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FORMULATION AND ANTIMICROBIAL INVESTIGATION OF A POLYHERBAL PREPARATION LOADED WITH ESSENTIAL OILS

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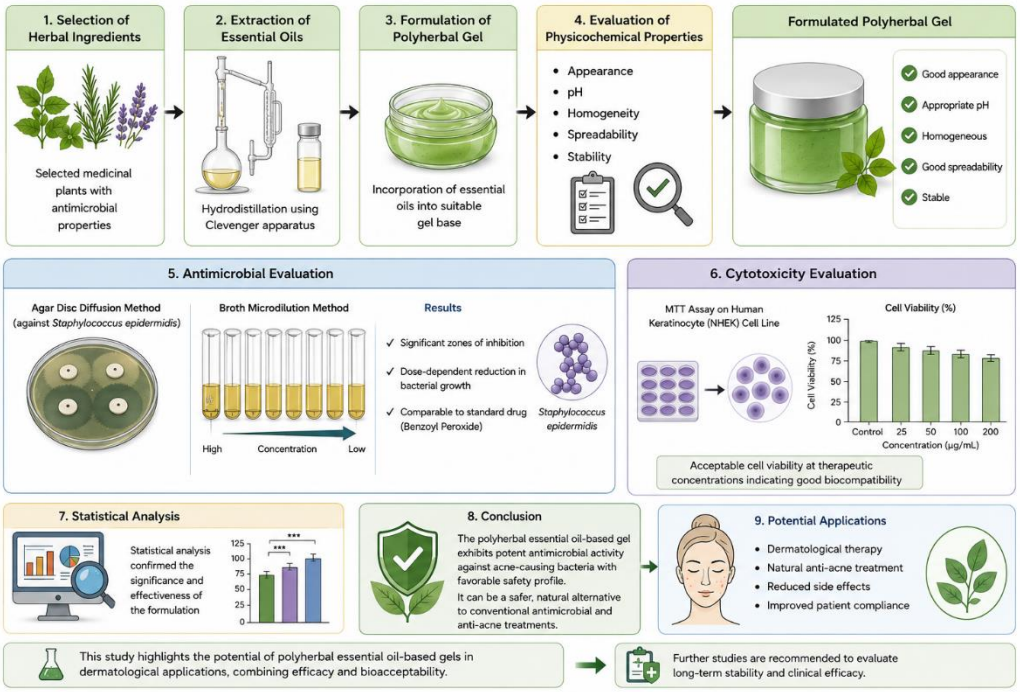
ABSTRACT

The present study was carried out to develop and evaluate a polyherbal gel containing essential oils for the treatment of acne and other microbial skin infections. Essential oils Carvacrol and thymol from Cymbopogon nardus (CNO) and Cymbopogon flexuosus (CFO) were extracted using the hydrodistillation method with a Clevenger apparatus. The extracted oils were assessed for their quality, antimicrobial activity, antioxidant potential, and safety profile. Both essential oils showed significant antibacterial activity against *Staphylococcus epidermidis*, a major microorganism associated with acne. Among the tested oils, CFO exhibited stronger antimicrobial activity than CNO, and the antibacterial effect increased with increasing concentration. A polyherbal gel containing both essential oils in a 1:1 ratio was successfully formulated and evaluated for various physicochemical parameters, including appearance, pH, homogeneity, viscosity, spreadability, and stability. The gel showed satisfactory characteristics suitable for topical application and remained stable under different storage conditions. Antimicrobial activity was evaluated using agar disc diffusion and broth microdilution methods, and the results were compared with the standard drug benzoyl peroxide. The formulated gel demonstrated enhanced antimicrobial activity and effective inhibition of microbial growth. In addition, the combined essential oil formulation exhibited higher antioxidant activity than the individual oils, suggesting a synergistic effect.The safety of the formulation was assessed using the MTT assay on Normal Human Epidermal Keratinocyte (NHEK) cell lines. The gel showed acceptable cell viability and lower cytotoxicity compared to the individual oils and standard drug. Overall, the developed polyherbal gel was found to be safe, stable, and effective, indicating its potential as a natural alternative for acne management and further clinical investigation.

