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DEVELOPMENT, OPTIMIZATION, AND EVALUATION OF A POLYHERBAL TOPICAL CREAM FOR ANTIMICROBIAL AND ANTI-INFLAMMATORY APPLICATIONS

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Keywords:

Polyherbal, Cream, Globule size, Anti-fungal, Antibacterial, C. *albicans*, *E .coli* and *Biofilm* and Time kill assay etc

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ABSTRACT: Microbial skin infections caused by bacterial and fungal pathogens remain a significant therapeutic challenge, highlighting the need for effective plant-based topical formulations. The present study aimed to develop and optimize an oil-in-water polyherbal cream containing extracts of *Cassia fistula*, *Delonix regia*, and *Parkinsonia aculeata*, and to evaluate its antimicrobial, anti-biofilm, and anti-inflammatory potential. Polyherbal cream formulations were prepared using an oil-in-water emulsification technique and optimized through a 3^2 factorial design by varying the oil phase and emulsifier concentrations. The optimized formulation (PHC-5) exhibited desirable physicochemical properties, including a mean globule size of 928.7±20.4 nm, polydispersity index 0.27±0.010, viscosity 6528.7±20.4 cps, and spreadability 41.54±2.95 g·cm/s, indicating suitable rheological behavior for topical application. Antimicrobial evaluation demonstrated that the optimized cream showed enhanced activity compared with the crude polyherbal extract. PHC-5 exhibited potent antifungal activity against *Candida albicans* with MIC 8.0±0.7 µg/mL and MBC 16.0±12.8 µg/mL, showing superior efficacy compared to activity against *Escherichia coli*. Additionally, the formulation significantly inhibited microbial adhesion and biofilm formation, while time-kill studies confirmed rapid and sustained microbial reduction. Overall, the optimized polyherbal cream demonstrated promising antimicrobial and anti-biofilm activity along with appropriate physicochemical characteristics, suggesting its potential as a natural topical alternative for the management of superficial microbial infections. Further *in-vivo* investigations are warranted to confirm its clinical applicability.

INTRODUCTION: Topical microbial infections caused by bacterial and fungal pathogens remain a significant dermatological concern, particularly with the growing prevalence of antimicrobial resistance. Conventional topical antibiotics and antifungal agents often produce adverse effects and reduced therapeutic efficacy, necessitating the exploration of plant-based alternatives with multitarget pharmacological activity [1].

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Medicinal plants rich in flavonoids, tannins, alkaloids, and terpenoids possess well-documented antimicrobial and anti-inflammatory properties and are widely used in dermatological formulations such as creams, gels, and ointments. *Cassia fistula*, *Delonix regia*, and *Parkinsonia aculeata* have individually demonstrated antimicrobial,

antioxidant, and anti-inflammatory activities attributable to diverse phytoconstituents [2]. Polyherbal formulations combining such extracts may provide synergistic therapeutic benefits and broader antimicrobial coverage compared with monoherbal preparations. Therefore, the present study aimed to formulate and optimize an oil-in-water polyherbal cream containing extracts of these three plants and evaluate its physicochemical characteristics, antimicrobial efficacy, and anti-biofilm potential for topical application [3-4].

Herbal medicines and their active phytoconstituents are increasingly explored for managing various diseases, including carcinoma, due to their broad pharmacological properties and minimal side effects [5-6]. Plant-derived compounds such as alkaloids, glycosides, tannins, flavonoids, vitamins, and minerals offer multimodal therapeutic effects, making them valuable in managing complex diseases [7]. Several herbal extracts have demonstrated significant antibacterial and antifungal potential, and herbal skin-care formulations such as creams, gels, lotions, soaps, and ointments have long been used to treat microbial skin disorders. Skin infections are predominantly caused by *Staphylococcus aureus*, fungi, and *Streptococcus* species. While conventional treatments often rely on antibiotics and antifungal agents like clotrimazole, herbal formulations offer a promising alternative or complementary approach [8].

Polyherbal formulations have been used for centuries in Ayurvedic, Chinese, and Unani systems of medicine, often proving more effective than monoherbal preparations due to synergistic interactions and broader therapeutic coverage [9]. Combinations of plant extracts and antibiotics have shown enhanced antimicrobial outcomes in various studies. While numerous reports exist on antimicrobial activities of individual herbs, scientific investigations on traditional polyherbal formulations remain limited [10-11]. Herbal remedies with antibacterial and antifungal properties, prepared from plant parts such as leaves, stems, roots, bark, and fruits, are widely utilized in topical formulations including creams, gels, lotions, soaps, and ointments [12]. These preparations have demonstrated effectiveness in treating skin infections caused by *Staphylococcus aureus*, *Streptococcus* species, and various fungi. Although conventional treatments rely on antibiotics and antifungal agents such as clotrimazole, herbal formulations offer a safer, cost-effective, and efficacious alternative [13-14]. *Cassia fistula* is traditionally used in dermatological applications and has demonstrated considerable antibacterial potential [15]. *Delonix regia* possesses documented anti-inflammatory, antimicrobial, hepatoprotective, and antioxidant activities [16]. *Parkinsonia aculeata* (Jerusalem thorn) is also known for its diverse pharmacological properties and widespread distribution [17]. Given the collective therapeutic potential of these three medicinal plants, the aim of the present study is to formulate and evaluate a polyherbal cream containing extracts of *Cassia fistula*, *Delonix regia*, and *Parkinsonia aculeata* for its antimicrobial and anti-inflammatory activity.

MATERIALS AND METHODS:

Materials: Cetyl palmitate and cetostearyl alcohol were employed as consistency-imparting agents, while Tween 80 and Span 60 were used as emulsifiers. Liquid paraffin and liquid palm oil served as the oil phase components for formulation development. Benzyl alcohol was utilized as a preservative, and rose oil (0.1% v/v) was incorporated as fragrance. Deionized water was used as the aqueous phase for preparation of the oil-in-water cream formulations. All chemicals and reagents used were of analytical grade and procured from approved commercial suppliers.

Collection and Authentication of Plant Material: "Leaves of *Cassia fistula Linn., Delonix regia, and Parkinsonia aculeata* were collected from the Ajara forest region (Kolhapur District, Maharashtra, India) in November 2024. The plant materials were identified and authenticated by Dr. S. S. Patil, Department of Botany, Shivraj Mahavidyalaya, Gadhinglaj, India. A voucher specimen (AMCP/COG/2024-07) has been deposited in the Department of Pharmacognosy herbarium for future reference.

Preparation of Ethanolic Extract: "Leaves of *Cassia fistula* Linn., *Delonix regia*, and *Parkinsonia aculeata* were washed with running tap water, shade-dried, and mechanically powdered. The powdered material was passed through sieve No. 44 and subjected to Soxhlet

extraction using 95% ethanol as the extraction solvent for 48 h in separate batches. The obtained extracts were cooled, filtered, and concentrated under reduced pressure using a rotary evaporator (Buchi, Flawil, Switzerland). The concentrated extracts were further dried and stored in airtight containers until further use [18].

Preliminary Phytochemical Estimation: *Cassia fistula Linn*, *Delonix regia* and *Parkinsonia aculeata* ethanolic extract were phytochemically examined for the presence of glycosides, Alkaloids, glycosides, saponins, carbohydrates, proteins and amino acids according to the standard chemical tests reported [19].

Preparation of CDP Polyherbal Cream: CDP polyherbal creams were prepared using an oil-in-water (O/W) emulsification method. The oil phase, consisting of cetyl palmitate, cetostearyl alcohol, Tween 80/Span 60 emulsifier blend (X_2), and the selected oil component (liquid paraffin or liquid palm oil; X_1), was accurately weighed and melted in a water bath maintained at 75 ± 2 °C. The aqueous phase was prepared separately by dissolving the combined dried plant extract (1 g) in approximately 20% of the required deionized water at 40 °C, followed by the addition of benzyl alcohol (1.5% w/w) as preservative. The remaining quantity of deionized water (up to the final formulation volume) was heated to 70 °C and added to the aqueous mixture. The preheated aqueous phase was gradually added to the oil phase at 70 °C under continuous stirring, followed by homogenization for 15 min to obtain a uniform emulsion. Formulations were prepared in multiple batches by varying the concentrations of the oil phase (X_1) and emulsifier mixture (X_2) according to the factorial design **Table 1**. The Tween 80: Span 60 ratio was adjusted based on the required hydrophilic–lipophilic balance (HLB) of the oil phase to ensure emulsion stability (0.4534:0.5466 for palm oil–based creams and 0.5923:0.4077 for liquid paraffin–based creams). Finally, rose oil (0.1% v/v) was incorporated as fragrance, and the formulation was mixed thoroughly until a homogeneous cream was obtained and allowed to cool to room temperature [20].

