Appearance
LF-1	4.22 ± 0.21	31± 0.5	1 ±0.25	Homogeneous-Monotonous
LF-2	4.17 ± 0.25	32± 0.5	1 ± 0.35	Homogeneous-Monotonous
LF-3	4.21 ± 0.17	32± 0.6	1 ± 0.22	Homogeneous-Monotonous
LF-4	4.14 ± 0.19	34± 0.3	1 ± 0.31	Homogeneous-Monotonous
LF-5	4.19 ± 0.26	35± 0.5	1 ± 0.30	Homogeneous-Monotonous
LF-6	4.25 ± 0.28	39± 0.5	1 ± 0.17	Homogeneous-Monotonous
LF-7	4.12 ±0.32	32± 0.4	1 ± 0.19	Homogeneous-Monotonous
LF-8	4.18 ± 0.25	34± 0.5	1 ± 0.21	Homogeneous-Monotonous
LF-9	4.11 ± 0.16	36± 0.2	1 ± 0.32	Homogeneous-Monotonous

Figure 3 presents a comparison of three key parameters – weight deviation (g, red columns), melting point (°C, blue columns), and fracture resistance (N, turquoise columns) – of nine formulations (LF-1 to LF-9). Weight deviation reflects the consistency of the formulations during the manufacturing process and hence their stability; the highest deviation was observed in formulation LF-6 at 4.25 g, while the lowest deviation was found in LF-9 at 4.11 g. ANOVA confirmed that there were statistically significant differences in weight deviation among the formulations ($p < 0.01$), and the difference between LF-9 and LF-6 was also found to be significant ($p < 0.05$). Melting point determines the physical properties of the formulations; LF-6 exhibited the highest melting point at 39 °C, while LF-1 had the lowest at 31 °C, and these differences were also found to be statistically significant (ANOVA, $p < 0.001$). Fracture resistance expresses the mechanical strength of the material; this value was found to be approximately 1 N for all analyzed formulations, and ANOVA showed that there was no statistically significant difference between the formulations in terms of this parameter ($p > 0.05$). In this context, the fact that LF-9 has the lowest weight deviation (significantly lower compared to LF-6) statistically supports that it is a more advantageous option in terms of production consistency and therefore potential stability.

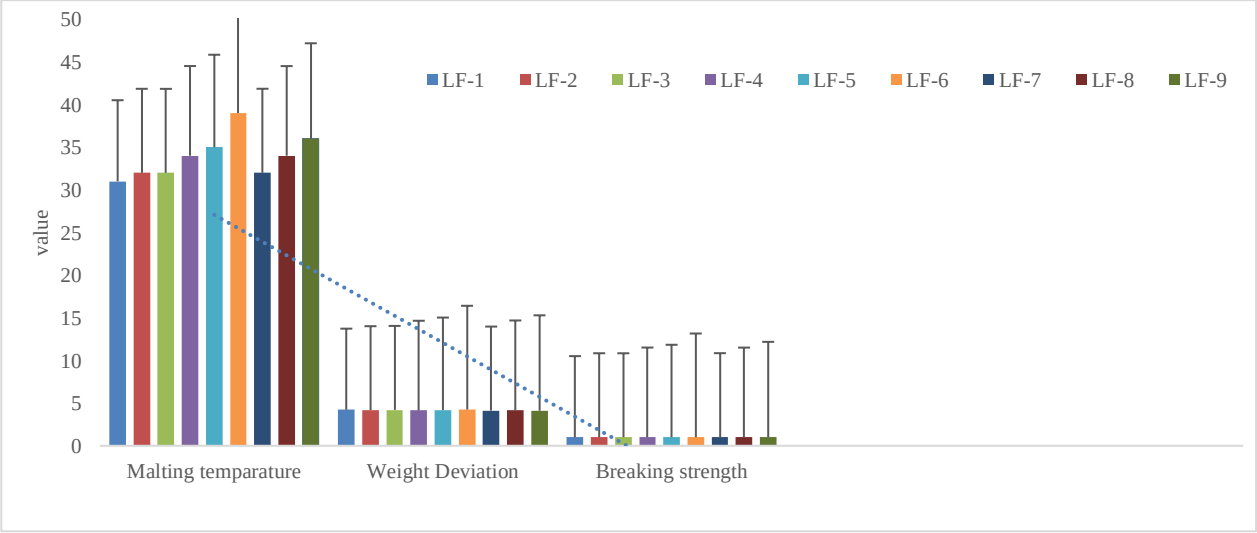

Figure 3. Lips Formulations Weight Deviation, Melting Temperature, and Breaking Strength

2.5. Margin of Safety (MOS) Results

Margin of Safety (MoS) analyses confirmed values> 100 (relative to PODsys) for all lip formulation components, indicating concentrations align with Generally Recognized as Safe (GRAS) standards and are acceptable for safe use (Figure 4).

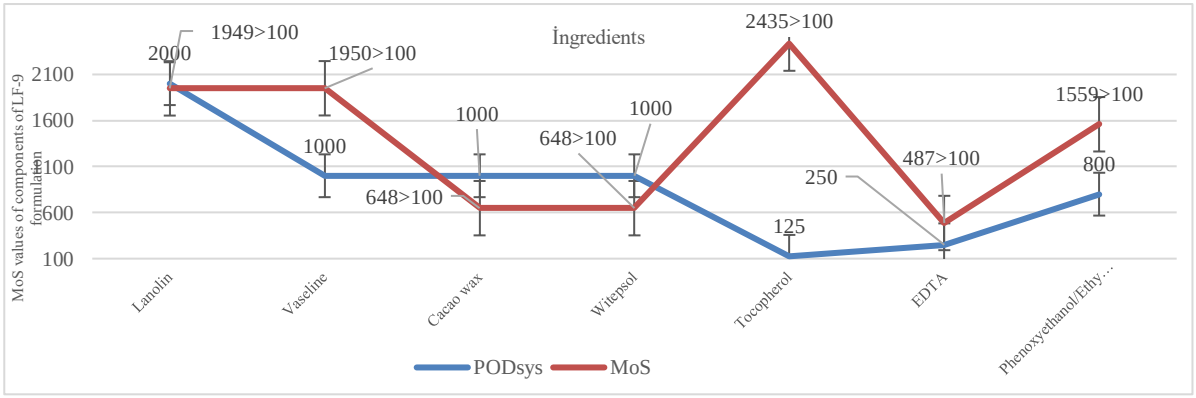

Figure 4. MoS values of components of the LF-9 formulation

2.6. Stability Results

The LF-9 formulation exhibited physicochemical stability over a six-month period when stored at room temperature (25 ± 2 °C) and under refrigeration (~4 °C), retaining properties specified in Table 4. Additionally, preservative efficacy tests conducted at 4°C and 24 ± 0.5 °C / 60 ± 2% RH confirmed the absence of microbial growth (Table 3).

