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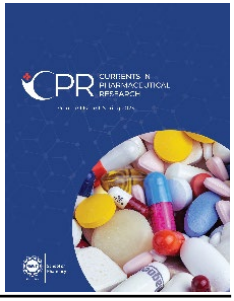

Title:	**Harnessing Synergistic Antimicrobial Potency: Clindamycin Phosphate and Aloe Vera Topical Gel for Advanced Acne Therapy**
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Harnessing Synergistic Antimicrobial Potency: Clindamycin Phosphate and Aloe Vera Topical Gel for Advanced Acne Therapy

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ABSTRACT

Topical drug delivery presents a promising approach for the treatment of dermatological conditions, such as acne vulgaris. This study examined the combination of clindamycin phosphate (C.P), a potent topical antibiotic, with *Aloe vera (A. vera)*, recognized for its antimicrobial and anti-inflammatory properties, to formulate topical gels for acne management. The principal aim of the study was to develop and assess topical gels that utilize the antimicrobial properties of C.P and *A. vera*. The study anticipated that this combination would enhance antimicrobial effectiveness, potentially improving acne treatment results and reducing hyperpigmentation. Utilizing the cold dispersion method, the study crafted topical gels incorporating a blend of excipients including Carbopol 940, oleic acid, glycerin, propylene glycol (PG), eucalyptus oil, methyl paraben, and propyl paraben. Optimization of viscosity, spreadability, and permeability was meticulously executed through Design Expert 11 software. The resultant optimized gels underwent rigorous *in-vitro* characterization to gauge their performance. Notably, these gels demonstrated desirable rheological attributes, boasting a pH of 7.1, spreadability and viscosity values of 32.9 g.cm/sec and 38.25 Pa.s, respectively. Furthermore, the drug content adhered to stringent USP standards (90%-110%), while *in-vitro* drug release studies depicted a commendable profile. Moreover, the gels exhibited noteworthy antioxidant activity and heightened antimicrobial efficacy against acne-causing bacteria. This compelling combination renders the formulated gels stable, reproducible, and skin-compatible, thus underscoring their potential as efficacious topical formulations for managing acne.

Keywords: Aloe vera (A. vera), anti-acne gel, antimicrobial, clindamycin phosphate (C.P), phytotherapy

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1.INTRODUCTION

Acne vulgaris is a prevalent skin disease worldwide, exerting substantial physical as well as psychological effects on affected individuals [1]. Despite the plethora of anti-acne modalities available, optimal management has been a challenge. Topical drug delivery is a promising avenue in dermal therapy, offering both localized and targeted treatments with improved patient compliance and lesser systemic side effects.

Current approaches, such as topical retinoids, benzoyl peroxide, and oral or topical antibiotics, mostly strive to mitigate acne-related pathological processes. Among these, clindamycin phosphate (C.P), an antimicrobial, has emerged as a cornerstone in management of acne [2].

Simultaneously, phytotherapy has emerged as an adjunct in treating acne. *Aloe vera (A. vera)*, a xerophytic plant, renowned for its antimicrobial potential, anti-inflammatory, and antioxidant properties, has gained considerable attention owing to its therapeutic potential in acne vulgaris management [3-6].

Focusing towards the complementary virtues of C.P and *A. vera*, the study hypothesized that when used in combination, enhanced therapeutic efficacy can be conferred in acne management. Thus, the current study attempted to combine the antibacterial properties of C.P and the potential of *A. vera* by formulating a topical gel to target acne vulgaris.

The current study incorporated C.P, an antibiotic, and *A. vera* powder as key therapeutic agents, harnessing their antimicrobial and anti-inflammatory properties, respectively. Additionally, to formulate the gel, Carbopol 940 was used as a polymer. Carbopol is a gelling agent owing to its highly efficient properties, its adaptable pH, clarity, and stability. Carbopol is suitable for gel formulations due to its exceptional thickening and suspending properties [7].

Oleic acid was utilized as a surfactant and permeation enhancer, leveraging its ability to self-assemble to form micelles and its advantage as an eco-friendly biodegradable surfactant [8]. Eucalyptus oil also served dual purposes of a flavor and penetration enhancer, enhancing the permeability of active ingredients by disrupting stratum corneum's lipid bilayer [9]. Glycerin acted as a plasticizer thus preserving moisture within the gel [10]. Moreover, propylene glycol (PG) was used as a solvent [11]. Finally, methylparaben and propylparaben acted as preservatives [12].

The study aimed to maximize the physicochemical properties of the prepared gels, paying special attention to viscosity, spreadability, and permeability, through careful formulation and *in-vitro* characterization. The main goal was to clarify how these formulations might help treat acne vulgaris and reduce related hyperpigmentation, expanding the arsenal of dermatological treatments.

2.METHODOLOGY

2.1. Chemicals

C.P (Hubei Yitai Co Limited, China), *A. vera* (Sigma, Pakistan), Carbopol 940 (Sigma Aldrich Chemie GmbH, USA), eucalyptus oil (Hemani Herbals), PG (DaeJung, Korea), glycerin (UNI-CHEM), sodium hydroxide (Sigma Aldrich, USA), methyl paraben and propyl paraben (Sigma, USA), Oleic Acid (UNI-CHEM, Pakistan), sodium dihydrogen phosphate, potassium dihydrogen phosphate (Sigma Aldrich Chemie GmbH), and distilled water (Research Lab, UCP).

2.2. Procedure

2.2.1.Formulation Strategy. The software Design Expert was used to design and optimize the topical anti-acne gel compositions (Table 1). Gels were prepared in accordance with the runs that Box Behnken Design (BBD) recommended. The BBD recommended a total of 14 runs while taking into account the independent variables, plasticizer (glycerin), permeation enhancer (eucalyptus oil), and surfactant (oleic acid). Design Expert used the quadratic model to perform mathematical modeling for the variables and computed replies.

$$Y= X_0 + X_1 + X_2 + X_3 + X_1X_2 + X_1X_3 + X_2X_3 + X_1^2 + X_2^2 + X_3 \quad (1)$$

Table 1. Composition Suggested by Design Expert

Formulation	Surfactant X_1 (mL)	Permeation Enhancer X_2 (mL)	Plasticizer X_3 (mL)
1	0.6	0.75	1.25
2	0.45	1	2.5
3	0.3	0.5	1.875
4	0.45	0.5	2.5
5	0.6	0.75	2.5
6	0.45	0.5	1.25

Formulation	Surfactant X_1 (mL)	Permeation Enhancer X_2 (mL)	Plasticizer X_3 (mL)
7	0.45	0.75	1.875
8	0.45	0.75	1.875
9	0.45	1	1.25
10	0.6	1	1.875
11	0.3	1	1.875
12	0.3	0.75	1.25
13	0.6	0.5	1.875
14	0.3	0.75	2.5

2.2.2. Formulation Method. 1% Carbopol was hydrated overnight in 100 mL of distilled water. The formation of Carbopol gel was achieved by adjusting its pH using a neutralizing agent, specifically NaOH. Subsequently, 1% of C.P and 2% of *A. vera* powder were dissolved in a solution containing 7.5% PG and incorporated into the prepared gel. To this mixture, glycerin, oleic acid, and eucalyptus oil were added. Additionally, preservatives, that is, 0.01% propyl paraben and 0.1% methyl paraben were mixed in the gel formulation after dissolving in 10 mL of distilled water at 70ºC [13]. The formulated gels were placed in hermetically sealed plastic containers for storage.