KEYWORDS: Polyherbal gel, Essential oils, Antimicrobial activity, *Staphylococcus epidermidis*, Cytotoxicity, MTT assay, Acne, Dermatological formulation.

Graphical Abstract



INTRODUCTION

Microbe is one of the most common chronic inflammatory skin disorders affecting millions of people worldwide. It mainly occurs during adolescence due to hormonal changes but may continue into adulthood in many individuals.**[1]** According to recent reports, acne affects nearly 85% of adolescents and young adults at some stage of their lives. The condition not only causes physical discomfort but also negatively impacts self-esteem, confidence, and overall quality of life.**[2]** Acne develops in the pilosebaceous units of the skin and is characterized by the formation of comedones, papules, pustules, nodules, and cysts. Several factors contribute to acne development, including excessive sebum production, follicular hyperkeratinization, microbial colonization, and inflammation. Because of its multifactorial nature, acne remains a challenging dermatological condition to manage effectively.**[3]** For many years, Cutibacterium acnes was considered the primary microorganism responsible for acne pathogenesis.**[4]** Recent studies have highlighted the important role of Staphylococcus epidermidis in acne progression and skin inflammation. The interaction between these microorganisms and the host immune response contributes significantly to the severity of acne lesions.**[5]** Controlling microbial growth has become an important strategy in acne

treatment. Currently, several conventional therapies are available for acne management, including topical antibiotics, benzoyl peroxide, retinoids, and oral medications.**[6]** Although these treatments are effective, their long-term use may lead to adverse effects such as skin irritation, dryness, allergic reactions, and the development of antimicrobial resistance. As a result, there is increasing interest in safer and more natural alternatives for acne treatment.**[7]** Herbal medicines and essential oils have gained considerable attention due to their antimicrobial, anti-inflammatory, and antioxidant properties. Essential oils obtained from medicinal plants contain various bioactive compounds that can inhibit the growth of acne-associated microorganisms while minimizing adverse effects.**[8]** Polyherbal formulations, which combine multiple herbal ingredients, may provide synergistic therapeutic effects and improved efficacy compared to single-component treatments.**[9]** The global demand for herbal and natural skincare products is increasing rapidly and is expected to grow significantly in the coming years due to consumer preference for safer and eco-friendly products.**[10]** The development of polyherbal essential oil-based formulations represents a promising approach for future dermatological applications. In this study, a polyherbal gel containing selected essential oils was formulated and evaluated for its antimicrobial activity against acne-associated microorganisms, particularly Staphylococcus epidermidis. **[11]** The study also assessed the physicochemical properties and cytotoxicity profile of the formulation to determine its suitability as a safe and effective natural anti-acne treatment.

MATERIALS AND METHODS

MATERIALS

The polyherbal gel formulation was prepared using selected essential oils possessing antimicrobial and anti-inflammatory properties. Carvacrol and thymol were used as the principal bioactive constituents due to their reported effectiveness against acne-associated microorganisms.**[12]** Chitosan of pharmaceutical grade was employed as the gelling and film-forming polymer. Acetic acid was used for polymer dissolution, while glycerol served as a plasticizer and humectant. Tween 80 was incorporated as a non-ionic surfactant to improve the dispersion of essential oils within the gel matrix. Ethanol of analytical grade was used as a solvent where required. Phosphate buffer solutions were prepared using analytical-grade phosphate salts, and all aqueous preparations were made using distilled water.

Preparation of Polyherbal Gel

Essential oils containing carvacrol and thymol were extracted by hydro distillation using a Clevenger apparatus. Chitosan was dissolved in 1% (v/v) acetic acid solution under continuous stirring until a clear solution was obtained. Glycerol and Tween 80 were added to the polymer solution, followed by the gradual incorporation of the extracted essential oils. The mixture was stirred continuously to obtain a homogeneous gel formulation. The prepared gel was stored in airtight containers for further evaluation.

