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Efficacy of *Ferula gummosa* Essential Oil in an Anti-Acne Cream: Evaluation of Antibacterial Activity, Sebum Reduction, and Sensory Properties

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ABSTRACT

Acne vulgaris is a prevalent skin condition affecting adolescents and young adults, with conventional treatments often leading to undesirable side effects. This study developed the formulation of an anti-acne cream based on *Ferula gummosa* essential oil and evaluated its physicochemical properties, antibacterial efficacy, and effects on sebum production. The formulated cream demonstrated favorable physicochemical properties, making it suitable for skin application. It exhibited strong antibacterial activity against both *Propionibacterium acnes* and *Staphylococcus epidermidis*. Significant reductions in sebum levels were observed in all areas of the face studied at the end of the trial. Participants rated the cream highly on several quality dimensions, although improvements in fragrance were suggested. The anti-acne cream containing galbanum essential oil shows significant potential in the treatment of acne vulgaris, combining effective sebum reduction and antibacterial activity with favorable user acceptability. This study supports further development and clinical testing of *F. gummosa* based formulations as a natural and effective treatment option for acne.
Keywords: Acne vulgaris, Galbanum based cream, *Propionibacterium acnes*, Sebum

INTRODUCTION

Acne vulgaris is the most prevalent chronic inflammatory skin disorder, profoundly impacting psychological health, manifesting as social phobia, isolation, and depression [1]. The etiology of acne involves increased sebum production induced by androgens, altered sebum composition, hyperproliferation of follicular keratinocytes, and colonization by microorganisms such as *Propionibacterium acnes*, *Staphylococcus epidermidis*, and *Malassezia furfur* within the pilosebaceous unit [2]. Traditional management strategies, primarily oral and topical antibiotics and retinoids, necessitate long-term administration and are associated with numerous adverse effects [3]. Thus, innovative therapeutic modalities are required to minimize side effects and enhance treatment efficacy.

The genus Ferula belongs to the family Apiaceae and contains 180-185 species distributed from central Asia westwards across the Mediterranean region to northern Africa [4]. In Flora Iranica, 30 species of Ferula have been mentioned in Iran, of which 15 species such as *F. gummosa*, *F. persica*, *F. aucheri* and *F. tabasensis* are native to Iran [4, 5]. *Ferula gummosa* Boiss. is a perennial and monocarpic plant that goes to the stem in the last year of growth (fourth to sixth year) and produces flowers and fruits [6]. This plant yields a latex known as galbanum, renowned for its broad pharmacological activities, including antimicrobial, disinfectant, and antidiabetic effects, attributed to its rich terpenoids content [7]. The essential oil (EO) of *F. gummosa* has demonstrated significant antimicrobial efficacy, surpassing traditional agents like 5% sodium hypochlorite and 0.2% chlorhexidine against pathogens such as *Enterococcus faeca*lis and *Candida albicans* [8]. Moreover, it has shown inhibitory effects against a spectrum of pathogenic bacteria including *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Salmonella enteritidis*, and *Listeria monocytogenes* [9,10]. Given its potent antimicrobial properties, *F. gummosa* presents a promising candidate for the development of novel acne treatments, especially in the context of rising antimicrobial resistance associated with conventional therapies.

The search for alternative acne treatments has directed attention towards *F. gummosa*, a plant native to Iran renowned for its extensive pharmacological portfolio. This plant's oleo-gum-resin, galbanum, contains a rich composition of bioactive terpenoids, including β-pinene, α-pinene, δ-3-carene and limonene [11, 12], which have demonstrated significant antimicrobial properties against a spectrum of pathogens that are relevant in acne pathogenesis [7]. These compounds disrupt microbial cell membranes and inhibit the synthesis of microbial toxins, effectively reducing the colonization of acne-inducing bacteria such as *Propionibacterium acnes* and *Staphylococcus epidermidis* [7, 9, 13]. Furthermore, the anti-inflammatory properties of these terpenoids could reduce the inflammatory response triggered by these bacteria within the pilosebaceous unit, a central pathogenic process in acne development. This dual action not only helps in mitigating the microbial load but also attenuates the inflammatory milieu that characterizes and exacerbates acne lesions. Moreover, studies have suggested that the sesquiterpenes in *F. gummosa* oil could modulate sebum production, addressing another crucial factor in acne pathophysiology [8]. These findings advocate for the integration of *F. gummosa* into dermatological research and development, aiming to exploit its bioactive compounds for novel topical therapies that target the key pathways implicated in acne.

Pharmacological agents that exhibit inhibitory effects against *Propionibacterium acnes* and *Staphylococcus epidermidis* are pivotal in the development of effective anti-acne therapies. Given the documented antimicrobial properties of *F. gummosa*, particularly its EO, the primary objective of this study was to develop a topical cream formulation aimed at treating acne vulgaris [14]. The study hypothesized that the EO of *F. gummosa*, rich in bioactive terpenoids such as β-pinene and limonene, would not only inhibit the growth of acne-related bacteria but also attenuate the inflammation associated with acne lesions. Initially, the antimicrobial efficacy of the EO was assessed

through in vitro studies to determine its inhibitory action against the targeted bacterial species. Subsequent in vivo studies were conducted to evaluate the clinical efficacy and safety of the formulated cream in managing acne symptoms in patients [15].

This research endeavors to harness the potent antimicrobial and anti-inflammatory properties of *F. gummosa* EO to innovate a novel topical treatment for acne vulgaris. By directly addressing both the bacterial proliferation and inflammatory aspects of acne through a natural formulation, this study aims to mitigate the reliance on traditional antibiotics and chemical agents, which are often marred by side effects and resistance issues. The development of such a treatment stands to not only broaden the therapeutic arsenal against acne but also offer a more sustainable and tolerable approach for patients suffering from this prevalent and distressing skin condition. Ultimately, the successful validation of *F. gummosa* EO as an effective anti-acne agent could pave the way for further exploration of plant-based therapeutics in dermatology, aligning with the growing demand for natural and holistic medical solutions.

MATERIALS AND METHODS

Raw Material

Ferula gummosa latex (oleo-gum resin) was collected from the natural habitat in Eqlid, Fars province, southwest of Iran (31°13′ N, 52°55' E) at an altitude of 2300 m above sea level, during September 2022. The collection process involved making precise incisions on the stem surface near the root of 4–6 years old plants to harvest latex, commonly known as galbanum. The latex was meticulously collected in clean stainless steel containers to prevent any contamination and preserve the integrity of the samples.

Essential Oil Isolation

The EO was extracted from the fresh latex of *F. gummosa* by water and steam distillation for five hours [16, 17]. The EO obtained was dehydrated with anhydrous sodium sulfate and stored in dark glass at 4-6°C until use.

