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Original Article

Design and Evaluation of Activated Charcoal-Enriched Antibacterial Soap Using a Design of Experiments (DoE) Approach

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ABSTRACT

Background: The formulation and optimization of an activated charcoal-based antibacterial soap were investigated to enhance cleansing efficacy and antimicrobial properties. The study was conducted at Chalapathi Institute of Pharmaceutical Sciences, Guntur, from 06 April 2024 to 31 January 2025, using a Box-Behnken Design (BBD) to optimize key formulation parameters. **Materials and Methods:** Activated charcoal and coconut oil were used as primary ingredients, with sodium hydroxide, Sodium Lauryl Sulfate (SLS), and stearic acid to improve lathering and hardness. BBD analyzed the impact of activated charcoal, SLS, and stearic acid concentrations on foam retention time and hardness. Statistical methods, including ANOVA and interaction profiling, were used. Physicochemical properties such as moisture content, free alkali, foam height, viscosity, pH, and Total Fatty Matter (TFM) were evaluated. Antimicrobial activity against *Bacillus subtilis* and *Escherichia coli* was determined using Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC). Patch testing confirmed dermatological safety. **Results:** The optimized soap exhibited 12.5% moisture content, 18% alcohol-insoluble matter, 2% free alkali, 13 cm foam height, 2500 cP viscosity, 82% TFM, and pH 6.1. Hardness was 62.78 N. MIC values were 50 µg/mL for *E. coli* and 25 µg/mL for *B. subtilis*, with significant bactericidal activity. No skin irritation was observed. **Conclusion:** The study successfully developed an antibacterial soap with optimized physicochemical properties, promising antimicrobial efficacy, and excellent skin compatibility, making it a potential candidate for personal care applications.

Keywords: Activated charcoal, Antimicrobial activity, Box-Behnken Design, Physicochemical evaluation.

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INTRODUCTION

The incorporation of activated charcoal in skincare formulations offers deep cleansing, detoxifying, and antimicrobial properties due to its highly porous structure, making it ideal for oily and acne-prone skin (Atolani *et al.*, 2016; Rambabu *et al.*, 2020). However, traditional formulations often face challenges such as inconsistent texture, poor lathering, and low hardness, affecting product performance and consumer satisfaction. This study employs the Box-Behnken Design (BBD) to optimize the formulation by systematically investigating the effects of temperature, stearic acid concentration, and SLS concentration on critical quality attributes like foam retention time and hardness (Singh *et al.*, 2011). Foam retention time indicates lather stability, essential for cleansing, while hardness reflects the soap's durability and structural strength (Stallings and Lupo, 2009; Levin *et al.*, 2010). By applying statistical modeling, this research aims to enhance both aesthetic and functional properties of activated charcoal soap, providing a scientific framework for developing high-quality skincare products with improved consistency and performance (Friedman and Wolf, 1996).

MATERIALS AND METHODS

Activated charcoal and Coconut oil was obtained from a retail outlet in Guntur, while sodium hydroxide, sodium lauryl sulfate, and stearic acid were obtained from Thermo Fisher Scientific India Pvt. Ltd., Bombay. Distilled water was procured from the Chalapathi Institute of Pharmaceutical Sciences laboratory. *Bacillus subtilis* (MTCC 441) and *Escherichia coli* (MTCC 1687)

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were obtained from the Microbial Type Culture Collection (MTCC), India.

Screening of Critical Factors for Soap Formulation

A preliminary screening study identified key formulation components and process parameters for DoE studies (Abdullah and Chin, 2010). Stearic acid ranged from 450-620 mg, SLS from 300-350 mg, and temperature from 40-50°C, based on initial trials to ensure optimal soap performance.

Formulation of Activated Charcoal Soap

Activated charcoal soap was prepared using pharmaceutical-grade ingredients following BBD optimization. Stearic acid was dissolved in coconut oil at 70°C. Lye solution was prepared by dissolving NaOH pellets in distilled water and added to the oil with continuous stirring (Afsar and Khanam, 2016). Activated charcoal was dispersed in the lye solution, while SLS was separately dissolved in water and incorporated into the mixture (Sucharita *et al.*, 2020). Essential oil was optionally added. The mixture was poured into molds, hardened for 48 hr, and cured for 14 days for stabilization (Ghanwat *et al.*, 2020; Manjusha *et al.*, 2019).