2.7. Gas Chromatography (GC) Results

In the study, GC analysis results identified beta citronellol (34.2%), geraniol (18.93%) and nonadecane (11.89%) as the dominant components in rose oil, while linalool (32.38%), linalyl acetate (28.1%) and terpinen-4-ol (5.91%) were identified as the main components in lavender oil (Table 4,5).

Table 3. Stability results: appearance, colour, odor, and microbiological growth results as of Day 0, Month 3, and Month 6

State of Stability	Control Period Month	Appearance	Colour	Smell	Microbiological growth (45°C)
Room (24 ± 0,5 °C/ RH % 60 ±2)	0th	Homogeneous	Specific	Specific	No
	3th	Homogeneous	Specific	Specific	No
	6th	Homogeneous	Specific	Specific	No
Refrigerator (4 ± 0,5°C)	0th	Homogeneous	Specific	Specific	No
	3th	Homogeneous	Specific	Specific	No
	6th	Homogeneous	Specific	Specific	No

2.8. Detection of Microorganisms

Microbiological testing (initial, 14, 28 days) consistently showed no microbial growth in the lip formulations, confirming preservative efficacy and the products' microbial safety and stability.

2.9. Antimicrobial Activity Results

L. angustifolia generally exhibited lower MIC values, whereas *R. damascena* L. demonstrated higher MIC values against specific microorganisms. The MIC values of *Lavandula angustifolia* L. extract against *E. coli, P. aeruginosa, E. faecalis, S. aureus*, MRSA, *B. cereus*, and *C. albicans* were 3.125, 6.25, 6.25, 12.5, 25, 1.562, and 3.125

µL/mL, respectively. Conversely, *R. damascena* L. extract yielded MIC values against the same bacteria of 1.562, 3.125, 3.125, 3.125, 12.5, 3.125, and 12.5 µL/mL, respectively (Figure 5).

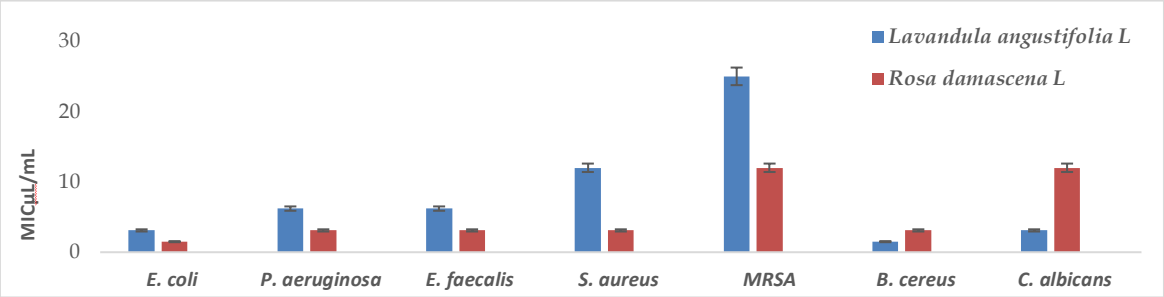

Figure 5. MIC Values of lavender and rose oils

3. DISCUSSION

The presence of heavy metal contamination in commercially available lip care products poses a significant public health concern. Specifically, the presence of heavy metals, including lead (Pb), cadmium (Cd), and arsenic (As), is particularly worrisome due to their potential for bioaccumulation with chronic use, potentially resulting in adverse toxicological effects. Empirical evidence from studies conducted by international regulatory bodies, such as the Food and Drug Administration (FDA), underscores the gravity of this issue by demonstrating the widespread occurrence of heavy metals in cosmetic products and their associated health risks [13]. Long-standing herbal therapy, including phytotherapy and aromatherapy using extracts/essential oils, has gained increased confidence due to technological advancements improving understanding of ingredient safety and efficacy. Research widely supports the benefits of herbal ingredients in skincare [14,15].

This approach reduces toxicity and ensures environmentally friendly production while maintaining optimal skin pH (4.5-6.5). This pH range is crucial for supporting skin barrier integrity and a healthy microbiome, essential for overall skin health [16].

This study developed an innovative, natural/sustainable lip care formulation optimized for pH 4.5-6.5. This range supports the skin's acid mantle, minimizes irritation, promotes a balanced microbiome, inhibits pathogen growth, and enhances product stability.

The formulation's primary constituents were selected based on their designated functions: The primary function of beeswax is as an occlusive agent, forming a protective barrier that mitigates transepidermal water loss and enhances moisture retention. The emollient (softening) properties of the constituents' lanolin, cocoa butter, and olive oil are well-documented, with lanolin also offering reparative benefits and cocoa butter providing significant moisturization. Beta vulgaris (beetroot) extract is also used to impart natural coloration. The essential oils comprising the formulation, including lavender, rose, rosemary, and clove, have been demonstrated to elicit a soothing and nourishing effect, in addition to their antimicrobial properties, which form the focal point of the present study. The antimicrobial efficacy of the blend is significantly attributed to compounds such as eugenol, particularly derived from sources such as clove oil. The primary function of eugenol in formulations is generally its potent antimicrobial action against bacteria and fungi, where it commonly serves as a protective or antiseptic role.

During the formulation optimization process, various ingredient ratios and combinations were tested to identify the best-performing formula. The selection of Formulation LF-9 as the optimal candidate was the result of a systematic evaluation process encompassing multiple analytical steps designed to compare the performance of nine formulations. Critical physicochemical characterization processes, specifically melting point determination and weight drift evaluation, determined LF-9's ideal thermal profile for application (36°C) and superior manufacturing consistency with minimal weight drift. Simultaneously, a comprehensive six-month stability evaluation process conducted under various storage conditions confirmed that the formulation maintained its physicochemical properties and microbiological integrity over time. Additionally, safety evaluation processes, including Margin of Safety (MoS) calculation for all components, established a significant margin of safety, while rigorous microbiological testing processes consistently demonstrated the absence of microbial contamination. Collectively, the superior and consistent results observed for LF-9

throughout these different analytical and evaluation processes supported its selection as the preferred formulation.

The results obtained are in line with findings from similar studies and demonstrate that effective and safe lip care products can be created using natural ingredients [17-24].

According to GC analysis results, the main components of rose oil are beta-citronellol, geraniol, and nonadecane, while the main components of lavender oil are linalool, linalyl acetate, and terpinen-4-ol. These components are consistent with those reported in the literature [25,26].

Differing chemical profiles between Lavender and Rose species may explain variations in essential oil antimicrobial activity, necessitating careful component selection for optimal formulation effectiveness [27]. Citronellal exhibits antimicrobial activity against microorganisms such as *E. coli*, *S. aureus*, and *C. albicans*, attributed to mechanisms including cell membrane disruption, enzyme inhibition, and biofilm formation prevention [28]. It is emphasized that geraniol has broad spectrum antimicrobial activity. It has been stated that it has the potential to reduce antibiotic resistance, especially on *P. aeruginosa* [29]. Beyond its direct antimicrobial effects, geraniol has been highlighted as having the potential to reduce antibiotic resistance in problematic pathogens such as Pseudomonas aeruginosa. Possible mechanisms underlying this effect include: disrupting the cell membrane and facilitating the entry of antibiotics into the cell, thus creating a synergistic effect; inhibiting efflux pumps, an important resistance mechanism in *P. aeruginosa* that expels antibiotics from the cell; and potentially interfering with other resistance and virulence factors such as biofilm formation or quorum sensing. These potential resistance-modifying properties of geraniol highlight the value of using natural compounds in controlling drug-resistant infections [30].