Physicochemical Evaluation

The formulated gel was evaluated for appearance, color, odor, pH, homogeneity, spread ability, and stability. The pH was measured using a calibrated digital pH meter. Spread ability was determined by the glass slide method, while homogeneity was assessed through visual inspection.

Antimicrobial Activity

The antimicrobial activity of the formulation was evaluated against *Staphylococcus epidermidis* using the agar disc diffusion and broth microdilution methods. The zones of inhibition produced by the formulation were measured and compared with those obtained using benzoyl peroxide as the standard reference drug. The minimum inhibitory concentration (MIC) was determined through serial dilution studies.

Cytotoxicity Study

The cytotoxicity profile of the polyherbal gel was assessed using normal human epidermal keratinocyte (NHEK) cell lines. Cell viability was determined by the MTT assay after exposure to different concentrations of the formulation. The percentage of viable cells was calculated and compared with untreated control cells.

Statistical Analysis

All experiments were performed in triplicate, and the results were expressed as mean ± standard deviation (SD). Statistical analysis was carried out using one-way analysis of variance (ANOVA), and a value of $p < 0.05$ was considered statistically significant. MTT, DMSO, PBS, and other analytical-grade chemicals were used for evaluation studies. All aqueous preparations were made using ultrapure Milli-Q water.

Instrumentation

A diverse range of advanced analytical and processing instruments was employed throughout the study. Quantitative absorbance measurements were performed using the IG-2100 UV-Vis spectrophotometer (Igene Labserve, India). FTIR spectral analysis for drug–excipient compatibility was carried out using the Bruker Alpha II FTIR system equipped with an ATR

module (Bruker, USA). Polyherbal preparation was facilitated using a Qsonica Q700 ultrasonic processor (Qsonica, USA), equipped with a 6 mm probe for efficient energy transfer. Particle size and zeta potential were analyzed using the Zetasizer Nano ZS (Malvern P analytical, UK), while morphological features of the Polyherbal were visualized through a JEOL JEM-2100 Transmission Electron Microscope (JEOL Ltd., Japan). Rheological measurements, including viscosity profiling, were conducted using a Brookfield DV-II+ Pro Viscometer coupled with a Helipath stand (AMETEK Brookfield, USA). pH values of the formulations were recorded using a FiveEasy™ pH meter (Mettler Toledo, USA), calibrated prior to each use with standard buffer solutions. For biological experiments, an Olympus CKX53 inverted microscope (Olympus Corp., Japan) was used to assess cellular morphology, and incubation was carried out in a Heracell™ VIOS 160i CO_2 incubator (Thermo Fisher Scientific, USA) under controlled conditions (5% CO_2, 37°C).