GC and GC-MS Analysis

GC conditions

The GC analysis was carried out on an Agilent Technologies 7890 GC, equipped with an FID detector and a non-polar DB-5 column (length 30 m, internal diameter 0.25 mm and stationary phase thickness 0.25 µm). The temperature of the injector and detector was set at 280 °C. The initial column temperature was 60 °C and programmed to rise to 220 °C at a rate of 3 °C/min, then to 260 °C at a rate of 20 °C /min and held at this temperature for 10 min. Nitrogen was used as the carrier gas at a flow rate of 0.7 mL/min. The relative content of each component in EO was determined by electronic integration of the peak area without internal standard or FID response factor correction [18, 19].

GC-MS Conditions

GC-MS analysis of EO was performed on an Agilent 7890A gas chromatograph coupled to an Agilent 5975C quadrupole mass spectrometer, equipped with a DB-5 column (length 30 m, internal diameter 0.25 mm and stationary phase thickness 0.25 µm). The column temperature was initially set at 60 °C, and then programmed to increase to 220 °C at a rate of 3 °C/min, then to 260 °C at a rate of 20 °C/min, and finally to remain at this temperature for 5 min. Injection chamber and transfer line temperatures were set at 260 °C and 280 °C respectively. The carrier gas was helium at a linear velocity of 30.6 cm/s. The scan time was 1s, the ionization energy was 70 eV, and the mass range scan was 30-400 a.m.u [12, 18-20].

Identification of EO Composition

The EO was diluted 1:10 with dichloromethane and 1 µl of the diluted sample was injected into the GC-MS. The constituents of EO were characterized by comparing their mass spectra with those stored in the GC-MS library [21]. The accuracy of the chemical compounds was then confirmed by comparison of their retention indices (RI) to those of authentic standards and by the co-injection of standards available in our laboratory [12, 17-20].

Cream Formulation Process

The anti-acne cream has been carefully designed to consist of two distinct phases: an aqueous phase and an oily phase, as detailed in Table 1. The formulation process is crucial to achieving a stable emulsion that ensures effective delivery of the active ingredients.

Oily Phase Composition and Preparation

The oily phase of the cream contained several lipophilic components: ethyl glycol monoacetate, stearic acid, grape seed oil, cetyl alcohol, glycerin monoacetate, lanolin, and propylparaben. Each ingredient was selected for its specific role: stearic acid as an emulsifier and thickener, grape seed oil for its antioxidant activity and to enhance skin absorption, cetyl alcohol as a texture enhancer, glycerin monoacetate for moisturizing effects, lanolin as a moisturizer and emollient, and propylparaben as a preservative to extend the shelf life of the product. The ingredients were combined and heated to 75°C in a water bath to facilitate melting and homogeneous mixing, ensuring that all components were evenly distributed throughout the mixture [22].

Aqueous Phase Composition and Preparation

Simultaneously, the aqueous phase was prepared by dissolving methylparaben and triethanolamine in distilled water. Methylparaben served as a preservative to inhibit microbial growth, while triethanolamine was used to adjust the pH of the emulsion to match that of human skin, thereby increasing the stability and skin compatibility of the cream. This mixture was also heated to 75°C to match the temperature of the oily phase, which is critical for achieving a stable emulsion during mixing [23].

Emulsification and Addition of Active Ingredients

After preparation of each phase, the oily phase was slowly added to the aqueous phase with continuous stirring at 75°C in a water bath. This gradual mixing under controlled conditions promotes the formation of a smooth and stable emulsion. Once the initial emulsion was

formed and homogenized, the mixture was allowed to cool. During the cooling process, 0.3% of *F. gummosa* EO was added to the cream. This EO, known for its antimicrobial and anti-inflammatory properties, is the active ingredient that targets acne-causing bacteria and inflammation. The addition at this stage helps to preserve the therapeutic properties of the EO, which can be sensitive to prolonged heat exposure [23].

Final Product Stabilization

The resulting cream was then stirred continuously during the cooling process to ensure that the emulsion remained stable and uniform until it reached room temperature. The final product was a homogeneous cream with improved penetration capabilities for effective delivery of *F. gummosa* EO to the skin, making it a potent topical treatment for acne vulgaris.

Table 1 Ingredients of the anti-acne cream formulated based on *F. gummosa* essential oil

Phases	Ingredients	Amount (%)
Oily	ethyl glycol monoacetate	1
	stearic acid	8
	grape seed oil	10
	cetyl alcohol	3
	glycerin monoacetate	3
	lanolin	2
	propylparaben	1
Aqueous	distilled water	Up to 70
	methylparaben	1
	triethanolamine	1
F. gummosa essential oil		0.3

Physiochemical Evaluation of the Cream

The physiochemical properties of the formulated anti-acne cream were evaluated to ensure product quality and efficacy. The parameters studied included appearance, homogeneity, pH, after feel, type of smear, and removal utilizing methods adapted from Sekar and Halim [24]. The cream's appearance was assessed for color and consistency, attributes that affect consumer compliance. Homogeneity was evaluated by visual inspection to ensure a consistent texture and absence of segregation, which is critical for consumer acceptance and therapeutic effectiveness. The pH of the cream was measured to confirm its suitability for use on skin, aiming for a range that minimizes irritation while maintaining efficacy [25]. The 'after feel'–how the cream feels on the skin after application–was analyzed for greasiness, tackiness, and absorbency, all of which impact the user experience. The type of smear, which describes how the cream spreads on application, and the ease of removal, which can affect the practical daily use of the product, was also examined. Additionally, the stability of the cream was tested by storing samples at 4°C and 25°C for a period of two months. This stability testing helps predict the shelf-life of the cream under different temperature conditions, ensuring the longevity and efficacy of the active ingredients over time.

Antimicrobial Activity

The antimicrobial efficacy of the cream was rigorously tested using the disk diffusion method, a standard technique for assessing the antibacterial properties of topical formulations. *Staphylococcus epidermidis* and *Propionibacterium acnes*, the bacteria predominantly involved in acne pathogenesis, were obtained from the Pasteur Institute of Iran, Tehran. These bacteria were cultured on nutrient agar plates to foster growth. Sterile disks impregnated with the cream containing galbanum EO 0.3% were placed on the agar surfaces. The plates were then incubated at 37°C, for 24 hours, simulating the ideal growth conditions for these skin bacteria.

The diameter of the inhibition zones around each disk was measured using a ruler, providing a quantitative measure of the antibacterial activity of the cream. Vancomycin served as a positive control due to its known efficacy against gram-positive bacteria, which benchmarks the cream's antibacterial potency. Conversely, disks containing only distilled water acted as negative controls to validate the test conditions. The reproducibility of the results was ensured by repeating this experiment three times, enhancing the reliability of the data obtained [26].