Although the direct antimicrobial activity of long-chain alkanes such as nonadecane is limited, it has been noted that they can increase the effectiveness of other antimicrobial compounds. It is reported to disrupt cell membranes, inhibit enzyme activity, and reduce biofilm formation [31].

It has been emphasized that linalool has broad-spectrum antimicrobial activity against *E. coli, S. aureus, B. cereus, C. albicans,* and many other microorganisms. Its mechanisms of action include cell membrane damage, enzyme inhibition, and DNA damage [32].

It is stated that linalyl acetate exhibits antimicrobial activity on its own, especially on *S. aureus* and *C. albicans*, and in some cases, it creates synergistic effects with traditional antibiotics [33].

Terpinen-4-ol is well documented to have strong antimicrobial activity, particularly against *S. aureus* (including MRSA), *C. albicans*, and other pathogens. It is reported to disrupt cell membranes, inhibit biofilm formation, and have immunomodulatory effects [34].

All lip formulations exhibited smooth, homogenous textures, indicating uniform component mixing and oil distribution. Melting time, a key physical property influencing these characteristics, is crucial; an ideal melting time enhances application (smooth spread, even layer), boosting ease of use and effectiveness. Consequently, optimizing melting behavior during design is essential for product performance and user satisfaction [35].

Witepsol, a commercial lipid base, possesses a more stable crystalline structure than cocoa butter. Its thermal stability (melting point 45-50°C) during formulation contributes to final product integrity [36].

Due to the proximity of lip temperature to body temperature, cocoa butter (particularly in LF-4, LF-5, and LF-6) was selected in conjunction with Witepsol (solid wax at room temperature), a constituent of the formulations. The low melting point of cocoa butter, in comparison to Witepsol, when exposed to elevated temperatures, can undergo a polymorphic transformation, as evidenced in the extant literature and Material Safety Data Sheets (MSDS) [37].

This polymorphic transformation can compromise the formulation's stability and efficacy. Furthermore, this behavior directly affects product distribution on the lips and the overall user experience. Therefore, it is essential to consider these factors carefully during formulation design and to conduct optimization studies [38,39].

Melting point analyses revealed that formulations LF-1, LF-2, LF-3, LF-7, and LF-8, all containing cocoa butter, exhibited melting points below body temperature, rendering them unsuitable for lip applications. Similarly, formulation LF-6, with a melting point of 39°C, was deemed inadequate for this purpose. In contrast, formulation LF-9, which melted at approximately 36°C, was considered appropriate for lip application. LF-9 was preferred due to its optimal cocoa butter/Witepsol ratio. Figure 4 highlights differing thermal resistances (e.g., LF-1 suited for lower temperatures, LF-6 for higher), critical for selecting formulations based on application environment. Fracture point analysis showed all formulations withstood 10 mg/30s, indicating

robustness. Comparable fracture strengths suggest similar durability and structural integrity across formulations. (Fig.4) [40].

The safety margin assessment results for the components of the LF-9 formulation indicate that they fall within the range of 250 and above, which is considered reliable according to Annex-3 of the Turkish Medicines and Medical Devices Agency (TİTCK) [41].

As depicted in Figure 5, an analysis of the Percentage of Dose System (PODsys) and MoS values for the components in formulation LF-9 revealed that all components exhibited MoS values exceeding 100. This finding indicates that the concentrations of the elements in the formulation provide a significant safety margin. Specifically, the high MoS values observed for components such as tocopherol, phenoxyethanol/ethylhexylglycerin, and petrolatum further support the safe use of these ingredients [40].

Studies have demonstrated the efficacy of *R. damascena* L. and *L. angustifolia* against multidrug-resistant clinical pathogens, with reported antimicrobial activity against *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa*, and *Candida albicans* [7,8,33,43].

In our antimicrobial assessment, the effects of *Lavandula angustifolia* L. and *Rosa damascena* L. essential oils on seven different microorganisms (*Escherichia coli, Pseudomonas aeruginosa*, *Enterococcus faecalis, Staphylococcus aureus*, MRSA, *Bacillus cereus, and Candida albicans*) were determined using a microdilution method. *L. angustifolia* oil exhibited the most potent antimicrobial activity, demonstrating MICs of 3.125, 1.562, and 3.125 µL/mL against *E. coli, B. cereus*, and *C. albicans*, respectively. *R. damascena* L. essential oil showed moderate antimicrobial activity, with MICs ranging from 1.562 to 12.5 µL/mL across the tested microorganisms. These findings support previous studies reporting the antimicrobial properties of *L. angustifolia* and *R. damascena* L. essential oils against various pathogens [44,46].

In addition to their antimicrobial/preservative action, *Lavandula angustifolia* L. and *Rosa damascena* L. essential oils contributed to the success of LF-9. This formulation met all performance criteria without adverse reactions. Formal testing confirmed its stability and superior attributes, including local effect, gloss, application ease, and smoothness. These results underscore the efficacy and safety of plant-derived components in lip care and highlight the potential of herbal alternatives for consumer products. This study has some limitations inherent to its scope. The formulation development and testing processes were conducted at a laboratory scale, which may not fully reflect the potential challenges of industrial production. While LF-9 was selected as a result of comparative analyses, extensive long-term (beyond six months) stability and in-depth evaluations were primarily focused on this single formulation. Importantly, the study did not include clinical efficacy trials or large-scale user perception and safety assessments (e.g., irritation potential), which are vital to demonstrating real-world performance and tolerability compared to existing products. Furthermore, due to the inherent variability of essential oil components, ensuring consistency in manufacturing processes is a challenge that must be addressed for commercial application.

4. CONCLUSION

Considering the potential risks associated with harmful chemicals and infections in synthetic lip care products, this study offers a natural alternative. The LF-9 formulation, developed using the antimicrobial properties of *Lavandula angustifolia* L. and *Rosa damascena* L. extracts sourced from Isparta, has successfully passed physicochemical and microbiological testing. This formulation not only provides deep nourishment to the lips but also offers protection against bacterial infections. This work represents a significant step toward the development of sustainable and safe products in the natural cosmetics sector. However, further evaluation of the product through broader clinical trials and investigation of its long-term usage effects are warranted.