RESULTS AND DISCUSSION

Method Creation and Optimization

Pre-formulation Studies and Analytical Method Development

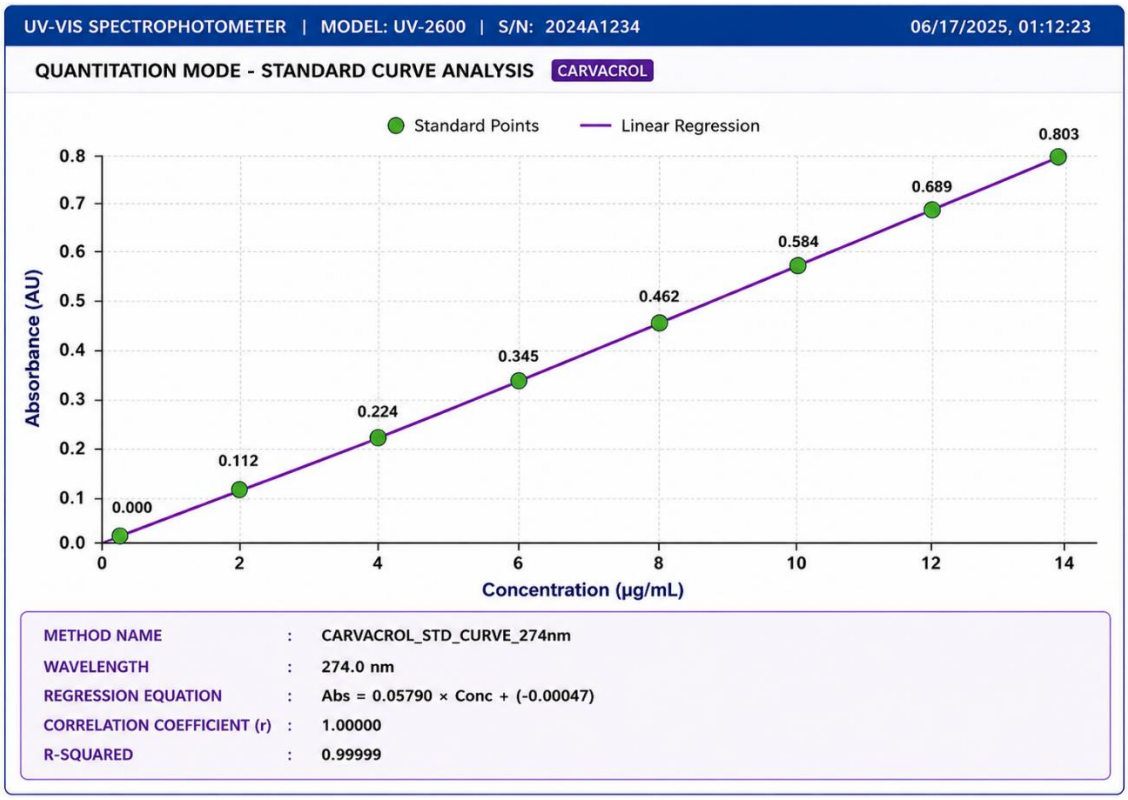
The physicochemical evaluation of carvacrol was conducted to confirm its purity, identity, and compatibility with formulation components. The investigations included melting point determination, UV-visible analysis, and infrared spectroscopy for compatibility assessment.

Melting Point

The melting behavior of carvacrol was assessed with a digital melting point instrument (Stuart SMP30). A capillary tube containing a small sample was subjected to gradual heating. The observed melting interval, 236–237 °C, matched reported reference data, thereby verifying the compounds purity.

Calibration Curve

A standard curve for carvacrol was prepared by making ethanol-based solutions at concentrations of 5, 10, 15, 20, and 25 µg/mL. Their absorbance values were recorded using a UV-Vis spectrophotometer (IGene Labserve, Model IG-2100). The absorbance values showed a linear correlation with concentration, and the regression coefficient (R^2) was above 0.999, indicating reliable quantitative performance of the method.



Determination of Maximum Absorbance (λmax)

To determine the maximum absorbance wavelength, a solution of carvacrol (10 µg/mL in ethanol) was scanned between 200 and 400 nm. The compound exhibited a prominent absorption peak at 275 nm, which was selected for all further spectrophotometric evaluations.