Subjects and Treatments

This study involved a carefully selected group of 29 volunteers, comprised of 15 men and 14 women, with an average age of 29.24 ± 1.13 years. Participants were chosen based on specific inclusion criteria, which included being between the ages of 18 and 38, having naturally oily skin, and possessing a skin phototype between II and V according to the Fitzpatrick scale

This scale helps to understand the skin's response to UV light and the potential risk of skin disease based on the melanin pigment in the skin.

Exclusion criteria were set to ensure the safety and reliability of the results. Individuals younger than 18 or older than 38, those currently using other acne medications, and those with known skin sensitivity to similar products were excluded. Prior to the main study, a patch test was conducted where a small amount of the cream was applied to the skin and left overnight to check for any adverse reactions. Participants who exhibited any signs of irritation or allergic reaction were promptly excluded from further stages of the trial.

The primary focus of the study was to measure the efficacy of a newly formulated anti-acne cream containing *F. gummosa* EO in reducing sebum production. Sebum levels were quantitatively assessed using a Sebumeter SM 815 [27], which provides a precise measure of sebum production in units of sebum per square centimeter (sebum/cm²). Measurements were taken from three distinct facial areas: the forehead, cheeks, and chin to ensure comprehensive results.

Baseline sebum measurements were recorded prior to the initiation of the treatment regimen. Subsequent measurements were then taken at intervals–on days 7, 14, 21, and 28 post-initial applications—to monitor the changes and effectiveness of the cream over time. The

treatment involved the application of the cream twice daily, every 12 hours, ensuring consistent dosing and maximizing therapeutic efficacy. The percentage change in sebum levels from baseline at each time point was calculated to assess the effectiveness of the cream. Ethical considerations were strictly adhered to throughout the study. Written informed consent was obtained from all participants, ensuring they were fully aware of the study's scope and any potential risks. The ethical approval for this study underscores the commitment to uphold the dignity and safety of the participants, aligning with international standards for clinical research.

Sensory Evaluation

For the sensory evaluation of the newly formulated anti-acne cream, we employed a modified version of the cosmetic product sensory evaluation questionnaire developed by Blaak et al. [28]. This comprehensive tool was adapted specifically to assess the sensory attributes of our anti-acne cream. The questionnaire covered a range of sensory perceptions and user experiences including tolerability, care effects (such as moisturization and oil control), skin feel, absorbability, and olfactory properties. Additional questions evaluated the users' overall attitude towards the product, their likelihood of recommending the cream to others, its effectiveness in reducing acne, and its impact on skin freshness.

Participants rated each attribute on a detailed seven-point Likert scale ranging from "strongly agree" to "strongly disagree," allowing for nuanced feedback on each aspect of the cream's sensory profile. This method provided a quantifiable measure of user satisfaction and product acceptability, which are critical in determining the potential success of the cream in a consumer market.

Statistical Analysis

The data collected from the sensory evaluation, as well as the sebum measurements, were statistically analyzed to ascertain the efficacy and acceptability of the cream. All results were expressed as mean ± SEM to provide a clear understanding of the data distribution and variability. The changes in sebum levels among the subjects were analyzed using the paired T-Test, a statistical method suitable for comparing two related samples - in this case, sebum levels before and after the cream application.

A significance level of $P < 0.05$ was established a priori, indicating that differences or effects would be considered statistically significant if the *p*-value obtained was less than 0.05. This threshold helps to minimize the probability of type I errors, thus ensuring that the observed effects are unlikely to be due to chance.

SPSS software (version 24), a robust tool for complex statistical data analysis, was used for data analysis. Graphical presentation of the data, including histograms and line graphs, was carried out using GraphPad Prism (version 8). These tools not only facilitated detailed analysis of the data, but also allowed for clear and effective presentation of the results, thereby enhancing the readability and scientific rigor of our findings.

RESULTS

Composition of Essential Oil

The EO was isolated from the oleo-gum resin of *F. gummosa* by water and steam distillation with a yield of 19.16% (w/w). In terms of appearance and sensory characteristics, the EO of *F. gummosa* was a colorless and transparent liquid with a turpentine odour and a green woody note.

According to the GC-MS analysis, 21 compounds were identified in the EO profile, which are listed in Table 2 in the order of their elution on a DB-5 column.

The analysis prominently highlighted β-pinene as the main bioactive compound, accounting for 68.8% of the total composition, which is known for its anti-inflammatory and antimicrobial properties (Table 2). Limonene, another significant component, constituted 15.6% of the oil, contributing to its aromatic quality and therapeutic potential, particularly in anti-acne applications (Table 2). Additionally, δ-3-carene, which made up 5.2% of the oil, contributes to the antimicrobial activity and may synergize with other components to enhance the therapeutic efficacy of the oil (Table 2). Minor but noteworthy constituents included α-pinene (3.4%) and myrcene (2.4%), both recognized for their roles in fragrance and potential health benefits such as analgesic and anti-inflammatory effects (Table 2). Two components identified at lower levels are *E,Z*-1,3,5-undecatriene (1.5%) and terpinolene (0.8%), each contributing as a fragrance (Table 2). It is interesting to note that 1,3,5-undecatriene ingredients at low levels cause a woody, green note in the EO (Table 2) [16].

Other compounds such as Z-β-ocimene, α-terpinyl acetate, γ-elemene, δ-cadinene, germacrene B, guaiol, β-eudesmol, and α-eudesmol were present at less than 0.5% but are critical in defining the therapeutic properties of the EO (Table 2).

The results highlight the predominance of β-pinene, limonene and δ-3-carene as the main components (Table 2, Fig. 1), which is consistent with the proposed use of the oil in dermatological applications, particularly for the treatment of conditions such as acne vulgaris due to its significant antimicrobial activity.

Table 2 Chemical compositions of the essential oil of *F. gummosa*

Compound	Amount (%)	Retention Index (RI)[a]	Retention Index (Adams)
α-thujene	t	931	930
α-pinene [b]	3.4	940	939
sabinene	t	978	975
β-pinene[b]	68.8	984	979
myrcene	2.4	991	990
α-phellandrene	t	1010	1002
δ-3-carene[b]	5.2	1017	1011
L-limonene[b]	15.6	1035	1029
Z-β-ocimene	0.2	1039	1037
terpinolene	0.8	1091	1088

E,*Z*-1,3,5-undecatriene	1.4	1163	1163
E,*E*-1,3,5-undecatriene	0.2	1170	1170
α-terpinyl acetate	0.3	1342	1349
γ-elemene	0.3	1435	1436
α-humulene	t	1460	1454
germacrene D	t	1488	1481
δ-cadinene	0.2	1525	1523
germacerene B	0.2	1558	1561
guaiol	0.4	1604	1600
β-eudesmol	0.2	1656	1650
α-eudesmol	0.3	1660	1653

