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"Be less curious about people and more curious about ideas."

Marie Curie

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Editor's Note

Dear Pharmaceutical Technology Researchers,

We are excited to be with you in this new issue of **JPharmTech**. The purpose of our journal is to inform the all pharmaceutical scientists about all latest and important research on drug development. **JPharmTech** will publish papers that focus on the research, development and evaluation of pharmaceutical delivery systems. The journal includes topics such as preformulation, drug delivery systems and targeting, formulation design, preparation, *in vitro* and *in vivo* characterization, pharmacokinetics, pharmacodynamics, biopharmaceutics and more.

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I want to thank you to the all of my friends who studied for those processes that are mentioned above. By the valuable contribution of our friends in the publishing team, this was a successful start for our journal.

Our main goals are having a strong international editor and referee team, to increase the recognition and index number of the **JPharmTech** and to be one of the most cited journals. That's why, your contribution and support to our journal with your papers and refereeing, are really important and valuable for us.

Thank you in advance & Best regards,

Müzeyyen Demirel

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- summarize previous research, providing the wider context and background and the importance of the current study,
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The Methods should describe clearly how you carried out your study. Provide sufficient details like study design and data collection to allow the work to be reproduced by an independent researcher. Methods that are already published should be summarized, and indicated by a reference.

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A combined Results and Discussion section is often appropriate; but optionally can be written separately. Provide a concise and precise description of the all experimental data, their interpretation as well as the experimental conclusions that can be drawn. Discussion should explore the significance of the results of the work, not repeat them. The P values and/or the specific statistical tests performed for each experiment should be included to the results and discussion section in the appropriate figure legend or main text. Results should include the name of the statistical test, followed by a colon, the test statistic and its value, degrees of freedom or sample size (depending on which is most appropriate for that test), and the P value, with indication if it is one-or two-tailed (unless you address this issue in the methods).

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- principles, relationships, and generalizations inferred from the results (but not a repetition of the results),
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Concept, Design, Supervision, Materials, Data Collection and/or Processing, Analysis and/or Interpretation, Literature Search, Writing, Critical Reviews. Initials of the contributing authors (i.e. AA, BB) should be given after these specified titles and all of them must be filled.

Acknowledgments

Acknowledgments should include sources of support, grants, disclaimers, names of those who contributed but are not authors, etc. The names of funding organizations should be written in full. If no funding or help has been provided for the research, please include the "None" statement.

Conflict of Interest Declaration

All authors must disclose any financial and personal relationships with other people or organizations that could inappropriately influence their work. If no conflict exists, the authors should include the "The authors declare no conflict of interest." statement under this section.

Ethics Committee Approval

When reporting experiments conducted with humans indicate that the procedures were in accordance with ethical standards set forth by the committee that oversees human experimentation. Approval of research protocols by the relevant ethics committee, in accordance with international agreements.

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Journal

[1] Ates M, Kaynak MS, Sahin S. Effect of permeability enhancers on paracellular permeability of acyclovir. *J Pharm Pharmacol*. (2016); 68(6): 781-790. https://doi.org//10.1111/jphp.12551

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Conference Paper

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RESEARCH ARTICLE

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Safety evaluation of different variants of a topically applied toilet bar soap range using skin irritancy testing methods in the Nigerian population

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ABSTRACT

Exposure to soaps can alter the physiological properties of the skin, such as pH, trans-epidermal water loss, and sebum levels, which are all determinants of skin irritability. We aimed to evaluate the safety of different variants of a topically applied toilet soap range using skin irritancy testing methods in the Nigerian population. Five variants of commercially available soap bars were used in this study. Each subject was patch tested using the standard methodology for application of patch test, with five variants of the proposed products. The pH, trans-epidermal water loss (TEWL), and sebum levels of the skin were determined before and after the wash test. Diffuse plaster erythema was observed in 15.4% of participants, with 18.5% complaining of itching on the plaster site. All five variants were negative for allergic contact dermatitis after 48 hours and 96 hours post-patch test. The soap range ensured that post washing pH of skin was maintained at 6.41 ± 0.07 for all participants in the study. Post washing, the sebum concentration on the skin was significantly reduced by 95.01% ± 0.07 ($p = 0.018$). The different variants of the topically applied soap bar were non - irritant, compatible with human skin, and showed no sign of allergic contact dermatitis.

1. INTRODUCTION

Contact dermatitis is an inflammatory skin condition caused by contact with chemicals (exogenous agents) that damage the skin either directly (irritant) or by specific sensitization (allergic). It is difficult clinically to differentiate between irritant contact dermatitis (ICD) and allergic contact dermatitis (ACD) [1]. ICD is a non-specific response of the skin to direct chemical damage, which releases the mediators of inflammation predominantly from the epidermal cells [2]. This may follow a single exposure or multiple exposures to a known irritant [2]. ACD is a type IV (delayed) hypersensitivity reaction affecting previously sensitized individuals, and it involves immunologic processes [1]. Three main pathophysiological changes include skin barrier disruption, epidermal cellular changes, and cytokine release [3]. Soaps and cleansers are common causes of skin irritation. Cleansers are composed of alkaline soaps or the less barrier-damaging synthetic detergents, known as syndets (synthetic detergents) [4,5]. Skin surfaces normally have acidic pH. Alkalis in many soaps and shampoos can cause skin irritation. Hence, the use of soaps with approximately pH 5.5 may prevent skin irritation [6]. Fragrances can also be potential irritants and sensitizers in cleansers/soaps. Fragrance mix was the most common sensitizing agent causing cosmetic allergy in a trial done at the dermatology outpatient clinic of the Lagos University Teaching Hospital, Nigeria [4-7].

Clinical features of irritant contact dermatitis include itching, pain, burning, stinging, and skin discomfort. Findings on examination of the skin include erythema (redness), mild oedema (swelling), and scaling. Chronic features include lichenification, scales, fissures, and ulceration [1]. Allergic reactions are the result of inflammation of parts of the body [8-10]. Although soap allergies rarely cause any serious medical problems, an allergic reaction to soap can cause severe discomfort which is preventable by simply stopping use of culprit soap and use of anti-inflammatory agents [10-13]. Allergic reactions may also be caused by sodium lauryl sulfate - an ingredient in soap that strips your skin of its natural oils [10]. Repeated itching can result in additional inflammation and irritation, increasing the intensity of the itch, leading to a condition

called lichen simplex chronicus or neurodermatitis. This presents initially with erythema (redness), excoriations, darkening of the affected portion, and consequently leathery (thickening) of the skin from prolonged itching [11,13-14].

Soap allergies can generally be diagnosed by the appearance of inflamed skin and by the history of recent changes in soaps or detergents used [15-18]. Confirmation of the diagnosis can be achieved using "patch" testing, in which patches containing chemicals suspected of causing the allergy are applied to the skin [16-18]. The patches are removed 48 hours later to see if an allergic reaction has developed; an additional examination 48 hours after patch removal can be performed to look for any delayed reactions [16]. The simplest way to treat an allergic reaction to a soap is to discontinue any newly introduced soaps or detergents and to revert to brands, which have not caused an allergic reaction. Antihistamines can be taken orally to relieve the symptoms [14]. Ointments that contain anti-inflammatory corticosteroids can relieve itching and inflammation. Calamine lotion, cold compresses, and milk/oatmeal baths may also relieve the itching [19].