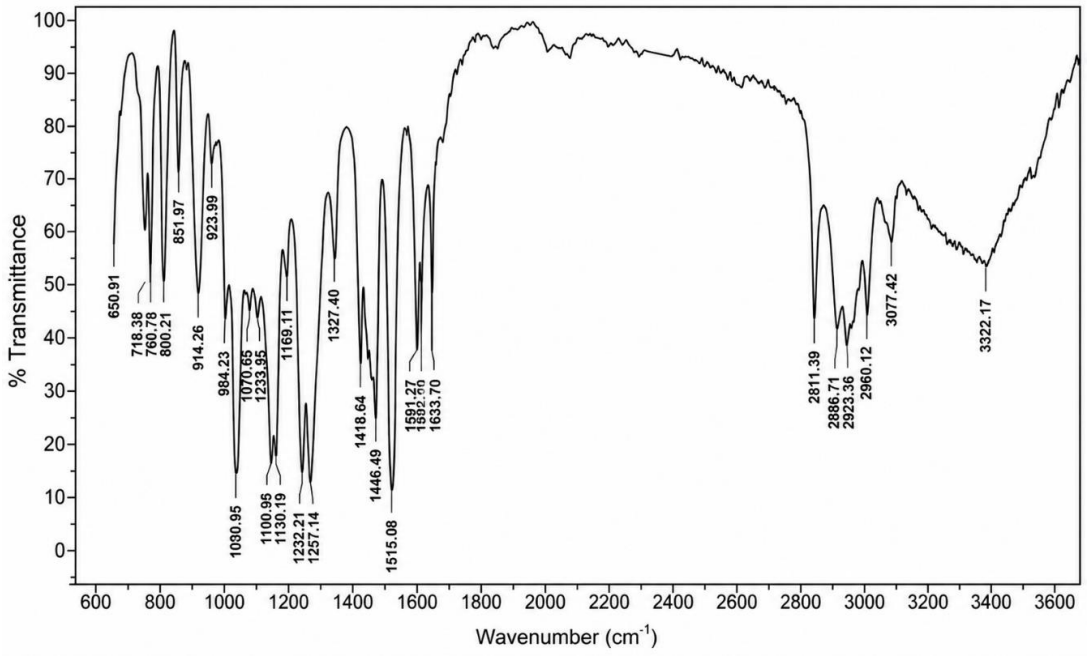
FTIR Compatibility with Excipients

Infrared spectroscopy was used to assess the compatibility of carvacrol with the selected formulation excipients. Spectra of carvacrol, individual excipients (Carbopol 940, Tween 80, propylene glycol, isopropyl myristate), and their physical mixtures were recorded using the Bruker Alpha II FTIR instrument with an ATR attachment. Characteristic peaks observed included hydroxyl stretching (around 3200–3400 cm^{-1}), aromatic ring vibrations (around 1600 cm^{-1}), and ether linkages (around 1260 cm^{-1}). These signals remained unaltered in the physical mixtures, indicating no significant interaction between carvacrol and the selected components.

FTIR ANALYSIS REPORT

FTIR analysis was carried out for each material separately, including Carvacrol, Silymarin, Propylene Glycol, Tween 80 (Polysorbate 80), Isopropyl Myristate, and Ethanol, along with their combined physical blend. Spectral data were recorded between 4000 and 400 cm^{-1}. The

study aimed to confirm the functional group peaks of individual substances and to evaluate whether mixing the components produced any shifts or indications of incompatibility.



Formulation Development and Optimization

Formulation of Carvacrol Polyherbal (F1–F5)

The Polyherbal formulations were developed using a systematic high-energy emulsification technique, ensuring fine droplet distribution and thermodynamic stability. Each formulation contained a fixed amount of the active compound, while the concentration of oil and surfactant systems varied to explore optimal composition. The detailed method is provided below in Table 5.1.

Table 2: Detailed Composition of Preliminary Carvacrol Polyherbal Formulations. (F1–F5)

Component (% w/w)	F1	F2	F3	F4	F5
Carvacrol (API)	1	1	1	1	1
Isopropyl Myristate (Oil)	14	9	4	9	9
Total Oil Phase	15	10	5	10	10
Deionized Water	65	70	75	70	70
Total	100	100	100	100	100

Step: Component Quantification

For the preparation of the Polyherbal formulations, the percentage composition of each component was first converted into actual weights, and all ingredients were accurately weighed using a calibrated analytical balance to ensure formulation precision. The oil phase

was prepared by transferring the required quantity of carvacrol into a clean beaker, followed by the addition of isopropyl myristate. The mixture was stirred continuously until a clear and homogeneous solution was obtained. Subsequently, each Polyherbal formulation (F1–F5) was incorporated separately into the pre-hydrated and neutralized gel matrix. The Polyherbal was added gradually under continuous gentle stirring using a glass rod or a low-speed mechanical stirrer to ensure uniform distribution throughout the gel base. Mixing was continued until a smooth, homogeneous, and visually stable Polyherbal was formed. The prepared formulations were then evaluated for their physical appearance, color, consistency, and viscosity.