Experimental Design: 3^2 full factorial designs were employed by the Design Expert software to optimize CDP polyherbal cream. The relationship between two independent variables, oil phase (X1) and emulsifier phase (X2), and three dependent variables, namely viscosity and spread ability, was studied at three levels per independent variable, namely high (+1), medium (-1) and low (-1). Table 1 shows a total of 9 trial runs that were produced using levels according to the Design Expert software. All the formulations developed as per the procedure were investigated viscosity (Y_1) and spreadability (Y_2) which was categorized as dependent or response variables. Various mathematical models referred to as "linear" and "two-factor" interaction. The responses utilized for evaluating the statistical significance of the created model as well as its terms were filtered out with ANOVA. The Design Expert® software produced 2D, 3D and perturbation graphs to examine the correlation and link between the dependent, independent, or other response variables. Finally, the required formulation CDP polyherbal cream was optimized using numerical and graphical optimization approaches based on properties such as viscosity and spreadability [21].

Evaluation of Polyherbal Cream:

Measurement of pH: pH of polyherbal cream was measured using digital pH meter. 1 gm of polyherbal cream was stirred in 100 ml of distilled water till it forms a uniform suspension. And pH of the dispersion was measured using digital pH meter [22].

Spreadability: Spreadability is a term expressed to stand for the extent of area to which the cream willingly spread on affected area or skin. It denoted in time (seconds) taken by two slides to slip off from the cream and located between the slides under the route of particular weight. Shorter the time taken for separation of two slides, better the spreadability [23].

Spreading is determined with the help of following formula,

$$S = M \times L / T$$

Where, S: Spreadability, M: Wight tide to upper Slide, L: Length moved on glass slide, T: Time taken to separate the slide completely from each other.

Viscosity Measurement: The viscosity of polyherbal cream was determined by using a Brookfield viscometer at 10-100 rpm and 37^{o}C. The equivalent dial reading on the viscometer was noted [24].

***In-vitro* Antibacterial Activity:**

Minimum Inhibitory Concentration (MIC): To evaluate the MIC, serial dilutions of each test samples, polyherbal extract (PH), PH-Cream, were prepared using Brain Heart Infusion (BHI) broth. Each formulation was subjected to 9–10 successive dilutions. A 5 µL aliquot of *Candida albicans* obtained from preserved stock cultures, was introduced into 2 mL of BHI broth. Subsequently, 200 µL of this bacterial suspension was dispensed into each dilution tube. After incubating for 24 hours, the tubes were assessed for turbidity, indicating bacterial growth [25].

Minimum Fungicidal Concentration (MFC): To distinguish whether polyherbal extract (PH), PH-Cream exhibited bactericidal or fungistatic effects against *Candida albicans*, the MFC assay was performed. From the MIC assay, 3–5 tubes showing sensitivity were selected, and their contents were aseptically plated onto nutrient agar and incubated for 24 hrs at 37°C. Then plates were examined for colony formation. The absence of visible bacterial growth indicated a bactericidal effect, whereas the presence of colonies suggested a bacteriostatic action of the tested formulations [26].

Minimum Inhibitory Concentration (MIC): MIC of the polyherbal extract (pH), pH-Cream has been assessed employing broth dilution method. Serial dilutions of each formulation were prepared in BHI broth, extending up to 9–10 dilution levels. A 5 µL aliquot from preserved stock cultures of *Escherichia coli* was inoculated into 2 mL of BHI broth. Subsequently, adding 200µL of this inoculated broth to each dilution tube. After incubation at 37°C for 24h, tubes were examined for turbidity, with lowest concentration demonstrating no visible growth recorded as the MIC [27].

Minimum Bactericidal Concentration (MBC): To distinguish whether polyherbal extract (PH), PH-Creamexhibited bactericidal or bacteriostatic effects against *Escherichia coli*, the MBC assay was performed. From the MIC assay, 3–5 tubes showing sensitivity were selected, and their contents were aseptically plated onto nutrient agar and incubated for 24 h at 37°C. Then plates were examined for colony formation. The absence of visible bacterial growth indicated a bactericidal effect, whereas the presence of colonies suggested a bacteriostatic action of the tested formulations [28].

Time Kill Assay (TKA): TKA was performed using the micro broth dilution technique to assess the fungicidal properties of the different formulations. Fungicidal cultures were incubated in microcentrifuge tubes containing polyherbal extract (PH), PH-Cream at control, MIC, and MFC levels. At specified time intervals (from 0 to 48 h), samples were taken, serially diluted, and spread on blood agar plates. Following a 48-hour incubation, bacterial colonies were enumerated with the help of a digital colony counter. The results were presented as $\log_{10}$ CFU/mL over time to generate time-kill curves [29].

Biofilm Reduction Crystal Violet Assay (BRCA): Biofilm formation was evaluated by inoculating bacterial suspensions, adjusted to a 0.5 McFarland standard, into Brain Heart Infusion (BHI) broth within the wells of a microtiter plate. The plate was incubated under aerobic conditions overnight to facilitate bacterial attachment. Following this, the plate was placed in an aerobic chamber and maintained at 37°C for 72 h to allow biofilm development on the well surfaces. After incubation, the medium was replaced with either fresh broth (control) or the test formulations at MFC and MIC levels. The plate has been then further incubated under the same conditions for 24 h. After treatment, the supernatants were carefully removed and the wells were gently washed utilizing PBS (pH 7.4) for removing planktonic cells. The connected biofilms were preserved with methanol for 15 min, dyed with 0.1% (w/v) crystal violet for 5 min, then rinsed using distilled water to remove color. The residual stain was solubilized in ethanol, and a microplate reader evaluate absorbance at 570nm to quantify biofilm biomass [30].

Statistical Analysis: Data was displayed as mean values and standard deviation (SD). One-way analysis of variance (ANOVA) was employed to

conduct statistical comparisons between groups, followed by Dunnett's multiple comparison test. Analyses were conducted utilizing GraphPad Prism 5.0 (GraphPad Software, La Jolla, CA, USA). P-values under 0.05 were statistically significant [31].

RESULTS AND DISCUSSION:

Preliminary Phytochemical Screening: The pH-extract has been preliminarily phytochemical investigated utilizing qualitative methods. The extract was found to contain various bioactive constituents, including phenolic compounds, alkaloids, tannins, flavonoids, Saponins, and glycosides. Current outcomes align with previous research that reported similar phytochemical profiles in pH-Extract [32-33]. Although quantitative results are not presented here, the presence of these constituents suggests a potential role in reducing and stabilizing silver ions at the time of nanoparticle synthesis.

Fitting of Data into the Model: The selection of the best fit model was based on the higher range of multiple correlation coefficients (R^2), predicted R^2 and adjusted R^2 values, and low values of standard deviation (SD) along with coefficients of variation (% CV) and predicted residual sums of the square (PRESS). The PRESS value indicated the model that best fit the data. The best-fit model was indicated by a smaller PRESS value. The most appropriate and acceptable fit model for all three responses is a quadratic model.