There has been little published on the incidence of irritant and allergic reactions to chemicals in bathing soaps as topical pharmaceutical over the counter products in Nigeria. Nonetheless, consumers and physicians continue to ascribe contact reactions to some soaps. The purpose of this study is to test the hypothesis that bathing soaps may cause skin irritation and allergic reactions. This study will also provide information on the effect of the soaps on skin pH, texture, sebum concentration, and water vapor transmission on the Nigerian skin.

2. MATERIALS AND METHODS

2.1. Materials

Five variants of commercially available soap bars (Code P toilet bar range), and an irritant reference, sodium lauryl sulfate (SLS) USP (Sigma Aldrich Merck KGaA, Darmstadt, Germany) were used in this study. The pH of 2% aqueous solutions in demineralized water for the soap chamber test was 7.9 for solid bar soap and of 10.5 for irritant reference sodium lauryl sulfate USP. The pH of demineralized tap water was neutral at 7.1

2.2. Methods

Individuals with healthy skin were invited to participate in the study. It was conducted for one week, from 8th October 2019 to 15th October 2019 at the Department of Pharmaceutics, Faculty of Pharmacy, College of Medicine campus, University of Lagos. The study area was Idi-araba area, Surulere Local government area Latitude: 6.5203° or 6° 31' 13" north and Longitude: 3.3538° or 3° 21' 13.8" east located in Nigeria, West Africa [20]. It is a cosmopolitan densely populated area of the commercial capital of Nigeria, i.e., Lagos State.

2.2.1. Study design

A prospective study aimed at assessing the presence or absence of skin irritation and allergy on the application of different variants of bathing soap on a pre-determined adult population. Ethical approval was obtained from the Human Research and Ethics Committee of Lagos University Teaching Hospital, Idi-araba Lagos, with Health Research Committee assigned No. ADM/DCST/HREC/APP/3148. Informed written consent was obtained from all subjects.

2.2.2. Inclusion criteria

All subjects must be at least 18 years old and must give a verbal and written informed consent.

2.2.3. Exclusion criteria

Non-consenting individuals, individuals younger than 18 years old, Pregnant subjects, individuals showing the presence of inflammatory skin conditions. Also excluded from the study were individuals with generalized pruritus from any cause; the skin of the back should not have been treated with a topical corticosteroid, and oral corticosteroids and cytotoxic drugs should not have been used one week before the test and for the duration of the study. Individuals showing the presence of sensory polyneuropathy (manifesting as biting, stinging, smarting, peppery sensations, anesthesia, hyperesthesia, hyperalgesia, etc.) and individuals with previous flares and history of atopic dermatitis were also excluded from the study.

2.2.4. Sample size calculation

Post-Hoc Power Analysis with Dichotomous Endpoint was utilized in obtaining the sample size. With a baseline incidence of irritancy at 25% [8,17], the probability of a type -I error, i.e., finding a difference when a difference does not exist, utilized an alpha cut-off of 5% (0.05) was evaluated alongside the probability of a type-II error, i.e., not detecting a difference when one actually exists. The Beta value related to study power (Power = 1 - β) was determined as a beta cut-off of 2% (0.02) this gave a sample size calculated as 49. The sample size utilized was 65 to allow for the subject decline in the middle of the research. Sixty-five healthy volunteers (males and females) between the ages of 19 and 53 years with the mean age of 32.98 years were invited to participate in the study.

2.2.5. Data collection

Prior written and verbal consent was taken from them after explaining the study to them. The volunteers filled a pre -study survey that contained their demographics, skin types, and likely background problems with the skin and bath related allergy. The tests were done in three categories: cosmetic irritancy test (patch test), repeat open application test, and wash off test. Each subject was assigned a number code which will be used to document result. The participants rested for at least 30 min at 28°C ± 1.5°C, at a relative humidity of 45% ± 2% RH, before the examination.

2.2.6. Patch test procedure (irritancy and allergy test)

Each subject was patch tested using a standard methodology for application of patch test, with five brands (A to E) of the proposed products, positive control, sodium lauryl sulfate, and negative control, demineralized water. The products were applied to the upper back in the Finn chambers secured with Scanpor tape. The readings were taken after 48 hours and seven days (delayed reaction). Each examination lasted for forty-five minutes to one hour after removal of the

patches. Results were graded according to the International Contact Dermatitis Research Group (ICDRG) standard. Dermoscopy was done to objectively document the erythema or skin changes after the removal of patches [15-18]. Positive reactions are those with at least an infiltrated erythema (one plus reaction).

2.2.7. Mild leave on cosmetic finished goods (open application test)

Products were applied behind the ears or beside the neck twice daily for 72 hours to one week; the site was examined for inflammation/redness on the last day. Dermoscopy was also done to objectively document erythema or skin changes.

2.2.8. Wash off test

Products were applied to one arm after wetting the arm and left on for 3 minutes, after which the hand was rinsed generously with water. The multiprobe adapter Cutometer® (Dual MPA 580 Courage+Khazaka electronic GmbH Mathias-Brüggen-Str. 91 50829 Köln, Germany) was used to measure the trans-epidermal water loss (tewameter), skin pH (pH meter) and sebum level in the skin (sebumeter) pre- and post-wash. Pre-wash and post-wash photography were taken during the patch test and open application test.

2.2.9. Statistical analysis

The data were presented as mean ± standard deviation of more than three experimental values for individual variables and analyzed by one-way ANOVA and Tukey's post hoc test. p-value ≤ 0.05 was considered statistically significant.

3. RESULTS AND DISCUSSION

The baseline skin evaluation for all participants was done before the commencement of the study, as shown in **Table 1**.

Table 1. *Evaluation of the baseline skin condition of the study subjects a representation of the Nigerian skin before the start of the study (n=65; *p<0.05)*

	Study Subjects	p-Value
Skin conditions (pre-wash)		
Dry skin	24.61%	
Very oily skin	20.00%	-
Normal	55.39%	
Problem with usual toilet soap		
No	96.92%	-
Yes	3.08%	
History of bath related itching of the skin		
No	81.54%	-
Yes	18.46%	
Average skin pH		
Pre-wash	5.71 ± 0.11	*0.041
Post-wash	6.33 ± 0.10	
Average trans-epidermal water loss		
Pre-wash	13.92 g/Hm^2 ± 0.21	0.052
Post-wash	12.18 g/Hm^2 ± 0.14	
Sebum skin content		
Pre-wash	9.00 µg/cm^2 ± 0.03	*0.018
Post-wash	1.27 µg/cm^2 ± 0.07	

About 96.92% of the participants had no problem with the current bathing soap they were using; a history of bath related itching of the skin in 18.5% of participants was noted. The general baseline condition of the population under study showed that 20% exhibited very oily skin, 24.61% had dry skin, and over half of the population had skin that was neither dry nor oily. There was a statistically significant difference pre- and post-wash for both the skin pH and skin sebum content p=0.041 and p=0.018, respectively. The results were reported as ±SD.

3.1. Patch Test Procedure (Irritancy and Allergy Test)

Diffuse plaster erythema was observed in 15.4% of participants, and 18.5% complained of itching on the plaster site. Dermoscopy readings on these sites were used to differentiate between plaster reactions and the presence of erythema, and they were carried by trained board-certified Dermatologists. All participants had negative patch test responses (allergic contact dermatitis) for the five variants of the soap tested at 48 hour and 96-hour reading. Irritant erythema was observed in all variants and reported in **Table 2**.