Table 3: Composition of Preliminary Polyherbal Formulations. (F1–F5)

Component	F1 (% w/w)	F2 (% w/w)	F3 (% w/w)	F4 (% w/w)	F5 (% w/w)
Carvacrol	1	1	1	1	1
IPM (Oil)	14	9	4	9	9
Total Oil Phase	15	10	5	10	10
Tween 80 (Surfactant)	10	10	10	13.3	13.3
Total Smix	20	20	20	20	20
Water q.s. to	100	100	100	100	100
Key Variable (Smix Ratio S:CoS)	01:01	01:01	01:01	02:01	02:01

Physicochemical Characterization of the Formulations

The prepared polyherbal gel formulations were subjected to physicochemical evaluation to assess their suitability for topical application. Visual examination was performed to evaluate color, appearance, homogeneity, transparency, texture, and the presence of any physical instability, including phase separation, creaming, or aggregation. The pH of each formulation was determined using a calibrated digital pH meter by direct measurement to ensure compatibility with the skin and minimize the risk of irritation upon topical administration. The observations obtained from these evaluations were used to assess the overall quality and stability of the developed polyherbal gel formulations.

***In-vitro* Antimicrobial Screening**

A preliminary antimicrobial screening using the agar disc diffusion method was conducted to evaluate the inhibitory potential of the essential oils obtained from the selected plant species against the test microorganism at different concentrations: 20, 40, 60, 80, and 100µg/ml. A representative image of the petri dishes displaying zones of inhibition is shown in Figures 5.1 and 5.2. The zone of inhibition was also recorded at different concentrations. Both the

essential oils exhibited good antimicrobial activity. The Zone of inhibition diameter of both the essential oils was measured from the antimicrobial assay performed in triplicate is shown in Table 5.3. Both the essential oils were evaluated to determine and quantify the most potent antimicrobial activity of the isolated essential oils. The prepared broth microdilution was measured by the absorbance (optical density OD_{600}), and then the percentage (%) growth inhibition of the essential oils (Eos) was calculated, as shown in Table 5.5. Both the EOs at the selected concentrations, 32µg/ml, 512µg/ml, and 1024µg/ml, showed good % growth inhibition. Among the essential oils, CFO exhibited the better antimicrobial activity compared to CNO, with a MIC value of 1024µg/ml. A representative graph of Concentration against percentage growth inhibition is shown in Figure 5.3. These two EOs were mixed into a 1:1 ratio and selected for further studies.

Figure.2: The Zone of inhibition diameter of CFO and CNO.

Table 3: The Zone of inhibition diameter of all the essential oils.

Sl. No.	Dose µg/ml	*Cymbopogon nardus*	*Cymbopogon flexuosus*	
1	20	7 mm	19 mm	**Benzoyl peroxide**
2	40	9 mm	13 mm	
3	60	12 mm	15 mm	
4	80	14 mm	17 mm	
5	100	16 mm	18 mm	22 mm

Table 4: The percentage (%) growth inhibition of all the essential oils (EOs).

Sample name	Conc. µg/mL	Absorbance	% Growth Inhibition
Control (Ve+)	0	0.82±0.03	0.00
Control (Ne-)	0	0.04±0.01	94.12
CNEO	30	0.22±0.01	72.61
CNEO	500	0.15±0.02	84.37

