



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 $95.01\% \pm 0.07$ ($p = 0.018$). The different variants of the topically applied soap bar were non-irritant, compatible with human skin, and showed no sign of allergic contact dermatitis.

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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 \pm 1.5°C, at a relative humidity of 45% \pm 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 (teometer), 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 \pm 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 \pm 0.11	
Post-wash	6.33 \pm 0.10	*0.041
Average trans-epidermal water loss		
Pre-wash	13.92 g/Hm ² \pm 0.21	
Post-wash	12.18 g/Hm ² \pm 0.14	0.052
Sebum skin content		
Pre-wash	9.00 μ g/cm ² \pm 0.03	
Post-wash	1.27 μ g/cm ² \pm 0.07	*0.018

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 \pm 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% \pm 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

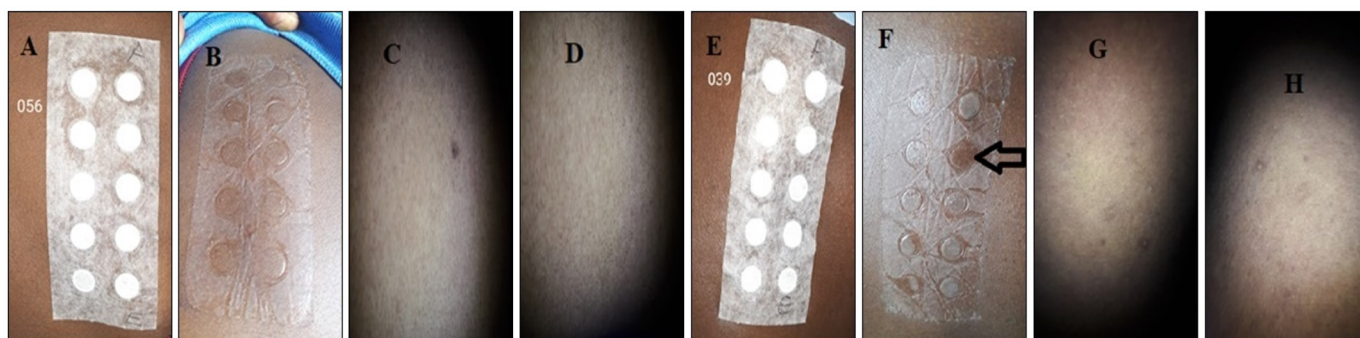


Figure 1. (A) Patch test for study participant No. 56 at Day 1 and (B) 96 hours post-test (C) Pre-wash dermoscope picture and (D) Post-wash dermoscope picture for participant No. 56; (E) Patch test for study participant No. 39 at Day 1 and (F) 96 Hours post-test with arrow showing slight erythema (G) 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 dehydroepiandrosterone 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/cm² ± 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/cm²; 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.

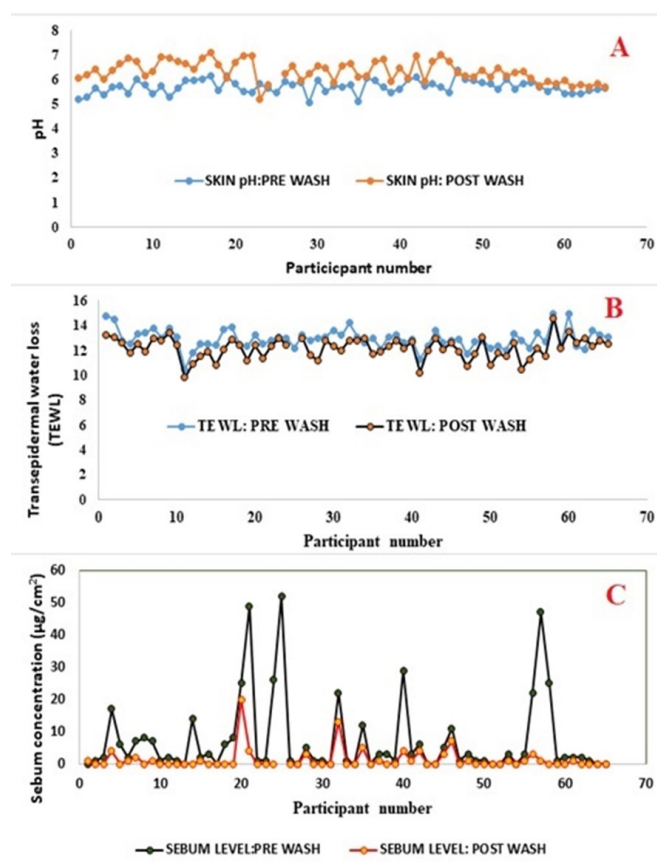


Figure 2. Pre-wash and Post-wash variation of (A) skin pH (B) trans-epidermal water loss (C) sebum concentration in a cross-section of the Nigerian population (n=65)

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/Hm², 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 $11.5 \pm 0.14 \text{ g/Hm}^2$ [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 $95.01\% \pm 0.07$ ($p=0.018$). 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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