5. MATERIALS AND METHODS

5.1. Materials

The formulation incorporated various components including cosmetic-grade *Rosa damascene* L (sourced from Talya, Turkey), clove oil (Clove Oil; Talya, Turkey), *Lavandula angustifolia* L. (Talya, Turkey), chitosan (Merck, Germany), lanolin (Galenik, Turkey), and ethylhexylglycerin (Ashland). The suitability of these ingredients for cosmetic use was ensured. Characterization and analysis were conducted using equipment such as a Milwaukee MW150 max pH meter (Szeged, Hungary), a PCE-RVI 10 rotational viscometer (Meschede, Germany), and an Elektromag M5040 PS electromagnetic stirrer (Çerkezköy, Turkey). In addition, a gas chromatography (GC) device with a flame ionisation detector (FID) was used to analyse volatile

components in rose and lavender oil samples. Undiluted samples were analysed using a suitable column and temperature program. The components were identified from the retention times of the peaks in the chromatogram, and their amounts were determined from the peak areas and their ratio to the total area (% area sum).

5.2. Method

5.2.1. Gas chromatography analysis

The separation and identification of volatile components found in lavender and rose essential oils are shown in Figures 6 and 7. Each peak in the chromatogram represents a different component in the oil. The X-axis represents the retention time (RT), which indicates the time it takes for the components to pass through the column, while the Y-axis shows the intensity of the signal detected by the detector and, therefore, the relative amount of each component.

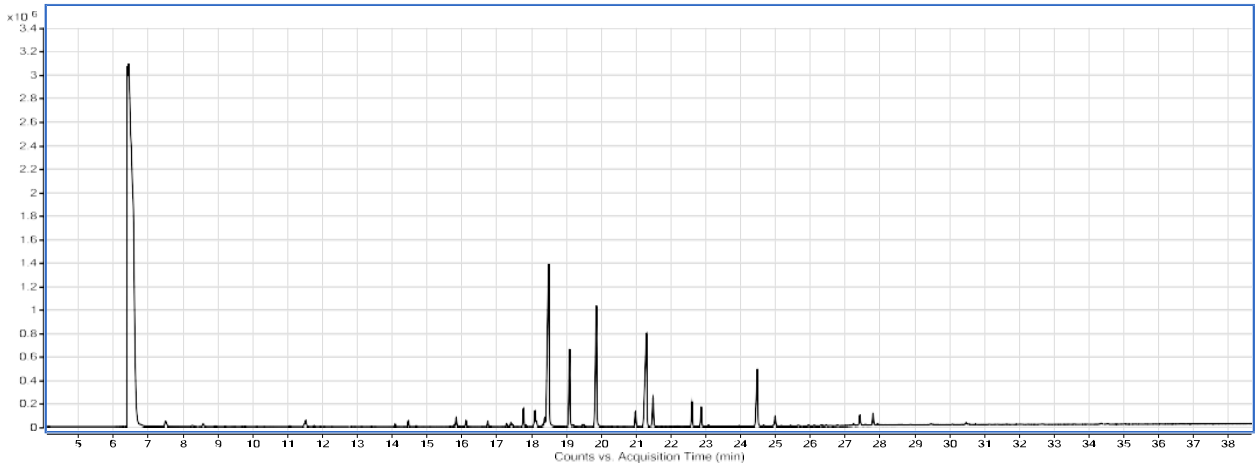

Figure 6. Gas Chromatography of *Rosa damascene* L. Essential Oil

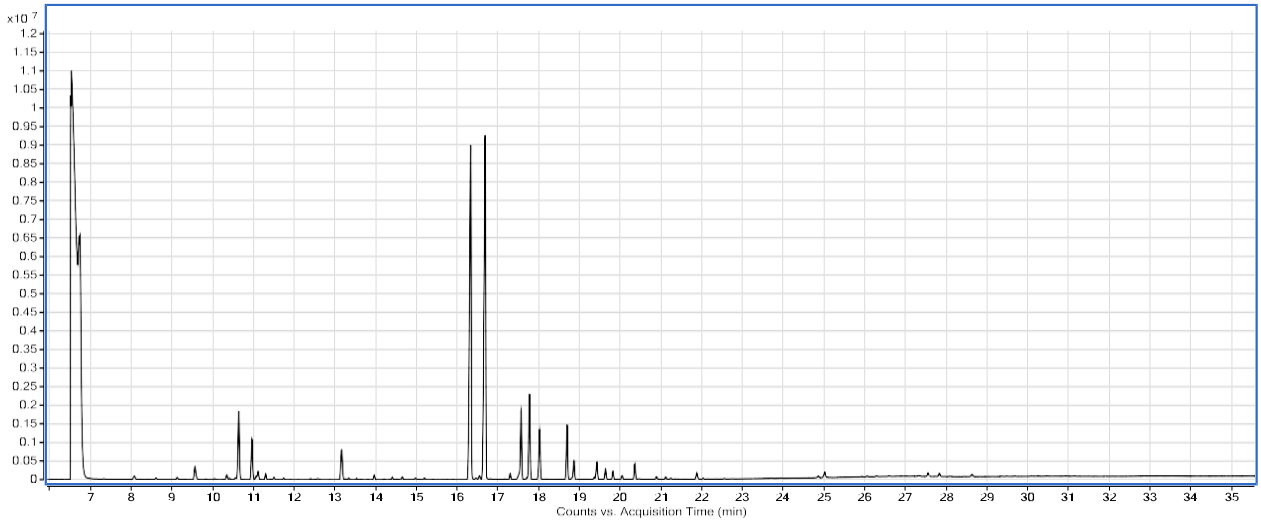

Figure 7. Gas Chromatography of *Lavandula angustifolia* Essential Oil

Analyses of *Rosa damascena L.* and *Lavandula angustifolia L.* essential oils were carried out by the gas chromatography (GC) method, and data were obtained using a flame ionization detector (FID) (Tables 4, 5).

Table 4. Component Analysis of *Rosa damascena* L. Essential Oil

Peak	Retention time	Area sum	%	Name
1	6.755	471806.94	1.41	ethanol
2	7.474	353844.26	1.06	alpha pinene
3	8.236	110153.21	0.33	beta pinene
4	8.552	184834.72	0.55	beta myrcene
5	11.458	60919.71	0.18	rose oxide A
6	11.734	38165.91	0.11	rose oxide B
7	14.055	66729.98	0.2	pentadecane
8	14.434	185584.76	0.56	linalool
9	15.807	231480.9	0.69	alpha guaiene
10	16.086	165861.6	0.5	trans caryophyllene
11	16.711	116077.83	0.35	citronellyl acetate
12	17.252	110975.85	0.33	e-citral
13	17.373	95657.19	0.29	alpha humulene
14	17.424	47895.25	0.14	alpha terpineol
15	17.735	389518.82	1.17	heptadecane
16	18.066	420978.98	1.26	germacrene-D
17	18.111	136291.3	0.41	z-citral
18	18.342	213879.09	0.64	geranyl acetate
19	18.462	11417304.61	34.2	beta citronellol
20	19.057	3210482.13	9.62	nerol
21	19.826	6318664.71	18.93	geraniol
22	20.946	441459.92	1.32	PEA
23	21.265	3968299.27	11.89	nonadecane
24	21.449	656168.08	1.97	nonadecene
25	22.566	489055.99	1.46	methyleugenol
26	22.829	389870.93	1.17	eicosane
27	24.438	1557468.88	4.67	heneicosane
28	24.952	255261.86	0.76	eugenol
29	27.376	206189.43	0.62	tricosane
30	27.756	254791.39	0.76	farnesol 3