2.3. Post-formulation Studies

2.3.1.Organoleptic Characterization. The organized arrangements of the topical gels were assessed for their physical characteristics including the color, odor, consistency, homogeneity, greasiness, and grittiness [14].

2.3.2. pH. A pH meter was employed to measure the pH of each of the 14 formulations after it had been calibrated using standard buffers.

2.3.3.Spreadability. 1 g of gel formulation was mounted on a slide and covered with another slide. 100 g weight was placed on the upper slide for 1 minute to form a uniform thin layer. The top movable slide was fastened to a thread carrying a weight of 30 g. Time taken by the upper slide to traverse 7.5 cm under the weight was checked and the following formula was used to calculate spreadability [15].

$$S=M/L \times T \quad (2)$$

2.3.4.Viscosity. The gel viscosity was checked by employing digital viscometer fitted with spindle size 4 and rotated at 12 rpm [16].

2.3.5.Drug Content. 1 g of topical gel was added in 100 mL distilled water with a magnetic stirrer and filtered. 0.9 mg/mL solution was prepared and again filtered through a syringe filter. Drug content for both C.P and *A. vera* was determined spectrophotometrically at wavelength of 210 nm and 330 nm.

2.3.6.*In-vitro* Assessment of Drug Permeation. Topical gel was placed in Franz diffusion (FD) cell (using SpectraPor: pore size 0.45 microns). A phosphate buffer was employed as receptor medium (pH 7.4). Water was coursed through the water bath to keep the temperature constant (37°C). Test samples, 5mL each, were withdrawn at time intervals of 15 min, 30 min, 1 hour, and upto 8 hours and supplanted with fresh media. Tests were spectrophotometrically evaluated at reasonable wavelength and the % drug permeation was determined [17].

2.3.7.FTIR Analysis of Formulation Components. FTIR was executed to elucidate the interactions between the ingredients in the prepared gel samples. The samples were placed on KBr discs and their wavelengths were obtained using infrared spectroscopy [18].

2.3.8.Antioxidant Activity (TAA). Total antioxidant capacity of the gels was estimated by 2, 2- diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay. A calibration curve % inhibition versus concentration of standard antioxidant (ascorbic acid) was plotted [19].

2.3.9.Antimicrobial Potential. The agar well diffusion assay was employed to assess the antimicrobial efficacy of gels. The *Cutibacterium acnes* cultures were used for this purpose. The diameter of zones of inhibition was observed following a 48-hour incubation period [20].

2.3.10. Stability Studies. This study was performed by storing the optimized gels in the stability chamber for 6 months at 40ºC/75% ± 5% RH [21].

3.RESULTS

The results of *in-vitro* characterizations were as follows.

3.1. Organoleptic Characterization

All the formulations were slightly white in color with characteristic minty smell. All formulations had thick consistency and were non-greasy and free of any aggregates or lumps. The prepared topical gels were slightly

white in color with a mint-like smell. Research has shown that the compound 1,8-cineole, found in eucalyptus oil, contributes to its distinctive minty and camphorous scent [22]. The whitish appearance of the gels resulted from the inclusion of C.P, which is naturally white [23]. Consistency plays a significant part in the effectiveness of anti-inflammatory and analgesic topical treatments. The gels developed in this study displayed favorable consistency, attributed to the incorporation of glycerin as a plasticizer [24]. Richard and colleagues have previously noted that glycerin can impact viscosity of topical formulations [25].

3.2. Numerical Optimization

The Design Expert performed numerical optimization by optimizing responses. The parameters for gel data optimization were determined by decreasing oleic acid (0.3 mL) and augmenting eucalyptus oil (1 mL) while keeping the quantity of glycerin between 1.25mL and 2.5mL. C.P permeability was between 80.23 and 92.42%. The penetration of *A. vera* was controlled between 80 and 91.05%. The gel's viscosity was controlled between 22.345 and 47.992 Pa.s, while its spreadability ranged from 16.07 to 25 g. cm/sec.

Table 2. Optimized Gel Formulation Composition

Chemicals	Concentration
Clindamycin Phosphate (C.P)	1g
Aloe vera (A. vera)	2g
Carbopol	3g
Propylene glycol (PG)	7.5mL
Eucalyptus Oil	0.99mL
Glycerin	1.276mL
Oleic Acid	0.408mL
Methyl Paraben	0.1g
Propyl Paraben	0.01g
Distilled Water	Q.s 100mL

Table 3 shows all predicted outcomes of the responses for the optimized formulation.

Table 3. Predicted Outcomes by Design Expert

Response	Carbopol Gel (CG)
Viscosity (Pa.s)	38.385
Spreadability (g.cm/sec)	32.815
Percentage Permeation of C.P(%)	85.497
Percentage Permeation of *A. vera (%)*	87.176

3.3. pH and Spreadability

The pH of the gel was determined to be between 6.5 to 7.2, which is close to the pH of the skin. The skin's pH typically falls within the range of 4.5 to 5.5, known as the acid mantle, which plays a vital role in preserving skin barrier function and preventing microbial overgrowth. Therefore, formulating products within a pH range that closely aligns with the skin's natural acidity is paramount for minimizing the risk of irritation, inflammation, and disruption of the skin barrier. pH also has some influence on bacterial growth that has been reported in various studies. The acidic environment created by the formulation can help inhibit *Cutibacterium acnes*, which tend to thrive in basic conditions [26]. Additionally, a pH-balanced formulation is less likely to disrupt the skin's microbiome, which plays a crucial part in preserving skin health and preventing acne development [27]. The spreadability lied between 32.9 g.cm/sec. Studies indicated that a good gel takes less time to spread on skin and has high spreadability [28]. The observed values for spreadability fall within a reasonable range. Higher spreadability values, such as those observed in this range, indicate that the gel can be applied smoothly and evenly onto the skin without excessive resistance. This characteristic is particularly advantageous in acne management, where consistent application over affected areas is necessary to target lesions and prevent new breakouts [29].