TABLE 1: FULL FACTORIAL DESIGN MATRIX SUMMARIZING THE LEVELS, FACTORS, AND RESPONSES OF 9 RUNS FOR OPTIMIZATION OF CDP POLYHERBAL CREAM

Batch code	Factor X_1	Factor X_2	Y_1	Y_2	Y3
PHC-1	5	3	5718.6 ± 18.9	42.68 ± 4.64	1118.6± 18.9
PHC -2	5	4	4221.5 ± 17.5	29.27± 4.13	821.5± 17.5
PHC -3	5	5	6087.5± 19.5	33.53 ± 3.82	987± 19.5
PHC -4	10	3	5991.5 ± 21.8	32.85 ± 3.72	1401.5± 21.8
PHC -5	10	4	6528.7 ± 20.4	41.54 ± 2.95	928.7± 20.4
PHC -6	10	5	5409.2± 21.3	28.25 ± 2.73	809.2± 21.3
PHC -7	15	3	6066.7 ± 34.1	36.23± 3.54	866.7± 34.1
PHC -8	15	4	3768.3 ± 28.8	31.43 ± 2.79	1768.3± 28.8
PHC -9	15	5	6430.5 ± 23.6	31.87 ± 2.61	1130.5 ± 23.6

Factor (Independent variables)	Actual levels		
	Low (-1)	Medium (0)	High (+1)
Factor X_1: oil phase (%w/v)	15	20	25
Factor X_2: emulsifier phase (%w/v)	8	10	12

Dependent variables or Responses	Constraint
Response 1: Viscosity (Y_1) (cps)	Maximize
Response 2: Spreadability (Y_2) (cm)	Maximize
Response 3: Globule size (Y3) (nm)	Minimize

Effect of Independent Variables on Viscocity (Y_1) of Polyherbal Cream: The level of the emulsifying phase was observed to correlate with a rise in viscosity. The viscosity was shown to reduce when the oil phase was raised, which was the opposite of what was seen for the oil phase level. The viscosity values are found in the range of 3768.3±28.8 cps to 6528.7±20.4 cps. The quadratic equation can be used to express the impact of the independent variables on the viscosity:

$$Y_1 = +6206.40- -104.43\ A+7.57\ B+1242.35\ AB-237.00A^2-702.30B^2 \ldots. (1)$$

Where Y_1 represents viscosity, X_1 denotes oil concentration, and X_2 indicates emulsifier concentration. The regression analysis showed that the oil phase exerted a significant negative effect ($p < 0.05$) on viscosity, whereas the emulsifier phase exhibited a significant positive effect ($p < 0.05$). Thus, viscosity decreased with increasing oil concentration and increased with higher emulsifier levels. The higher coefficient value of X_1 suggested that oil concentration had a greater influence on viscosity compared with the emulsifier concentration.

The quadratic model was statistically significant with an F-value of 35.35, indicating only a 0.72% probability that the observed value occurred due to noise. Significant model terms included X_1, X_2, X_1X_2 and X_1^2 ($p < 0.05$). The predicted R^2 (0.8169) was in reasonable agreement with the adjusted R^2

(0.9555), confirming a good model fit. The Adeq Precision value of 16.19 (>4) indicated an adequate signal-to-noise ratio, supporting the use of the model for navigating the design space. The effects of independent variables on viscosity were further illustrated through three-dimensional response surface and perturbation plots **Fig. 1A** and **1B**, demonstrating the dominant influence of oil and emulsifier concentrations on viscosity. Significant interaction and quadratic terms (AB and B^2) further confirmed the reliability of the model, while removal of non-significant terms ($p>0.10$), maintaining hierarchy, could improve overall model predictability.

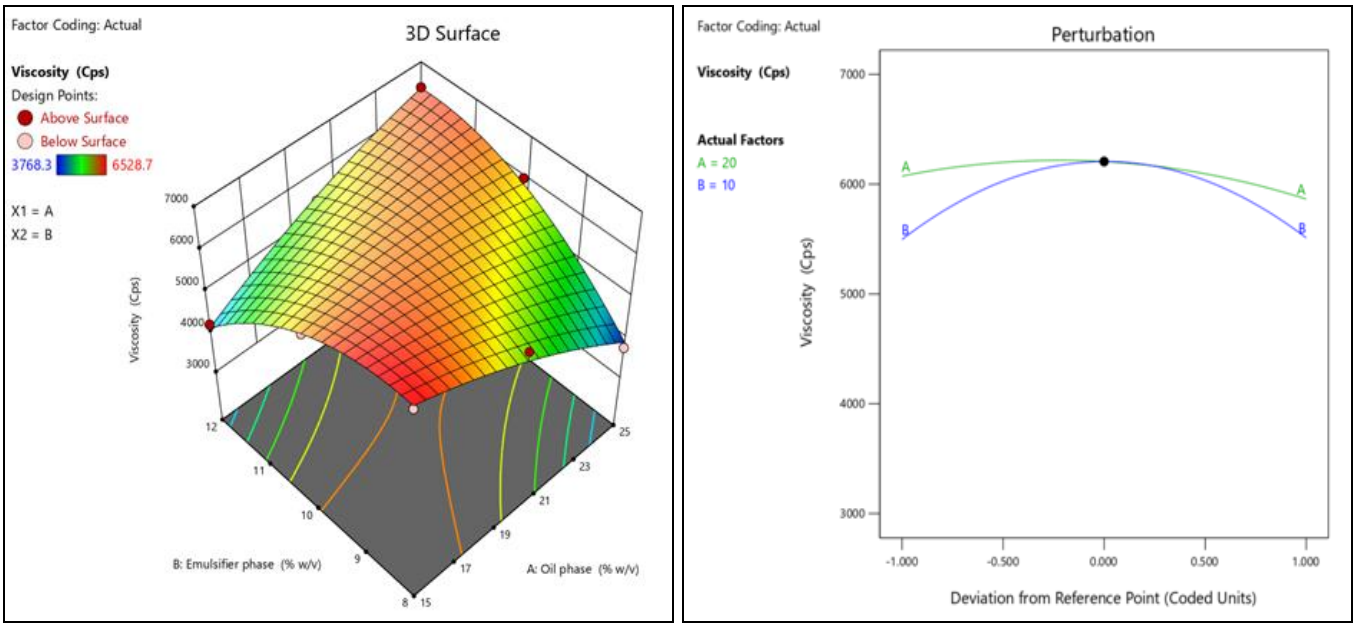

FIG. 1: (A) 3D-RESPONSE SURFACE (B) PERTURBATION PLOT SHOWING THE EFFECT OF AMOUNT OF OIL AND AMOUNT OF EMULSIFIER PHASE ON VISCOSITY OF OPTIMIZED PHC-5 RESPECTIVELY

Effect of Independent Variables on Spredability (Y_2) of Polyherbal Cream: Spreadability decreased with an increase in the emulsifying phase. The efficacy of the developed polyherbal cream could be greatly determined by its spread ability. spreadability determined for all polyherbal cream are shown in **Table 1**. The spreadability is found to be in the range of 29.27± 4.13 to 42.68 ± 4.64 %. The effect of the independent variables on the spreadability can be explained using the linear equation.

$$Y_2 = +34.18-1.81\ A-4.38\ B+3.18\ AB\ldots\ (2)$$

Where Y_2 represents spreadability, X_1 denotes oil concentration, and X_2 indicates emulsifier concentration. The regression equation demonstrated that the oil phase exerted a positive effect on spreadability, whereas the emulsifier phase showed a negative effect, indicating that spreadability increased with increasing oil concentration and decreased with increasing emulsifier concentration. The quadratic model was statistically significant with an F-value of 9.76 ($p < 0.05$). Among the model terms, X_1, X_2, and X_2^2 were significant factors influencing spreadability. The Adeq Precision value of 9.267 (>4) indicated an adequate signal-to-noise ratio, confirming that the model could be effectively used for navigation within the design space.

Although the predicted R^2 (0.3016) was not in close agreement with the adjusted R^2 (0.7666) (difference > 0.2), suggesting possible model limitations or data variability, the model remained useful for preliminary optimization and required confirmation runs for validation.

Three-dimensional response surface and perturbation plots **Fig. 2A** and **2B** illustrated that spreadability increased with increasing oil concentration and decreased with higher emulsifier levels. Significant model terms included B and AB, whereas non-significant terms ($p > 0.10$) could be reduced, while maintaining model hierarchy, to further improve predictability and robustness.