[a]The retention indices (RI) of the compounds were determined by co-injection of a homologous series of n-alkanes C_8–C_{24} on the DB-5 column; [b] The authentic standards available were used for co-injection / comparison analysis

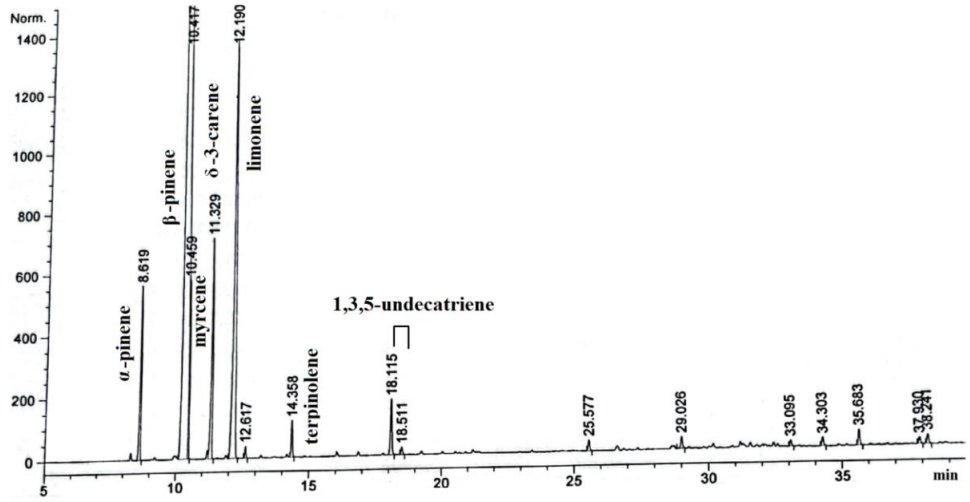

Fig. 1 GC chromatogram of the essential oil of *F. gummosa*

Comprehensive Physicochemical Characterization of Anti-Acne Cream

The formulation of the anti-acne cream was systematically evaluated for its physicochemical properties to ensure therapeutic efficacy and user acceptability. The results of these evaluations, which are critical to understanding the interaction of the cream with the skin and its overall usability, are summarized in Table 3.

Homogeneity and Appearance

The cream exhibited excellent homogeneity, with no visible signs of separation or inconsistency, which can often lead to uneven distribution of active ingredients during application. The consistent appearance and unchanged color throughout the study period also demonstrates to the stability of the product under different storage conditions.

Measurement of pH

The cream's pH was precisely measured at 5.23, which is within the ideal range for skin products, typically between 4.5 and 5.5 [29]. This level of acidity is crucial for maintaining the skin's natural barrier function and microbial flora, minimizing irritation and enhancing the skin's natural defense against pathogenic bacteria [25, 29].

Viscosity and Spreadability

Viscosity, measured at 337,700 cps, indicates the robust and stable consistency of the cream, which is essential to ensure the sustained release of active ingredients. High viscosity is indicative of the cream's ability to form a coherent film on the skin, providing a protective barrier and prolonged interaction of the active ingredients with the skin surface. Spreadability was quantified at 3.127 g.cm/s, demonstrating the ease of application of the cream- a critical factor in user compliance and satisfaction. The spreadability test reflects the mechanical resistance of product to shear forces during application, a key factor in the user's sensory experience.

Sensory Evaluation: Odor and After-Feel

The cream was reported to have a pleasant odor, an attribute that significantly enhances the desirability and acceptability of topical applications. The after-feel was described as emollient and slippery providing moisturization without leaving an undesirable residue. This after-feel is particularly beneficial for acne-prone skin, which often requires a balance between moisturization and non-occlusive formulations.

Ease of Removal and Type of Smear

Ease of removal is a critical attribute, especially for products used in combination with other skincare routines. The cream was easily rinsed off, facilitating its use as part of a broader skincare regimen and allowing reapplication or use of other products without residue build-up. The non-greasy smear quality of the cream ensures that it can be used under makeup or sunscreen, enhancing its versatility and appeal to users seeking a treatment-compatible with different skin products.

Table 3 Physiochemical properties of anti-acne cream formulated based on *F. gummosa* essential oil

pH	Viscosity (cps)	Spread-ability (g.cm/s)	Homogeneity	Appearance	Odor	After feel	Type of smear	Removal
5.23	337700	3.127	good	no change	good	emollient and slipperiness	non-greasy	easy

Antibacterial Efficacy of an Anti-Acne Cream Formulated with *F. gummosa* EO

The experimental results underscore a significant enhancement in antibacterial activity when *F. gummosa* EO is incorporated into the anti-acne cream. For *S. epidermidis*, the presence of the EO increased the inhibition zone to 9.92 ± 1.17 mm, compared to a considerably smaller zone of 6.32 ± 0.95 mm observed with the EO-free formulation. This demonstrates a substantial improvement in bacterial suppression, which is crucial given the role of *S. epidermidis* in skin infections and its potential to exacerbate acne.

Similarly, against *P. acnes*, the cream with EO produced an inhibition zone of 8.17 ± 0.92 mm, significantly larger than the 3.37 ± 0.82 mm observed for the cream without EO. This marked efficacy against *P. acnes* highlights the potential of the cream to directly target and reduce the bacterial load associated with acne, thereby mitigating one of the primary causative factors of this skin condition.

Visual and Quantitative Confirmation

The disk diffusion assay provided clear visual confirmation of these quantitative findings, with significantly larger zones of inhibition for the cream containing *F. gummosa* EO. This visual evidence corroborates the numerical data and illustrates the superior antibacterial performance of the cream. Such visual assessments are pivotal as they provide an immediate empirical confirmation of the cream efficacy.

Therapeutic Implications

The pronounced antibacterial activity of the cream with *F. gummosa* EO suggests significant therapeutic potential for the treatment of acne-prone skin. By effectively controlling the bacterial growth that contributes to acne pathology, this formulation could serve as a powerful tool in acne management strategies. The results not only validate the use of *F. gummosa* in topical applications, but also emphasize the importance of bioactive natural compounds in enhancing the efficacy of dermatological products (Table 4, Fig. 2).