3.4. Viscosity

The viscosity of formulated gel was 35.6 Pa.s to 56.26 Pa.s. The gel viscosity plays a vital role in controlling various factors, such as drug permeation, gel strength, spreadability, and pourability. The viscosity of gels was consistent with that of most anti-acne topical gels. Studies showed that the hydroxyl groups in glycerin interact with the polymers to form hydrogen bonds. The more the concentration of plasticizer more would be the network connectivity and hydrogen bonding [30].

Christensen et al. [31] suggested that the intramolecular hydrogen bonding leads to an increased viscosity. This leads towards the inference that addition of glycerin in a gel can increase its viscosity. It was observed that the formulations with higher quantity of glycerin showed a higher viscosity than other formulations. A study performed by Sant et al. [32] showed that oleic acid imparted a significant increase in the viscosity of the gelling systems. Moreira et al. [33] also reported an increase in the viscosity of the gels with an increased concentration of oleic acid. Oleic acid induced network formation by stimulating the physical interaction between the molecules of gels [34]. A direct relation was found between the concentration of oleic acid and the viscosity of prepared gels.

3.5. Drug Content

The drug content of all the formulations of Carbopol topical gels was found to be between 90% - 110%. It was concluded that the results of drug content of active ingredient C.P and *A. vera* were in compliance with USP specifications.

3.6. FTIR of Formulation Ingredients

Figure 1(a). represented the IR spectra of Carbopol 940. Peaks were seen at 1710 cm^{-1}, showing C=O group. Peaks at 810 cm^{-1} showed strong C-H bending and at 2950 cm^{-1} indicated C-H stretching of alkane group. Absorption band at 1175 cm^{-1} represented C-O-C linkage [35].

Figure 1(b). depicted the IR spectra of C.P. Peaks at 3280 and 3382 cm^{-1} showed O–H; at 1085 and 1145 cm^{-1} due to C–O cyclic ether stretching in the galactose sugar group; at 1670 cm^{-1} due to N–C=O stretching of amide carbonyl group; at 1265 and 1310 cm^{-1}, indicating S–C–H bending of C1–SCH3 group and at 860 cm^{-1} due to C–Cl stretching Chloro group. Peaks were also seen at 1452 cm^{-1}, showing C–N stretching of pyrrolidine group and finally at 2950 cm^{-1} due to C–H stretching of C4–alkyl group [36].

Figure 1(c). elucidated the IR spectra of *A. vera* powder showing broad bands around 3000–3500 cm^{-1} due to stretching of O−H group present in mannose and galacturonic acid, and to phenolic groups in anthraquinones. Peak at 1650 cm^{-1} showed C=O in *A. vera*; at 1100–1245 cm^{-1} owing to C-O-C of acetyl groups, indicating bioactive polysaccharides, (acemannan and glucamannans). The spectral region between 800 and 900 cm^{-1} showed C-O bonds stretching vibrations associated to polysaccharides and sugars in *A. vera* [37].

Figure 1(d). showed FTIR analysis of formulated topical gel. Peaks were found at 3300 and 3400 cm^{-1} due to medium N–H stretching and between 1650 and 2000 cm^{-1} due to weak C−H bending. Peaks at 1465 cm^{-1} due to medium C−H bending indicated a presence of an alkane, and at 845 cm^{-1} showing C=C bending.

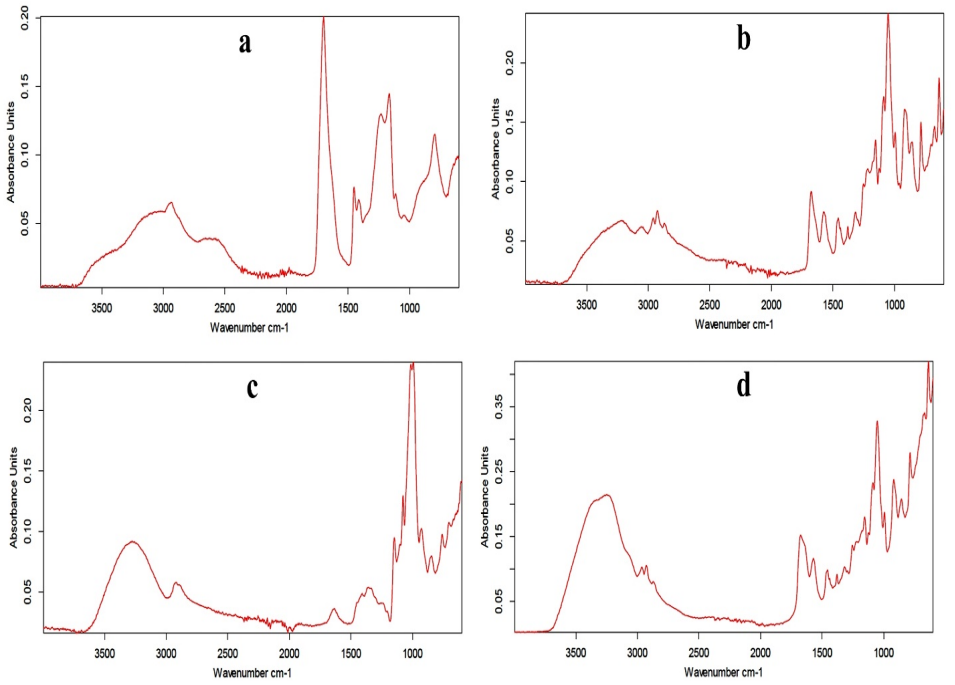


Figure 1. FTIR of a) Carbopol b) C. P c) *A. vera* d) Gel

3.6.1. *In-vitro* Assessment of Drug Permeation. The permeation of C.P and *A. vera* from gel at 8-hour interval was 85% and 87.5%, respectively. Herman et al. [38] suggested that high concentration of eucalyptus oil increases the penetration of drugs through the skin barrier. Another study showed that eucalyptol, a constituent of eucalyptus oil, elevated the amount of drug diffusion through skin [39]. Oleic acid used in the formulation also acted as a permeation enhancer and showed a significant impact on the permeation of active ingredients. Abd et al. [40] 2018 developed a formulation using eucalyptol and oleic acid as permeation enhancers and reported that both substances showed increased permeation of active ingredient.

3.7. Characterization Tests of Optimized Gel

Table 4. Results of Characterization Tests

Parameter	Result
Appearance	Slightly white color and mint-like odor
pH	7.1
Viscosity (Pa.s)	38.25
Spreadability (g.cm/sec)	32.9
Drug Content (%)	91.5%

3.7.1.*In-vitro* Drug Permeation Study. The current study observed that the permeation of the active ingredients through the optimized gel reached 80% (Figure 2). Furthermore, the research also revealed that the inclusion of eucalyptus oil and oleic acid significantly augmented the permeation ability of the both active ingredients. These findings align with prior research; for instance, Herman A. noted a direct relationship between the eucalyptus oil concentration in formulations and drug penetration into the skin barrier. Additionally, eucalyptol, a major component comprising approximately 90% of eucalyptus oil facilitated percutaneous absorption of the drug. Another study demonstrated that eucalyptol and oleic acid notably enhanced the percutaneous absorption.