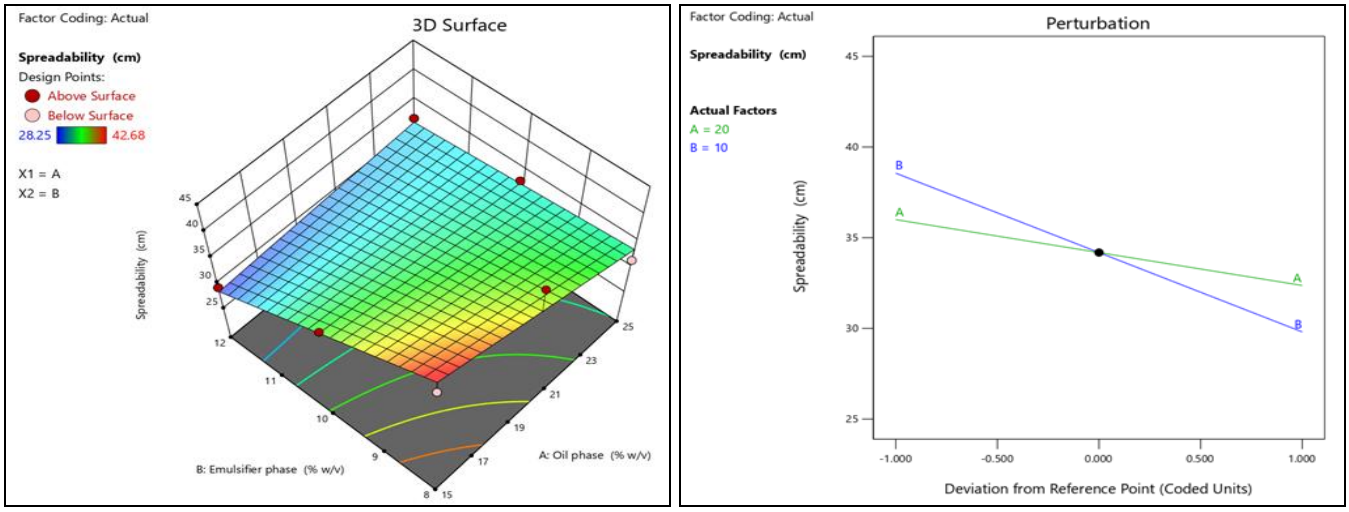

FIG. 2: (A) 3D-RESPONSE SURFACE (B) PERTURBATION PLOT SHOWING THE EFFECT OF AMOUNT OF OIL AND AMOUNT OF EMULSIFIER PHASE ON SPREDABILITY OF OPTIMIZED PHC-5 RESPECTIVELY. EFFECT OF INDEPENDENT VARIABLES ON GLOBULE SIZE (Y_3) OF PHC

The lower globule size was observed for PHC-2 (821.5±17.5 nm) while the highest globule size was obtained for PHC-8 (1768.3± 28.8 nm). The effect of independent variables on the globule sizecan be described by the following linear equation.

Globule size (Y2) =+964.22+280.57**A**+-175.73 **B**-132.65AB+181.27 A^2+11.07 B^2

Where Y_3 represents globule size, X_1 denotes the oil percentage, and X_2 indicates the emulsifier percentage. The regression equation revealed that the oil phase exerted a positive effect on globule size, whereas the emulsifier phase showed a negative effect, indicating that globule size increased with increasing oil concentration and decreased with increasing emulsifier concentration. Response surface and perturbation plots **Fig. 3A** and **3B** further confirmed that globule size markedly decreased as the emulsifier concentration increased from 8–12%, while it increased with oil concentration from 15–25%. The perturbation plot demonstrated steep slopes for both factors A (oil) and B (emulsifier), confirming them as critical variables influencing globule size. The small difference between the predicted R^2 (0.9608) and adjusted R^2 (0.9899) (<0.2) indicates good model agreement. The Adeq Precision value of 36.547 (>4) demonstrated an adequate signal-to-noise ratio, confirming the suitability of the model for design navigation. The model was statistically significant with an F-value of 158.02, indicating only a 0.08% probability that the result occurred due to noise. Model terms A, B, AB, and A^2 were significant ($p < 0.05$), while non-significant terms ($p > 0.10$) could be removed to improve model predictability.

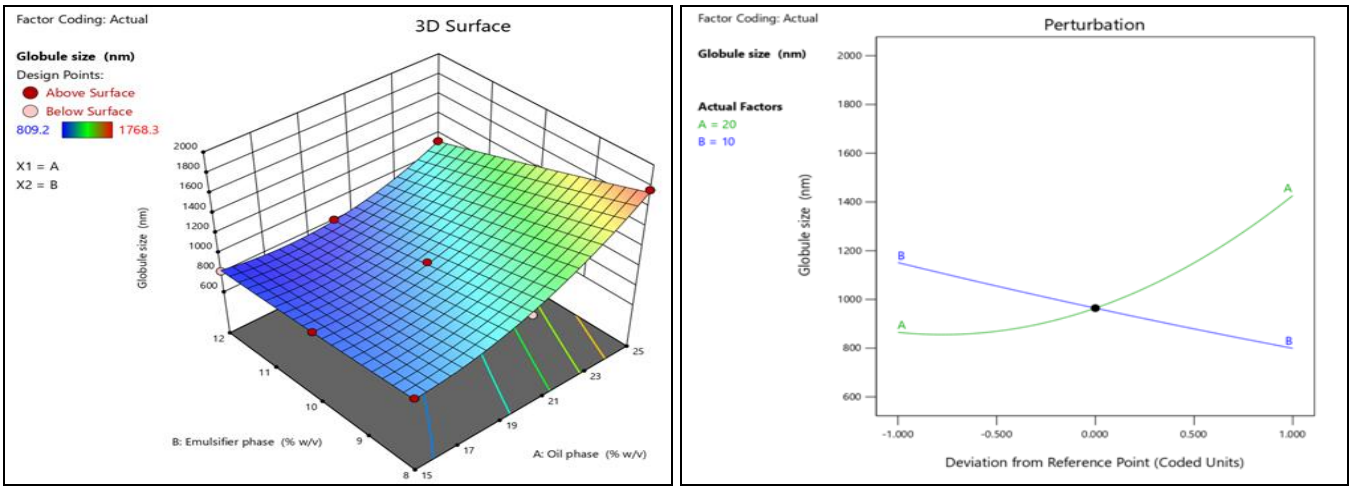

FIG. 3: (A) 3D-RESPONSE SURFACE (B) PERTURBATION PLOT SHOWING THE EFFECT OF AMOUNT OF OIL AND AMOUNT OF EMULSIFIER PHASE ON GLOBULE SIZE OF OPTIMIZED PHC-5 RESPECTIVELY

Optimization of Polyherbal Gel: Constraints were applied to the independent variables (viscosity and % spread ability–maximize and globule size – minimize) to ascertain the optimized polyherbal cream (PHC-5) in the design space and numerical optimization process. The amounts of lipid (10%) and emulsifier (4%) have viscocity-6528.7 ± 20.4, spreadability-41.54 ± 2.95 and globule size-928.7± 20.4 nm considered as optimized PHC batch and used for further evaluation [37].

Globule Size and PDI: The lower globule size was observed for PHC-2 (821.5± 17.5 nm) while the highest globule size was obtained for PHC-8 (1768.3± 28.8 nm). Results of globule size (928.7± 20.4) optimized PHC-5 was shown in **Fig. 4.**