Optimization of Soap Formulation Using DoE

Experimental Design

A Box-Behnken Design (BBD) with 3 factors at 3 levels was applied using JMP Statistical Software (version 17.0.0) to optimize activated charcoal soap formulation. The design included 15 runs with 3 center points, replicated once, totaling 30 experimental runs. This model assessed the main effects, interactions, and quadratic influences of formulation variables on soap quality (Borhan *et al.*, 2014). The statistical software generated nonlinear quadratic equations to predict and optimize the response outcomes (Das *et al.*, 2023; Shaviklo and Rafipour, 2014). The general form of the equation for the two responses (Y_1: Hardness and Y_2: Foam Retention Time) is:

$$Y=S0+S1X1+S2X2+S3X3+S4X1X2+S5X1X3+S6X2X3+S7X12+S8X22+S9X32$$

Where Y=Response, S_0=Intercept, S_1 to S_9=Regression coefficients, X_1, X_2, X_3=Independent variables (Sodium Lauryl Sulfate, Stearic Acid, and Temperature) denoted by coded values of +1 and -1 for high and low values, respectively (Table 1). X_1^2, X_2^2, X_3^2=Quadratic terms, indicating the curvature of the response surface for each factor. X_1X_2, X_1X_3, X_2X_3=Interaction terms, representing the interaction effects between the factors.

The model equations help in predicting the response behavior and optimizing the formulation conditions.

pH Determination

The pH of each soap bar was measured by dissolving 1 g of the formulation in 100 mL of distilled water. After filtration, the pH of the obtained filtrate was promptly assessed using a calibrated pH meter (Eriksson *et al.*, 1998).

Moisture Content Determination

The gravimetric method was used to determine the moisture content of the soap samples. Accurately weighed 10 g of the formulation was kept in a pre-weighed petri dish and subjected to a hot air chamber at 115°C for 30 min (Muteki *et al.*, 2007). The samples were periodically withdrawn, cooled in a desiccator, and weighed until a constant weight was achieved. The percentage of moisture content was determined using the following equation.

$$\text{Moisture Content (\%)}=\text{Initial weight}-\text{Final weight/Initial weight}\times 100$$

Determination of Alcohol-Insoluble Content

To assess the alcohol-insoluble portion, 5 g of soap was dissolved in 50 mL of warm ethanol, filtered through pre-weighed filter paper, dried at 105°C for 30 min, cooled in a desiccator, and reweighed (Muteki *et al.*, 2007). The alcohol-insoluble content (%) was calculated using the following equation:

$$\text{Alcohol-Insoluble Content (\%)}=\text{Final weight-Initial weight of filter paper/Sample weight Samp}$$

Determination of Percentage Free Alkali

Free alkali content was determined by titration. 5 g of soap was refluxed with 50 mL of neutralized alcohol for 30 min, cooled, and titrated with 0.1 N HCl using phenolphthalein indicator until the pink color disappeared (Bornare *et al.*, 2021). The percentage of free alkali was then calculated using the following formula:

$$\text{Free Alkali (\%)}=V\times N\times 40.00/W\times 100$$

Where V=Volume of 0.1 N HCl used (mL), N=Normality of HCl, W=Weight of the soap sample (g) and 40.00=Molecular weight of NaOH (if sodium-based soap) or 56.10 for KOH (if potassium-based soap).

Determination of Foam Height

The foam height of the soap was measured using the cylinder shake method. Around 1 g of soap was dissolved in 50 mL of distilled water, shaken in a 100 mL cylinder for 2 min, and left undisturbed for 10 min. Foam height was recorded, and the average was calculated from three repetitions (Bornare *et al.*, 2021).

Determination of Foam Retention Time

The foam retention time of the soap was evaluated by analyzing its stability over time. A 1% soap solution (25 mL) was shaken 10 times in a cylinder, and the initial foam height was recorded. Foam height was measured every 5 min until collapse. The average retention time was calculated from repeated trials (Bornare *et al.*, 2021).

Determination of Viscosity

The viscosity of the soap was evaluated using a Brookfield viscometer. A 1% soap solution was prepared and examined with Spindle 63 at different rotational speeds (rpm) under room temperature conditions (Pandey *et al.*, 2014). The viscosity readings were recorded, and the experiment was repeated to ensure accuracy. The average viscosity was reported in centipoise (cP).