5.2.2. Formulation study

In a water bath, the solid components of the formulation, waxes and fats (e.g., Cocoa Butter, Witepsol), were heated until they melted completely. Then, liquid oils (e.g., Sweet Almond Oil, Olive Oil, Rosemary Oil) and other fat-soluble ingredients (e.g., Lanolin, Vaseline) were added, and the mixture was heated until it became homogeneous. The mixture was carefully mixed to ensure homogeneity and to minimize the formation of air bubbles. After the mixture was removed from the stove or the temperature was slightly lowered, the heat-sensitive components, essential oils (Lavender, Rose Extract, Clove), colorants (Pigment), antioxidants (Tocopherol - Vitamin E), and other active ingredients were added and gently mixed. The homogeneous mixture obtained was poured into pre-greased lip balm molds and cooled in an ice bath for approximately 30 minutes to allow the structure to set quickly. The completely hardened lip balms were removed from the molds and packaged. The ingredients and quantities of the different formulations (LF-1 to LF-9) are detailed in Table 6. Each ingredient in the formulation was selected to play a specific role to enhance the performance of the product (moisturization, protection, structure, antimicrobial effect, etc.). For example, cocoa butter and Witepsol provide structure and melting point, lanolin and petrolatum soften the skin and form a barrier; vitamin E provides antioxidant protection.

Table 5. Component Analysis of *Lavandula angustifolia* Essential Oil

Peak	Retention time	Area sum	%	Name
1	8.073	123595.07	0.4	alpha pinene
2	8.603	52351.35	0.17	camphene
3	9.122	69062.57	0.23	beta pinene
4	9.559	456523.86	1.5	beta myrcene
5	10.338	137311.24	0.45	dl-limonene
6	10.632	1766534.08	5.79	cis-ocimene
7	10.956	1035890.86	3.39	delta 3 carene
8	11.061	66310.21	0.22	gamma terpinene
9	11.107	247048.35	0.81	3-octanone
10	11.292	127358.97	0.42	hexyl acetate
11	11.494	48178.02	0.16	paracymenene
12	11.732	40286.52	0.13	alpha terpinolene
13	13.955	91209.42	0.3	hexyl butyrate
14	16.317	9883373.81	32.38	linalool
15	16.534	91799.91	0.3	camphor
16	16.675	8576486.84	28.1	linalyl acetate
17	17.285	105314.84	0.35	alpha santelene
18	17.554	1268798.01	4.16	lavandulyl acetate
19	17.759	1804589.12	5.91	terpinen 4-ol
20	18.007	1014212.14	3.32	trans caryophyllene
21	18.68	1021859.36	3.35	trans beta farnesene
22	18.847	400235.84	1.31	lavandulol
23	19.41	354328.04	1.16	alpha terpineol
24	19.622	195572.33	0.64	endoborneol

5.2.3. Physicochemical studies

As part of the physicochemical characterization of the formulations, the pH of each formulation was measured using a pH meter (Mettler). Viscosity analysis was performed with a rotary viscometer. Additionally, the formulations were visually evaluated for homogeneity, phase separation, color changes, and spreadability.

5.2.4. Weight deviation control

The tests were conducted following the method outlined in the British Pharmacopoeia (BP) 2002. In this study, twenty different lip products were weighed to determine their average weight. For the weight deviation test, the requirement was that at least eighteen of the weighed products should not deviate by more than 5% from the average weight. Additionally, a maximum of two products was permitted to deviate by more than 7.5%. All tests were performed three times to ensure repeatability and reliability.

5.2.5. Appearance control

The study was conducted to identify potential defects such as crystal formation, mold, or fungal contamination on the surfaces of lip products. The homogeneity of the products was evaluated by examining both the surfaces and cross-sections

5.2.6. Organoleptic properties

The organoleptic properties of the prepared herbal lip care products were evaluated based on parameters such as color, odor, and fullness.

5.2.7. Determination of melting time

Melting point/time was determined using open-ended glass capillary tubes (n=5) filled to ~10 mm with the lip product. After equilibration, tubes were subjected to specified temperatures. The objective was a formulation stable at room temperature yet melting upon lip application.

Table 6. Lip formulation components

Ingredients (g)	Function(s)	LF-1	LF-2	LF-3	LF-4	LF-5	LF-6	LF-7	LF-8	LF-9
Lavandula angustifolia Oil	Antimicrobial, Fragrance, Soothing	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Rosa damascena Extract	Antimicrobial, Fragrance, Soothing	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20
Cocoa Butter	Emollient, Occlusive, Thickener	2.50	5.00	10.00	-	-	-	1.25	2.50	5.00
Witepsol	Solid Base/Hardener, Thickener (Lipid Base)	-	-	-	2.50	5.00	10.00	1.25	2.50	5.00
Chitosan	Film Forming, Potential Antimicrobial, Viscosity	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
Lanolin	Emollient, Occlusive, Restorative	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00
Vaseline	Emollient, Occlusive, Restorative	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Sweet Almond Oil	Emollient, Skin Conditioner	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Olive Oil	Emollient, Skin Conditioner	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Rosemary Oil	Antioxidant, Antimicrobial, Fragrance	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Clove Oil	Antimicrobial, Fragrance, Antioxidant, (Topical Analgesic) (Main active ingredient: Eugenol)	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Tocopherol (Vitamin E)	Antioxidant, Skin Conditioner	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20
Ascorbic Acid (Vitamin C)	Antioxidant, (pH Regulator)	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20
EDTA	Chelating Agent (Stability)	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Phenoxyethanol Ethylhexylglycerin	Protective System	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Pigment	Colorant	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05

5.2.8. Breaking point test

The structural integrity of the lip product was evaluated through a mechanical strength test. This involved subjecting 10-gram samples, positioned horizontally with a 1-inch overhang, to incrementally increasing weight until breakage occurred within a 30-second timeframe. The breaking point was defined as the weight that induced this failure. Each sample was tested in triplicate. This methodology is a common

practice for assessing the structural resilience of lip products and their resistance to fracture under normal usage conditions [10].

5.2.9. pH parameters

To determine the pH value of the lip care product, samples were maintained within a temperature jacket (Labo Instrument, Turkey) at a constant temperature of 34 ± 2 °C. pH measurements were performed using a Milwaukee MW150 pH meter. Considering the skin pH range is 4.5-6.5, the product was targeted to exhibit a pH within these values. All tests were conducted in triplicate.