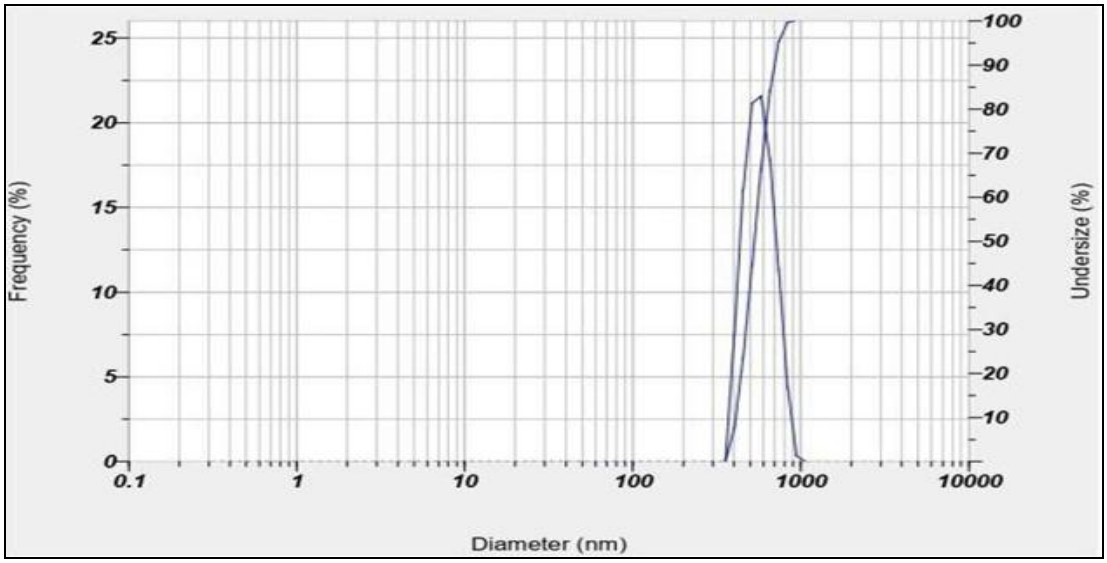

FIG. 4: GLOBULE SIZE OF OPTIMIZED PHC-5 FORMULATION

***In-vitro* Antimicrobial Study:**

MIC and MFC of pH-Extract and pH-Cream: MIC and MFC were evaluated for pH extract, pH-Cream, against *Staphylococcus aureus* and *Candida albicans* using the standard broth dilution method. These parameters are vital indicators of antimicrobial efficacy, where MIC refers to the lowest concentration inhibiting visible bacterial growth, and MFC refers to the concentration that kills ≥99.99% of the initial inoculum. Significant inhibitory activity against *Candida albicans* has been demonstrated by pH extract, pH-Cream. The MIC values of pH-Extract, and pH-Cream on *Candida albicans* were found to be 31.25±2.8 µg/mL and (8.0±0.7 µg/mL) respectively. MFC is the content at which 99.99 viable organisms are eliminated in contrast to the initial inoculums for a short time 65. The MFC values of pH-Extract, and pH-Cream **Fig. 5** on *Candida albicans* were found to be (62.8±3.7µg/mL) and 16.0±12.8 µg/mL respectively. The MIC of pH-Extract and pH-Cream against *E. coli* has been seen to be 85.4±4.9 µg/mL, and 62.5±4.2 µg/mL, correspondingly. In contrast, the pH extract and pH-Cream showed higher MBC values of 142.3±6.8 µg/mL and 125±4.6 µg/mL, for *E. coli*, **Fig. 6**. This improvement suggests the effective synergistic antibacterial action between the phytochemicals in pH-Extract and the intrinsic antimicrobial properties of PH-Cream [38].

Results of antimicrobial study are shown in **Table 2**. The polyherbal cream developed from *Cassia fistula*, *Delonix regia*, and *Parkinsonia aculeata* demonstrated significantly superior antifungal activity when compared to the corresponding polyherbal extract. This enhancement arises primarily from the formulation advantages offered by a semisolid topical base.

While the crude extract contains potent antifungal phytochemicals including anthraquinones, flavonoids, tannins, alkaloids, and phenolic compounds their therapeutic potential is limited by poor skin permeation, rapid degradation, and insufficient retention time when applied directly in solution form. Incorporating these bioactive compounds into a cream base improves their solubility, prevents oxidative degradation, and ensures a controlled, sustained release at the site of

application. Thus, the findings strongly support that formulation into a topical cream markedly enhances the antifungal efficacy of the polyherbal combination, making it a more effective therapeutic candidate for fungal skin infections [39-41].

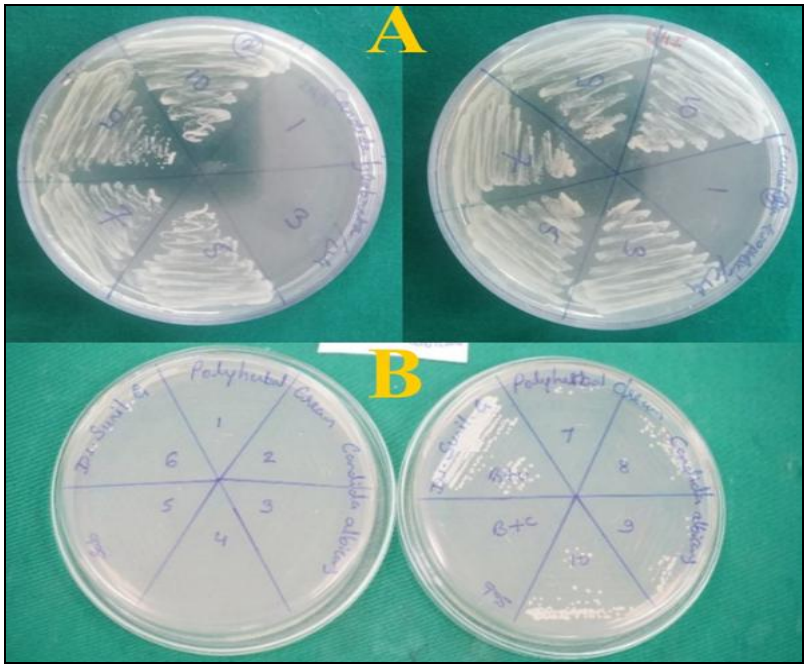

FIG. 5: MFC CONCERTATION OF A) PH-EXTRACT B) OPTIMIZED pHC-5 ON *CANDIDA ALBICANS*

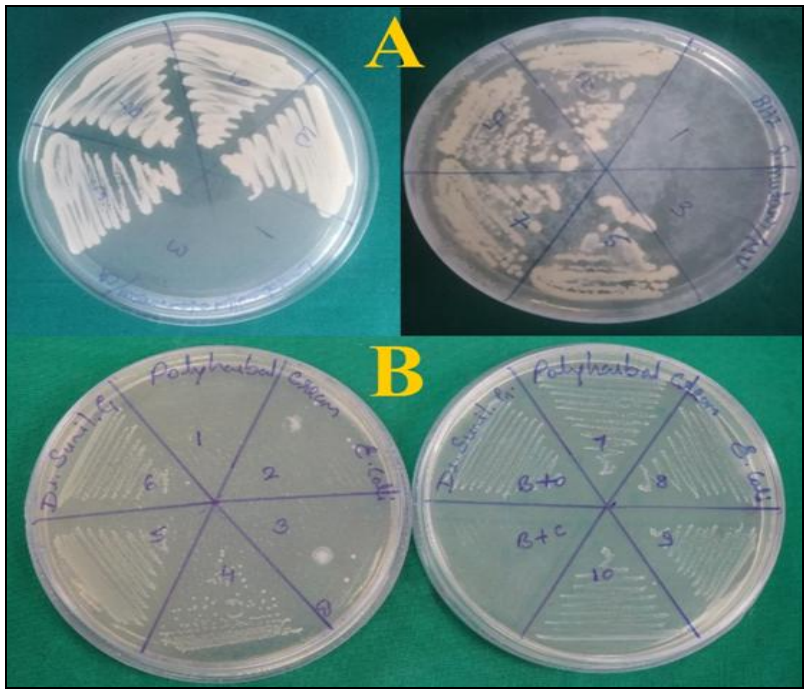

FIG. 6: MBC CONCERTATION OF A) PH-EXTRACT B) OPTIMIZED pHC-5 ON *E. COLI*

TABLE 2: MIC VALUE OF PH EXTRACT AND pH- CREAM ON *C. ALBICANS* AND *E. COLI*

Sr. no.	Test Material	MIC (µg/mL)	MBC (µg/mL)
	C. albicans		
1	pH Extract	31.25±2.8	62.8±3.7
2	pHC-5	8.0±0.7	16.0±12.8
	E. coli		
1	PH Extract	85.4±4.9	142.3±6.8
2	pHC-5	62.5±2.5	125±4.6

All values represent mean ± standard deviation (n=3)

Time-Kill Assay: The time-kill kinetic assay was performed to evaluate the bactericidal efficacy of PH extract, PH-Cream against *Candida albicans* over a time-course, at both MIC and MFC concentrations. The results demonstrated a time- and concentration-dependent bactericidal activity, with PH-Cream exhibiting the most rapid and effective killing profile, particularly at MFC levels. At MIC concentrations, bacterial growth was inhibited progressively with increased incubation time, indicating a bacteriostatic effect. However, at MFC concentrations, complete fungal elimination (100% kill) was observed within 48 hours **Fig. 7.** The improved antifungal efficacy is attributed to the inherent mechanisms of PH-cream, including disruption of fungal membranes, generation of reactive oxygen species (ROS), and inhibition of DNA replication and protein synthesis [42-45].