Table 2. *Evaluation of the skin reaction of the subjects after patch test and open application test (n=65)*

Soap Variants	% of study Subjects exhibiting skin irritancy		
	Irritant erythema after 48 h (After patch test)	Irritant erythema after 96 h (After patch test)	Allergic contact dermatitis (After open application test)
A	18.4	6.2	0
B	20.0	4.6	0
C	16.9	4.6	0
D	15.4	4.6	0
E	20.0	9.2	0
Tap water	1.53	0	0
1% SLS	0	0	0

3.2. Mild Leave on Cosmetic Finished Goods (Open Application Test)

The products were applied behind the ears or beside the neck twice daily for 72 hours to one week. On examination of the areas using dermoscopy at 48 hours and 72 hours, there was no evidence of allergic contact dermatitis in all soap variants.

3.3. Wash Off Test

The Patch test for study participant No. 56 at Day 1- and 96-hours post-test showed no reaction for erythema and contact dermatitis (**Figure 1A-D**) with the dermoscope picture being clear of xerotic changes pre- and post-wash. At 96 hours post-test wash slight erythema was observed in some participants (**Figure 1E**).

The wash-off tests had its most pronounced results when the variation of sebum content was measured. Sebum concentration on the skin was significantly reduced by 91.54% ± 0.2 (p=0.018) (**Figure 2C**). Trans-epidermal water loss was reduced in all participants post-wash (**Figure 2B**) with the skin remaining well hydrated after washing with the

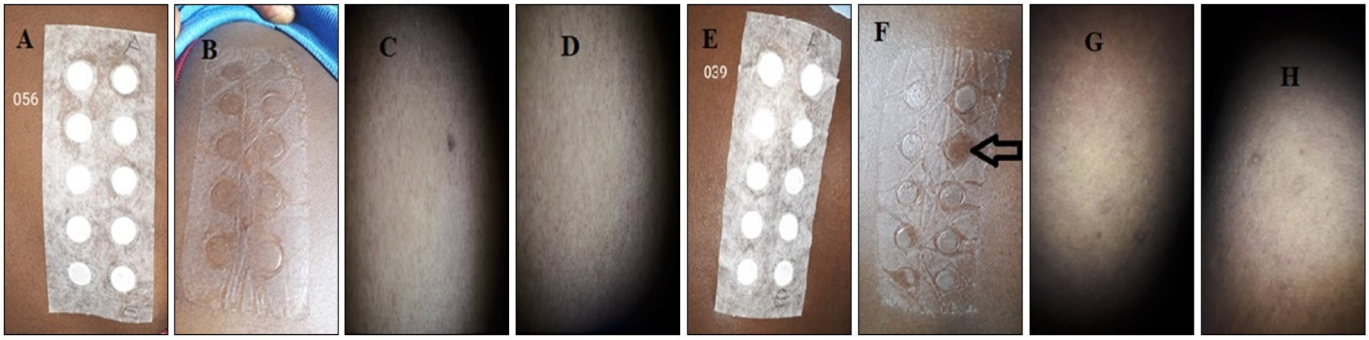

Figure 1. *(A) Patch test for study participant No. 56 at Day 1 and (B) 96 hours post-test (C) Pre-wash dermoscope picture and (D) Post-wash dermoscope picture for participant No. 56; (E) Patch test for study participant No. 39 at Day 1 and (F) 96 Hours post-test with arrow showing slight erythema (G) Pre-wash dermoscope picture and (H) Post-wash dermoscope picture for participant No. 39*

soap. In more than 67% of the study participants, the sebum was completely stripped from the skin hence leaving the skin without any of the natural oily skin moisturizers produced by sebaceous glands.

Skin irritancy is a measure of the suitability of a topically applied soap on the skin of an individual. The occurrence of skin reactions following the use of cosmetics is the primary cause of the lack of use of specific brands. Skin reactions ranging from itching, redness, provocation, or exacerbation of atopic dermatitis and xerosis are commonly observed when certain cosmetics are topically applied on sensitive skin. Most of the respondents in this study exhibited normal skin type with an average pH of 5.71; this value increased post-wash with all five variants of the soap used. The elevated skin surface pH is as a result of the decrease in epidermal expression of Na/H^+ exchange 1, which regulates skin surface pH, this is altered after washing with soap to ensure that the skin maintains a pH, which is close to neutral [21,22].

Sex hormones such as testosterone (in males and females), etiocholanolone in females, and dehydroepi-androsterone in males influence sebum production. These levels occur between the ages of 18-50 years with peak levels at 24 years of age and a steady decline after 60 years [23]. Skin sebum content was 9.0 µg/cm^2 ± 0.03 (pre-wash) without a significant difference between the male and female participants. About 20.0% of the participants had oily skin with sebum content in the range of 22-49 µg/cm^2; these participants were in the age range of 25 to 30 years old, these results are in consonance with previous studies of Caucasian skin types [23]. Post wash with all the variants of the soap saw an average of 91.54 ± 0.27% reduction of sebum on the skin of the respondents, with over 67.8% of the respondents showing a 100% clearance of sebum from the surface of the skin (**Figure 2C**). The soaps had a skin oil stripping effect, and the total elimination of sebum leads to dryness of the skin surface. The inclusion of moisturizers in the soaps would ensure the maintenance of a minimal amount of sebum on the skin after washing. *Xerosis cutis* is the medical term for abnormally dry skin. Dry skin is common, especially in older adults. It is usually a minor and temporary problem, but it may cause discomfort. The skin needs moisture to stay smooth. Xerotic changes were seen on the skin of 21.5% of all participants, washing with all variants of the soaps enhanced these changes, due to stripping of the skin of its sebum content. The soap had a drying effect on the skin, which may not be compatible with optimal skin health. Dermoscopy showed diffuse erythema, perifollicular cast/ pigmentation, as well as background erythema in a total of six respondents; post wash results, however, showed increasing erythema in only two participants.

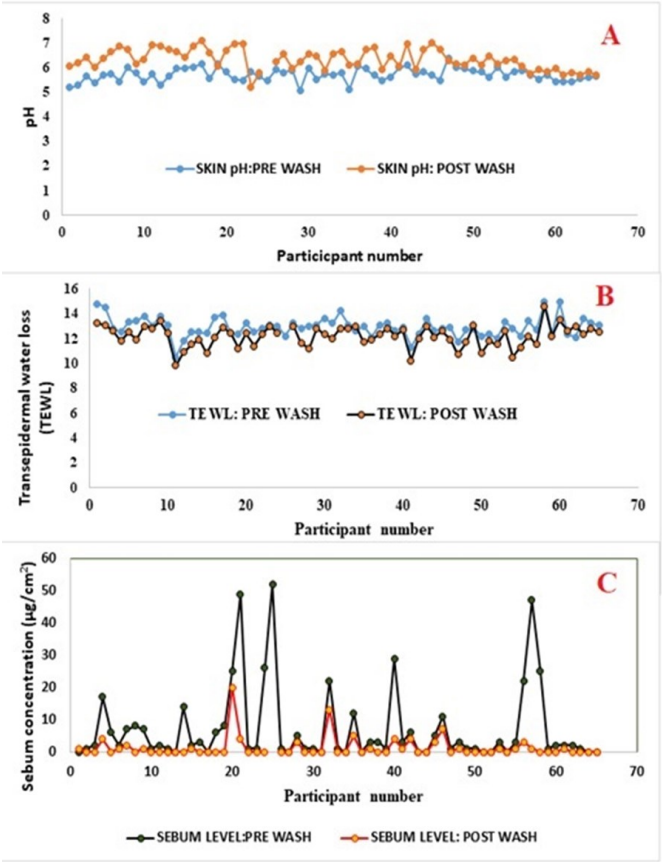

Figure 2. *Pre-wash and Post-wash variation of (A) skin pH (B) trans-epidermal water loss (C) sebum concentration in a cross-section of the Nigerian population (n=65)*