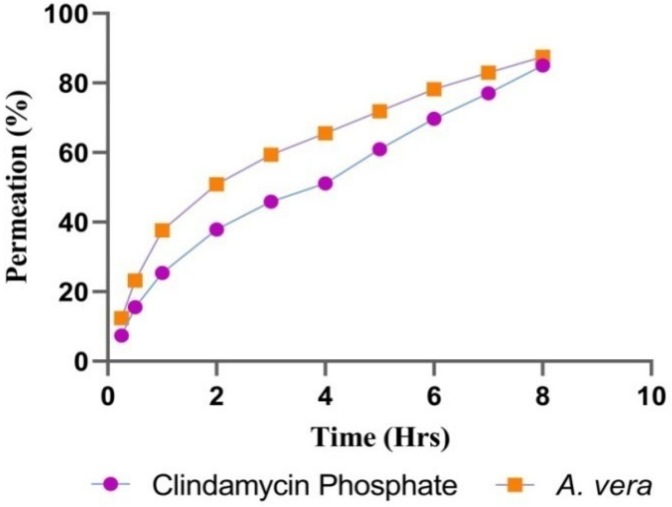


Figure 2. *In-vitro* Assessment of Permeation of Active Ingredients in Optimized Gel

3.7.2.Antioxidant Activity (TAA). DPPH radical scavenging assay is one of the rapid evaluation test for hydrogen/electron-donating activity of different antioxidants. *A. vera*, featured prominently as an active ingredient in the formulated gels of this study, exhibited significant antioxidant activity. This was attributed to its phenolic and flavonoid contents, which directly contribute to its efficacy in scavenging DPPH radicals. Research underscores the potential of *A. vera's* antioxidant properties in the treatment of various skin and systemic diseases [41]. The current study employed DPPH assays to confirm *A. vera's* robust antioxidant profile. The gel formulations demonstrated concentration-dependent scavenging of DPPH radicals. Both control (ascorbic acid) and test formulations exhibited notable DPPH radical reduction with increasing concentrations as shown in Figure 3 and Figure 4. Previous investigations highlighted that non-flavonoid polyphenols are predominant in *A. vera*, constituting a substantial portion of its total polyphenolic content [42]. Additionally, another study corroborated *A. vera* gel's capacity to effectively scavenge DPPH and ABTS in a concentration-correlated manner, consistent with the *in-vitro* findings [43].

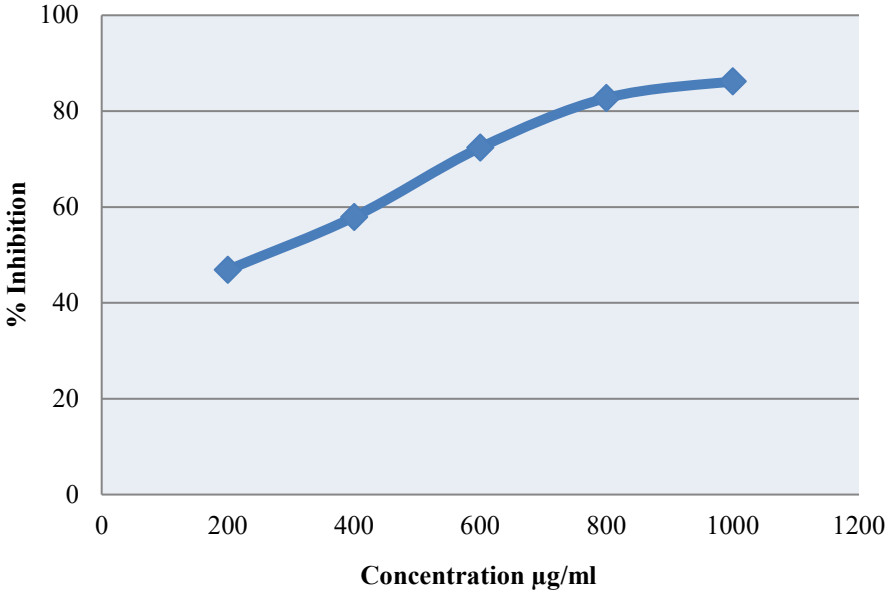


Figure 3. TAA of Ascorbic Acid (Standard)

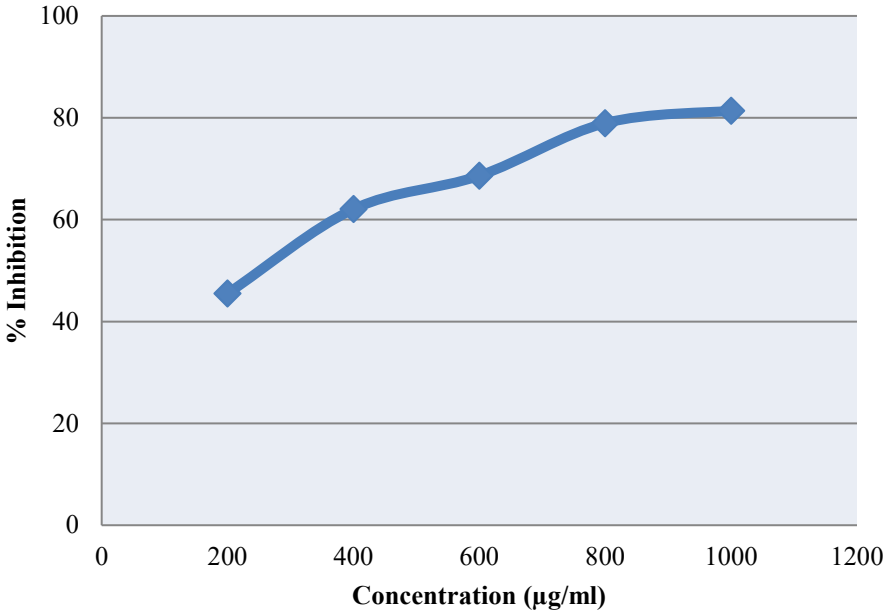


Figure 4. TAA of Optimized Gel

3.7.3.Antimicrobial Activity. Figure 5. depicted the antimicrobial activity of gels. The zones of inhibition determined in this study are detailed in Table 5. Testing of the antimicrobial efficacy of the gel containing *A. vera* as a sole ingredient revealed comparable inhibitory effects to those of the commercially available C.P formulation (Clinagel). Furthermore, the combination gel incorporating both *A. vera* and C.P exhibited superior inhibitory activity compared to the marketed C.P preparation.