At MIC concentrations, a gradual decline in fungal viability was observed, indicating a bacteriostatic effect that intensified with time. The improved performance of pH-Cream over pH extract suggests a synergistic effect between the bioactive phytochemicals from *C. fistula*, *D. regia*, and *P. aculeata*, which are known to enhance membrane permeability and induce oxidative stress in microbial cells [46-48]. Moreover, the incorporation of pH-Extract into a cream base may facilitate sustained drug delivery and intimate contact with the infected site, thereby improving therapeutic outcomes in the management of microbial infections. These findings align well with the MIC and MFC results **Table 2**, further supporting the potent and prolonged antimicrobial potential of PH-Cream as a novel topical antimicrobial strategy.

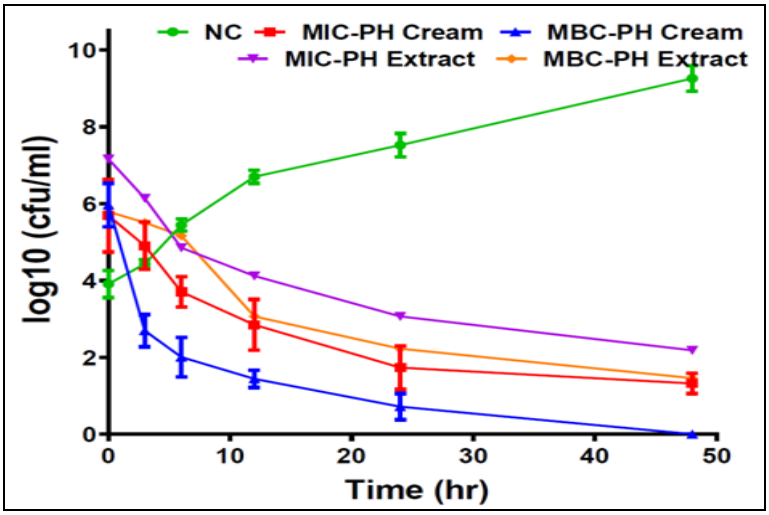

FIG. 7: TIME KILL CURVE SHOWING TIME DEPENDENT FUNGAL INHIBITION AND BACTERICIDAL ACTIVITY OF PH EXTRACT AND pH-CREAM (pHC-5) ON *CANDIDA ALBICANS*. Data are mean ± SD of three independent experiments. MIC minimum inhibitory concentration, MBC minimum fungicidal concentration

Biofilm Reduction Assay (Crystal Violet Assay): Biofilm formation by *C. Albicans* is a critical factor in its pathogenicity and drug resistance. The biofilm matrix acts as a protective barrier, limiting the penetration of drugs and shielding embedded fungal cells [49]. The crystal violet assay was employed to assess the ability of PH, and PH-Cream to disrupt pre-formed *C. albicans* biofilms. The results showed that both MIC and MFC concentrations of PH, and PH-Cream significantly reduced biofilm biomass in a dose-dependent manner, with maximum reduction at MIC concentrations **Fig. 8.** Interestingly, biofilm inhibition was more effective at MIC than at MFC, possibly due to the sublethal stress imposed by MIC concentrations, which affects quorum sensing and adhesion without immediate cell lysis [50]. The biofilm reduction assay has displayed a prominent effect of MIC and MFC concentration of PH, and PH-Cream on *C. albicans*, cells **Fig. 8**. Significant ($p < 0.001$) cells reduction (84.21±3.57%) was observed with pH at MIC and at MFC, the cell reduction was 62.54±4.57%. In the case of PH-Cream, the significant ($p < 0.001$) cell reduction observed at MIC and MBC was up to 60.24±3.64% and 45.32±2.78 % respectively. The high biofilm reduction capacity of PH-Cream formulation indicates their potential to disrupt the protective matrix and reduce bacterial adhesion. This effect is primarily attributed to Penetration of PH-Cream

into the EPS (extracellular polymeric substance) of the biofilm, Interruption of fungal signalling (quorum sensing) pathways, and Inhibition of surface protein-mediated adhesion, which is essential for biofilm development [51-55]. These findings suggest that PH-Cream formulations not only inhibit planktonic fungal growth but also impair biofilm-associated resistance mechanisms, making them promising agents for treating chronic and biofilm-mediated infections.

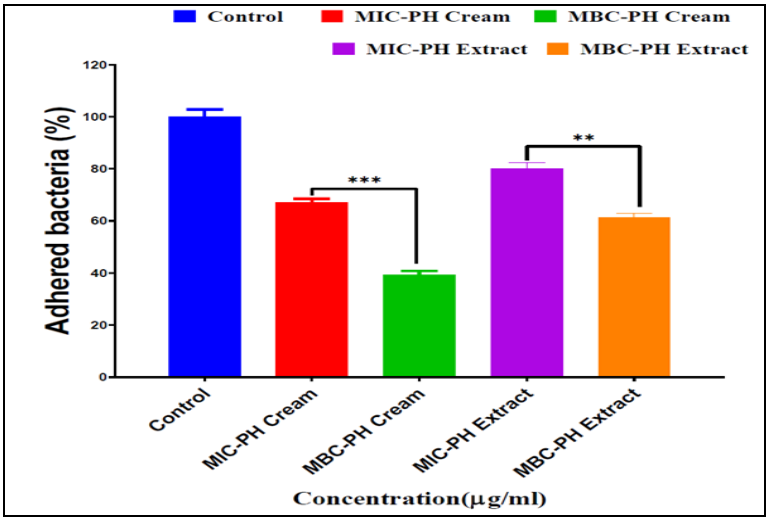

FIG. 8: MIC MINIMUM INHIBITORY CONCENTRATION, MBC MINIMUM FUNGICIDAL CONCENTRATION; B) EFFECT OF pH-EXTRACT, AND PH-CREAM (pHC-5) ON THE FUNGAL INHIBITION ON MICROTITRE PLATE. Data are mean ± SD of three independent experiments. Significant difference specified as ***p < 0.001, **p < 0.01, *p < 0.05 and ns- not significant between control versus treated samples.

***In-vitro* Anti-inflammatory Activity:**

Egg Albumin Denaturation Method (Protein Denaturation): The inhibition of protein denaturation assay demonstrated a concentration-dependent anti-inflammatory response for the polyherbal extract (pH-Extract), polyherbal cream (pH-Cream), and the standard drug acetylsalicylic acid. Among the tested samples, pH-Cream exhibited significantly higher inhibitory activity compared with pH-Extract at all tested concentrations ($p<0.05$). At 100 µg/mL, pH-Cream showed 60.60 ± 10.42% inhibition, which was significantly greater than pH-Extract (53.60 ± 0.73%) and comparable to acetylsalicylic acid (55.16±0.85%), indicating statistically non-significant difference between pH-Cream and the standard ($p>0.05$) **Fig. 9**. Similarly, at intermediate and lower concentrations (3.125–50 µg/mL), pH-Cream consistently produced significantly higher inhibition than the extract ($p < 0.05$). The IC_{50} values further supported these findings, where pH-Cream exhibited a significantly lower IC_{50} (61.54 ± 9.54 µg/mL) than pH-Extract (92.34 ± 7.35 µg/mL) ($p < 0.01$) and an IC_{50} comparable to acetylsalicylic acid (64.35 ± 7.35 µg/mL) ($p>0.05$). The enhanced anti-inflammatory activity of pH-Cream may be attributed to improved dispersion and increased availability of phytoconstituents within the emulsified system, facilitating greater interaction with inflammatory protein substrates [56-62]. Overall, these results indicate that the optimized polyherbal cream possesses statistically significant anti-inflammatory activity comparable to the standard drug and represents a promising topical therapeutic candidate.