5.2.10. Calculation of margin of safety (MoS)

The systemic exposure dose (SED) was estimated using equation 1, and the margin of safety (MoS) values were estimated using equation 2. The MoS of the lip formulation was determined with respect to skin surface area of 4.8 cm² and application frequency of two times per day as recommended by Cosmetics Guideline3. The daily exposure level was found to be 0.057 g/day. The systemic exposure dose (SED) was calculated using formula (1), and the margin of safety (MoS) values were calculated using formula (2).

$$SED\ (mg/gün) = \frac{DAa\ (\mu g/cm^2) \times 10^{-3}\ mg/\mu g \times SSA\ (cm^2) \times F\ (gün^{-1})}{60} \quad \text{(Eq.1)}$$

$$MoS = PODsys\ /\ (SED * \%Cons.) \geq 100\ (erişkinler\ için) \quad \text{(Eq.2)}$$

Formula 1, used to calculate the systemic exposure dose (SED), includes the applied dermal dose (DA_a) (μg/cm²), the application area (skin surface area, SSA) (cm²), and the application frequency per day (day^{-1}) as parameters. Formula 2, used to calculate the margin of safety (MoS), includes the point of departure for systemic effects (PoDsys) and the concentration percentage (% Cons.).

5.2.11. Stability of lip care product formulation

This evaluation was conducted following the guidelines established by the International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) and the cosmetics regulation stipulated by the Turkish Medicines and Medical Devices Agency (TİTCK). Within this framework, the products underwent storage at two distinct temperature conditions: refrigerated conditions, maintained at approximately 4 °C, and ambient conditions, controlled at 25 ± 2 °C, for six months. Throughout stability studies, assessments were periodically performed to monitor the products' physical characteristics, including appearance, color, pH value, viscosity, and microbiological growth status.

5.3. Microbiological Evaluation

5.3.1. Preparation of samples

To prevent contamination during microbiological assays, all containers used were disinfected by immersion in 70% ethanol. For specimen preparation, a one-gram sample of the lip product was mixed with 9 ml of sterile phosphate-buffered saline (PBS) that contained 0.1% polysorbate 80. This mixture was then dissolved in a controlled water bath for 10 to 15 minutes. The resulting suspension underwent serial dilution, using sterile PBS with 0.1% polysorbate 80 as the diluent. The initial suspension was labeled as the 10^1 dilution, and further dilutions from 10^1 to 10^3 were created for the quantitative analysis of aerobic mesophilic bacteria and fungi [11].

5.3.2. Investigation of aerobic mesophilic microorganisms

The pour plate method was used to detect and quantify aerobic mesophilic microorganisms. A 1 mL aliquot from each sample was added to a sterile petri dish and mixed with 20 mL of agar medium using the

pour technique. The plates were incubated at 32.5 ± 2,5 °C for 3 to 5 days, after which aerobic mesophilic bacteria were enumerated for each sample [12].

5.3.4. The investigation of yeast and mold

Yeast and mold populations were quantified using the pour plate technique. For each sample, a 1 mL aliquot was aseptically dispensed into a sterile Petri dish, followed by the addition of 20 mL of Sabouraud dextrose agar (SDA). The agar and aliquot were thoroughly combined before solidification. The plates were incubated at 22.5 ± 2,5 °C for 5-7 days. After incubation, colony-forming units (CFUs) of yeast and mold were counted and recorded for each sample, providing a quantitative measure of the fungal population [11].

5.3.5. The investigation of Escherichia coli

The pour plate method was utilized to detect the presence of *Escherichia coli*. A 1 mL aliquot from each sample was carefully transferred to a sterile petri dish using aseptic techniques. Next, 20 mL of MacConkey Agar (MCA) medium (Merck, Germany) was added to the petri dish in its molten form and mixed thoroughly with the aliquot. The inoculated plates were then incubated for 18 to 72 hours at a temperature ranging from 30 to 35°C. After the incubation period, brick-red colonies that developed around the bile sediment were evaluated as indicators of the presence of *E. coli* [12].

5.3.6. The investigation of Staphylococcus aureus

To determine the presence of *Staphylococcus aureus,* a one-ml aliquot, derived from each specimen, was aseptically transferred onto Baird Parker Agar (Merck, Germany) medium. The inoculated plates were subsequently incubated within a temperature range of 30 to 35 °C for 18 to 72 hours. Following the incubation period, the observation of black, shiny colonies surrounded by clear zones was considered indicative of *S. aureus* presence. The enumeration of *S. aureus* detected in each specimen was performed, and the resulting data were documented [12].

5.3.7. The investigation of Pseudomonas aeruginosa

To ascertain the presence of *Pseudomonas aeruginosa,* a one-ml aliquot, derived from each specimen, was aseptically transferred to 20 ml of Cetrimide Agar (Merck, Germany) medium. The inoculated plates were then subjected to incubation within a temperature range of 30 to 35 °C for A duration ranging from 18 to 72 hours.. Following the incubation period, the observation of green colonies on the medium, if present, was considered indicative of *P. aeruginosa* presence. The enumeration of *P. aeruginosa* detected in each specimen was performed and the resulting data were documented [12].

5.3.8. The investigation of Candida albicans

To determine the presence of *Candida albicans*, a one-ml aliquot, derived from each specimen, was aseptically transferred onto Sabouraud Dextrose Agar (SDA) medium. The inoculated plates were subsequently incubated at a temperature of 25 °C for 5 to 7 days. Following the incubation period, the observation of white to beige colonies on the medium was considered presumptive evidence for *C. albicans* presence. The enumeration of *C. albicans* detected within each specimen was performed, and the resultant data were documented [11]. In instances where microbial growth is detected as a result of microbiological assays, the concentration of colony-forming units (CFU) arising within each specimen is calculated using the following formula (Eq.3).

$$CFU/ml = \frac{\text{Total number of colonies obtained} \times \text{Dilution factor}}{\text{Sample volüme (ml)}} \quad \text{(Eq.3)}$$

5.4. Antimicrobial Activity

The antimicrobial activity of the oils was evaluated in vitro against a panel of microbial strains, encompassing Gram-negative bacteria (*E. coli* ATCC 25922, *P. aeruginosa* ATCC 27853), Gram-positive bacteria (*B. cereus* ATCC 11778, *E. faecalis* ATCC 29212, *S. aureus* ATCC 25923, MRSA ATCC 43300), and the fungal pathogen *C. albicans* ATCC 90028. To determine the MIC values at which the oils exhibited antibacterial

activity, a microdilution method was employed. In this method, 96-well microplates were prepared. Serial two-fold dilutions were performed by adding 100 µL of the sample to wells containing Mueller Hinton Broth (MHB) medium. Subsequently, 5 µL of a bacterial suspension, prepared to a 0.5 McFarland turbidity (10^8/mL), was added to each well. The microplates were then incubated overnight at 35°C. Following incubation, the microplates were assessed. The MIC was defined as the lowest concentration at which no microbial growth was observed.