Various studies indicated that high antimicrobial activity in topical agents is important in the treatment of *acne vulgaris* [44]. The current study focused on the formulation of a gel having high antimicrobial activity. For this purpose, antimicrobial agents C.P and *A. vera* were used. Studies showed that polysaccharides present in *A. vera* gel could stimulate phagocytic leucocytes to destroy bacteria. Pyrocatechol (a hydroxylated phenol) also has toxic effects on various microorganisms [45].

The current study showed that the gels containing *A. vera* as a single antibacterial agent had a good antimicrobial activity, while the gels containing C.P and *A. vera* showed a high inhibitory effect. Additionally, it

was noted that the gels had a higher antimicrobial activity in comparison to marketed Clindacin gel containing C.P.

Table 5. Anti-microbial Potential

Formulation	Inhibition Zone (cm)
Standard Gel (Clinagel)	2.5
Gel with *A. vera*	2.9
Gel with C.P and *A. vera*	3.2

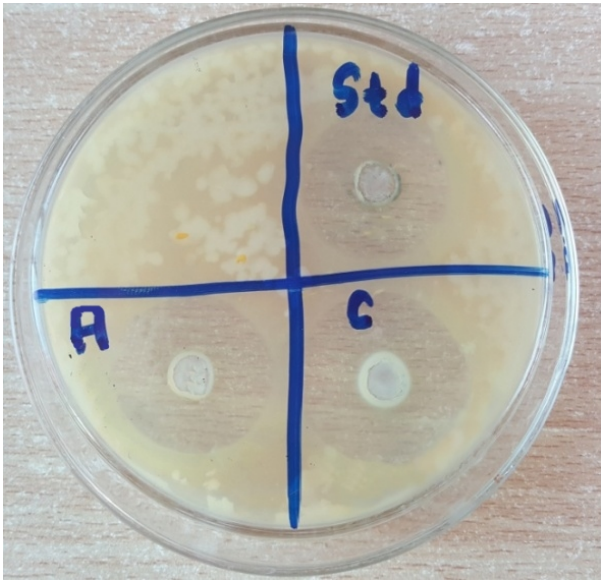


Figure 5. Antimicrobial Potential of Gels A) With *A. vera* C) with C.P and *A. vera* Std) Control

3.7.4.Stability Study. Table 6. indicated the results of stability studies after a period of 6 months. The physical appearance, homogeneity, and texture of gels also remained the same as week zero. The viscosity of gels was found to be between 30 Pa.s – 40 Pa.s.

Stability study in pharmaceutical products is important as it provides information regarding the influence of time and environmental factors on the shelf life and quality of the product [46]. In the current study, topical gels were subjected to accelerated stability testing (45ºC/75% RH) for a period of 1 month and the results showed no significant changes in the topical gels. This could be attributed to the addition of paraben preservatives that have properties against various microbes [47]. *A. vera* itself has

antioxidants and antimicrobial effects that may improve the shelf life of gels [48].

Table 6. Results of Stability Studies after 6 Months

Parameters	Carbopol Gel (CG)
Appearance	Whitish
Odor	Minty
pH	7.2
Spreadability (g.cm/sec)	31.25
Viscosity (Pa.s)	38.1
Drug Content (%)	91

3.7.5.Response Surface Methodology.

3.7.5.1. In-vitro Permeation of Active ingredients. *In-vitro* permeation studies of active ingredients revealed notable insights into the factors influencing the permeation of C.P and *A. vera*. Graphical representations depicted in Figures 6 and 7 showcased the impact of surfactant oleic acid and permeation enhancer eucalyptus oil on the permeation process. Specifically, eucalyptus oil exhibited a significant enhancement in drug permeation from the topical gels. Furthermore, the presence of glycerin also contributed to increased drug permeation, although to a lesser extent compared to other factors.

Comparative analysis between the effects of surfactant and permeation enhancer on drug permeation underscored the superior influence of the surfactant in enhancing permeability. This observation aligns with findings by Hmingthansanga et al. [49], who emphasized permeation enhancing capability of oleic acid. Notably, approximately 75% of eucalyptus oil comprises 1,8-cineole, a cyclic terpene known to alter the lipid structure of the stratum corneum, thereby facilitating the penetration of both polar and nonpolar drugs [50].