Table 4 The antibacterial activity of the formulated cream

Pathogen	cream without *F. gummosa* essential oil				
	The diameter of zone inhibition (mm)				Mean±SD
	Rep 1	Rep 2	Rep 3	Rep 4	
S. epidermidis	6.8	5.3	7.4	5.8	6.32 ± 0.95
P. acnes	4.1	3.5	2.2	3.7	3.37 ± 0.82
	cream with *F. gummosa* essential oil (mm)				
S. epidermidis	11.2	9.8	10.3	8.4	9.92 ± 1.17
P. acnes	9.3	7.2	8.5	7.7	8.17 ± 0.92

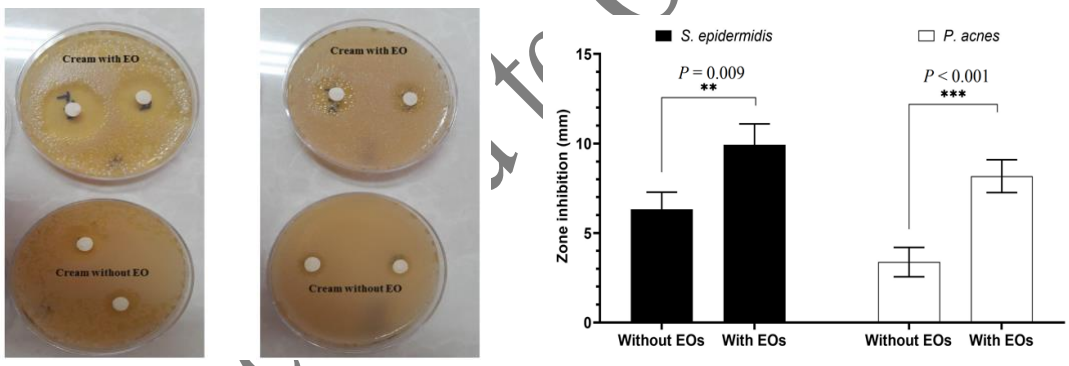

Fig. 2 The mean comparisons of the diameter zone inhibition of cream with or without *F. gummosa* EO against *S. epidermidis* and *P. acnes* evaluated by disk diffusion assay

Long-Term Effects of Anti-Acne Cream on Sebum Production

Forehead Region Results

The longitudinal assessment began with an initial sebum level measurement of 248.2 ± 44.96 µg/cm² in the forehead area. During 28-day treatment period, there was a marked reduction in sebum production, reaching a final level of 100.22 ± 10.98 µg/cm². The decrease was progressive and statistically significant at each weekly interval, with p values decreasing over time ($P = 0.036$ on day 7, $P = 0.008$ on day 14, and $P < 0.001$ on days 21 and 28). This trend highlights the consistent performance of the cream in reducing sebum production in one of the most sebum-prone areas of the face.

Cheek Region Results

In the cheek area, baseline sebum levels were recorded at 178.22 ± 29.48 µg/cm². Following the application of the anti-acne cream, a gradual decline in sebum levels was observed, culminating in a significant reduction to 122.1 ± 14.75 µg/cm² by the end of the study. The reductions became statistically significant midway through the study, with notable decreases marked on days 14 ($P = 0.049$) and 28 ($P = 0.018$). These findings highlight the cream's effectiveness in controlling sebum levels in the cheek region, which is critical in managing the spread and severity of acne.

Chin Region Results

The chin started with a sebum level of 183.25 ± 21.59 µg/cm², which was reduced to 126.11 ± 17.48 µg/cm² by day 28. The decrement in sebum levels was consistent and statistically significant on days 7 ($P = 0.046$), 21 ($P = 0.041$), and 28 ($P = 0.038$), reflecting the sustained effect of the cream throughout the treatment period (Fig. 3a). This reduction is particularly relevant given the density of sebaceous glands in the chin area, making it a critical target for effective acne treatment.

These detailed results indicate that the anti-acne cream, bolstered by the natural efficacy of *F. gummosa* EO, significantly and progressively reduces sebum production across different facial areas. By effectively lowering sebum levels, the cream not only addresses one of the fundamental factors contributing to acne formation, but also helps maintain skin health over time. This comprehensive analysis of the cream's performance on different areas of the face confirms its potential as a valuable addition to acne treatment regimens, offering a natural and effective solution to combat excess sebum secretion (Fig. 3b, 3c).

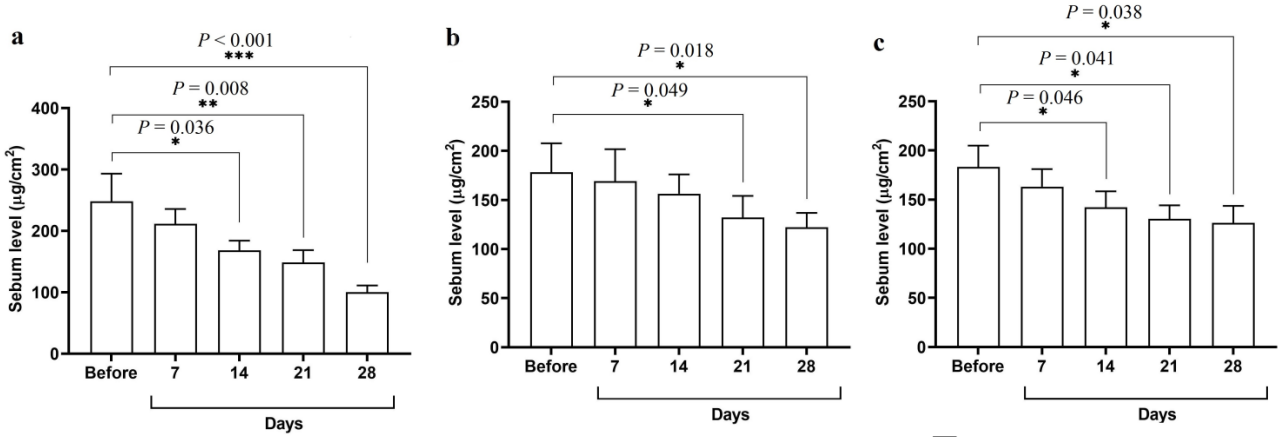

Fig. 3 The sebum levels of subjects used the formulated anti-acne cream with *F. gummosa* EO in the forehead (a), cheek (b) and chin (c) areas. The cream was used twice a day by the volunteers (n=25)

Comprehensive Sensory Evaluation of Anti-Acne Cream

In a comprehensive evaluation of the sensory attributes of an anti-acne cream formulated with *F. gummosa* EO, participants provided feedback after 28 days of use, which was analyzed to assess the overall efficacy of the cream and user satisfaction. The sensory attributes measured included tolerability, care effects, skin sensation, absorbency, odour, overall impression, likelihood to recommend, efficacy in reducing acne, and skin freshness.

Participants reported high tolerability with a mean score of 1.69, indicating that the cream was gentle and did not cause irritation, making it suitable for sensitive skin-a crucial factor in acne treatment products. The skincare benefits were also favorably reviewed, with a mean score of 1.82, reflecting the cream's ability to improve skin texture and hydration. This is important for acne treatments as effective moisturization can alleviate the dryness often associated with acne therapies.