Determination of the Hardness of the Soap

The hardness of the soap was measured using a TA.HDplus Texture Analyzer with a 500N load cell and P/2:2 mm probe at 25°C. The probe penetrated 8 mm at 10 mm/s, and the maximum force (N) was recorded. Results were presented as mean±SD from triplicate measurements (Jacob and Chandy, 2019).

Total Fatty Matter (TFM) Determination

TFM was measured by dissolving 10 g of soap in 150 mL distilled water, followed by the addition of 20 mL of 15% sulfuric acid. After heating, 7 g of beeswax was added to solidify the fatty acids. The cooled fatty acid cake was dried and weighed (Muteki *et al.*, 2007). TFM was calculated using the formula:

$$TFM=(A-X)/W\times100$$

where A is the total weight of wax and fatty acids, X is the weight of wax alone, and W is the initial weight of the soap sample (Jacob and Chandy, 2019).

Determination of Skin Irritation

The soap was applied to a small skin area for 10 min and observed for redness, itching, or inflammation. Absence of irritation classified the soap as non-irritant (Ahmed *et al.*, 2021).

Determination of MIC and MBC

The MIC was determined by the broth microdilution method, where bacterial suspensions of *Escherichia coli* and *Bacillus subtilis* were inoculated into serial dilutions of the soap and incubated at 37°C for 24 hr. The lowest concentration without visible growth was noted as the MIC (Sindhu *et al.*, 2019). The MBC was identified by subculturing from wells without growth onto agar plates, with the lowest concentration causing 99.9% bacterial reduction considered the MBC (Kathuria and Singh, 2015).

RESULTS

Optimization of Activated Charcoal Soap Formulation Using Box-Behnken Design

Activated charcoal soap formulation was optimized using BBD by evaluating the effects of SLS concentration (X1), stearic acid concentration (X2), and temperature (X3) on hardness (Y1) and Foam Retention Time (FRT) (Y2). A total of 15 experimental runs were conducted (Table 2). Hardness ranged from 25.3 to 109.87 N, with higher stearic acid and lower temperatures contributing to greater hardness. Foam retention time varied between 2.7 and 3.9 min, indicating that SLS and temperature significantly influenced foam stability (Figure 1). The models exhibited good predictive capability, with R^2 values of 0.83 for hardness and 0.54 for foam retention time. The quadratic polynomial equations for the responses were:

$$Y1=42.51+9.26X1+24.21X2+1.45X3+7.99X1X2+2.96X1X3+3.89X2X3+30.36X12+12.41X22-12.4132$$

$$Y2=3.25+0.11X1+0.04X2+0.03X3-0.03X1X2+0.02X1X3-0.02X2X3-0.02X12+0.15X22-0.15X32$$

Interaction plots (Figure 2) revealed that SLS and stearic acid had a synergistic effect on hardness, while their combined influence on foam retention time was more complex. The 3D surface plots (Figure 3) highlighted that hardness increased with higher stearic acid and lower temperatures, while FRT was predominantly influenced by SLS concentration. The prediction profiler (Figure 4) identified the optimal formulation as 350 mg SLS, 450 mg stearic acid, and 47.18°C temperature, achieving 62.15 N hardness and 3.73 min FRT with a high desirability score of 0.999752. This optimized formulation is expected to balance both hardness and foam retention, making it suitable for further validation.

Physicochemical Characterization of the Formulated Soap

The antibacterial soap was evaluated for quality, stability, and skin compatibility. The pH (6.1) was within the acceptable range, ensuring effective cleansing without irritation. Moisture content

Table 1: Variables and their levels in BBD.

Factors	Levels	
	Low (-)	High (+)
Independent variables		
Sodium Lauryl Sulaphate (mg) X1	300	350
Staeric Acid (mg) X2	450	620
Temperature (°C) X3	40	50
Dependent variables		
Hardness (N) Y1	Maximize	
Foam Retention Time (min) Y2	Maximize	