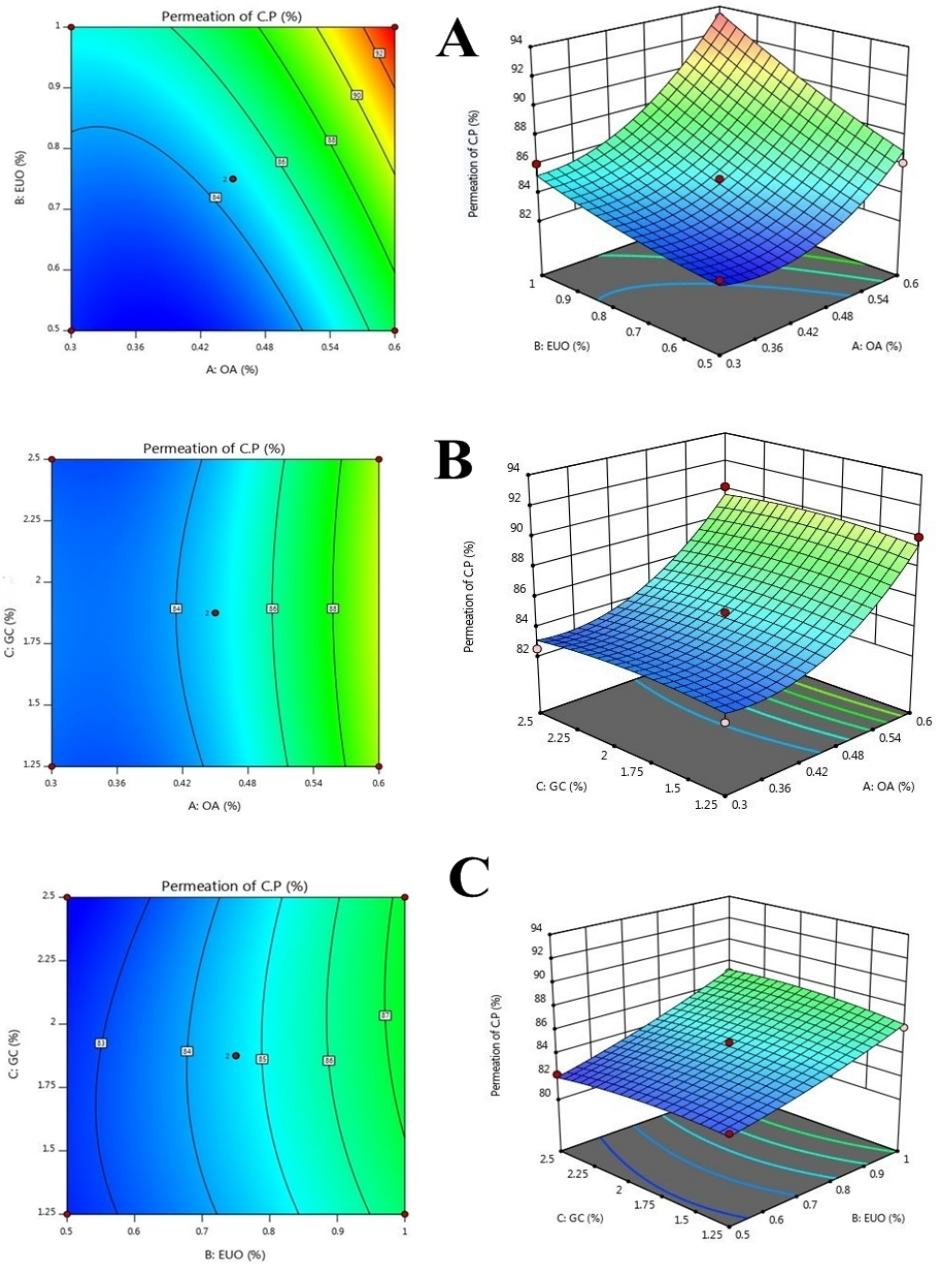


Figure 6. 3D Surface and Contour Plots Depicting the Influence of EUO, OA, and GC on Permeation of C.P

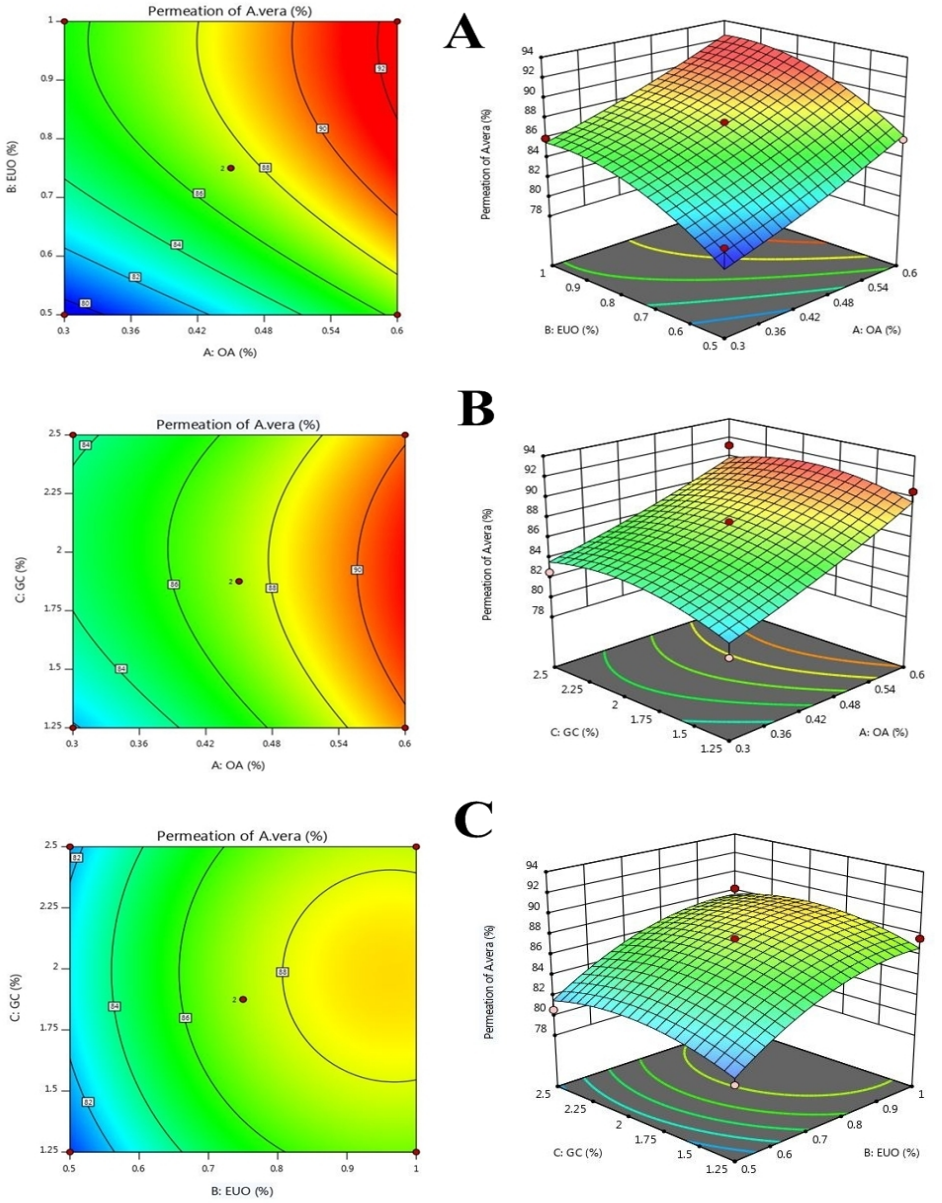


Figure 7. 3D Surface and Contour Plots Depicting the Influence of EUO, OA, and GC on Permeation of *A. vera*

Tables 7 and 8 represent the variability of permeation of active ingredients C.P and *A. vera*, respectively.

Table 7. Variability in C.P Permeation

Terms	Degree of Freedom	*F*-Value	*p*-Value	Significance
Model	9	18.42	0.0065	Yes
X_1	1	92.86	0.0006	Yes
X_2	1	49.14	0.0022	Yes
X_3	1	0.0028	0.9602	No
X_1X_2	1	4.52	0.1007	No
X_1X_3	1	0.0277	0.8759	No
X_2X_3	1	0.5096	0.5148	No
X_1^2	1	16.15	0.0159	Yes
X_2^2	1	0.4326	0.5467	No
X_3^2	1	0.6048	0.4802	No

Table 8. Variability in A. vera Permeation

Terms	Degree of Freedom	*F*-Value	*p*-Value	Significance
Model	9	5.12	0.0652	No
X_1	1	22.93	0.0087	Yes
X_2	1	18.38	0.0128	Yes
X_3	1	0.4964	0.5200	No
X_1X_2	1	0.0009	0.9780	No
X_1X_3	1	0.1324	0.7344	No
X_2X_3	1	0.0032	0.9579	No
X_1^2	1	0.0989	0.7688	No
X_2^2	1	2.46	0.1919	No
X_3^2	1	1.71	0.2609	No

3.7.5.2. Viscosity. Viscosity of the prepared gels as shown in Figure 8 showed that different variables had diverse effects on the viscosity. Upon the addition of glycerin and oleic acid, the gels' viscosity was augmented, while it diminished on the addition of eucalyptus oil.