The sensation of the cream on the skin was particularly well received (mean score of 1.62), with participants noting that the cream felt soothing and non-irritating upon application. Absorbability was another strong point (mean score of 1.82), as the cream absorbed quickly and cleanly into the skin without leaving a greasy residue, an important feature for users who may layer other skincare or cosmetic products over the acne treatment.

However, the assessment of the cream's aroma indicated some room for improvement (mean score of 2.90), suggesting that while the fragrance did not detract significantly from the overall experience, it was less pleasing compared to other aspects. Enhancing the fragrance could therefore potentially elevate the product's overall acceptance and satisfaction.

The overall impression of the cream was very positive (mean score of 1.38), indicating that most participants found the product aesthetically pleasing and effective. This was supported by a high likelihood of recommending the cream to others (mean score of 1.41), demonstrating confidence in its effectiveness and a willingness to endorse the product.

The cream's efficacy in reducing acne was underlined by a mean score of 1.55, showing that participants observed visible improvements in their acne symptoms over the course of the study. Additionally, the cream contributed to a feeling of skin freshness (mean score of 1.75), which helps in maintaining a clean and revitalized appearance.

This in-depth sensory analysis demonstrates that the anti-acne cream not only meets key functional and aesthetic expectations, but also highlights specific areas for further improvement. The insights gathered from participant feedback provide a valuable foundation for ongoing product development, with a particular focus on improving the fragrance to enhance user experience and satisfaction. Overall, the cream shows great promise as an effective and user-friendly option in the acne treatment market.

Table 4 Rating the formulated anti-acne cream with *F. gummosa* essential oil by subjects based on the Likert scale (1 = "strongly agree" to 7 = "strongly disagree")

Parameter	Mean	SD	1	2	3	4	5	6	7
Tolerability	1.69	0.13	13	12	4	-	-	-	-
Care effects	1.82	0.13	10	14	5	-	-	-	-
Skin sensation	1.62	0.16	17	8	2	2	-	-	-
Absorbability	1.82	0.11	8	18	3	-	-	-	-
Smell	2.9	0.24	6	3	12	5	2	1	-

Impression	1.38	0.12	20	8	1	-	-	-	-
Recommendation	1.41	0.10	18	10	1	-	-	-	-
Reducing acne	1.55	0.11	15	12	2	-	-	-	-
Skin freshness	1.75	0.14	13	10	6	-	-	-	-

DISCUSSION

Acne vulgaris is a prevalent skin disorder that notably affects individuals during adolescence through to adulthood, particularly between the ages of 20 to 40. The psychological impact of this condition is significant, often leading to isolation, diminished self-confidence, and in severe cases, depression. Given the adverse effects associated with conventional pharmacological treatments, there is a growing interest in exploring alternative therapies that utilize medicinal plants. In this context, our study focused on the potential of *F. gummosa* galbanum EO in formulating an anti-acne cream. On the basis of GC-MS analysis, 21 compounds were identified in the oil, with β-pinene (68.8%), limonene (15.6%), and δ-3-carene (5.2%) being the predominant components. Notably, the substantial concentration of limonene might be particularly significant due to its well-documented antimicrobial and anti-inflammatory properties, which are crucial in acne treatment [30].

Our findings align with existing research that underscores the therapeutic potential of EOs in dermatological applications. However, they also emphasize the complexity of using natural products where variability is inherent due to natural and procedural factors. This study contributes to the growing body of evidence supporting the use of *F. gummosa* in topical treatments and opens avenues for further research to explore its full potential in acne management strategies, aiming to provide a natural, effective, and well-tolerated alternative to synthetic drugs [22, 31, 32].

Acne vulgaris is prominently associated with the colonization of *P. acnes* and *S. epidermidis* on the skin. These bacteria are integral to the pathogenesis of acne, contributing significantly to the inflammatory processes observed in the condition [33]. Notably, the emergence of antimicrobial-resistant strains of these bacteria across various global regions has been well-documented, posing substantial challenges to traditional acne management strategies and underscoring the urgent need for novel therapeutic approaches [34, 35].

The potent antibacterial properties of *F. gummosa* EO has been highlighted in previous studies, suggesting its utility as an alternative treatment option. For instance, Eftekhar et al. demonstrated that the EO of *F. gummosa* exhibited superior antibacterial activity against standard strains of *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli*, and *Enterococcus faecalis* compared to conventional antibiotics such as ampicillin and erythromycin [8]. This finding is particularly significant, as it illustrates the oil's capacity to outperform established antimicrobials, suggesting its potential role in overcoming antibiotic resistance [36, 37]. In our study, we explored the efficacy of an anti-acne cream formulated with *F. gummosa* EO specifically against *P. acnes* and *S. epidermidis*. The results from disk diffusion assays confirmed that the cream possesses strong antimicrobial effects against these acne-related pathogens. The integration of *F. gummosa* EO into dermatological products could therefore represent a significant advancement in the treatment of acne. This approach leverages the natural antibacterial properties of the oil and offers a promising solution to the growing issue of antimicrobial resistance, aligning with the ongoing search for more sustainable and effective therapeutic strategies in dermatology [31].

Sebum production is a critical factor in the pathogenesis of acne, with elevated levels directly contributing to the development and exacerbation of the condition. Increased sebum not only clogs pores but also provides an enriched environment that fosters the growth of acne-causing microorganisms such as Propionibacterium acnes. Consequently, strategies aimed at reducing sebum secretion are pivotal in managing acne effectively [1, 38]. In this study, we quantitatively assessed the impact of an anti-acne cream formulated with *F. gummosa* EO on sebum production in three key facial areas: the forehead, cheeks, and chin. Measurements taken before and after a 28-day treatment period demonstrated significant reductions in sebum levels across all examined sites. Specifically, the data revealed a marked decrease in sebum production, aligning with the hypothesized benefits of the EO in modulating sebaceous gland activity.

The reduction in sebum levels suggests that the *F. gummosa* EO possesses specific properties that may inhibit sebaceous gland activity or alter lipid synthesis pathways, thereby mitigating one of the primary triggers of acne flare-ups. The efficacy of the cream in reducing sebum production not only supports its potential as a therapeutic agent but also highlights its role in addressing the overproduction of sebum–a key factor in acne pathophysiology. Moreover, by decreasing the availability of lipids from sebum, the cream likely contributes to an unfavorable environment for the proliferation of acne-related bacteria, further aiding in the reduction of acne symptoms. This dual action–direct reduction of sebum production and indirect suppression of bacterial growth–enhances the cream's therapeutic potential, making it a promising candidate for inclusion in acne treatment regimens [2, 39].