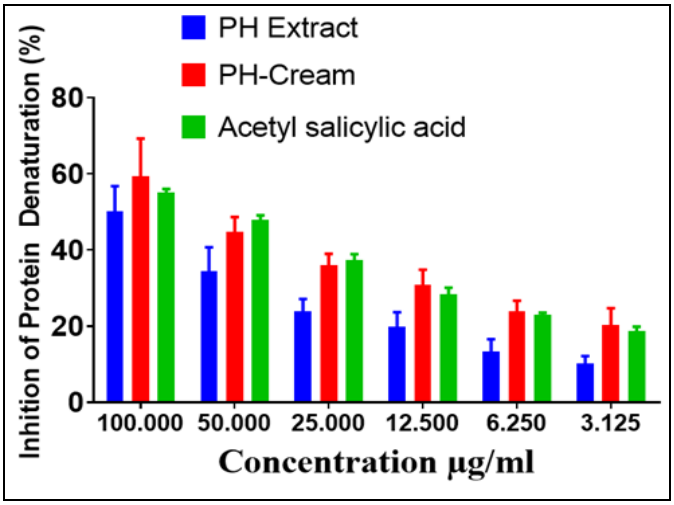

FIG. 9: COMPARISON BETWEEN %INHIBITION OF PROTEIN DENATURATION BY PH EXTRACT, pH-CREAM (pHC-5) AND STANDARD ACETYL SALICYLIC ACID

CONCLUSION: The present study successfully developed and optimized an oil-in-water polyherbal cream containing extracts of *Cassia fistula*, *Delonix regia*, and *Parkinsonia aculeata* using a 3^2 factorial design approach. Formulations prepared with palm oil as the oil phase and Tween 80/Span 60 as the emulsifying system demonstrated acceptable physicochemical characteristics suitable for topical application. Among the developed batches, the optimized formulation (pHC-5) exhibited desirable properties, including viscosity 6528.7±20.4 cps, spreadability 41.54±2.95 g·cm/s, and globule size 928.7 ± 20.4 nm, which were consistent with the predicted optimization outcomes, confirming the robustness of the experimental design. Biological evaluation revealed that the optimized cream showed significant antimicrobial activity, particularly against *Candida albicans*, demonstrating superior efficacy compared with the crude extract and notable activity against *Escherichia coli*.

The enhanced antimicrobial performance, together with appropriate rheological and stability characteristics, indicates that the developed polyherbal cream can serve as a promising natural topical alternative to conventional synthetic antimicrobial formulations. Nevertheless, comprehensive *in-vivo* pharmacological and clinical investigations, along with long-term stability and safety assessments, are essential to further validate its therapeutic potential for the management of microbial infections.

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CONFLICT OF INTEREST: Nil

REFERENCES:

1. Kareru PG, Keriko JM, Kenji GM, Thiong'o GT, Gachanja AN and Mukiira HN: Antimicrobial activities of skincare preparations from plant extracts. Afr J Tradit Complement Altern Med 2010; 7(3): 214-218.
2. Rahmani AH: *Cassia fistula* Linn: Potential candidate in the health management. Pharmacogn Res 2015; 7(3): 217-224.
3. Duraipandiyan V and Ignacimuthu S: Antibacterial and antifungal activity of *Cassia fistula* L. J Ethnopharmacol 2007; 112(3): 590-594.
4. Singh S and Kumar SN: Introduction to genus Delonix. World J Pharm Pharm Sci 2014; 3(6): 2042-2055.
5. Starr F, Starr K and Loope L: *Parkinsonia aculeata*. Plants of Hawaii Reports 2003.
6. Zambare KK, Kondapure AA, Reddy KV, Thalkari AB, Karwa PN and Nikam YP: Pharmacological applications of *Parkinsonia aculeata*. Res J Pharm Technol 2021; 14(3): 1767-1770.
7. Anitha J and Miruthula S: Anti-inflammatory analysis of Cassia fistula fruit pulp extracts. Int J Pharmcog 2014; 1: 207-215.
8. Kulkarni A, Govindappa M, Chandrappa CP, Ramachandra YL and Koka PS: Phytochemical and antimicrobial activities of Cassia fistula. Adv Med Plant Res 2015; 3(1): 8-17.
9. Nagpal MA, Nagpal N, Rahar S, Shah G, Swami G and Kapoor R: Phytochemical investigation of Cassia fistula leaves. Pharmacogn J 2011; 3(26): 61-69.
10. Suhane N, Shrivastava RR and Singh M: Gulmohar ornamental plant with medicinal uses. J Pharmacogn Phytochem 2016; 5(6): 245-248.
11. Oyedeji OA, Azeez LA and Osifade BG: Chemical composition of *Delonix regia* seeds. S Afr J Chem 2017; 70: 16-20.
12. Shabir G, Anwar F, Sultana B, Khalid ZM, Afzal M and Khan QM: Antioxidant and antimicrobial attributes of *Delonix regia*. Molecules 2011; 16(9): 7302-7319.
13. Sammour RH and El-Shanshoury AER: Antimicrobial activity of legume seed proteins. Bot Bull Acad Sin 1992; 33: 185-190.
14. Sharma S and Vig AP: Antioxidant properties of Parkinsonia aculeata leaves. Sci World J 2013; 2013: 604865.
15. Al-Youssef HM and Hassan WH: Antimicrobial activity of *Parkinsonia aculeata* essential oils. Merit Res J Med Med Sci 2015; 3(4): 147-157.
16. Gupta MK, Kenganora M, Banerjee A, Saini L and Kumar V: Pharmacognostic evaluation of *Parkinsonia aculeata* bark. J Pharm Sci Biosci Res 2011; 1: 86-92.
17. Hassan WH, Abdelaziz S and Al-Yousef HM: Chemical composition of *Parkinsonia aculeata* fraction. Arab J Chem 2019; 12(3): 377-387.
18. Chellathurai BJ, Anburose R, Alyami MH, Sellappan M, Bayan MF and Chandrasekaran B: Polyherbal topical gel for acne. Gels 2023; 9(2): 163.
19. Bhinge SD, Bhutkar MA, Randive DS, Wadkar GH, Todkar SS and Kakade PM: Antimicrobial polyherbal gel formulation. Ann Pharm Fr 2017; 75(5): 349-358.
20. Gautam SS and Dwivedi S: Antimicrobial activity of *Delonix regia* leaf extracts. Eur J Mol Clin Med 2020; 7(11): 1-6.
21. Mwangi RW, Macharia JM, Wagara IN and Bence RL: Medicinal properties of *Cassia fistula*: Review. Biomed Pharmacother 2021; 144: 112240.
22. Kumawat RB, Kaushik P and Meena G: Antimicrobial screening of phytochemicals. Int J Adv Sci Technol 2020; 29(3): 11347-11358.
23. Abdelaziz S, Al-Yousef HM, Al-Qahtani AS, Hassan WH, Fantoukh OI and El-Sayed MA: Phytochemical profile of