CNEO	1000	0.08±0.03	90.46
CFEO	30	0.20±0.03	76.83
CFEO	500	0.13±0.02	78.59
CFEO	1000	0.06±0.01	95.12

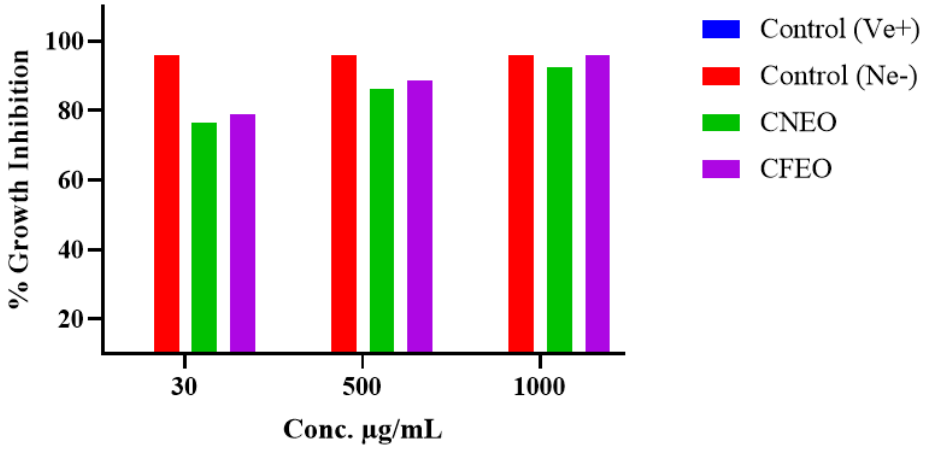


Figure 3: Graph of Concentration against % Growth Inhibition.

Table .5. All the measurements for various parameters were performed in triplicate.

Time (Day)	Herbal Gel	Refrigerated Condition (25°C ± 2°C)			Room Temperature (4°C ± 2°C)		
		pH	Viscosity	Appearance	pH	Viscosity	Appearance
0	CEO	5.61±0.07	18,430±350	Clear	5.61±0.07	18,430±350	Clear
30	CEO	5.59±0.07	18,420±230	Clear	5.59±0.07	18,417±345	Clear
60	CEO	5.58±0.02	18,415±220	Clear	5.57±0.05	18,410±250	Clear
90	CEO	5.57±0.04	18,410±240	Clear	5.53±0.08	18,406±224	Slight Turbidity

***In-vitro* Cytotoxicity Assay**

The cytotoxicity of CEO and EOHG was evaluated using the MTT assay on Normal Human Epidermal Keratinocyte (NHEK) cells to assess their safety. Both samples showed cell viability comparable to the untreated control at concentrations ranging from 5–100 µg/mL. Cell viability decreased in a dose-dependent manner at higher concentrations, reaching 65.77% for CEO and 76.63% for EOHG at 400 µg/mL. Since cell viability remained above 50%, the CC50 value could not be determined and was estimated to be greater than 400 µg/mL, indicating a high selectivity index (>100). Overall, EOHG exhibited lower cytotoxicity and better biocompatibility than CEO and the standard drug benzoyl peroxide, suggesting its suitability for topical anti-acne applications.