Glycerin, a widely used nonaqueous solvent favors hydrogen bonding and has favorable physical properties, making it a useful solvent for numerous formulations. Pham et al. highlighted the hydrogen bonding capacity of glycerol [51]. This engenders network formation in the gel, elevating viscosity on incorporation of glycerin [52].

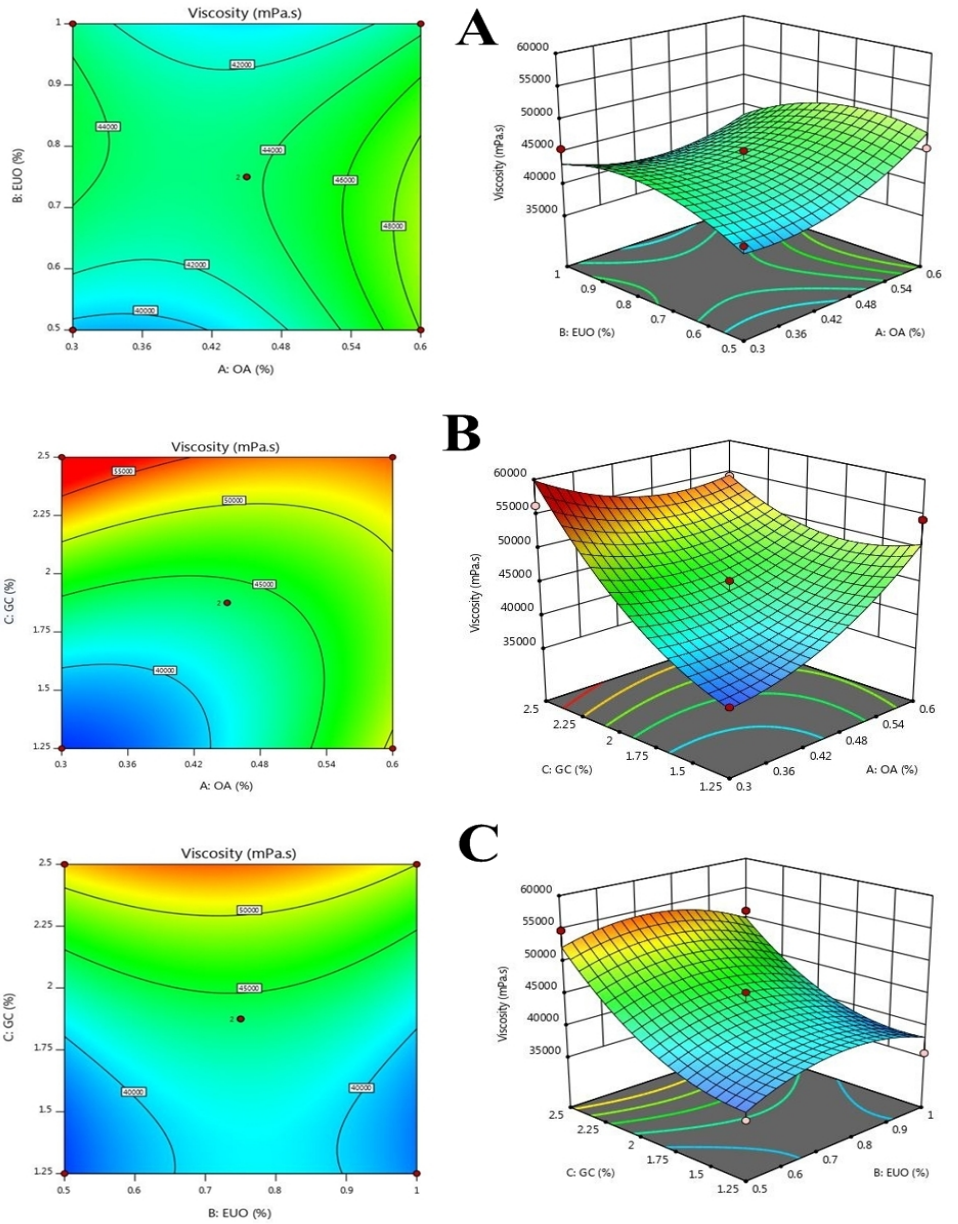


Figure 8. 3D Surface and Contour Plots Depicting the Influence of EUO, OA, and GC on Viscosity

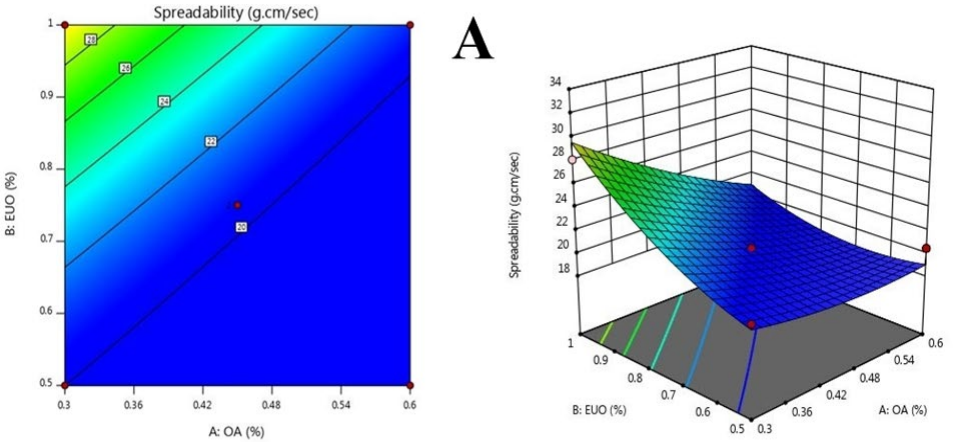
Table 9 shows the variability in viscosity of the gel formulation.

Table 9. Variability in Viscosity

Terms	Degree of Freedom	*F*-Value	*p*-Value	Significance
Model	9	4.80	0.0726	No
X_1	1	2.59	0.1831	No
X_2	1	0.0280	0.8753	No
X_3	1	24.72	0.0076	Yes
X_1X_2	1	1.19	0.3364	No
X_1X_3	1	6.32	0.0658	No
X_2X_3	1	0.2020	0.6764	No
X_1^2	1	1.81	0.2498	No
X_2^2	1	2.14	0.2172	No
X_3^2	1	2.97	0.1600	No

3.7.5.3. Spreadability. Figure 9 illustrates the determination of the effect of various variables on gel spreadability. The overall impact was predominantly positive which suggests that changes in formulation components could enhance spreadability. Eucalyptus oil acted as a key contributor, exerting a positive influence on gel spreadability [53].