Skin dryness in aged Caucasians and African Americans have been seen to be higher than that in the Chinese population [24,25]. A previous study showed no difference in TEWL between adult males and females at around 40 years of age [25]. This present study showed that the participants exhibited an average TEWL of 13.92 ± 0.21 g/ Hm^2, which is in consonance with previous literature in comparison with Caucasians, Chinese, and African

Americans [25]. There was a slight decrease in TEWL post-wash with soap (**Figure 2B**). Several factors are contributing to the reduced TEWL, and these include a reduction in natural moisturizers in SC, i.e., sebum, which aids regulation of TEWL, utilization of medication like antiretroviral as well as ingestion of herbal products [26]. Trans-epidermal water loss is critical in human cutaneous functions such as regulating epidermal proliferation, differentiation, or inflammation; hence utilization of cleansing agents should be able to moderate TEWL (**Figure 2B**). Post-wash TEWL is reduced (**Figure 2B**), but not so much as to be able to hinder skin functions such as epidermal proliferation, thereby preventing inflammation. For healthy skin, average TEWL should be at least 11.5 ± 0.14 g/Hm^2 [25,27], and the average TEWL from the study participants pre- and post-wash met this criterion. This direct link between sebum concentration and TEWL is critical in designing soap formulations that will ensure a protective sebum layer on the skin post-wash to enable the skin to perform its functions efficiently.

4. CONCLUSION

The different variants of the topically applied soap bar were found to be non – irritant and compatible with human skin, and they did not cause allergic contact dermatitis. The soap variants can be safely used on the human body for cleansing purposes where washing with fluids such as water is necessary to avoid retention of the soap on the skin surface, hence reducing the likelihood of irritant erythema. The soap range ensured that the post-wash pH of the skin was maintained at 6.41 ± 0.07 for all participants, and the sebum concentration on the skin was significantly reduced by 95.01% ± 0.07 (p=0.018). Sebum skin-stripping can be reduced by increasing the moisturizer content of the soap formulation, hence protecting the skin from xerosis. This research also gives insight into the characteristics of the African skin with respect to pH, sebum content, and TEWL and how all these parameters are affected by washing with bathing soaps.

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CONFLICT OF INTEREST DECLARATION

The authors report no conflict of interest. The authors alone are responsible for the content and the writing of the paper.

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Development and optimization of antioxidant polyherbal cream using artificial neural network aided response surface methodology

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ABSTRACT

Artificial Neural Networks have become phenomenal in drug modeling, predicting experimental outcomes, and optimization studies during product development. This research investigates artificial neural networks (ANN) to optimize a novel formulation of Polyherbal face cream composed of *Cymbopogon citratus*, *Hibiscus sabdariffa*, and *Ocimum gratissimum* extracts. The central composite design was used to develop a framework for the products to be studied, describing the independent variables as the oil phase concentration and the emulsifying agents used in the formulation. The dependent variables/responses are viscosity, spreadability index, and particle size of the cream. The obtained responses were then optimized using ANN models. The validity of the statistical models used for predicting the observed responses was confirmed by carrying out three experimental confirmation runs at the identified optimum conditions. The viscosity of the cream formulation decreased with an increase in the amount of oil phase. However, there was a strong correlation between the amount of emulsifying phase in the emulsifier product and the viscosity or the cream. The particle sizes did not vary greatly between the various formulations regardless of the concentration of plant extract. Formulation of Polyherbal face cream composed of *Cymbopogon citratus*, *Hibiscus sabdariffa*, and *Ocimum gratissimum* extracts was shown to have significant antioxidant activity. A prediction of an optimized formulation was made using ANN modeling, and it was shown to have comparable results with the predicted values.

1. INTRODUCTION

The use of artificial neural network models to derive response surface plot has found increasing use in optimization modeling. They are of great use for data sets that do not possess linear relationships. Ilomuanya *et al*. used a central composite design to optimize formulations of hydrogels that had wound healing properties due to infused extracts of *Tetracarpidium conophorum* [1]. Plant extracts have been known to contain antioxidants, which are beneficial to skin health. Antioxidants are among the most significant substances to help protect and often correct skin damage from free radicals. Free radicals are the very elements that age, destroy, and damage our skin. These harmful elements attack our skin, causing wrinkles, age spots, and deterioration of collagen. Edeoga et al. showed that they contain tannins, flavonoids, with traces of other terpenoid compounds on the evaluation of the aqueous extract of the leaves of Ocimum gratissimum [2]. Akinmoladun *et al.* [3] further confirmed the phytochemical constituent and antioxidant activity of extract from the leaves of *Ocimum gratissimum*. Chiu *et al.* demonstrated that the aqueous extract of basil has antioxidant activities which beneficial effects of *Ocimum gratissimum* aqueous extract on rats with (Carbon tetrachloride) CCl_4-induced acute liver injury [4]. The antioxidant and cytoprotective activity of *Ocimum gratissimum* extracts against hydrogen peroxide-induced toxicity in human $HepG_2$ cells of the liver has also been established [4]. The inhibitory effect of aqueous extracts of two varieties (red and white) of *Hibiscus sabdariffa* (Roselle) calyces on carbohydrate hydrolyzing enzymes (α-amylase and α-glucosidase) have been evaluated to determine a possible mechanism for their anti-diabetes properties. The antidiabetic property of Roselle was a direct result of its antioxidant properties [5]. *Cymbopogon citratus* (lemongrass) is a potent antimicrobial and antioxidant, natural bioproduct widely used in food preservation as an alternative to synthetic compounds [6]. Oxygenated monoterpenoids are the major constituents of *Cymbopogon citratus*. The antimicrobial activity of the essential oil of *Cymbopogon citratus* against different Gram-positive and Gram-negative pathogenic bacteria, yeasts, and filamentous fungi has been evaluated by Carmo *et al.* [6]

Commercially available topical product formulations with medicinal properties that contain natural products in various

proportions are not optimized. There is the variability of treatment outcomes comparing brands composed of similar herbal extracts and composition. This study targets the development of a novel and optimized formulation of face creams containing extracts from Roselle (*Hibiscus sabdariffa*), Lemon grass (*Cymbopogon citratus*), and Basil (*Ocimum gratissimum*) leaf extracts utilizing artificial neural networks.

2. MATERIALS AND METHODS

2.1. Materials

Benzyl alcohol (Long-Range Europe Ltd., Bedfordshire, UK), Rose Oil (Shubham Natural Products Fragrance and Exports, Uttar Pradesh, India), Cetostearyl alcohol (Ampluschem Co. Ltd., Bangkok, Thailand), Cetyl Palmitate (Strahl and Pitsch LLC, NY, USA), and Liquid Paraffin (Sigma Aldrich, St Louis Missouri, USA), Refined Palm Olein (Raffles oil LFTZ Enterprise Lagos), Tween 80 and Span 60 (obtained from Sigma Aldrich, St Louis Missouri, USA) was used for this research. All other chemicals and reagents used were of analytical grade.