The results of this investigation highlight the critical role of sebum modulation in the management of acne and substantiate the efficacy of *F. gummosa* EO as a pivotal component in innovative acne treatment strategies. By targeting sebum production, which is central to acne's pathogenesis, this study demonstrates the oil's capacity to directly influence a primary causal factor in acne development. The use of *F. gummosa* EO not only addresses this key pathogenic mechanism but also introduces a natural and potentially less irritating alternative to conventional acne medications, which are often associated with significant adverse effects.

Additionally, the sensory evaluation of the anti-acne cream enriched with *F. gummosa* EO provided further insights into its acceptability and efficacy. Utilizing a modified version of the questionnaire developed by Blaak et al. [28], the study assessed various quality dimensions such as tolerability, care effects, skin sensation, absorbability, overall impression, willingness to recommend, effectiveness in reducing acne, and skin freshness. The results were overwhelmingly positive, indicating high levels of patient satisfaction across these metrics. Participants reported that the cream performed well in all assessed categories, affirming its favorable profile in terms of sensory attributes and functional benefits. The only exception was the cream's aroma, where the feedback suggested room for improvement [40-42]. This aspect of the sensory evaluation underscores the importance of fragrance in the overall user experience and highlights an area for potential enhancement to increase consumer satisfaction.

These findings collectively reinforce the therapeutic potential of *F. gummosa* EO in acne management. By providing a treatment that not only effectively reduces sebum production but also meets user expectations for sensory quality, the study advances the development of

holistic acne solutions. Such treatments promise enhanced patient compliance and satisfaction, which are crucial for the long-term success of any dermatological therapy.

The integration of *F. gummosa* galbanum EO into an anti-acne cream has demonstrated promising results in addressing two fundamental aspects of acne treatment: reduction of sebum production and inhibition of bacterial growth. The study's findings support the oil's potential as an efficacious alternative to traditional acne medications, which are often associated with adverse effects and antibiotic resistance [37,43]. Moreover, the sensory evaluations indicate a high degree of user satisfaction, particularly in terms of skin tolerability, moisturization, and overall effectiveness, although further refinement in fragrance could enhance consumer acceptance. These results not only underscore the therapeutic value of *F. gummosa* EO but also highlight the need for additional research to fully elucidate its mechanisms of action and long-term benefits. By focusing on natural, less irritating ingredients, this study contributes to the broader dermatological field, suggesting a shift towards more sustainable and patient-friendly treatment modalities for acne and other skin conditions [15].

CONCLUSION

In conclusion, this study establishes the therapeutic potential of *F. gummosa* essential oil in treating acne, highlighting its ability to significantly reduce sebum production and inhibit the growth of key acne-related bacteria, *Propionibacterium acnes* and *Staphylococcus epidermidis.* The antimicrobial efficacy of the cream, driven by major components like β-pinene, limonene, δ-3-carene and α-pinene, suggests a viable alternative to traditional acne treatments that often face challenges such as antibiotic resistance. While the overall sensory evaluation was positive, further refinement of the product's fragrance could enhance consumer satisfaction. These promising results support continued research into *F. gummosa* essential oil for its integration into dermatological practice as an effective natural acne treatment.

Conflict of Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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REFERENCES

1. Zaenglein A.L., Pathy A.L., Schlosser B.J., Alikhan A., Baldwin H.E., Berson D.S., Bowe W.P., Graber E.M., Harper J.C., Kang S., Keri J.E., Leyden J.J., Reynolds R.V., Silverberg N.B., Stein Gold L.F., Tollefson M.M., Weiss J.S., Dolan N.C., Sagan A.A., Stern M., Boyer K.M., Bhushan R. Guidelines of care for the management of acne vulgaris. Journal of the American Academy of Dermatology. 2016;74(5):945-973.e33.
2. Cong T.X., Hao D., Wen X., Li X.H., He G., Jiang X. From pathogenesis of acne vulgaris to anti-acne agents. Archives of Dermatological Research. 2019;311(5):337-349.
3. Oge L.K., Broussard A., Marshall M.D. Acne vulgaris: Diagnosis and treatment. American Family Physician. 2019;100(8):475-484.
4. Mozaffarian V. A dictionary of Iranian plant names: Latin-English-Persian: Farhang Mo'aser. 1996.
5. Mozaffarian V. The family of Umbelliferae. Iran- Keys and Distribution. Research Institute of Forests and Rangelands Press, Tehran, 1983, pp. 114-116.
6. Panahi M., Rezaee, M.B., Jaimand K. A review of phytochemistry and phylogeny that aid bio-prospecting in the traditional medicinal plant genus Ferula L.(Apiaceae) in Iran. Journal of Medicinal plants and By-Products. 2020;9(2):133-148.
7. Mahboubi M. *Ferula gummosa*, a traditional medicine with novel applications. Journal of Dietary Supplements. 2016;13(6):700-718.
8. Eftekhar F., Yousefzadi M., Borhani K. Antibacterial activity of the essential oil from *Ferula gummosa* seed. Fitoterapia. 2004;75(7):758-759.
9. Fatemikia S., Abbasipour H., Saeedizadeh A. Phytochemical and acaricidal study of the galbanum, *Ferula gummosa* Boiss. (Apiaceae) essential oil against tetranychus urticae koch (tetranychidae). Journal of Essential Oil Bearing Plants. 2017;20(1):185-195.
10. Jalili-Nik M., Soukhtanloo M., Javanshir S., Jahani Yazdi A., Esmaeilizadeh M., Jafarian A.H., Ghorbani A. Effects of ethanolic extract of *Ferula gummosa* oleo-resin in a rat model of streptozotocin-induced diabetes. Research in Pharmaceutical Sciences. 2019;14(2):138-145.
11. Mofasseri M., Tofighi Z., Pirali Hamedani M., Hadjiakhoondi A., Tavakoli S., Moein Z., Baharipour Z. Remarkable variation in phytochemicals of *Ferula gummosa* Bioss. essential oils collected from different parts of Iran. Research Journal of Pharmacognosy. 2022;9(4):29-38.
12. Vaez Shahrestani A., Azimi R., Abdousi V., Mirza M., Ghanbari Jahromi M. Evaluation of the physical and chemical properties of the essential oil obtained from the oleo-gum-resin of two populations of *Ferula gummosa* from Isfahan and Fars provinces. Eco-phytochemical Journal of Medicinal Plants. 2024;12(1):103-119.
13. Moon S.H., Roh H.S., Kim Y.H., Kim J.E., Ko J.Y., Ro Y.S. Antibiotic resistance of microbial strains isolated from korean acne patients. The Journal of Dermatology. 2012;39(10):833-837.
14. Mohammadhosseini M., Venditti A., Sarker S.D., Nahar L., Akbarzadeh A. The genus Ferula: Ethnobotany, phytochemistry and bioactivities – A Review. Industrial Crops and Products. 2019;129:350-394.
15. Asnaashari S., Kazemnezhad M., Masoud F., Javadzadeh Y. An overview on the anti-acne properties of herbal essential oils. Journal of Herbal Medicine. 2023;38:100642.