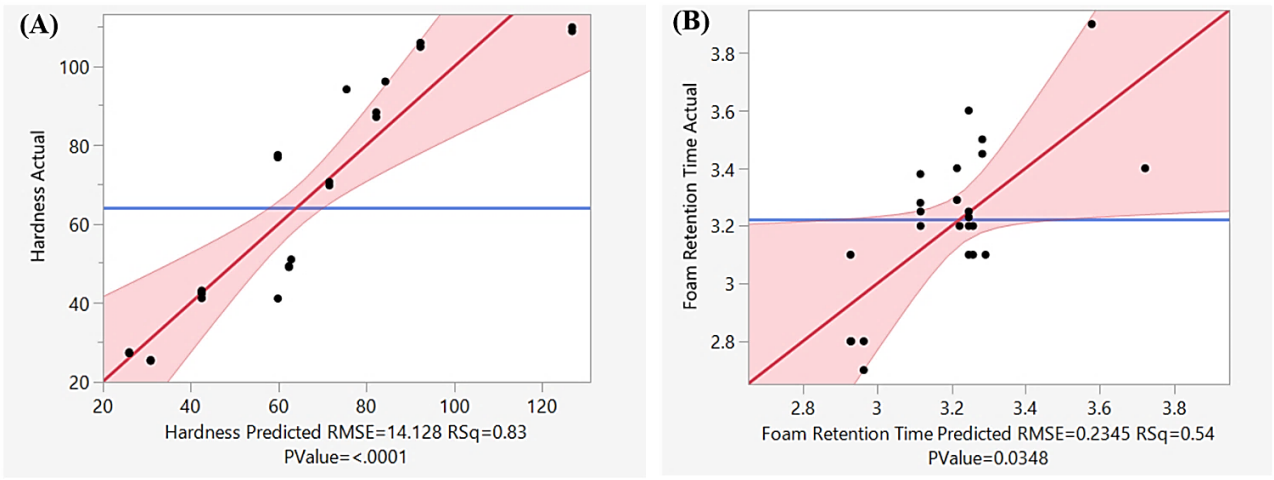

Figure 1: Actual vs Prediction Plots (A) Hardness (B) Foam retention time showing the correlation between experimental and predicted values.

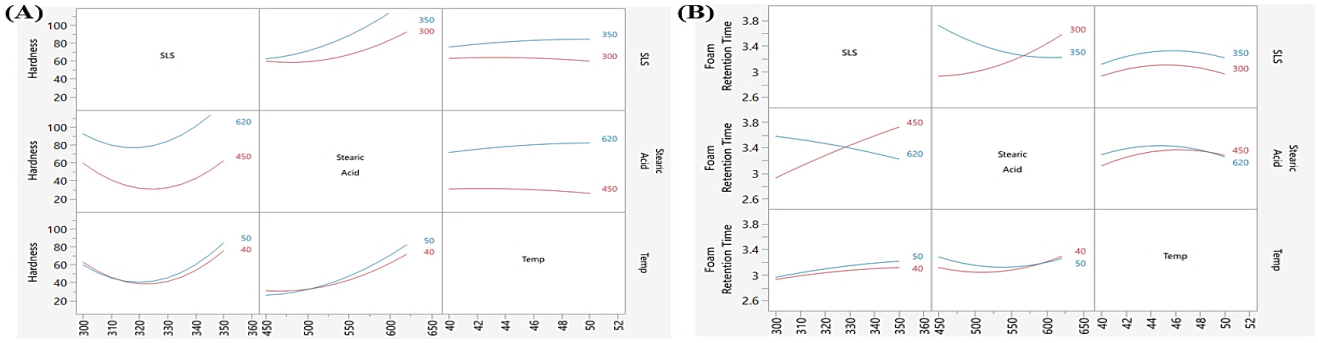

Figure 2: Interaction Profiles (A) Hardness (B) Foam retention illustrating the effects of formulation variables on the response parameters.

Table 2: Experimental outcomes for various formulations designed using the CCD approach.

Runs	SLS (mg) (X_1)	Stearic Acid (mg) (X_2)	Temp (°C) (X_3)	Hardness (N) Y1	FRT (min) Y2	Replicate (Y1)	Replicate (Y2)
1	0	+	-	70.63	3.1	69.8	3.1
2	0	0	0	43.16	3.1	43.1	3.1
3	+	+	0	109.87	3.2	108.91	3.2
4	0	-	-	25.5	3.25	25.3	3.2
5	-	+	0	105.94	3.9	104.91	3.9
6	-	0	+	41.2	2.8	41.1	2.7
7	0	+	+	88.29	3.2	87.13	3.1
8	+	-	0	49.05	3.4	49.3	3.4
9	0	0	0	41.2	3.23	42.41	3.2
10	0	-	+	27.46	3.5	27.23	3.45
11	-	-	0	77.49	2.8	76.87	3.1
12	+	0	+	96.13	3.4	96.12	3.29
13	0	0	0	42.4	3.25	42.8	3.6
14	+	0	-	94.17	3.38	94.12	3.28
15	-	0	-	51.01	2.8	51	2.8