Conversely, oleic acid and glycerin exerted a diminishing effect on spreadability. While they may offer viscosity enhancement and emollient properties, their impact on spreadability requires careful consideration [54].



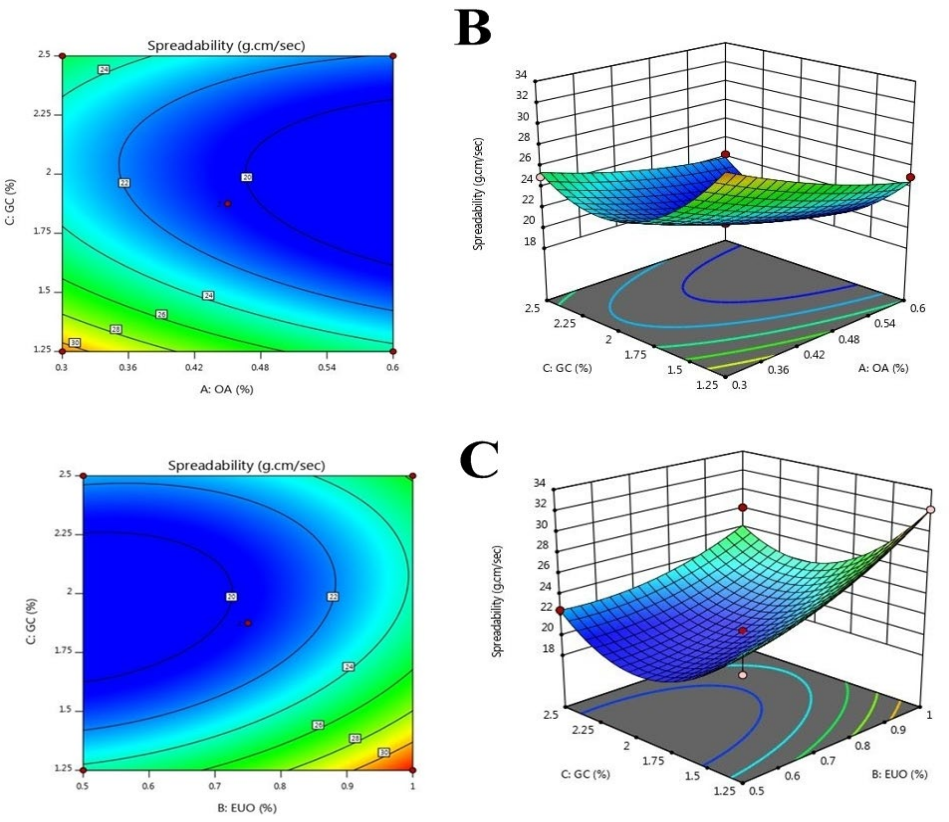


Figure 9. 3D Surface and Contour Plots Depicting the Influence of EUO, OA, and GC on Spreadability

The comprehensive analysis of the variability in gel formulations is presented in Table 10.

Table 10. Variability in Spreadability

Terms	Degree of Freedom	*F*-Value	*p*-Value	Significance
Model	9	4.80	0.0726	No
X_1	1	2.59	0.1831	No
X_2	1	0.0280	0.8753	No
X_3	1	24.72	0.0076	Yes
X_1X_2	1	1.19	0.3364	No
X_1X_3	1	6.32	0.0658	No
X_2X_3	1	0.2020	0.6764	No
X_1^2	1	1.81	0.2498	No

Terms	Degree of Freedom	*F*-Value	*p*-Value	Significance
X_2^2	1	2.14	0.2172	No
X_3^2	1	2.97	0.1600	No

3.7.6.Mathematical Modeling.

Permeation of C.P = 84.64 + 3.17 +2.31+ 0.0175 + 0.99 + 0.0775 + 0.3325 +2.09 + 0.3425 + 2.09 + 0.3425 - 0.40 (5)

Permeation of *A. vera* = 87.35 + 3.47 + 3.1 + 0.51 – 0.03 – 0.3725 – 0.0575 +0.36 -1.79 -1.5 (6)

Viscosity = 43755.5 + 2198.75 – 228.63 + 6796.88 – 2110.25 – 4859.25 - 869 +2908 -3163.25 + 3724.25 (7)

Spreadability = 20.45 - 2.42 + 2.87 – 1.96 -1.92 + 0.6475 – 1 + 0.625 + 1.29 + 4.57 (8)

The polynomial equation revealed that factors X1 and X3 contributed to an increase in gel viscosity, whereas factor X2 led to a decrease. The positive coefficient of X_0 indicated an overall positive response. Specifically, oleic acid (X1) and glycerin (X3) enhanced viscosity due to their thickening properties in the gels. Conversely, eucalyptus oil was found to reduce the viscosity of the topical formulations.

Factor X2 positively influenced spreadability, whereas X1 and X3 had a negative impact, likely due to the inverse relationship between spreadability and viscosity. The mathematical models employed to analyze permeation patterns suggested a uniformly positive outcome. Each factor, denoted as X_1, X_2, and X_3, appears to positively influence the permeation process of both C.P and *A. vera.* Across all tested combinations, there was a discernible enhancement in the permeation of C.P when incorporated into CGs. Furthermore, a comparative assessment between surfactant and permeation enhancer effects on drug permeation underscores the superior influence of the surfactant in augmenting the permeability.

Nevertheless, the oleic acid and eucalyptus oil both have drug penetrating capabilities and their suitable concentration in the topical gels might provide desired therapeutic concentration across the skin.

4.CONCLUSION

Topical gels containing C.P and *A. vera* were prepared successfully by dispersion method. The characterization tests were performed on the

prepared formulations and all tests were in favor of the claims of this research. The current study determined that the prepared topical gels had a high antimicrobial activity against acne causing bacteria. The antioxidant assay of the gels showed a high antioxidant capacity. The evaluation of organoleptic properties, pH, viscosity, spreadability, *in-vitro* permeation study, skin irritation test, and stability study proved that the formulated gels turned out to be stable and reproducible. Furthermore, *in-vivo* inflammatory studies would be recommended to take this research further.

Author Contribution

Tayyaba Rana writing-original draft. **Muhammad Zaman** Supervision **Aiman Mahmood** writing-original draft. **Aneeqa Saleem Sufyan** writing-review & editing. **Zahbia Roohani** writing-review & editing.

Conflict of Interest

The authors of the manuscript have no financial or non-financial conflict of interest in the subject matter or materials discussed in this manuscript.

Data Availability Statement

Data supporting the findings of this study will be made available by the corresponding author upon request.

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