2.2. Methods

2.2.1. Preparation of the extract

Leaves of Lemongrass (*Cymbopogon citratus*) were freshly harvested from Odogbolu Local Government Area; latitude: 6° 49' 59.99" N, longitude: 3° 45' 59.99" E (Ogun State, Nigeria). Sweet Basil (*Ocimum gratissimum*) was freshly harvested late in the evening from a farm in Ojo Local Govt. Area; latitude: 6.4581° N, 3.2020° E (Lagos State, Nigeria). The dried calyces of Red Roselle (*Hibiscus sabdariffa*) were purchased from the local Market at Ikorodu (Lagos State, Nigeria). These herbs were identified at the Department of Botany, University of Lagos, Lagos, Nigeria. Voucher specimens assigned reference numbers LUH 7954, LUH 7955, and LUH 7956, respectively, were deposited in the institutional herbarium of the University of Lagos for reference.

One kg each of leaves of Lemongrass (*Cymbopogon citratus*); leaves of Sweet Basil (*Ocimum gratissimum*) and dried calyces of Red Roselle (*Hibiscus sabdariffa*) collected and washed in running water. The plant materials were sun-dried for seven days before milling into powder with a clean Pellet-type Hammer mill (Model MKHM500 C Opa-Locka, FL 33054). Then 500 g of the dried powder from each plant was macerated in 1000 mL of distilled water for 24 h at room temperature to obtain the aqueous extracts and then was lyophilized to obtain a dry solid residue using the method of Ademiluyi and Oboh [5]. The freeze-dried extract was then stored in amber glass bottles in a desiccator at 4°C.

2.2.2. Formulation of polyherbal creams

The oil phase of liquid paraffin or palm oil (X_1), Tween 80/Span 60 combinations (X_2), Cetostearyl alcohol, and cetyl palmitate were melted together in a water bath (**Table 1**), heated to 75°C. The aqueous phase was prepared by dissolving 0.25% w/w of the dried plant extracts in about 20% of the required volume of deionized water for the cream formulation at about 40°C. Benzyl alcohol 1.5% w/w was then added to the aqueous mixture. The resulting mixture was then placed on a water bath, and more deionized water was added to it, up 50% of the final volume. Both phases were mixed at 70°C and homogenized using a portable hand blender, Kenwood Triblade System Immersion Blender HB711M 700W (Kenwood Corp.Hachioji, Tokyo) at 85 rpm for 15 minutes. This process was replicated for different cream batches after varying the concentrations of Tween 80/ Span 60 (X_2) and the Oil Phase (X_1) see **Table 2**.

Table 1. *Coded and actual values for oil and emulsifying phases of cream formulations*

Independent Variables (%)	Sym.	Coded and Actual Levels (% w/w)				
		-1.414	-1	0	1	1.414
Oil Phase						
Liquid paraffin Palm oil	X_1	25	26.5	30	33.5	35
Emulsifier Phase						
Tween 80 Span 60	X_2	7	7.6	9	10.4	11

The ratio of emulsifier amounts to be used for the cream formulation to produce a stable cream was determined after having considered the HLB values of the oil phase components. The Tween 80:Span 60 ratio was calculated as: 0.5923:0.4077 (for Liquid Paraffin Based Cream); and 0.4534:0.5466 (for the Palm Oil Based Cream). Rose oil

Table 2. *Physicochemical characteristics of the formulations in response to variation in the oil phase and emulsifying phase (all values are reported as ± SD, n=3)*

Run	Factor 1 Oil Phase (% w/v)	Factor 2 Emulsifier Phase (%)	Response 1 Viscosity (centipoise)	Response 2 Spreadability (average % change in diameter)	Response 3 Particle Size (µm)	pH	pH (after 28 days)	Sensitivity (Draize test)
S1	26.5	7.6	5668.1	44.19	0.120	5.06 ± 0.03	5.05 ± 0.02	NR
S2	33.5	7.6	4362.2	28.78	0.114	4.76 ± 0.08	4.77 ± 0.09	NR
S3	25.0	9.0	5368.0	27.09	0.113	5.00 ± 0.01	5.00 ± 0.01	NR
S4	30.0	9.0	6480.0	40.30	0.140	4.66 ± 0.13	4.65 ± 0.16	NR
S5	30.0	9.0	8020.0	32.77	0.150	5.74 ± 0.02	5.75 ± 0.01	NR
S6	33.5	10.4	6002.0	33.57	0.104	4.65 ± 0.16	4.62 ± 0.19	NR
S7	30.0	9.0	8290.2	40.73	0.166	5.48 ± 0.01	5.49 ± 0.05	NR
S8	35.0	9.0	7924.1	49.83	0.085	4.60 ± 0.25	4.60 ± 0.18	NR
S9	30.0	7.0	5936.5	34.15	0.171	4.91 ± 0.01	4.91 ± 0.21	NR
S10	26.5	10.4	9014.3	34.03	0.144	5.01 ± 0.02	5.00 ± 0.01	NR
S11	30.0	11.0	8068.0	38.09	0.106	4.70 ± 0.10	4.69 ± 0.11	NR

(0.1%v/v) utilized as the perfume in the formulation was added into the cream, and the resulting formulation was thoroughly mixed to obtain a homogenous mix.

2.2.3. Experiment design

A two-factor Central Composite Design (CCD) was used to develop the experimental design to study the response pattern and determine the optimum combination of variables to maximize the chosen responses. The independent variables investigated were the oil phase and emulsifying phase concentrations. The responses or dependent variables investigated were viscosity, spreadability, and particle size. The experimental design was developed using the data in **Table 1** with Design Expert® software version 7.0.0 (Stat-ease, Inc. Minneapolis, USA). The values of the independent variables were calculated using **Equation 1** [5].

$$x_i = \frac{X_i - X_0}{\Delta X_i} \quad (1)$$

Where x_i and X_i are the coded and actual values of the independent variable, respectively. X_o is the actual value of the independent variable at the center point, and ΔX_i is the step-change in X_i.

2.2.4. Artificial neural network modeling

Multilayer Full Feed Forward (MFFF) and a Multilayer Normal Feed Forward (MNFF) were evaluated to determine which was more suitable. For the training algorithm, several options were considered, and they include Incremental Back Propagation (IBP), Batch Back Propagation (BBP), Quick Propagation (QP), Generic Algorithm (GA), and Levenberg-Marquadt (LM) Algorithm. For each of these learning algorithms, 70% of the experimental data was used for training the network, 15% was used for validating the model, and the remaining 15% was then used for testing the network. Validation of the data predicted by the ANN model was done by using the trained network to generate output responses for the last 15% of input data without knowledge of their actual responses, and the generated responses were then compared with their actual responses [7].