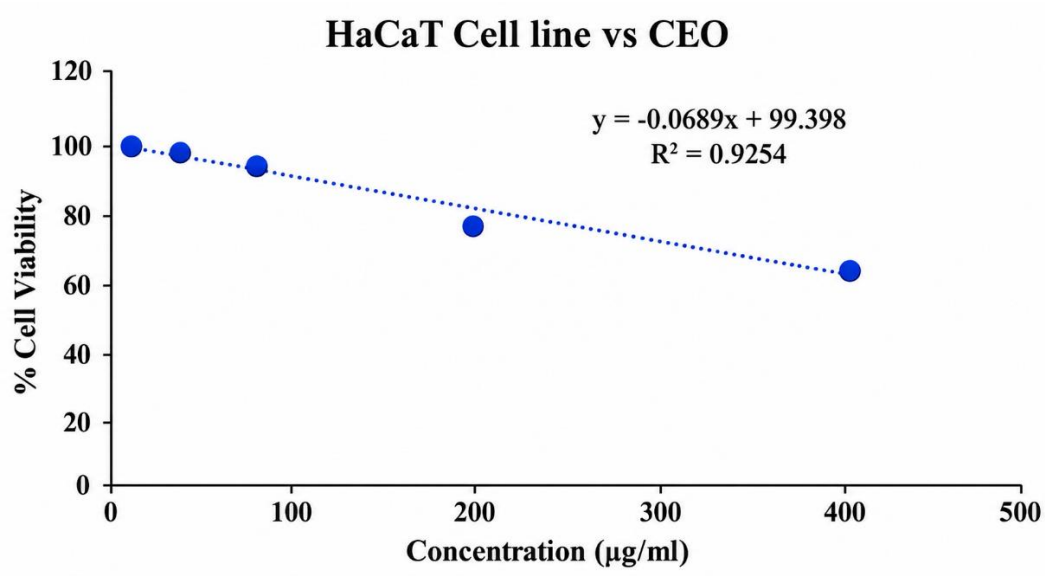
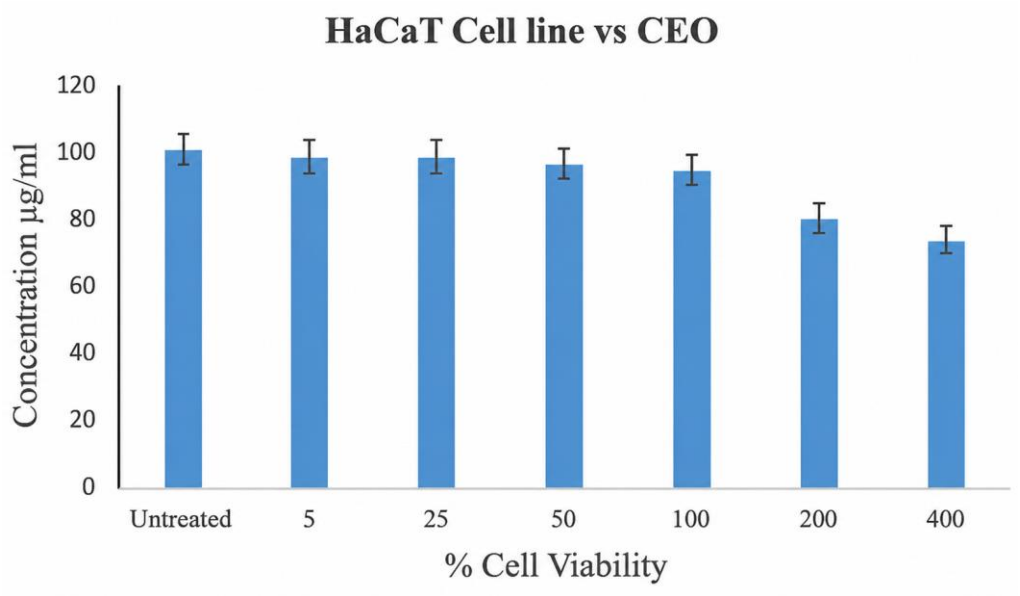


Figure 6: The Linear regression equation of the NHEK Cell line vs CEO.



CONCLUSION

The present study successfully developed and evaluated a polyherbal gel formulation containing essential oils with promising antimicrobial properties. The formulated gel exhibited satisfactory physicochemical characteristics, including appropriate pH, good homogeneity, acceptable appearance, and stability, indicating its suitability for topical application. Antimicrobial studies demonstrated significant inhibitory activity against *Staphylococcus epidermidis*, suggesting its potential effectiveness in managing acne-associated microbial infections and safety profile of essential oils obtained from *Cymbopogon nardus* (CNO) and *Cymbopogon flexuosus* (CFO). The essential oils were extracted

successfully and showed good quality and purity. Both oils exhibited significant antibacterial activity against *Staphylococcus epidermidis*, which is one of the major bacteria involved in acne formation. Among the two oils, CFO showed better antimicrobial activity than CNO. The results also showed that antibacterial activity increased with increasing concentration of the oils. The combined essential oil formulation demonstrated stronger antioxidant activity than the individual oils, indicating a synergistic effect. A herbal gel containing both oils in a 1:1 ratio was prepared successfully and showed suitable physicochemical properties such as good pH, viscosity, and appearance for topical use. Stability studies confirmed that the gel remained stable under different storage conditions. The formulated gel showed enhanced antimicrobial activity and lower cytotoxicity compared to the individual oils and standard drug. Overall, the developed herbal gel can be considered a safe, stable, and promising natural formulation for acne treatment and may be useful for further in-vivo and clinical studies.

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CONFLICT OF INTEREST

The authors announce that there is no disagreement of interest associated with this research work.

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Nil

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