5.5. Statistical Analysis

Experimental data were collected by the Randomized Block Design (RBD) with three replications (n=3) and the differences between the physicochemical properties (weight deviation, melting point, fracture resistance) of the formulations (LF-1 to LF-9) were examined by one-way analysis of variance (ANOVA) at $p < 0.05$ significance level. ANOVA revealed that there were statistically significant differences among the formulations for weight deviation and melting point parameters ($p < 0.01$ and $p < 0.001$, respectively), but there was no significant difference in fracture resistance ($p > 0.05$). For the parameters showing significant differences, the differences between the specific means were determined and these differences are indicated with different letters in the relevant tables/figures.

Acknowledgements: This study was funded by the Scientific and Technological Research Council of Turkey (TUBITAK) Tubitak-2209/A Grant No 1919B012311654. We would also like to thank Sebat Rose Oil and Essential Oils Cosmetics Construction. Machinery. Tur. Industry. Trade Ltd. Co.

Author contributions: Concept- B.I., A.A.K.; Design- B.I., A.A.K.; Supervision – B.I.; Resources.; B.I.; Materials- B.I., A.A.K., C.A., Ş.A., H.Y., B.Z.; Data Collection and/or Processing - B.I., A.A.K; Analysis and/or Interpretation - B.I., A.A.K.; Literature Search -B.I.; Writing-B.I.; Critical Reviews - B.I., A.A.K.

Conflict of interest statement: The authors declared no conflict of interest in the manuscript.

REFERENCES

[1] Wang M, Zhang L, Hao H, Yan M, Zhu Z. Applications of Engineered Skin Tissue for Cosmetic Component and Toxicology Detection. Cell Transplant. 2024; 33:1-24. https://doi.org/10.1177/09636897241235464
[2] Sasounian R, Martinez RM, Lopes AM, Giarolla J, Rosado C, Magalhães WV, Innovative Approaches to an Eco-Friendly Cosmetic Industry: A Review of Sustainable Ingredients. Clean Technol. 2024; 6(1):1-23. https://doi.org/10.3390/cleantechnol6010011.
[3] Xie M, Jiang Z, Lin X, Wei X. Application of plant extract cosmetics in the field of anti-aging. J Dermatol Sci Cosmetic Technol. 2024; 1(2): 100014. https://doi.org/10.1016/j.jdsct.2024.100014
[4] Annapurna Veludurthi P, Kumar Vadaga AA. Comprehensive Review of Formulations, Ingredients, Advances, and Future Perspectives in Lipcare Cosmetics. J Pharm Insight Res. 2024; 02(03):123-128. https://doi.org/10.5281/zenodo.11527489
[5] Bibow A, Oleszek, W. Essential Oils as Potential Natural Antioxidants, Antimicrobial, and Antifungal Agents in Active Food Packaging. Antibiotics 2024;13: 1168. https://doi.org/10.3390/antibiotics13121168
[6] Vora LK, Gholap AD, Hatvate NT, Naren P, Khan S, Chavda VP. Essential oils for clinical aromatherapy: A comprehensive review. J Ethnopharmacol. 2024;330(10): 118180. https://doi.org/10.1016/j.jep.2024.118180
[7] Cheng BCY, Fu XQ, Guo H, Li T, Wu ZZ, Chan K. The genus Rosa and arthritis: Overview on pharmacological perspectives. Pharmacol Res. 2016; 114:219-234. https://doi.org/10.1016/j.phrs.2016.10.029
[8] Speranza B, Guerrieri A, Racioppo A, Bevilacqua A, Campaniello D, Corbo MR. Sage and Lavender Essential Oils as Potential Antimicrobial Agents for Foods. Microbiol Res (Pavia). 2023; 14(3): 1089-1113. https://doi.org/1010.3390/microbiolres14030073
[9] British Pharmacopoeia Commission, British Pharmacopoeia, 13th Edition, 2002.
[10] Kathleen P, William M. Martindale: the complete drug reference. Br J Clin Pharmacol. 2000; 49(6): 613. https://doi.org/10.1046/j.1365-2125.2000.00206.x
[11] Bashir A, Lambert P. Microbiological study of used cosmetic products: highlighting possible impact on consumer health. J Appl Microbiol. 2020; 128: 598-605. https://doi.org/10.1111/jam.14479
[12] Onurdağ FK. Microbiological Investigation of Used Cosmetic Samples. Hacettepe Univ J Fac Pharm. 2010; 1: 1-16.
[13] Habib U, Pervaiz A, Murtaza A, Hussain A, Sarwar H, Ulah M, Ayub MA, Ahmad N, Bashir M. Assessing The Cumulative Effects of Heavy Metal Exposure From Cosmetic Products On Local Consumers. J Mountain Area Res. 2024; 9: 149-167. https://doi.org/10.53874/jmar.v9i0.195