To evaluate the predictive capability of the ANN models, and to determine the efficiency of the modeling tool, the predicted responses of each model were compared with experimental responses. The predictive capability of each model was assessed using the coefficient of determination (R^2 value), root mean square error (RMSE), absolute average deviation (AAD), and mean absolute deviation (MAD) as shown in **Equations 2** to **5** [7,8].

$$R^2 = 1 - \sum_{i=1}^{n} \left[\frac{(y_{exp} - y_{pred})^2}{(y_{exp} - y_{exp,ave})^2} \right] \quad (2)$$

$$RMSE = \left[\frac{1}{n} \sum_{i=1}^{n} (y_{pred} - y_{exp})^2 \right]^{1/2} \quad (3)$$

$$AAD(\%) = \left[\frac{1}{n} \sum_{i=1}^{n} \left(\frac{y_{exp} - y_{pred}}{y_{exp}} \right) \right] \times 100 \quad (4)$$

$$MAD = \left[\frac{1}{n} \sum_{i=1}^{n} (y_{pred} - y_{exp})^2 \right]^{1/2} \quad (5)$$

where,
n is the number of points
y_{pred} is the predicted value obtained from the model
y_{exp} is the actual value
$y_{exp,ave}$ is the average of the actual values.

The coefficient of determination (R^2) indicates the degree of fit for the model [8]. The closer the R^2 value is to 1, the better the model fits the actual data. R^2 is a measure of the reduction in the response variability by using the repressor variables in the model, while RMSE and AAD are direct methods for describing deviations. The RMSE, AAD, and MAD between predicted and experimental values must be as small as possible [8].

2.2.5. Physical evaluation/organoleptic characteristics

All prepared formulations were observed for homogeneity and phase separation by visual appearance and touch. The appearance of the cream was judged by its color, opalescence, roughness, and other organoleptic properties were observed [9,10]. The Draize test was used to evaluate skin sensibility. (Ethical approval Protocol Number CMUL/HREC/10/18/454). The creams were evaluated for their creaming index using **Equation 6**.

$$\% CI = \frac{CC}{CT} \times 100 \quad (6)$$

where CC is the total height of the cream layer (showing phase separation), and CT is the total height of the emulsion layer (without phase separation) [9]. The creams were evaluated for spreadability, pH, particle size, and viscosity [9,10]. Utilizing the method of Fahimi *et al.*, the creams were tested on Day 1 and 30 days after preparation for microbial growth in tryptone soy agar plates [9].

2.2.6. Evaluation of the antioxidant activity

The free radical scavenging activity of all the extracts was evaluated by 1,1-diphenyl-2-picryl-hydrazyl (DPPH) according to the previously reported method by Shen *et al.* [11]. The capability of scavenging the DPPH (% inhibition) radical was calculated by using **Equation 7**.

$$\text{DPPH scavenging effect} = \frac{(A_0 - A_1)}{A_0} \times 100 \quad (7)$$

where, A_0 is the absorbance of the control reaction (Methanol), and A_1 is the absorbance in the presence of all the formulations or for that of the reference. All the tests were performed in triplicates.

2.2.7. Statistical analysis

The experimental design was developed using the data in **Table 1** with Design Expert® software version 7.0.0 (Stat-ease, Inc. Minneapolis, USA). The data were expressed as mean ± SEM and analyzed by one-way ANOVA, followed by Dunnett's test. The data were considered statistically significant at $p<0.05$.

3. RESULTS

3.1. Physical Evaluation/Organoleptic Characteristics

All the formulations had a cosmetically appealing appearance and smooth texture, and they were all homogenous with no signs of phase separation. All products

had a pseudoplastic behavior (as shear increased, the viscosity of formulation decreases), with the particle size of the globules ranging from 0.2 to 0.4µm. The particle sizes did not vary markedly for the various formulations. Thus, given varying concentrations of the oil phase and emulsifying phase, the particle sizes of the creams are comparable. The pH of the cream was evaluated immediately (i.e., 24 h post-production) and after storage for three weeks. The pH values were found to be relatively constant, with little variation in their values after three weeks (**Table 2**).

3.2. Evaluation of Creaming Indices

The various formulations of the cream on observation at 24 h, seven days, and 15 days after formulation were found to exhibit creaming of various levels (**Figure 1**). However, Samples S1 and S3 were found to have the highest creaming index. These corresponded to formulations with the lowest amounts of oil phase (26.5% and 25%, respectively). It also showed that as the oil phase reduced, there were more significant changes in creaming and cream stability after seven days and 15 days. This effect was most marked for formulations with lower amounts of emulsifier and oil phase (S1) and evident in formulation with low emulsifier alone (S9), which showed better stability (creaming) due to the higher amount of oil phase present. The formulation containing the lowest emulsifier and oil phase (S3) showed the highest creaming (**Table 2**).

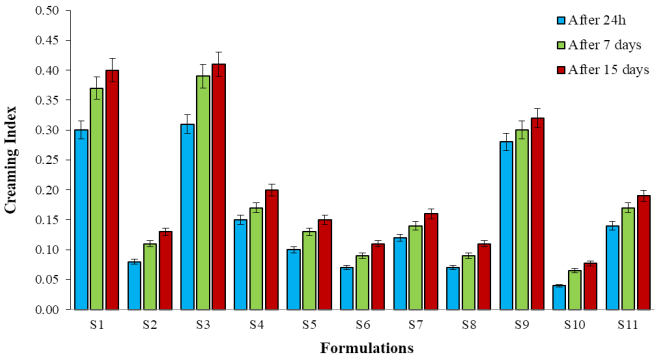

Figure 1. *Evaluation of creaming index of the polyherbal cream formulations (n=3)*

3.3. Microbial Limit Test

The creams were microbiologically tested using tryptone soy agar on day 1 and day 30. On observation of the prepared plates after incubation for 48 h, it was discovered that there was no growth of bacteria on the prepared plates. On repeating the tests after 15 days, all the samples except S2 were found to have no growth on the plates.

3.4. Evaluation of the Antioxidant Activity

The absorbance of the various extracts was obtained using a UV-VIS Spectrophotometer (Mettler Toledo AVI 195-1100nm). 800 µg/mL of the extract had a DPPH radical scavenging activity of 71.23% against control % inhibition of reference 98.01%, as shown in **Figure 2**.

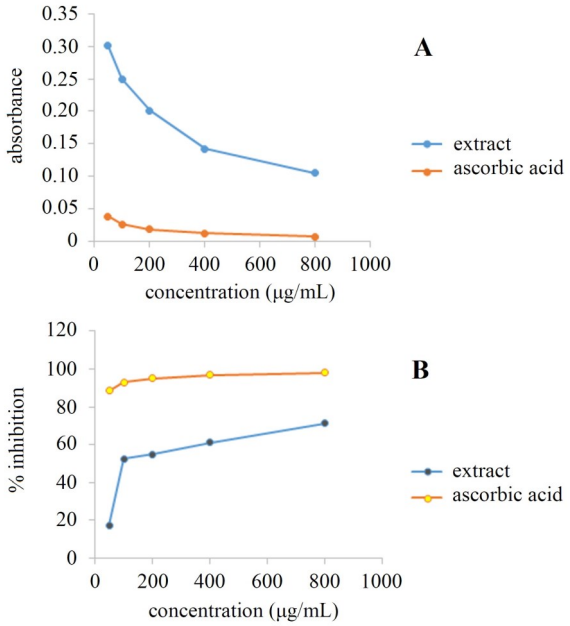

Figure 2. *Plot of absorbance of extracts and ascorbic acid at various concentrations (A) and evaluation of DPPH radical scavenging activity of extract (B) (n=3)*