16. ISO 14716:1998(E). Oil of galbanum (Ferula galbaniflua Boiss. et Buhse).
17. Jalali H.T., Petronilho S., Villaverde J.J., Coimbra M.A., Domingues M.R.M., Ebrahimian Z.J., Silvestre A.J., Rocha S.M. Deeper insight into the monoterpenic composition of Ferula gummosa oleo-gum-resin from Iran. Industrial Crops and Products. 2012;36(1):500-507.
18. Solouki A., Zare Mehrjerdi M., Aliniaeifard S., Azimi R. Postharvest light and temperature elicitors improve chemical composition and level of essential oils in basil (*Ocimum basilicum* L.) through boosting antioxidant machinery. Postharvest Biology and Technology. 2023;199:112279.
19. Mahdi Navehsi F., Abdossi V., Abbaszadeh B., Azimi R., Dianat M. Efect of gamma rays on the essential oil and biochemical characteristics of the *Satureja mutica* Fisch & C. A. Mey. Scientific Reports. 2024;14:7581.
20. Abbaszadeh B., Layeghhaghighi M., Azimi R., Hadi N. Improving water use efficiency through drought stress and using salicylic acid for proper production of *Rosmarinus officinalis* L. Industrial Crops and Products. 2020;144:111893.
21. Adams R.P. Identification of Essential Oil Components by Gas Chromatography-Mass Spectroscopy, 4th Edition, Allured Publishing Corporation, Carol Stream, Illinois, 2017.
22. Draelos Z.D. Cosmetic Dermatology: Products and Procedures. John Wiley & Sons, 2021.
23. Garg T., Rath G., Goyal A.K. Comprehensive review on additives of topical dosage forms for drug delivery. Drug Delivery. 2015;22(8):969-987.
24. Sekar M., Halim F.H.A. Formulation and evaluation of natural anti-acne cream containing syzygium samarangense fruits extract. Annual Research and Review in Biology. 2017;17(3):1-7.
25. Lukic M., Pantelic I., Savic S.D. Towards optimal pH of the skin and topical formulations: from the current state of the art to tailored products. Cosmetics. 2021;8(3):69.
26. Abbey T.C., Deak E. What's new from the CLSI subcommittee on antimicrobial susceptibility testing M100. Clinical Microbiology Newsletter. 2019;41(23):203-209.
27. Crowther, J.M. Method for quantification of oils and sebum levels on skin using the Sebumeter. International Journal of Cosmetic Science. 2016;38(2):210-216.
28. Blaak J., Keller D., Simon I., Schleißinger M., Schürer N.Y., Staib P. Consumer panel size in sensory cosmetic product evaluation: A pilot study from a statistical point of view. Journal of Cosmetics, Dermatological Sciences and Applications. 2018;8(3):97-109.
29. Segger D., Aßmus U., Brock M., Erasmy J., Finkel P., Fitzner A., Heuss H., Kortemeier U., Munke S., Rheinländer, T., Schmidt-Lewerkühne H., Schneider W., Weser G. Multicenter study on measurement of the natural pH of the skin surface. International Journal of Cosmetic Science. 2008;30(1),75.
30. Proença A.C., Luis A., Duarte A.P. The role of herbal medicine in the treatment of acne vulgaris: A systematic review of clinical trials. Evidence-Based Complementary and Alternative Medicine. 2022;(1):2011945.
31. Kousar S., Nadeem F., Khan O., Shahzadi A. Chemical synthesis of various limonene derivatives: A comprehensive review. International Journal of Chemical and Biochemical Sciences. 2017;11:102-112.
32. Boaro C.S.F., Vieira M.A.R., Campos F.G., Ferreira G., De-la-Cruz-Chacón I., Marques M.O.M. Factors Influencing the Production and Chemical Composition of Essential Oils in Aromatic Plants from Brazil. In: Essential Oil Research, Trends in Biosynthesis, Analytics, Industrial Applications and Biotechnological Production, Springer Nature. 2019, pp. 19-47.
33. Eady E.A. Ingham E. *Propionibacterium acnes*-friend or foe? Reviews in Medical Microbiology. 1994;5(3):163-173.
34. Claudel J.P., Auffret N., Leccia M.T., Poli F., Corvec S., Dréno B. *Staphylococcus epidermidis*: A potential new player in the physiopathology of acne? Dermatology. 2019;235(4):287-294.
35. Bagatin E., Rocha M.A.D.d., Freitas T.H.P., Costa C.S. Treatment challenges in adult female acne and future directions. Expert Review of Clinical Pharmacology. 2021;14(6): 687-701.
36. Utegenova G.A., Pallister K.B., Kushnarenko S.V., Özek G., Özek T., Abidkulova K.T., Kirpotina L.N., Schepetkin I.A., Quinn M.T., Voyich J.M. Chemical composition and antibacterial activity of essential oils from Ferula L. Species against methicillin-resistant *Staphylococcus aureus*. Molecules. 2018;23(7):1679.
37. Pajohi Alamoti M., Bazargani-Gilani B., Mahmoudi R., Reale A., Pakbin B., Di Renzo T., Kaboudari A. Essential oils from indigenous Iranian plants: A natural weapon vs. multidrug-resistant *Escherichia coli*. Microorganisms. 2022;10(1):109.
38. Eichenfield D.Z., Sprague J., Eichenfield L.F. Management of acne vulgaris – A Review. The Journal of the American Medical Association. 2021;326(20):2055-2067.
39. Yang J.H., Yoon J.Y., Kwon H.H., Min S., Moon J., Suh D.H. Seeking new acne treatment from natural products, devices and synthetic drug discovery. Dermato-Endocrinology. 2017;9(1): e1356520.
40. Ghasemi Y., Faridi P., Mehregan I., Mohagheghzadeh A. *Ferula gummosa* fruits: An aromatic antimicrobial agent. Chemistry of Natural Compounds. 2005;41(3):311-314.
41. Mohammadhosseini M., Nekoei M. Chemical compositions of the essential oils and volatile compounds from the aerial parts of *Ferula ovina* using hydrodistillation, MAHD, SFME and HS-SPME methods. Journal of Essential Oil Bearing Plants. 2014;17(5):747-757.
42. Li X., He C., Chen Z., Zhou C., Gan Y., Jia Y. A review of the role of sebum in the mechanism of acne pathogenesis. Journal of Cosmetic Dermatology. 2017;16(2):168-173.
43. Talebi Kouyakhi E., Naghavi M.R., Alayhs M. Study of the essential oil variation of *Ferula gummosa* samples from Iran. Chemistry of Natural Compounds. 2008;44(1):124-126.