(12.5%) balanced hardness and longevity, while alcohol-insoluble content (18%) contributed to structural integrity. Free alkali content (2%) confirmed the formulation's mildness. Foaming properties showed 3 min 53 sec foam retention and 13 cm foam height, indicating good stability. The soap solution's viscosity (2500 cP) ensured optimal consistency. Mechanical strength (62.78 N) confirmed durability, and the TFM value (82%) indicated high quality. A skin irritation test showed no adverse effects, confirming dermatological safety. Evaluation results are summarized in Table 3.

MIC and MBC Study

The MIC values obtained were 25 µg/mL for *B. subtilis* and 50 µg/mL for *E. coli*, indicating a stronger inhibitory effect on the Gram-positive strain. The MBC values, expressed in CFU/mL, were 1.2×10^3 for *E. coli* and 8.5×10^2 for *B. subtilis*, demonstrating significant bactericidal activity. These results confirm the formulation's effectiveness against both Gram-positive and Gram-negative bacteria. Furthermore, the soap formulation exhibited effective bacterial inhibition at relatively low concentrations, supporting its potential application as an antibacterial cleansing agent. A summary of MIC and MBC reports are presented in Table 4.

DISCUSSION

The optimization of the antibacterial soap formulation using the Box-Behnken Design highlighted the significant effects of SLS, stearic acid, and temperature on foam retention time and hardness. The statistical analysis provided critical insights into the influence of these independent variables on the final product characteristics. The hardness of the soap formulation was primarily influenced by the concentration of stearic acid, as observed from the polynomial equation and interaction profiles. Stearic acid acts as a hardening agent in soap formulations, contributing to the structural integrity and mechanical strength of the soap bars. The significant *p*-value (<0.0001) confirmed that the contribution of stearic acid to hardness was statistically significant (Yetukuri *et al.*, 2024). Additionally, the quadratic effect of stearic acid further reinforced its impact, indicating that both low and high concentrations play a vital role in determining the final hardness (Poyrazoglu and Onal, 2021). SLS, as a surfactant, significantly affected foam retention time, which is a crucial parameter for consumer satisfaction and cleansing efficacy. The interaction between SLS and stearic acid showed a synergistic effect, where higher concentrations of both components resulted in improved foam stability. The *p*-value of 0.0348 for foam retention time indicated that the model could significantly predict the effect of formulation variables on this parameter. The non-linear interaction between temperature and stearic acid highlighted the importance of processing conditions in achieving the desired product characteristics (Kim, Kim and Cho, 2019). The surface plots and interaction profiles provided visual evidence of these complex relationships, aiding in the understanding of the formulation's behavior. The physicochemical characterization of the optimized formulation demonstrated its suitability for antibacterial applications. The pH of 6.1 falls within the acceptable range for antibacterial soaps, ensuring effective cleansing without causing skin irritation. The moisture content of 12.5% contributed to the soap's stability, preventing excessive softness or cracking.