3.5. Modeling and Analysis Using ANN

3.5.1. Validation of ANN model results

The values of viscosity, spreadability, and particle size predicted by the optimum ANN model are presented in **Table 3** alongside the experimental values for comparison. There was reasonable agreement between the model predictions and the experimental values. The comparison of the experimental values of the response and those predicted by the ANN model showed that there was an acceptable level

Table 3. *Comparison of experimental results with ANN predicted results*

Run	Input Factors		Responses					
			Viscosity (cP)		Spreadability (%)		Particle size (µm)	
	X_1	X_2	Experiment	ANN	Experiment	ANN	Experiment	ANN
S1	30.0	9.0	6480	6997	40.30	39.93	0.14	0.15
S2	30.0	7.0	5936	5936	34.15	34.15	0.17	0.17
S3	33.5	7.6	4362	4362	28.78	28.78	0.12	0.12
S4	26.5	7.6	5668	5668	44.19	44.19	0.12	0.12
S5	35.0	9.0	7924	7924	49.83	49.83	0.09	0.09
S6	25.0	9.0	5368	5368	27.09	27.09	0.11	0.11
S7	30.0	9.0	8020	6997	38.77	39.93	0.15	0.15
S8	33.5	10.4	6002	6002	33.57	33.57	0.11	0.11
S9	30.0	9.0	6490	6997	40.73	39.93	0.17	0.15
S10	30.0	11.0	6568	6568	38.09	38.09	0.11	0.11
S11	26.5	10.4	8014	8014	34.03	34.03	0.14	0.14

of fit between the experimental and model-predicted results. The goodness of fit of the ANN models for viscosity, spreadability, and particle size are shown in **Table 4**. A good fit was obtained between the model prediction and the experimental observations. This claim is supported by the high R^2 and adjusted R^2 values. Beyond that, the error terms (RMSE, AAD, and MAD) were relatively small compared to the mean of the observations [1,7].

Table 4. *The goodness of fit statistics for ANN models*

Parameter	Response		
	Viscosity (cP)	Spreadability (%)	Particle size (µm)
R^2	0.8850	0.9949	0.9304
R^2 Adj	0.7700	0.9898	0.8608
Mean	6439	37.2300	0.1300
RMSE	396.98	0.4732	0.0055
AAD	0.0259	0.0053	0.0196
MAD	884	5.1872	0.02182

3.5.2. Optimization of input factors and responses

The results of optimization carried out using genetic algorithm (GA), particle swarm optimization (PSO), and rotation inherit optimization (RIO) are summarized in **Table 5**. From the results, there was virtually no difference between the values obtained from the three optimization methods. The optimal values of viscosity, spreadability, and particle size obtained from the genetic algorithm were 8355.86 cP, 47.01%, and 0.12µm. These were obtained with oil and emulsifying phase levels of 27.86% and 11% (**Table 6**).

Table 5. *Summary of optimization results*

Variable	GA	PSO	RIO
Oil phase (%)	27.86	27.86	27.87
Emulsifying phase (%)	11.00	11.00	11.00
Maximum viscosity (cP)	8355.86	8355.91	8355.87
Maximum spreadability (%)	47.01	46.98	47.02
Maximum particle size (µm)	0.12	0.12	0.12

Table 6. *Comparison of predicted and actual optimized results*

Results	Predicted Optimized Values	Observed Optimized Values
Viscosity (cP)	8355.86	8312.67
Spreadability (%)	47.01	46.24
Particle Size (µm)	0.12	0.117

The response surface plots presented in **Figure 3A** to **Figure 3D** show the relationship between the responses (viscosity, spreadability, and particle size) and the input factors (oil phase and emulsifying phase). Utilizing the architecture of the optimal ANN for predicting viscosity, spreadability, and particle size viscosity was found to increase with an increase in the level of the emulsifying phase, as shown in **Figure 3A**. The reverse was observed to the oil phase level as the viscosity was observed to decrease when the oil phase was increased. High levels of emulsifying phase combined with low oil phase levels were found to yield high viscosity values. Spreadability decreased with an increase in the emulsifying phase, as shown in **Figure 3B**.

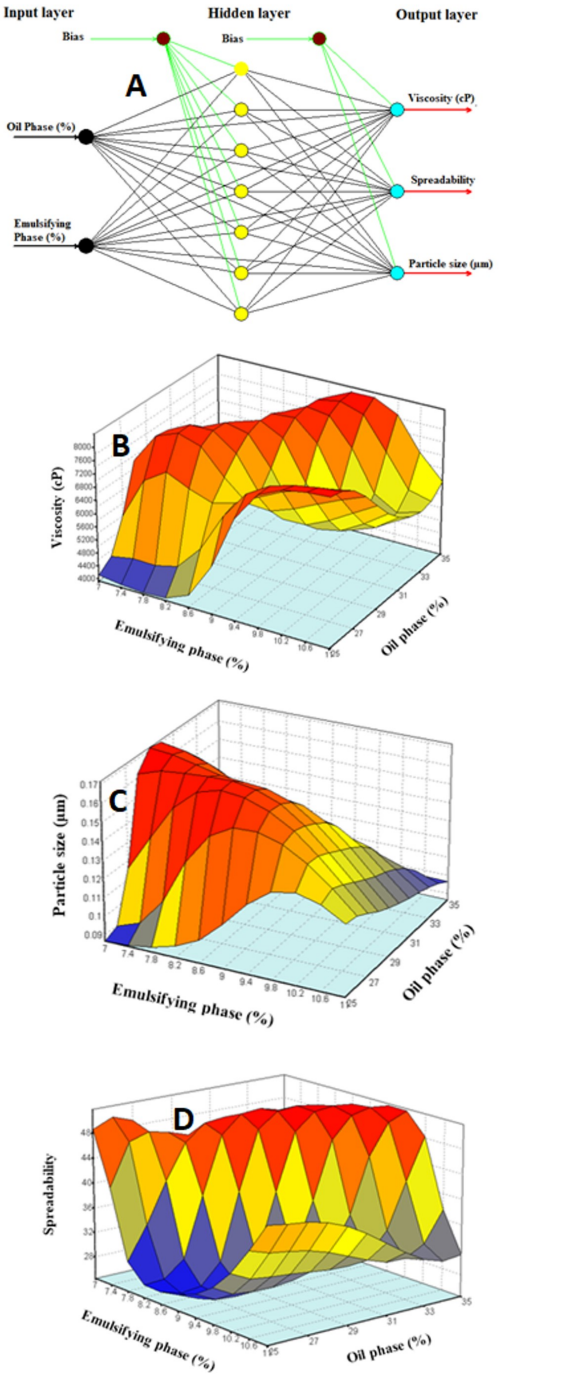

Figure 3. *Architecture of the optimal ANN for predicting viscosity, Spreadability and Particle Size (A); response surface plot showing the effect of oil phase and emulsifying phase on viscosity (B); response surface plot showing effect of oil phase and emulsifying phase on particle size (C); response plot showing effect of oil phase and emulsifying phase on spreadability (D).*

Similarly, spreadability also decreased with an increase in the oil phase level, although its effect was not significant compared to the emulsifying phase. Particle size increased with an increase in the emulsifying phase level, as seen from **Figure 3C**. On the other hand, the utilization of intermediate oil phase levels resulted in high particle size values.