Parkinsonia aculeata. Saudi Pharm J 2020; 28(9): 1129-1137.
24. Sharma S and Vig AP: Phytochemical screening of *Parkinsonia aculeata*. Biomed Res Int 2014; 2014: 756184.
25. Shehu HA, Mukhtar AG, Adetoyinbo II, Ojo AO and Mus'ab UA: Antibacterial activity of Cassia fistula leaf extracts. J Pharmacogn Phytochem 2020; 9(3): 1779-1783.
26. Bhokare P, Khadke A, Kulkarni S and Kuchekar G: Antibacterial activity of *Delonix regia* extracts. J Pharmacogn Phytochem 2018; 7(3): 904-909.
27. Qureshi SP; Antibacterial activity of *Parkinsonia aculeata* leaf extract. Int J Res Biosci Agric Technol 2017; 5(2): 667-670.
28. Bhalodia NR and Shukla VJ: Antibacterial and antifungal activities of *Cassia fistula*. J Adv Pharm Technol Res 2011; 2(2): 104-109.
29. Kamba AS and Hassan LG: Microbial screening of *Parkinsonia aculeata* leaves. Int J Drug Dev Res 2010; 2(1): 1-7.
30. Chatur VM, Ansari NM, Joshi SK and Walode SG: Formulation and evaluation of polyherbal cream. J Drug Deliv Ther 2022; 12(4): 112-115.
31. Balouiri M, Sadiki M and Ibnsouda SK: Methods for evaluating antimicrobial activity. J Pharm Anal 2016; 6(2): 71-79.
32. Sathishkumar M, Sneha K, Won SW, Cho CW, Kim S and Yun YS: Green synthesis of silver nanoparticles. Colloids Surf B Biointerfaces 2009; 73(2): 332-338.
33. Galatage ST, Trivedi R and Bhagwat DA: Characterization of camptothecin and anticancer potential against prostate cancer. Future J Pharm Sci 2021; 7(1): 104. doi:10.1186/s43094-021-00259-3.
34. Kodoli RS, Galatage ST, Killedar SG, Pishwikar SA, Habbu PV and Bhagwat DA: Hepatoprotective activity of Phyllanthus niruri endophytes. Future J Pharm Sci 2021; 7(1): 97. doi:10.1186/s43094-021-00252-w.
35. Killedar SG, Bhagwat DA, Choudhari A, Saboji JK, Chougule PC and Galatage ST: Microsponge of amphotericin B for topical delivery. Res J Pharm Biol Chem Sci 2019; 10(1): 1288-1300.
36. Galatage ST, Manjappa AS, Kumbhar PS, Salawi A, Sabei FY and Siddiqui AM: Silver nanoparticles using *Emilia sonchifolia* for treatment of bloodstream diseases. Ann Pharm Fr 2023; 81(4): 653-666. doi:10.1016/j.pharma.2023.03.003.
37. Galatage ST, Hebalkar AS, Gote RV, Mali OR and Killedar SG: Silver nanoparticles by green synthesis: Overview. Res J Pharm Technol 2020; 13(3): 1503-1510.
38. Galatage ST: Development of microparticles of sumatriptan succinate *via* nasal route. Int J Pharm Sci Res 2019; 10(9): 4194-4200.
39. Sathishkumar M, Sneha K, Won SW, Cho CW, Kim S and Yun YS: Green synthesis of silver nanoparticles using cinnamon bark extract. Colloids Surf B Biointerfaces 2009; 73(2): 332-338. doi:10.1016/j.colsurfb.2009.06.005.
40. Hakkim FL, Bakshi HA, Khan S, Krishnamoorthy R. Green synthesis of silver nanoparticles using *Ocimum sanctum.* Pharmacogn J 2020; 12(2): 367-372. doi:10.5530/pj.2020.12.61.
41. Krishnaraj C, Jagan EG, Rajasekar S, Selvakumar P, Kalaichelvan PT and Mohan N: Synthesis of silver nanoparticles using *Acalypha indica* leaf extract. Colloids Surf B Biointerfaces 2010; 76(1): 50-56. doi:10.1016/j.colsurfb.2009.10.008.
42. Veerasamy R, Xin TZ, Gunasagaran S, Xiang TFW, Yang EFC and Jeyakumar N: Biosynthesis of silver nanoparticles using mangosteen leaf extract. J Saudi Chem Soc 2011; 15(2): 113-120. doi:10.1016/j.jscs.2010.06.007.
43. Zhang XF, Liu ZG, Shen W and Gurunathan S: Silver nanoparticles: Properties and therapeutic approaches. Int J Mol Sci 2016; 17(9): 1534. doi:10.3390/ijms17091534.
44. Agnihotri S, Mukherji S and Mukherji S: Size-controlled silver nanoparticles and antibacterial efficacy. RSC Adv 2014; 4(8): 3974-3983. doi:10.1039/C3RA44507K.
45. Duran N, Marcato PD, De Souza GIH, Alves OL and Esposito E: Antibacterial effect of silver nanoparticles produced by fungal process. J Biomed Nanotechnol 2007; 3(2): 203-208.
46. Li WR, Xie XB, Shi QS, Zeng HY, Ou-Yang YS and Chen YB: Antibacterial activity of silver nanoparticles on *Escherichia coli*. Appl Microbiol Biotechnol 2010; 85(4): 1115-1122. doi:10.1007/s00253-009-2159-5.
47. Kumbar VM, Peram MR, Kugaji MS, Shah T, Patil SP and Muddapur UM: Effect of curcumin on biofilm formation. Odontology 2021; 109(1): 95-103. doi:10.1007/s10266-020-00538-7.
48. Girilal M, Fayaz AM, Elumalai LK, Sathiyaseelan A, Gandhiappan J and Kalaichelvan PT: Comparative stress physiology of silver nanoparticles. Colloid Interface Sci Commun 2018; 24: 30-35. doi:10.1016/j.colcom.2018.04.003.
49. Masum MM, Siddiqa MM, Ali KA, Zhang Y, Abdallah Y and Ibrahim E: Biogenic synthesis of silver nanoparticles using *Phyllanthus emblica*. Front Microbiol 2019; 10: 820. doi:10.3389/fmicb.2019.00820.
50. Saravanakumar K, Chelliah R, Shanmugam S, Varukattu NB, Oh DH and Kathiresan K: Green synthesis of nanosilver from *Gardenia jasminoides*. J Photochem Photobiol B. 2018; 185: 126-135. doi:10.1016/j.jphotobiol.2018.06.006.
51. Femi-Adepoju AG, Dada AO, Otun KO, Adepoju AO, Fatoba OP. Green synthesis of silver nanoparticles using Gleichenia pectinata. Heliyon 2019; 5(4): 01543. doi:10.1016/j.heliyon.2019.e01543.
52. Femi-Adepoju AG, Adepoju AO, Fatoba PO and Olayemi VT: Biosynthesis and antimicrobial potency of silver nanoparticles. Int J Curr Res 2018; 10(12): 76390-76398.
53. Shahzad M, Millhouse E, Culshaw S, Edwards CA, Ramage G and Combet E: Dietary polyphenols inhibit pathogen growth and biofilm formation. Food Funct 2015; 6(3): 719-729. doi:10.1039/C4FO00794H.
54. Kala BS, Gunjan C, Disha N and Shobha P: Treatment of periodontal disease—a herbal approach. Int J Pharm Sci Rev Res 2015; 33(2): 118-122.
55. Galatage ST, Manjappa AS, Bhagwat DA, Trivedi R, Salawi A and Sabei FY: Oral self-nanoemulsifying drug delivery systems for fosfestrol. Eur J Pharm Biopharm 2023; 187: 89-103. doi:10.1016/j.ejpb.2023.10.013.
56. Tan J: Dapsone 5% gel: A new option in topical therapy for acne. Skin Therapy Lett 2012; 17(8): 1-3.
57. Galatage ST, Hebalkar AS, Gote RV, Mali OR and Killedar SG: Silver nanoparticles by green synthesis: An overview. Res J Pharm Technol 2020; 13(3): 1503-1510. doi:10.5958/0974-360X.2020.00271.2.
58. Yadav V, Galatage ST, Manjappa AS, Salawi A, Peram MR and Nadaf SJ: Green synthesis of silver nanoparticles from Clematis gouriana. The Microbe 2024; 5: 100197. doi:10.1016/j.mbce.2024.100197.
59. Galatage ST: Development and characterization of microparticles of sumatriptan succinate. Int J Pharm Sci Res 2019; 10(9): 4194-4200. doi:10.13040/IJPSR.0975-8232.10(9).4194-00.

60. Galatage ST, Manjappa AS, Waghmode RR, Harale SS, Katkar RB and Desai SA: Role of drug repurposing in cancer treatment. In: Drug Repurposing—Advances, Scopes and Opportunities. Intech Open 2023. doi:10.5772/intechopen.108034.
61. Galatage ST, Manjappa AS, Katkar RB, Shinde SA, Phalake RA and Kadam RJ: Exploring anticancer potential of camptothecin. Int J Pharm Sci Nanotechnol 2024; 17(1): 7153-7160. doi:10.37285/ijpsn.2024.17.1.8.
62. Kumar B, Sharma P, Gupta VP, Khullar M, Singh S and Dogra N: Synthesis and evaluation of pyrimidine bridged combretastatin derivatives. Bioorg Chem 2018; 78: 218-230. doi:10.1016/j.bioorg.2018.02.003.

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