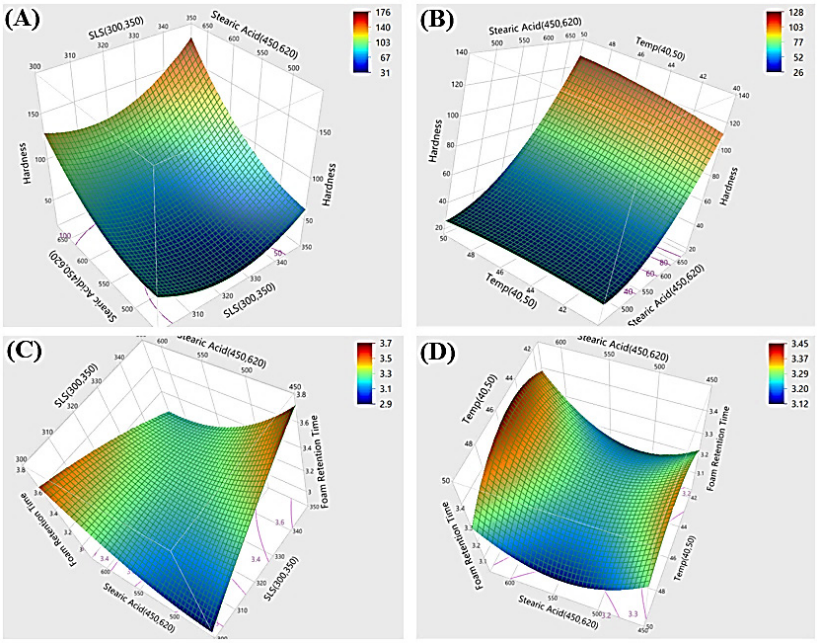

Figure 3: 3D graphs illustrating the effects of independent factors on responses: (A) Effect of SLS and Stearic Acid on Hardness, (B) Effect of Stearic Acid and Temperature on Hardness, (C) Effect of SLS and Stearic Acid on Foam Retention Time, and (D) Effect of Stearic Acid and Temperature on Foam Retention Time.

The alcohol-insoluble content of 18% reflected the presence of essential soap components that enhance structural integrity. The free alkali content of 2% was well within the safe limits, confirming the formulation's mildness and suitability for daily use. The foaming properties of the soap, with a foam height of 13 cm and a retention time of 3 min and 53 sec, were consistent with high-quality commercial soaps. These properties are essential for consumer satisfaction, as they enhance the perception of cleansing efficacy (Lawal, Adesina and Ogunniyi, 2017). The viscosity of 2500 cP provided optimal rheological characteristics, ensuring ease of application and uniform spreading. The hardness value of 62.78 N confirmed the soap's mechanical strength, making it durable and resistant to breakage during use and storage. The TFM of 82% indicated that the formulation contained a high proportion of fatty matter, contributing to its cleansing and emollient properties. The skin irritation test demonstrated the dermatological safety of the formulated soap, with no signs of irritation observed during the study. This finding is particularly important for personal care products, as skin compatibility is a critical factor for consumer acceptance. The absence of irritation further supports the suitability of the formulation for regular use. The antibacterial efficacy of the soap was evaluated through MIC and MBC studies against Gram-positive and Gram-negative bacteria. The MIC values of 25 µg/mL for *B. subtilis* and 50 µg/mL for *E. coli* demonstrated a stronger inhibitory effect on the Gram-positive strain, which is consistent with previous reports on the antimicrobial activity of activated charcoal. The lower MIC for *B. subtilis* may be attributed to the differences in cell wall composition between Gram-positive and Gram-negative bacteria. The MBC values further confirmed the bactericidal potential of the soap, with 8.5×10^2 CFU/mL for *B. subtilis* and 1.2×10^3 CFU/mL for *E. coli*. The antibacterial activity of the formulated soap can

Table 3: Evaluation parameters for the activated charcoal soap formulation.

Evaluation Parameter	Methodology	Results
pH	Measured using a pH meter.	6.1
Moisture Content (%)	Gravimetric method; drying at 115°C for 30 min.	12.50%
Alcohol-Insoluble Content (%)	Soap dissolved in warm ethanol, filtered, and residue dried at 105°C.	18%
Free Alkali (%)	Titration with 0.1N HCl.	2%
Foam Retention Time (min:sec)	Cylinder shake method; foam height measured over time.	3 min 53 sec
Foam Height (cm)	1g soap dissolved in 50mL water; shaken for 2 min, measured after 10 min.	13 cm
Viscosity (cP)	Measured using Brookfield viscometer (Spindle 63).	2500 cP
Hardness (N)	Measured using TA.HDplus Texture Analyzer with a 500N load cell.	62.78 N
Total Fatty Matter (TFM) (%)	Soap dissolved, acidified, and fatty acids weighed.	82%
Skin Irritation	Applied to the skin for 10 min, checked for irritation.	Non-irritant