[14] Atanasov AG, Zotchev SB, Dirsch VM, Orhan IE, Banach M, Rollinger JM. Natural products in drug discovery: advances and opportunities. Nat Rev Drug Discov. 2021; 20(3):200–216. https://doi.org/10.1038/s41573-020-00114-z
[15] Gonçalves S, Gaivão I. Natural Ingredients in Skincare: A Scoping Review of Efficacy and Benefits. Biomed Biopharm Res. 2023; 20(2):143-160. https://doi.org/10.19277/bbr.20.2.328
[16] Lukić M, Pantelić I, Savić SD. Towards optimal pH of the skin and topical formulations: From the current state of the art to tailored products. Cosmetics. 2021; 8(3): 69. https://doi.org/10.3390/cosmetics8030069
[17] Kaczmarczyk D, Strub DJ, Polowy A, Wilk KA, Lochyński S. Selected Essential Oils in Cosmetic Emulsions: Process Oriented Stability Studies and Antimicrobial Activity. Nat Vol Essent Oils. 2015; 2(4): 27-39.
[18] Shkreli R, Terziu R, Memushaj L, Dhamo K. Formulation and stability evaluation of a cosmetics emulsion loaded with different concentrations of synthetic and natural preservative. Hist Stud Phys Biol Sci. 2022; 5(1):38-51. https://doi.org/10.62400/jbs.v5i1.6373
[19] Visht S, Salih SS, Mohammed DA, Abduljabbar AA, Hama SJ, Khudhair IA. Formulation and Evaluation of Lip Balm Using Different Herbal Pigments. Pharmacogn Res. 2024; 16(2):367-375. https://doi.org/10.5530/pres.16.2.46
[20] Fajdek-Bieda A, Pawlin´ska J, Wróblewska A, Z˙wierełło W, Łus´ A. Antibacterial and Preservative Potential of Eugenol and Isoeugenol in Cosmetics: A Natural Solution for Product Stability. Appl Sci. 2025; 15:2129. https://doi.org/10.3390/app15042129
[21] Nong Y, Maloh J, Natarelli N, Gunt HB, Tristani E, Sivamani RK. A review of the use of beeswax in skincare. J Cosmetic Dermatol. 2023; 22:2166–2173. https://doi.org/10.1111/jocd.15718
[22] Purnamawati S, Indrastuti N, Danarti R, Saefudin T. The Role of Moisturizers in Addressing Various Kinds of Dermatitis: A Review. Clin Med Res. 2017; 15: 3-4: 75-87. https://doi.org/10.3121/cmr.2017.1363
[23] Chouhan S, Sharma K, Guleria S. Antimicrobial Activity of Some Essential Oils—Present Status and Future Perspectives. Medicines 2017; 4: 58. https://doi.org/10.3390/medicines4030058
[24] Zia P, Sunita M, Sneha S. Extraction of Natural Colour from Beet Root (Beta vulgaris), Its Phytochemical Analysis and Antibacterial Activity. EAS J Nutr Food Sci. 2021; 3: 4. 80-84. https://doi.org/84.10.36349/easjnfs
[25] Raeber J, Favrod S, Steuer C. Determination of Major, Minor and Chiral Components as Quality and Authenticity Markers of Rosa damascena Oil by GC-FID. Plants. 2023; 12: 506. https://doi.org/10.3390/plants12030506
[26] Jung J, Sewenig S, Hener U, Mosandı A. Comprehensive authenticity assessment of lavender oils using multielement/multicomponent isotope ratio mass spectrometry analysis and enantioselective multidimensional gas chromatography–mass spectrometry. Eur Food Res Technol. 2005; 220:232–237. https://doi.org/1010.1007/s00217-004-1049-4
[27] Husnu Can, Baser K, Buchbauer G. Handbook of Essential Oils: Science, Technology, and Applications. CRG. 2010.
[28] Lopez GP, Roque LB, Igal K, Espinosa EG, Bellotti N. Citronellol-functionalized natural silica: a biogenic approach for antifungal and antibacterial material applications. Front Chem. 2025; 13:1535787. https://doi.org/10.3389/fchem.2025.1535787
[29] Chen W, Viljoen AM, Geraniol – A review update. South Afr J Bot. 2022; 2022 (150):1205-1219. https://doi.org/10.1016/j.sajb.2022.09.012.
[30] Patel K, Panchal R, Sakariya B, Gevariya M, Raüyani R, Soni R, Goswami D. Combatting antibiotic resistance by exploring the promise of Quorum Quenching in targeting bacterial virulence. Microbe J. 2025; 6: 100224. https://doi.org/10.1016/j.microb.2024.100224
[31] Rybczyńska-Tkaczyk K, Grenda A, Jakubczyk A, Kiersnowska K, Bik-Małodzińska M. Natural Compounds with Antimicrobial Properties in Cosmetics. Pathogens. 2023; 15,12(2):320. https://doi.org/10.3390/pathogens12020320
[32] Tang T, Zhong W, Tang P, Dai R, Guo J, Gao Z. Linalool's Multifaceted Antimicrobial Potential: Unveiling its Antimicrobial Efficacy and Immunomodulatory Role Against Saprolegnia parasitica. BioRxiv. 2024; 600497. http://biorxiv.org/lookup/doi/10.1101/2024.06.24.600497
[33] Truong S, Mudgil P. The antibacterial effectiveness of lavender essential oil against methicillin-resistant Staphylococcus aureus: a systematic review. Front Pharmacol. 2023; 7(14):1306003. https://doi.org/10.3389/fphar.2023.1306003
[34] Maquera Huacho PM, Rodriguez Herrero E, Verspecht T, Pauwels M, Marcantonio E, Palomari Spolidorio DM. Terpinen-4-ol and carvacrol affect multi-species biofilm composition. Biofouling. 2019; 35(5):561-572. https://doi.org/10.1080/08927014.2019.1630386
[35] Mawazi SM, Redzal NAA, Othman N, Alolayan SO, Lipsticks History. Formulations and Production: A Narrative Review. Cosmetics.2022; 9(1): 25. https://doi.org/10.3390/cosmetics9010025
[36] Adegboye T, Itiola O. Physical and Release Properties of Metronidazole Suppositories. Trop J Pharm Res. 2008; 7 (1): 887-896. https://doi.org/10.4314/tjpr.v7i1.14673
[37] Şekeroğlu G, Kaya A. Effects of shear and cooling rates on the crystallization behavior of cocoa butter. Harran Tarım ve Gıda Bilimleri Derg. 2021; 25(2): 120-130. https://doi.org/10.29050/harranziraat.843455
[38] Declerck A, Nelis V, Danthine S, Dewettinck K, Vander Meeren P. Characterisation of fat crystal polymorphism in cocoa butter by time-domain nmr and dsc deconvolution. Foods. 2021; 10(3): 520. https://doi.org/10.3390/foods10030520

[39] Stobbs JA, Pensini E, Ghazani SM, Leontowich AFG, Quirk A, Tu K. Phospholipid Self-Assembly in Cocoa Butter Provides a Crystallizing Surface for Seeding the Form V Polymorph in Chocolate. Crystal Growth Des. 2024: 2024 (24): 2685-26699. https://doi.org/10.1021/acs.cgd.3c01130
[40] Velasco MVR, Fernandes AR, Ferrera M, Claudinéia D, Sales A, Pinto O. Stability evaluation of organic Lip Balm. Brazil J Pharm Sci. 2013; 49(2):293. https://doi.org/10.1590/S1984-82502013000200011
[41] Türkiye İlaç ve Tıbbi Cihaz Kurumu Klinik Araştırmalar. Türkiye İlaç ve Tıbbi Cihaz Kurumu Klinik Araştırmalar Daire Başkanlığına Yapılan Klinik Araştırma Başvurularına İlişkin Kılavuz. Türkiye İlaç ve Tıbbi Cihaz Kurumu Klinik Araştırmalar. 2024. https://www.titck.gov.tr/faaliyetalanlari/ilac/klinik-arastirmalar
[42] Rico F, Mazabel A, Egurrola G, Pulido J, Barrios N, Marquez R. Meta-Analysis and Analytical Methods in Cosmetics Formulation: A Review. Cosmetics. 2024; 11(1): 1. https://doi.org/10.3390/cosmetics11010001
[43] Gismondi A, Di Marco G, Redi EL, Ferrucci L, Cantonetti M, Canini A. The antimicrobial activity of Lavandula angustifolia Mill. essential oil against Staphylococcus species in a hospital environment. J Herb Med. 2012; 26: 100426. https://doi.org/10.1016/j.hermed.2021.100426
[44] Jiang Y, Wu N, Fu YJ, Wang W, Luo M, Zhao CJ. Chemical composition and antimicrobial activity of the essential oil of Rosemary. Environ Toxicol Pharmacol. 2011; 32: 63–68. https://doi.org/10.1016/j.etap.2011.03.011
[45] Swamy MK, Akhtar MS, Sinniah UR. Antimicrobial properties of essential oils plant against human pathogens and their mode of action: An updated review. Evid-Based Complement Altern Med. 2016; 20:3012462. https://doi.org/10.1155/2016/3012462
[46] Tullio V, Roana J, Cavallo L, Mandras N. Immune Defences: A View from the Side of the Essential Oils. Molecules. 2023; 28(1): 435. https://doi.org/10.3390/molecules28010435435