4. DISCUSSION

Despite the multitude of alternatives available at present, the interest in natural product cosmeceuticals is increasing. The medicinal plants present great potential in developing such new products considering different beneficial properties

such as antioxidant effects, stimulation of collagen synthesis, improvement of the skin elastic properties, photoprotection, and moisturizing. Besides, natural ingredients are well tolerated, and the incidence of side effects to herbal extracts is extremely rare [11,12]. They are usually derived from extensive research and, since there are of mostly natural origin, do not have associated side effects synthetic products have, such as irritancy and carcinogenicity. The Draize test carried out showed the cream formulations had no sensitizing effects like erythema and oedema.

The free radical scavenging property of the cream was analyzed. In **Figure 2A**, the absorbance of the extract decreased as the concentration of the extract increased. This could be attributed to the effect of the antioxidants present in the extracts (lemongrass, basil, and roselle aqueous extracts). These antioxidants reacted with the free radicals of DPPH; they thus depleted the amount of the DPPH radicals present in the reaction mixture as shown in **Figure 2B**, which illustrates the percentage inhibition of the extracts as concentration increased. However, the antioxidant potential of the extracts, as seen from **Figure 2B**, is not as high as that of the reference used (Ascorbic acid). Still, it showed marked free radical scavenging properties.

Cetyl palmitate and cetostearyl alcohol were chosen for their thickening properties as well as inherent emulsifying ability. The percent used (1%) was arrived at after trial and error using initially higher concentrations in formulating sample batches. Initial products formed at higher concentrations of Cetylpalmitate and Cetostearyl alcohol were too viscous and presented difficulty in emulsification with the hand blender used for this work. The plant extracts used all have been shown to have various degrees of antioxidant, anti-aging, and antimicrobial properties [3-4,12-14]. However, in these studies, the cold water extracts of the plants were found to have moderate to low antimicrobial properties. The finding of this work agreed with their findings. The concentration of the crude extracts used is low 0.25%, and as such, their antimicrobial properties will be at a minimum. This necessitated the use of preservatives.

The emulsifiers (span 60 and tween 80) chosen for the work have also been shown to form stable cream formulations that are non-irritant and non-toxic at use concentrations used for the work [13]. During the emulsification of the cream, some other factors which were not taken into consideration in this study might have had some undetermined influence on the properties of the cream formulation of interest (particle size, spreadability, and viscosity). Such factors include the duration of emulsification, the temperature at which the oil and aqueous phases were mixed before emulsification, the distance the homogenizer was immersed into the formulation during emulsification, and the speed of the blending equipment rotor blades. However, to reduce the influence of these factors, these attributes were kept as much as possible constant, and each cream formulation was prepared in triplicate to reduce error due to unforeseen variability.

The central composite design methodology was found not to provide a good prediction of an optimized product using experimentation data. Thus, artificial neural networks software was used to design an optimization model and predict an optimized product and also to generate response surface plots showing the relationship between the various independent variables and the dependent variables. The optimization predictions were confirmed by preparing in triplicate cream formulations using the recommended values of the independent variables. The average responses from the trials obtained were found to be comparable with predicted optimization results. This thereby confirms that the formulation has been optimized. The optimized formulation was also found to possess a low creaming index, no variation in pH on standing, and other physicochemical properties on storage.

The response surface plots show the relationship between the responses (viscosity, spreadability, and particle size) and the input factors (oil phase and emulsifying phase). Viscosity was found to increase with an increase in the level of the emulsifying phase. This could be attributed to the availability of the emulsifier to form a micellar layer around the oil globules and thus stabilize the formed emulsion. A minimum amount of surfactant is needed to cover the smallest possible droplets, protecting them against coalescence and Ostwald ripening. But, as already noticed by Nikovska *et al.* [15], there is an optimum value of emulsifier required for emulsion stability. This optimum value is attributed to the competing role of repulsive structural versus attractive depletion forces. At low micellar concentrations, the depletion interactions between droplets result in lower stability, while at higher micellar concentrations, the structural forces induce a repulsive energy barrier, which enhances stability. This is evident from the study as for the increase in emulsifier concentration.

The reverse was observed concerning the oil phase level as the viscosity was observed to decrease when the oil phase was increased. The increased oil phase and reduced emulsifier concentration amounted to reduced emulsification on agitation. The creaming behavior correlates with the reports from Nikovska *et al.* [15], where the emulsions with higher viscosity show better emulsion stability against creaming. Therefore, at low oil concentration, the viscosity of the emulsion is low, droplet aggregation and floc formation are enhanced, and creaming is rapid since the weakly flocculated network simply collapses under its own weight [16]. On the contrary, in the emulsions containing higher oil phase concentrations, droplets are more densely packed, which increases emulsion viscosity, enhances the inter droplet interactions and network formation, and thus lowers the creaming rate [14].

Spreadability decreased with an increase in the emulsifying phase. Similarly, spreadability also decreased with an increase in the oil phase level, although its effect was not significant compared to the emulsifying phase. The above factors have already been shown to cause an increase in viscosity of the cream formulation. Thus, it can be said that as the viscosity of the preparation increased, the spreadability of the formulation decreased. This agreed with the findings of Inoue *et al.* [17], which showed that differences in the oil and water content of cream formulations affected the physicochemical properties of a cream, such as skin penetrability, viscoelasticity, flattening, and internal structure.

Particle size increased with an increase in the level of the emulsifying phase, as seen. On the other hand, intermediate levels of the oil phase resulted in high values of particle size. This agreed with Nikovska *et al.* [15] in establishing that droplet/particle aggregation was dependent on surfactant availability. However, this was up to an optimum value, which was predicted using anterior neural networks to be at optimum values of 0.12 µm. Other factors, such as homogenizer emulsification speed, temperature, polymer-surfactant interactions, and duration of emulsification, have been identified in the literature to affect the particle size of the final product [18]. Polydispersity/droplet size distribution also like viscosity, has also been shown to be related to the stability of the cream. As the oil phase increased, the particle size of droplets was found to increase. This is due to the increased coalescence which occurred in the formulation. Thus, the droplets formed were bigger. Temperature, duration of emulsification, and emulsification speed could be other factors that could influence the particle size.

The predicted values for the optimized cream formulation considered the various factors already discussed above (viscosity, spreadability, and particle size). The predicted values did not differ significantly from the experimentally obtained values. The formulated cream was of moderate viscosity and on examination for creaming showed no creaming after seven days. Some creaming was observed after 15 days. The value of the emulsifier predicted from optimization is 11%, which is the maximum percentage of the emulsifier used in the experimental design. However, there is a possibility that increasing the amount of the emulsifier will lead to products of superior stability profile, viscosity, and spreadability.

5. CONCLUSION

Formulation of the polyherbal face cream containing *Cymbopogon citratus, Hibiscus sabdariffa,* and *Ocimum gratissimum* extracts were prepared and were shown to have significant antioxidant activity. Central Composite Design was used to prepare a framework for the formulation design. This framework was then used to prepare various cream formulations using palm oil as the oil base and Tween 80/ Span 60 as the emulsifying phase. Using advanced neural network modeling, a prediction of an optimized formulation was made. The optimized values for oil and emulsifying phase were found to be 27.86% and 11%, respectively. The predicted responses were found to be: spreadability indices, 47.01%, particle size, 0.12 µm, and viscosity of 8355.85 cp. The optimized formulation was prepared and was shown to have comparable results with the predicted values.

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CONFLICT OF INTEREST DECLARATION

The authors report no conflict of interest. The authors alone are responsible for the content and the writing of the paper.

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