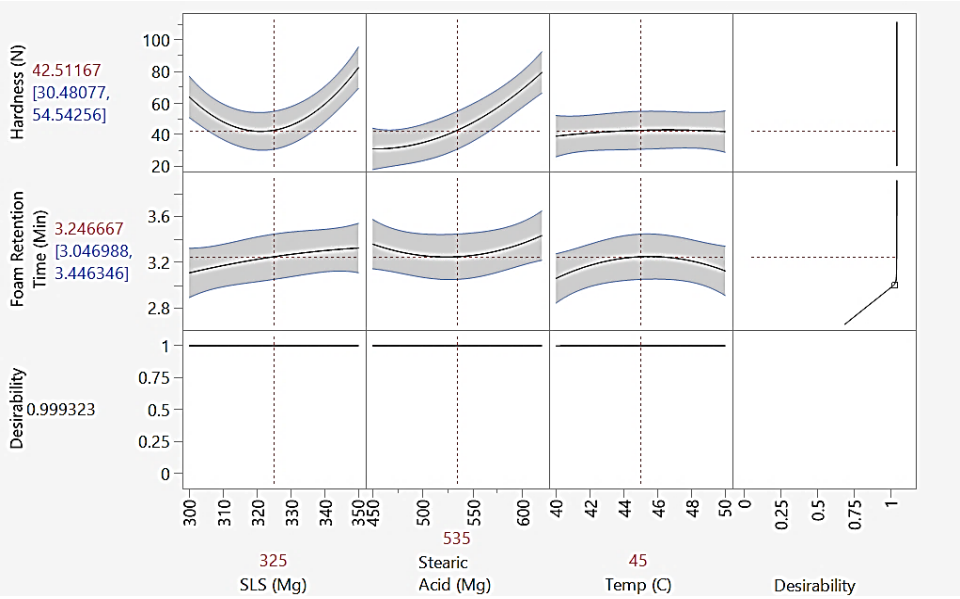

Figure 4: Prediction Profiler of optimized Formulation: Illustrating the relationship between formulation variables and the predicted response outcomes for the optimized formulation.

Table 4: MIC and MBC values of the optimized Formulation against selected microbial strains.

Bacterial Strain	MIC (µg/mL)	MBC (CFU/mL)
Escherichia coli	50	1.2×10^3
Bacillus subtilis	25	8.5×10^2

be attributed to the presence of activated charcoal, which is known for its adsorptive and antimicrobial properties (Fereydoonzadeh and Nahrani, 2022). Activated charcoal enhances the cleansing efficacy of the soap by adsorbing impurities and toxins from the skin surface (Chaudhary and Bano, 2020). Additionally, its porous structure facilitates the entrapment of microbial cells, contributing to the bactericidal effect. The synergistic action of activated charcoal and the soap base components results in a formulation with both cleansing and antibacterial properties. The overall findings of this study highlight the potential of activated charcoal-based soap as an effective antibacterial personal care product. The optimized formulation demonstrated excellent physicochemical properties, antimicrobial efficacy, and dermatological safety, making it a promising candidate for further development and commercialization. Future studies could focus on long-term stability testing, sensory evaluation, and clinical efficacy trials to further validate the product's performance and consumer acceptability. The incorporation of natural essential oils or herbal extracts could also be explored to enhance the soap's antimicrobial activity and aesthetic appeal (Singh *et al.*, 2021). These advancements could contribute to the development of multifunctional personal care products that meet the growing demand for natural and eco-friendly formulations.

CONCLUSION

The present study successfully optimized and characterized an activated charcoal-based antibacterial soap formulation using the Box-Behnken Design. Statistical analysis of formulation variables and their interaction profiles demonstrated significant effects on key physicochemical attributes, ensuring optimal product performance. The optimized formulation exhibited a moisture content of 12.5%, foam height of 13 cm, viscosity of 2500 cP, and total fatty matter of 82%, indicating favorable stability and cleansing properties. Antibacterial evaluation through MIC and MBC studies confirmed significant inhibition against both Gram-positive and Gram-negative strains, with MIC values of 25 µg/mL and 50 µg/mL, respectively, and MBC values of 8.5×10^2 CFU/mL and 1.2×10^3 CFU/mL, respectively. These findings highlight the potential of the developed soap as an effective antibacterial cleansing product, supporting its further investigation for commercial and dermatological applications.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

BBD: Box-Behnken Design; **DoE:** Design of Experiments; **MIC:** Minimum inhibitory concentration; **MBC:** Minimum bactericidal concentration; **SLS:** Sodium lauryl sulfate; **CFU:** Colony Forming Unit; **MTCC:** Microbial Type Culture Collection and Gene Bank; **NaOH:** Sodium hydroxide; **HCl:** Hydrochloric acid; **KOH:** Potassium Hydroxide; **RPM:** Rotation per minute; **cP:** Centipoise; **TFM:** Total Fatty Matter; **FRT:** Foam retention time.

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